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RARE DE NOVO AND TRANSMITTED COPY NUMBER VARIATIONS IN AUTISM SPECTRUM DISORDERS: IMPLICATIONS FOR FUNCTIONAL NETWORKS OF GENES INVOLVED IN NEUROGENESIS, NEURONAL METABOLISM, SYNAPTIC FUNCTION, NEUROIMMUNITY, INTRACELLULAR SIGNALING AND CHROMATIN REMODELING

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To my husband

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INTRODUCTION

1. Autism Spectrum Disorders (ASD)

1.1 Classification and clinical diagnosis

Autistic behaviors were independently identified as recognizable syndromes in the early 20th century by Heller [Heller, 1908] and, subsequently, by Kanner [Kanner, 1943] and Asperger [Asperger, 1944]. In particular, Prof. Kanner described autism in 1943 in 11 children manifesting withdrawal from human contact as early as age 1 year postulating origins in prenatal life [Kanner, 1943]. In the Diagnostic and Statistical Manual of Mental Disorders IV-Text Revised (DSM-IV-TR) [Task Force on DSM-IV, 2000] the autism diagnosis spans a broad continuum of what are collectively known as Autism Spectrum Disorders (ASD) or Pervasive Developmental Disorders (PDD). ASD include several conditions, namely full-syndrome autism (Autistic Disorder or Idiopathic Autism) [MIM 209850], Childhood Disintegrative Disorder, Asperger Syndrome (AS) and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS) [Task Force on DSM-IV, 2000].

The latest estimates put the population prevalence of ASD at approximately 1 in 110 [Autism and Developmental Disabilities Monitoring Network, 2009]. Incidence appears to be independent of ancestry and demographics, when similar rates being found on a global scale when the same diagnostic tools are used [Fombonne, 2009]. ASD show a 4:1 male to female gender bias, which may rise to 11:1 when considering Asperger disorder [Gillberg *et al*., 2006].

Although heterogeneous, ASD are united by a combination of three core behavior symptoms: a) impaired language and communication; b) deficiencies in social interaction; c) restricted interest and repetitive stereotypic behavior [Task Force on DSM-IV, 2000]. Symptoms of ASD usually begin in early childhood with evidence of delayed development before age 3 years, although prospective studies of children at higher risk have shown that deficits in social interaction and communication may be starting in the first 6-12 months of life [Pizzarelli and Cherubini, 2011]. The fully-autistic patients show impairments in all the three areas of behavior previously described, whereas AS patients have deficits in social interaction and behavior but normal cognitive development and language skills. A diagnosis of PDD-NOS is instead placed in those patients who meet the diagnostic criteria for autism, but with a later onset, or in those patients who show two out of the three core behavior symptoms [Task Force on DSM-IV, 2000]. Furthermore, about 50-70% of children with autism are identified as intellectually disabled by nonverbal IQ testing and approximately 25% develop seizures [Baird *et al*., 2006; Tuchman and Rapin, 2002]. Autism can be considered complex due to the presence of dysmorphic features (25-30%) and/or microcephaly (5-15%) or macrocephaly (30%), or essential (i.e., absence of physical abnormalities and microcephaly).

Other common pathological disturbances may be present including anxiety, sensorial abnormalities, gait and motor disturbances, sleep disturbances and comorbidity with psychiatric disorders such as attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD) and mood disorder [Geschwind, 2009].

Rating scales helpful in establishing the diagnosis are Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) in combination with clinical presentation [Lord *et al*., 1989, 1994].

1.2 Etiopathogenesis

1.2.1 Genetic architecture in Autism Spectrum Disorders

The etiology of ASD is complex and encompasses the roles of genes, the mitochondria, the environment, and the immune system. However, there is strong evidence for the importance of complex genetic factors comprised of different form of genetic variations in the etiology of ASD. Twin and family studies have, indeed, established the preponderant genetic basis of autism and indicate that the heritability of autism is over 90% [Abrahams e Geschwind, 2008; Freitag, 2007; Monaco and Bailey 2001; Muhle *et al*., 2004; Persico e Bourgeron, 2006], which is the highest heritability value so far associated with a neuropsychiatric disorder [Schaaf and Zoghbi, 2011]. Furthermore, the recurrence risk for ASD varies by gender for the second child to be affected (4% if the first child affected is a female and 7% if a male), whereas the recurrence rate increases to 25-30% if the second child is also diagnosed with ASD [Constantino *et al*., 2010; Ozonoff *et al*., 2011; Rosenberg *et al*., 2011].

The genetic causes of ASD can be classified as follows (**Figure 1**):

1) ASD-related monogenic syndromes;
2) rare chromosomal abnormalities;
3) rare copy number variations (CNVs);
4) rare gene mutations;
5) common genetic variants.

1) Approximately 10% of patients with ASD have an identifiable Mendelian condition or genetic syndrome (**Fig. 1**) [Devlin and Scherer, 2012]. This means that these patients are characterized by a complex phenotypic picture, showing autistic traits as well as craniofacial dysmorphism and/or congenital malformations. Unlike idiopathic autism, syndromic ASD have a sex ratio M:F of 1:1.

Among these syndromes the most frequent are Fragile X syndrome (~1-2% of ASD cases), Tuberous sclerosis (~1%), PTEN macrocephaly syndrome (1%), and Rett syndrome (~0.5-1%). In detail, Fragile X syndrome is caused by expansion of the CGG trinucleotide repeat in the *FMR1* gene to the full mutation size of 200 or more CGG repeats. Molecular studies indicate that FMR1

may cause the autism phenotype via two mechanisms: RNA toxicity to the neurons and gene silencing that affects neuronal connectivity [Hagerman *et al*., 2008; Handa *et al*., 2005; Schenck *et al*., 2003].

The *PTEN* (phosphatase and tensin homolog) gene was initially described as a tumor suppressor gene associated with a broad group of disorders referred to as PTEN hamartoma tumor syndrome. More recently, *PTEN* mutations have been associated with autism and macrocephaly [Butler *et al*., 2005; Buxbaum *et al*., 2007; Delatycki *et al*., 2003; Parisi *et al*., 2001; Zori *et al*., 1998], and PTEN is recognized to play an important role in brain development, neuronal survival and synaptic plasticity.

Rett syndrome was initially classified by the DSM-IV as a pervasive developmental disorder (PDD) and it is the only PDD for which a specific genetic etiology has been identified [Amir *et al*., 1999]. Ninety-six percent of individuals with classic Rett syndrome have mutations in the X-linked *MECP2* gene [Moretti and Zoghbi, 2006] and *MECP2* mutations have been reported in approximately 1% of children diagnosed with autism [Lintas and Persico, 2009; Moretti and Zoghbi, 2006]. Evidence of variable expression of MeCP2 in the brains of individuals with both autism and Rett syndrome and evidence that MeCP2 deficiency can reduce expression of the genes *UBE3A* and *GABRB3*, which are implicated in autism, indicate some causal relationship between the two disorders [Samaco *et al*., 2004, 2005].

Other rare syndromes associated with ASD (<1%) are, for example, Neurofibromatosis type I, Sotos, Timothy, and Joubert syndromes. Moreover, in a recent review over than 103 disease genes were identified among subjects with ASD or autistic behavior [Betancur, 2011]. These genes have all been previously implicated in ID, thus suggesting that these two neurodevelopmental disorders share common genetic basis.

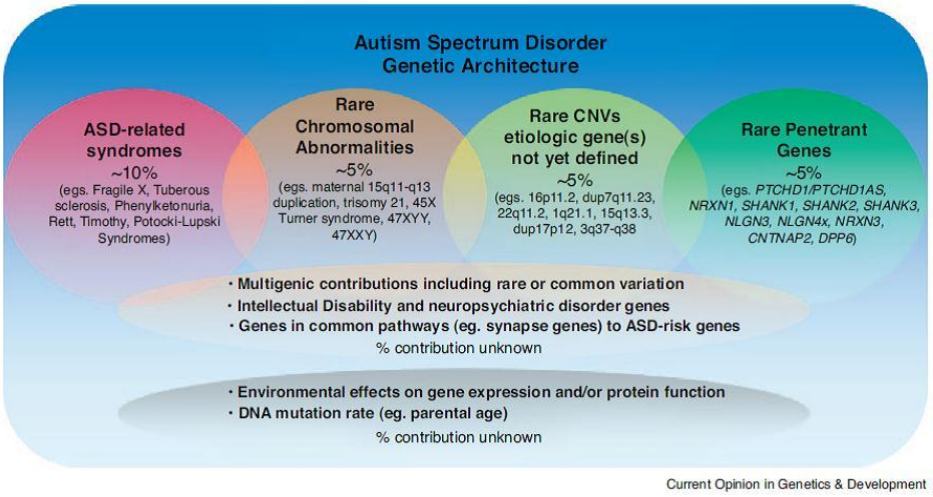

Fig. 1. Genetic architecture in ASD. Four groupings are shown of rare genetic risk factors and their estimated contribution to ASD. Genetic contributions to ASD can also arise from direct or indirect effects on genes and proteins by environmental influences [Devlin and Scherer, 2012].

2) High resolution karyotyping reveals cytogenetically chromosome rearrangements in ~5% of individuals with ASD (**Fig. 1**). Interestingly, the largest unbalanced chromosomal anomalies were found in syndromic-ASD [Devlin and Scherer, 2012; Jacquemont *et al*., 2006; Miles *et al*., 2005]. Although cytogenetic abnormalities on almost every chromosome have been found in autism, only a few occur commonly enough to be possible loci for autism genes [Reddy, 2005; Vorstman *et al*., 2006; Wassink *et al*., 2001]. Among them, the most common cytogenetic abnormality found in individuals with ASD (3-5%) is the maternally derived 15q11-q13 duplication of the Prader-Willi/Angelman syndrome critical region, which is generally the result of a *de novo* supernumerary isodicentric 15q chromosome and less commonly the result of a maternally derived interstitial 15q duplication. The maternally derived 15q11-q13 interstitial duplication is a highly penetrant cause of autism, whereas the paternally derived duplication has little or no phenotypic effect, indicating the significance of genomic imprinting of this region [Hogart *et al*., 2010].
Other aneuploidies in ASD include trisomy 21 [Kent *et al*., 1999], 45,X Turner syndrome [Skuse 2000], deletions of 2q37, 18q, 22q13.3, Xp22.3, and the sex chromosome aneuploidies 47,XYY, 47,XXY [Gillberg, 1998; Jha *et al*., 2007; Manning *et al*., 2004; Marshall *et al*., 2008; Vorstman *et al*., 2006; Shinawi *et al*., 2009a].

3) Rare *de novo* and inherited CNVs can also contribute to the genetics of ASD in as many as ~5-7% of ASD cases reported of unknown cause [Christian *et al*., 2008; Devlin and Scherer, 2012; Gilman *et al*., 2011; Kumar *et al*., 2008; Levy *et al*., 2011; Marshall *et al*., 2008; Sanders *et al*., 2011; Sebat *et al*., 2007; Weiss *et al*., 2008]. The yield was higher in those patients identified as having "syndromic" autism [Jacquemont *et al*., 2006]. These CNVs are typically too small to be detected by karyotyping and can involve a single gene acting much as a sequence-level mutation, or they can encompass several genes as part of a genomic disorder [Lee and Scherer, 2010]. Furthermore, larger CNVs often affect recurrent genomic regions leading to well recognizable microdeletion/microduplication syndromes.
Screening for CNVs by using array technologies such as array comparative genomic hybridization (aCGH with BAC or oligonucleotide clones) and SNP array, has proven to be a rapid method to detect both large and small changes associated with ASD susceptibility. In studies of idiopathic ASD, the most common recurrent anomaly is a ~600 kb microdeletion/microduplication of chromosome 16p11.2 (0.8%) [Kumar *et al*., 2008; Marshall *et al*., 2008; Weiss *et al*., 2008]. Of note, this CNV is also observed in ASD cases with additional dysmorphology [Fernandez *et al*., 2010; Shinawi *et al*., 2010], in a variety of other disorders including schizophrenia (SCZ), bipolar disorder (BD), seizures, ADHD, and dyslexia, as well as in apparently unaffected family members; thus, interpretation of the significance of this anomaly can be difficult [McCarthy *et al*., 2009; Rosenfeld *et al*., 2010; Shinawi *et al*., 2010].

The 15q13.3 microdeletion syndrome is characterized by a highly variable phenotype and incomplete penetrance, including ID, seizures, subtle facial dysmorphism ad neuropsychiatric disorders such as ASD [Ben Shachar *et al*., 2009; Miller *et al*., 2009; Pagnamenta *et al*., 2009; Sharp *et al*., 2008]. The reciprocal duplication have also been reported in association with ASD/autistic features [Guilmatre *et al*., 2009; Miller *et al*., 2009; Szafranski *et al*., 2010; van Bon *et al*., 2009]. Both the deletion and duplication span the *CHRNA7* gene, a candidate for epilepsy [Shinawi *et al*., 2009b].

Recently, it has been underlined the importance of the 7q11.23 locus in ASD pathogenesis. Indeed, while the deletions of this region cause Williams-Beuren syndrome, a contiguous gene syndrome which is comorbid with ASD [Challman *et al*., 2003; Gillberg and Rasmussen, 1994; Gosch and Pankau, 1994; Herguner and Mukaddes, 2006; Klein-Tasman *et al*., 2009; Lincoln *et al*., 2007; Reiss *et al*., 1985], the reciprocal duplication is responsible for the 7q11.23 microduplication syndrome, which has been reported, among others, in patients with ASD and severe language delay [Berg *et al*., 2007; Depienne *et al*., 2007; Kirchhoff *et al*., 2007; Qiao *et al*., 2009; Stankiewicz and Lupski, 2010; Van der Aa *et al*., 2009].

The impact of CNV on ASD pathogenesis will be deeply covered in the next chapter.

4) CNV screening and direct sequencing of candidate genes are rapidly identifying genes for further characterization in relation to ASD, that overall contribute to a ~5% of the ASD genetic architecture (**Fig. 1**) [Devlin and Scherer, 2012]. These approaches have implicated, among others, genes encoding proteins for synaptogenesis such as *NRXN1* [Ching *et al*., 2010; Kim *et al*., 2008; Szatmari *et al*., 2007], *NRXN3* [Vaags *et al*., 2012], *NLGN3* [Jamain *et al*., 2003], *NLGN4* [Jamain *et al*., 2003; Laumonnier *et al*., 2004], *SHANK2* [Berkel *et al*., 2010, 2012; Leblond *et al*., 2012], and *SHANK3* [Durand *et al*., 2007; Moessner *et al*., 2007] as affecting ASD risk. Both neurexins (NRXN1 and NRXN3), which are located at the pre-synaptic plasma membrane, and neuroligins (NLGN3 and NLGN4), that are located at the post-synaptic plasma membrane, are adhesion molecules that contribute to synapse formation by directly interaction. The SKANK proteins act, instead, as scaffolding proteins at the post-synaptic density. Some rare, highly penetrant mutations in these genes appear as sufficient to be monogenic causes of ASD, including cases of syndromic autism [Baris *et al*., 2007; Berkel *et al*., 2010; Betancur, 2011; Ching *et al*., 2010; Dhar *et al*., 2010; Durand *et al*., 2007; Gauthier *et al*., 2009; Glessner *et al*., 2009; Guilmatre *et al*., 2009; Jamain *et al*., 2003; Kim *et al*., 2008; Manning *et al*., 2004; Marshall *et al*., 2008; Moessner *et al*., 2007; Pinto *et al*., 2010; Prasad *et al*., 2000; Szatmari *et al*., 2007].

More than 100 genetic and genomic loci have been reported in subjects with ASD, showing the success of ongoing efforts but also underscoring the fact that whole-exome and whole-genome sequencing will be critical approaches for identifying ASD genes and loci [Betancur, 2011].

Recently, a few papers reported the utility of whole-exome sequencing in pinpointing the contribution of single nucleotide variants (SNVs) to the risk of ASD. Four studies published in the current year have looked for *de novo* mutations and a fifth for recessive mutations. A role for *de novo* mutations in ASD has been, indeed, suggested by previous CNV screenings and smaller-scale exome sequencing studies. In 2011 O'Roak *et al.* [O'Roak *et al*., 2011] sequenced 20 individuals with sporadic ASD and their parents and identified four potentially causative *de novo* events in *FOXP1*, *GRIN2B*, *SCN1A*, and *LAMC3*, thus showing that family-based exome sequencing was a powerful approach for identifying new candidate genes for ASD.

More recently, Iossifov *et al*. [Iossifov *et al*., 2012] sequenced 343 family "quads" (i.e. the parents of a single child with ASD and his/her unaffected sibling), Sanders *et al*. [Sanders *et al*., 2012] 238 families, including 200 quads, O'Roak *et al*. [O'Roak *et al*., 2012] 189 trios (i.e., a child with ASD and his/her parents), and Neale *et al*. [Neale *et al*., 2012] 175 trios. Sequencing data from healthy parents and siblings allow the *de novo* point mutation rate to be estimated as $2x10^{-8}$ per base per generation, a value only slightly higher than that previously reported. Interestingly, the mutation rate was comparable between patients and unaffected siblings, although a shift in the mutation spectrum towards mutations that were predicted to disrupt protein function was found in the probands. Furthermore, it has been reported that most *de novo* mutations have a paternal origin and there is an increase in the number of mutations with paternal age [O'Roak *et al*., 2012].

In terms of the biology of ASD, a significantly enriched connectivity among the proteins encoded by the genes harboring *de novo* missense or nonsense mutations (~45% of *de novo* deleterious variants affects brain-expressed genes) as well as excess connectivity to either prior ASD genes of major effect or genes previously implicated in other neurodevelopmental disorders, has been reported, thus indicating that a subset of observed events are relevant to ASD risk. In particular, analysis of *de novo* variations provided evidence in favor of a few genes as genuine autism risk genes, namely *CHD8* and *KATNAL2* [Neale *et al*., 2012; O'Roak *et al*., 2012], *SCN2A* [Sanders *et al*., 2012], and *NTNG1* [O'Roak *et al*., 2012]. Of note, O'Roak *et al*. [O'Roak *et al*., 2012] identified a network linked to β-catenin and chromatin remodeling whereas Iossifov *et al*. [Iossifov *et al*., 2012] found out an enrichment of genes regulated by the Fragile-X-syndrome associated FMR1 protein. Of note, anomalies in all these pathways have been previously associated with ASD.

Finally, Chahrour *et al*. [Chahrour *et al*., 2012] used homozygosity analysis to identify probands from nonconsanguineous families that showed evidence of distant shared ancestry, suggesting potentially recessive mutations. Whole-exome sequencing of 16 probands revealed validated homozygous, potentially pathogenic recessive mutations that segregated perfectly with disease in 4/16 families. The candidate genes (*UBE3B*, *CLTCL1*, *NCKAP5L*, *ZNF18*) encode proteins involved in proteolysis, GTPase-mediated signaling, cytoskeletal organization, and other pathways

already implicated in ASD pathogenesis. Furthermore, it has been reported that neuronal depolarization regulated the transcription of these genes [Chahrour *et al*., 2012].

5) Although genetic linkage studies performed over the last fifteen years in multiplex families have suggested many chromosomal regions as well as single genes as susceptibility loci for ASD [Barret *et al*., 1999; IMGSAC, 1998, 2001; Persico *et al*., 2001; Philippe *et al*., 1999, 2002; Yonan *et al*., 2003; etc.], current evidences are tenuous for individual common variants that affect risk of ASD. Indeed, four large, independent genome-wide association studies (GWAS) have been reported so far, two assayed half-million single nucleotide polymorphisms (SNPs) each and detected a significant association at two different loci: 5p14.1 [Wang *et al*., 2009] and 5p15.2 [Weiss *et al*., 2009], and two assayed one million SNPs and reported a significant association for a SNP located at 20p12.1 [Anney *et al*., 2010, 2012]. Furthermore, in one recent study, defects in frontal lobe circuit connectivity have been associated with a SNP in the *CNTNAP2* gene [Scott-Van Zeeland *et al*., 2010], a putative ASD risk gene, subsequently confirmed by Anney *et al*. [Anney *et al*., 2012]. Thus, the overall data predict that while the existence of common variants affecting the risk of ASD is almost assured, their individual effects are modest and their collective effects could be smaller than that for rare variations [Anney *et al*., 2012].

1.2.2 Mode of inheritance

Despite the heritability of autistic disorder is now widely recognized, the mode of inheritance is not yet fully understood as a single model is likely not applicable in all patients. The most accepted model until recently is the polygenic/multigenic model (multiple-hit hypothesis), according to which the disease was due to the combined effect of a series of low-penetrance variants (SNPs, CNVs, inversions) in genes/susceptibility loci, initially estimated at around 10-20, not necessarily shared among different patients and belonging to a very large pool of genes [Folstein and Rosen-Sheidley, 2001]. Such variations are not to be considered causative *per se* but may confer an increased risk if inherited in particular combinations [Persico and Bourgeron, 2006]. Differences in combination and amount of these inherited mutations and/or the different interplay with the environment would lead to the onset of a fully-autistic or an ASD phenotype (**Fig. 2**).

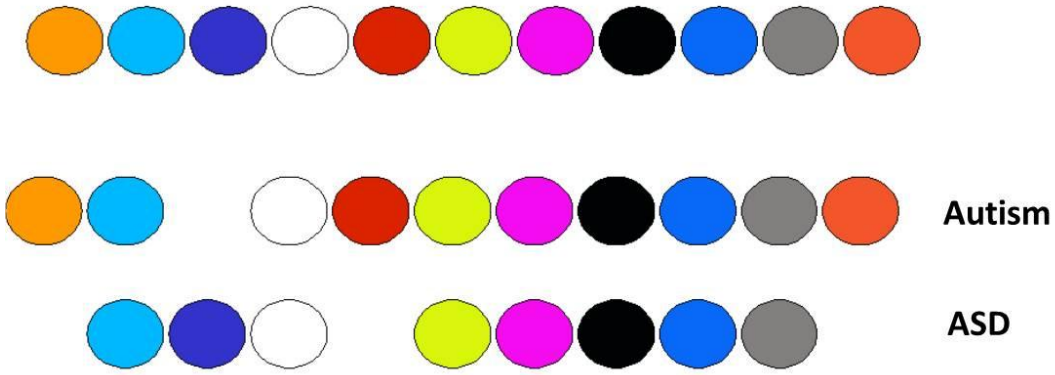

Fig. 2. Schematic view of the Polygenic-Multigenic inheritance model. There are several susceptibility loci in the genome which can be inherited in different amounts and combinations leading to a more or less severe autistic phenotypes, once exceeded the susceptibility threshold.

In 1999 Risch *et al*. [Risch *et al*., 1999] performed a genome-wide linkage study using a sample of 90 families, and, for the first time, proposed a model of heritability based on the existence of at least 10 susceptibility loci, which did not exclude a much highly heterogeneous etiology, in some cases also of Mendelian type. Recently, the number of loci potentially involved in ASD has grown to a few hundreds (130-400) [Gilman *et al*., 2011; Levy *et al*., 2011; Sanders *et al*., 2011].

However, there are many exceptions to the polygenic/multigenic model, represented by those patients in whom the onset of ASD is due either to a single, highly penetrating gene mutation, as in the case of syndromic and not syndromic monogenic ASD that follow a Mendelian genetics, or to a chromosomal rearrangement which can be considered causative *per se*. In addition, CNV screening of large ASD cohorts by using high-throughput, high resolution technologies, which have been performed during the last few years, highlights the importance of rare *de novo* and inherited CNVs of high penetrance in the etiology of these disorders [Gilman *et al*., 2011; Levy *et al*., 2011; Sanders *et al*., 2011], giving rise to a paradigm shift away from common variant model of ASD genetic architecture (based on low penetrating variants) to one suggesting a role for multiple rare and distinct genetic risk factors (with a higher penetrance), known as oligogenic heterozygosity model [Levy *et al*., 2011], which does not exclude, however, a modulation of the phenotype by common susceptibility genetic variants.

1.2.3 Mitochondrial abnormalities in Autism Spectrum Disorders

Inborn errors of metabolism may contribute to at least 5% of cases with ASD [Manzi *et al*., 2008]. Deficiency of certain enzymes in metabolic disorders leads to an accumulation of substances that can cause toxic effects on the developing brain, contributing to ASD. Indeed, a large proportion of patients with syndromic-ASD shows signs of dysfunction of mitochondrial energy metabolism including (a) high levels of lactate, pyruvate and alanine in the blood, urine and/or cerebrospinal

fluid (b) carnitine deficiency in serum (c) increased oxidative stress [Dhillon *et al*., 2011; Palmieri and Persico, 2010]. In the mitochondria ATP production, free oxygen radicals and reactive oxygen species (ROS) are produced and then normally removed from the cells by anti-oxidant enzymes. When the production of ROS and free radicals exceeds the limit, oxidative stress occurs leading to cell death by apoptosis or necrosis [Kannan and Jain, 2000]. Since brain cells have limited antioxidant activity, a high lipid content and high requirement for energy, it is more prone to the effects of oxidative stress [Juurlink and Paterson, 1998].

Coleman and Blass were the first to link to bioenergy metabolism disturbances with ASD. They reported lactic acidosis in four children with autism [Coleman and Blass, 1985]. Later, László *et al*. [László *et al*., 1994] reported increased serotonin, lactic acid and pyruvate levels in children with autism. Lombard then proposed that mitochondrial oxidative phosphorylation defects could cause abnormal brain metabolism in children with autism, leading to lactic acidosis and decreased serum carnitine levels [Lombard, 1998]. Additionally, muscle biopsies studied by Tsao and Mendell [Tsao and Mendell, 2007] and Shoffner *et al*. [Shoffner *et al*., 2010] in autistic patients showed single or combined defects in complex I, II, III, IV, and V.

These biochemical abnormalities are associated with variable phenotypes, which generally include complex neurological clinical features, congenital malformations and/or dysmorphism. In some ASD patients mutations or genomic rearrangements involving nuclear or mitochondrial genes, which encode mitochondrial enzymes, have been identified [Dhillon *et al*., 2011]. However, in most cases the genetic cause remains unknown although recent evidence emerged from the analysis of post-mortem autistic brains suggests that mitochondrial dysfunction represents a downstream effect of an immune system deregulation or a wrong calcium signaling [Palmieri and Persico, 2010].

Of note, early screening and treatment of these conditions may have a positive impact in preventing disease progress [Dennis *et al*., 1999; Dhillon *et al*., 2011].

Summing up, in most patients with idiopathic ASD (>70%) the genetic cause remains unknown (**Fig. 3**).

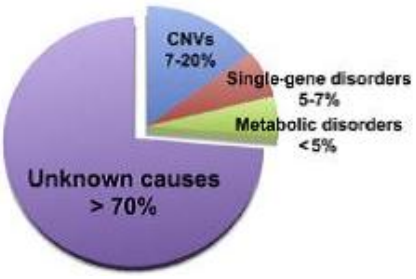

Fig. 3. Genetic causes of ASD which have been classified independently from the presence of a syndromic phenotypes [Schaaf and Zoghbi, 2011].

1.2.4 Immune dysfunctions in Autism Spectrum Disorders

Among the large number of ASD candidate genes recently uncovered by using genome-wide scanning technologies, several play important roles in immune function. For example, proteins encoded by *MET*, *PTEN*, *TSC1* and *TSC2*, have a major role in regulating interleukin (IL)-12 production from myeloid cells [Fukao *et al*., 2002], whereas the major histocompatibility complex type 2 (MHC-II) haplotypes [Lee *et al*., 2006; Torres *et al*., 2002], as well as complement 4B (C4B) [Odell *et al*., 2005], and macrophage inhibitory factor (MIF) [Grigorenko *et al*., 2008] are important in directing and controlling immune responses.

Several evidences suggest that ASD symptoms may be related to immune dysfunction [Careaga *et al*., 2010; Enstrom *et al*., 2009; Korade and Mirnics, 2011], supporting the importance of all arms of the immune system in immune regulation within the central nervous system (CNS) to maintain a healthy neuro-immune environment, that may be dysfunctional in ASD (**Fig. 4**). Indeed, several immune proteins function within the nervous system as mediators of normal neurodevelopment [Deverman and Patterson, 2009]. Cytokines, such as TNF-a, IL-1b, the TGF-b family of molecules, mediate direct effects on neuronal activity. For example, TNF-a inhibits neurogenesis and promotes neuron death, and plays an important role in synaptic pruning [Cacci *et al*., 2005; Stellwagen and Malenka, 2006; Widera *et al*., 2006]. Other neuropoeitic cytokines, such as IL-1b and IL-6, also exert varied effects on neuronal survival, proliferation, synapse formation, migration, and differentiation, thus suggesting that cytokines are both necessary for normal neurodevelopment and behavior and that any perturbation in the cytokine network can impact neurodevelopment (**Fig. 4**).

Moreover, microglial cells, that are the resident mononuclear phagocytic cells of the CNS, participate in immune surveillance of the CNS as well as in synaptic pruning in normal neurodevelopment [Bessis *et al*., 2007], through the production of inflammatory cytokines and the generation of reactive oxygen species (ROS) within the CNS [Garden and Moller, 2006; Hanisch and Kettenmann, 2007] (**Fig. 4**). The phagocytosis of dead or dying neurons by microglia is believed to be a normal and relatively non-inflammatory function [Bessis *et al*., 2007]. Furthermore, genetic abnormalities in microglia can result in profound effects on behavior.

Interesting findings from animal models suggest that neurogenesis is modulated by the interaction between T cells and CNS [Ziv *et al*., 2006; Ziv and Schwartz, 2008]. Altered T cell activation in ASD may therefore directly affect the course of neurodevelopment. In addition, increased levels of complement proteins can participate in synaptic scaling, opsonizing synapses and targeting them for removal by phagocytic microglia (**Fig. 4**).

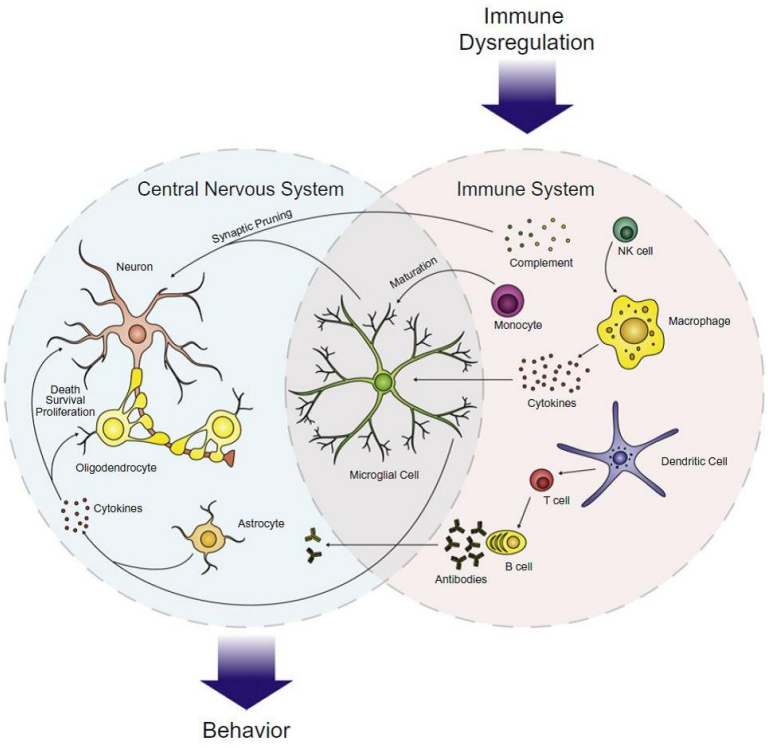

Fig. 4. Immune dysfunction in ASD involves a network of interactions between several cell types, from the innate and adaptive arms of the immune system [Onore *et al*., 2012].

Numerous immunological anomalies (both in the CNS and in the periphery) involving inflammation, cytokines, immunoglobulins, and cellular activation have been noted in individuals with autism. In detail:

- Neuroinflammation. A marked ongoing neuroinflammation, including microglia activation and increased inflammatory cytokine and chemokine production (e.g., IL-1b, IL-6, IL-12p40, TNF-a, CCL-2), has been reported in postmortem brain specimens from individuals with ASD [Li *et al*., 2009; Morgan *et al*., 2010; Vargas *et al*., 2005]. Furthermore, expression profiling of postmortem brain tissue from ASD individuals revealed increased transcript levels of several immune system associated genes, and gene co-expression networks showed abnormalities in cortical patterning [Voineagu *et al*., 2011]. These findings have been associated with changes in microglia and immune activation.

- Altered cytokine/chemokines/complement/adhesion molecule/growth factor protein profiles. Increased plasma levels of pro-inflammatory cytokines (IL-1b, IL-6, IL-8 and IL-12p40) as well as of chemokines have been reported in ASD [Ashwood *et al*., 2011b,d; Grigorenko *et al*., 2008; Kajizuka *et al*., 2010], which positively correlate with worsening in poor communication and social interaction behaviors.

- Altered immunoglobulin levels and auto-antibodies anti-CNS. Decreased total levels of IgM and IgG classes of immunoglobulin have been reported, with lower levels found to correlate with more aberrant behaviors [Heuer *et al*., 2008]. In addition, antibodies reportedly reactive to human and non-human primate brain and CNS proteins have also been described in children and adults with ASD. They include antibodies against serotonin receptors [Singh *et al*., 1997a], myelin basic protein [Singer *et al*., 2006; Singh *et al*., 1993; Vojdani *et al*., 2002], heat shock proteins [Evers *et al*., 2002], and glial filament proteins [Singh *et al*., 1997b]. The real role of these auto-antibodies in ASD is unknown and it hasn't been demonstrated yet whether any of these antibodies induce cellular damage or have any pathological consequence. However, it has been demonstrated that, irrespective of the target epitope, antibodies from ASD subjects bind specifically to cerebellar interneurons and Golgi type II cells in tissue obtained from rhesus macaque monkeys [Wills *et al*., 2009, 2011], thus leading, alternatively, to decreased or increased cellular activity. Furthermore, complement proteins can bind to auto-reactive antibodies, that is another mechanism which may lead to cell damage or death [Gasque *et al*., 2002], and an increase in complement proteins has been reported in sera from children with ASD.

- Anomalies in adaptive cellular response. Atypical adaptive T cell responses are observed in individuals with ASD [Ashwood *et al*., 2011c]. For example, a predominance of IL-4^+ IFN-γ^- T cells was observed in the circulating $CD4^+$ T cell population [Gupta *et al*., 1998], with a bias towards a T_H2 phenotype. An increased production of the pro-inflammatory cytokine TNF-a was also found, that is consistent with an activated T_H2 immune response in humans. Of note, TNF-a production was associated with increased stereotypical behavior, a hallmark symptom of ASD [Ashwood *et al*., 2011c]. Increased T cell activation may also be linked with decreased apoptosis leading to the survival of activated cells [Ashwood *et al*., 2011a], thus supporting a chronic inflammation as seen in chronic inflammatory conditions such as Crohn's disease [Monteleone *et al*., 2006].

Moreover, anomalies in circulating levels of soluble adhesion molecules P-Selectin, L-Selectin and PECAM-1 have been observed in patients with ASD. These proteins control the passage of T cells across endothelial barriers, thus mediating T cell/CNS interactions. In high functioning individuals with ASD levels of sPECAM-1, sP-Selectin and sL-selectin were decreased compared with controls [Iwata *et al*., 2008; Tsuchiya *et al*., 2007] and lower levels of P-Selectin were associated with more impaired social skills [Iwata *et al*., 2008], thus suggesting that modulating immune cell access to the brain in ASD may influence abnormal social interactions.

- Anomalies in innate cellular response. Atypical natural killer (NK) cell activity has been described in patients with ASD in terms of reduced lytic activity, as well as an increasing number of circulating monocytes [Sweeten *et al*., 2003]. These atypical monocyte responses are intriguing, and indicate abnormal myeloid involvement in ASD. Hyperactivation of myeloid cells in ASD is

implicated in both the periphery and CNS, as increased infiltration of monocytes and perivascular macrophages are observed in brain specimens from individuals with ASD [Vargas *et al*., 2005].

Collectively, evidence of atypical cytokine production, altered T cell activation and potential impaired apoptotic activity suggest there is a predisposition to chronic inflammation which could negatively affect healthy cognitive development in ASD. Furthermore, it is well known the role of the blood brain barrier in regulating the interaction between immune cells and CNS, as explained in **Fig. 5**, which can be compromised in autistic patients [Goines and Van de Water, 2010].

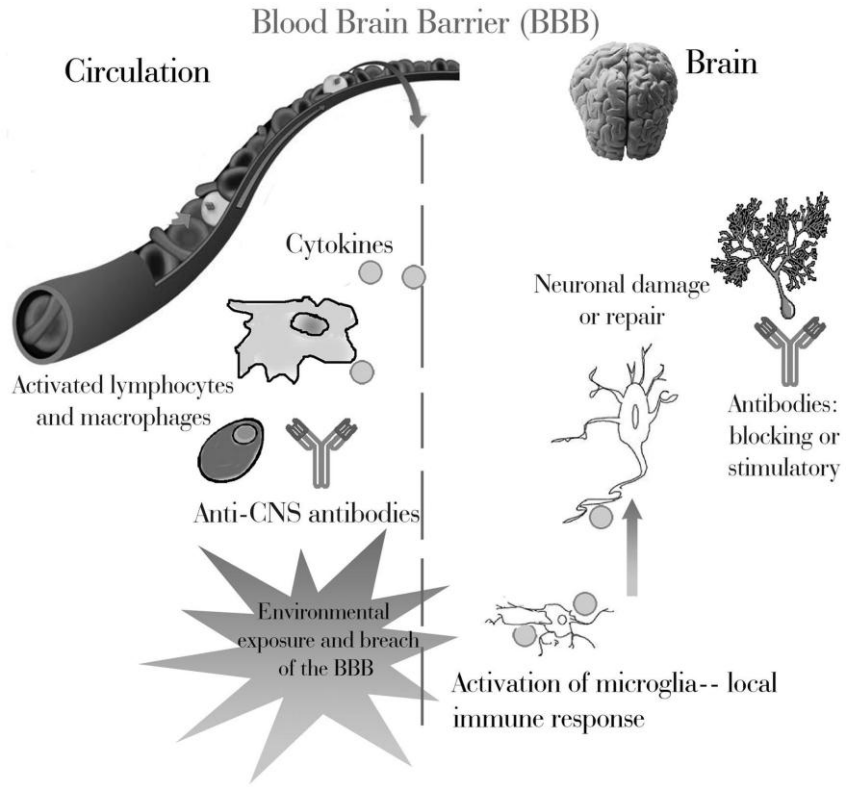

Fig. 5. Interactions between the Immune and Central Nervous Systems (CNS) in Autism Spectrum Disorders During postnatal life, an intact blood brain barrier (BBB) limits the entry of immune species into the brain (left). Lymphocytes, macrophages, various cytokines, and antibodies are generally maintained in the periphery. However, the blood brain barrier is permeable during fetal development and can be compromised by infections and environmental exposures throughout life, and the absence of a complete barrier allows immune components access to the brain. Individuals with autism (right) show alterations in BBB permeability which lead to increased pro-inflammatory cytokines in the brain, as well as activation of resident immune cells known as microglia. Additionally, antibodies that target brain tissues have been described in both children with autism and their mothers [Goines and Van de Water, 2010].

1.2.5 Environmental factors

The absence of a known genetic cause in most ASD cases, and the incomplete penetrance of known genetic risk factors, suggests that environmental factors are linked with the causation of ASD. In particular, in some individuals with ASD the development of the disorder seems to depend on the interactions between a "vulnerable" genome and unfavorable environmental factors (pollutants and neurotoxins, viral infections, and maternal factors), which may alter the neurodevelopment [Herbert *et al*., 2006; Newshaffer *et al*., 2007] (**Fig. 6**).

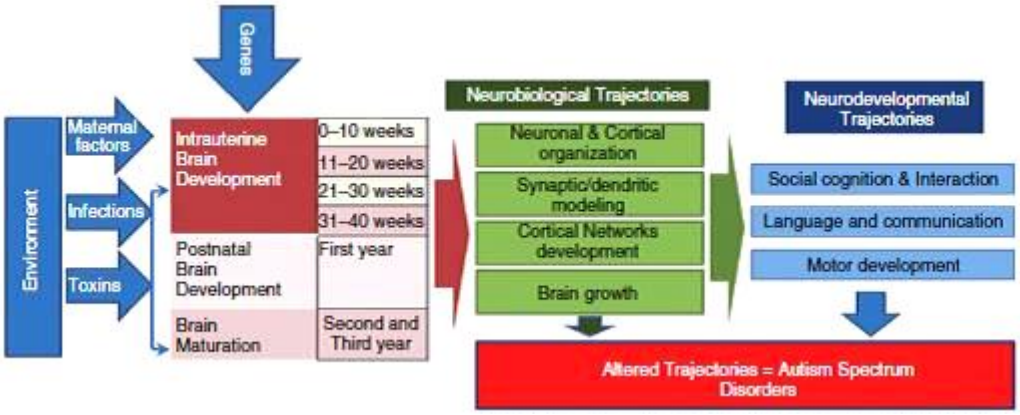

Fig. 6. Genetic and environmental factors that may influence cerebral development during fetal and early postnatal life, potentially implicated in ASD pathogenesis [Pardo and Ebehart, 2007].

Among these factors there are toxins, such as lead and mercury, that, during CNS development in utero, could cause damage to the fetus brain, especially in the early weeks of pregnancy [Bernard *et al*., 2001; Geier *et al*., 2009]. Indeed, in the United States it was estimated that about 60,000 children a year are born with neurological problems, including ASD, due to exposure to methyl-mercury in utero [Mutter *et al*., 2005], which is thought to alter the chromatin state inducing epigenetic modifications [Arai *et al*., 2011]. It has been hypothesized that children with anomalies in the system of detoxification, who were exposed to the adverse effects of mercury, have an increased risk to develop ASD compared to healthy controls, since the toxin is retained in the body for longer time by failing to be completely eliminated [Holmes *et al*., 2003]. A treatment with chelating has, indeed, demonstrated that these children eliminate much mercury through the urine, compared with controls [Bernard *et al*., 2001; Holmes *et al*., 2003].

Growing research has highlighted maternal immune activation, especially during the first or second trimesters of pregnancy, as one potential environmental factor that increases the risk for ASD [Patterson, 2009]. It has been reported an increased risk for ASD in association with mothers that required hospitalization for a viral infection in the first trimester of pregnancy, or a bacterial infection in the second trimester of pregnancy [Atladottir *et al*., 2010], suggesting that bacterial and viral infections may confer different risks depending on gestational age.

Furthermore, epidemiological data from large population based studies show increased rates of autoimmune disorders (up to 46%) in the families of individuals with ASD [Atladottir *et al*., 2009; Braunschweig *et al*., 2008; Croen *et al*., 2005]. Two independent studies have shown that self-maternal antibodies can recognize and interact with specific protein epitopes of the fetal brain, suggesting a potential inflammatory process that leads to the production of antibodies directed to the developing brain [Braunschweig *et al*., 2008; Croen *et al*., 2008; Singer *et al*., 2009].

Therefore, during the early stages of embryonic life the mother's immune system may act negatively against fetal proteins by altering pathways of the CNS neuronal development, thereby increasing the risk of developing ASD [Minshew and Williams, 2007; Zimmerman *et al*., 2007]. In experiments using anti-brain protein reactive antibodies from mothers who have children with ASD, administration

of these antibodies mediate behavioral changes and neuro-pathology in the offspring of pregnant dams [Singer *et al*., 2009].

2. Copy number variations (CNVs) in human genome

With the advent and application of high-resolution, genome wide analyses it has been demonstrated that the human genome is a highly dynamic structure that shows significant variations on a large scale compared to the current reference genomic sequence and that the human species is much more genetically variable than previously appreciated. The genomes of two unrelated individuals may, indeed, differ from each other with respect to the number of copies of thousands of loci - Copy Number Variations, CNVs [Iafrate *et al*., 2004; Perry *et al*., 2008; Redon *et al*., 2006 ; Sebat *et al*., 2004; Sharp *et al*., 2005; Wong *et al*., 2007] - and to the presence of structural rearrangements such as polymorphic balanced inversions [Antonacci *et al*., 2009; Kidd *et al*., 2008; Tuzun *et al*., 2005].

The term CNV refers to a DNA segment of at least 1 kb, for which differences in the number of copies have been observed by comparing two or more genomes. These quantitative variations may occur as acquisition of genetic material (insertions or duplications) and losses (deletions or null genotypes) in relation to the reference genomic sequence. Approximately 11,700 CNVs are known so far which involve at least 1,000 genes, and cover 12-15% of the genome, thus significantly contributing to the genotypic and phenotypic variability of the population [Carter, 2007; Merikangas *et al*, 2009; Stankiewicz and Lupski, 2010]. CNV size can vary greatly in the order of kilobases (kb) or megabases (Mb) and, generally, they are not identified by conventional cytogenetics, but through methods that use high-resolution array technology, such as SNP arrays (Single Nucleotide Polymorphism arrays) and array-CGH (array-based comparative genomic hybridization).

The best estimates currently available suggest that, considering the genetic difference in terms of total number of base pairs between two individuals chosen at random, CNVs contribute about twice as much as SNPs [Korbel *et al*., 2007; Tuzun *et al*., 2005].

Studies performed over the past five years have proven the critical role played by structural genetic variants (most of which are in the form of changes in the number of copies) in modulating gene expression and the "disease" phenotype. For example, rare CNVs, that affect genes implicated in neurodevelopmental pathways, are involved in the onset of schizophrenia [Kirov *et al*., 2009; Stefansson *et al*., 2008; Vrijenhoek *et al*., 2008; Walsh *et al*., 2008], bipolar disorder [Lachman, 2008], attention deficit hyperactivity disorder [Bateman and Gull; 2011; Elia *et al*., 2011a and b; Jarick *et al*., 2012; Lionel *et al*., 2011; Williams *et al*., 2012], intellectual disability [Shoukier *et al*., 2012; Utine *et al*., 2012], and autism spectrum disorders [Glessner *et al*., 2009, Marshall *et al*., 2008, Sebat *et al*., 2004; Weiss *et al*., 2008]. Furthermore, the few available association studies have demonstrated the importance of CNVs as common predisposing factors, by identifying specific CNVs able to confer a different risk for the development and progression, for example, of

HIV infection [Gonzalez *et al*., 2005], autoimmune diseases [Fanciulli *et al*., 2007; McKinney *et al*., 2008; Yang *et al*., 2007] and asthma [Brasch-Andersen *et al*., 2004; Ivaschenko *et al*., 2002].

2.1 Copy number variations in Autism Spectrum Disorders

Submicroscopic CNVs, *de novo* and inherited, are emerging as an important category of genetic risk for ASD with a different impact depending on the type of CNV identified [Bremer *et al*., 2011; Pinto *et al*., 2010; Sebat *et al*., 2007]. Screening for CNVs has proven to be one of the more successful strategy for the discovery of ASD candidate loci over the past five years [Cook and Scherer, 2008; State, 2010]. Furthermore, the same or overlapping CNVs are being identified as risk factors across a few neurodevelopmental disorders, indicating that some ASD loci are likely pleiotropic with variable expressivity [Cook and Scherer, 2008; Guillmatre *et al*., 2009; Lionel *et al*., 2011].

The increased resolution of the array-based approaches suggests that the proportion of ASD cases (both idiopathic and syndromic) that may be ultimately attributed to a rare structural variant is around 10-20%, a percentage higher than that of 6-7% obtained by conventional cytogenetic analysis [Abrahams and Geschwind, 2008; Bremer *et al*., 2011; Cuscò *et al*., 2009; Marshall *et al*., 2008; Pinto *et al*., 2010]. Furthermore, the *de novo* CNV rate in ASD is roughly three to seven times than that in controls [Levy *et al*., 2011; Marshall *et al*., 2008; Pinto *et al*., 2010; Sanders *et al*., 2011; Sebat *et al*., 2007] and has been reported to be higher in simplex (low-risk) compared to multiplex families (high-risk) [Marshall *et al*., 2008; Sebat *et al*., 2007] although this is not always the case [Pinto *et al*., 2010].

In addition, rare *de novo* deletions show a higher frequency compared to duplications and an increased frequency of rare *de novo* large CNVs has been observed in ASD female patients compared to male patients, findings that allow to speculate on a possible explanation of the sex ratio asymmetry observed in ASD [Levy *et al*., 2011]. It has been suggested, in fact, that due to the natural resistance of a female to suffer from genetic forms of ASD, "disruptive" genomic events with a high penetrance and involving a great number of genes are needed for ASD onset [Gilman *et al*., 2011; Levy *et al*., 2011].

In some patients multiple *de novo* variants have been detected presenting with a more complex and syndromic form of ASD. Furthermore, the observed rare *de novo* and inherited variants implicate the same genes, indicating that transmitted variants are clearly risk factors in some families and may display incomplete penetrance [Fernandez *et al*., 2010; Vaags *et al*., 2012]. The evidence of a higher frequency of rare inherited CNVs in ASD patients than in general population suggests that this type of variations, considered individually, increases the susceptibility to the disease rather than being the direct cause [Bremer *et al*., 2011], thus supporting the existence of an oligogenic heterozygosity model of inheritance in multiplex cases, in particular in high-functioning ASD

[Schaaf *et al*., 2011]. In addition, rare inherited duplications have been found more frequently than deletions [Levy *et al*., 2011; Marshall *et al*., 2008; Pinto *et al*., 2010; Sanders *et al*., 2011; Sebat *et al*., 2007; Zhao *et al*., 2007]. Although many CNVs act in an apparently dominant manner, some transmission is clearly recessive (e.g. CNVs affecting *PCDH10* and *NHE926*) in consanguineous ASD families with rare homozygous deletions [Chahrour *et al*., 2012; Morrow *et al*., 2008].

The penetrance of a CNV for ASD depends on the dosage sensitivity and function of the gene(s) affected [Cook and Scherer, 2008]. Some CNVs affecting single (e.g., *SHANK* deletions) or multiple (e.g., 16p11.2 deletions) genes are likely sufficient to cause ASD on their own and represent highly penetrant forms of the disorder. These CNVs are typically *de novo* in origin, cause a more severe phenotype (some individuals have two or more *de novo* CNVs and a severe clinical presentation), and are more prevalent in sporadic forms of ASD. Recent CNV studies estimate that there are as many as 300 *de novo* risk loci related to ASD across the genome [Anney *et al*., 2010, 2012; Levy *et al*., 2011; Marshall *et al*., 2008; Pinto *et al*., 2010; Sanders *et al*., 2011; Sebat *et al*., 2007; Weiss *et al*., 2009].

Other CNVs may contribute to the phenotype but in most cases would require other genetic or non-genetic factors in order to reach the threshold of an ASD diagnosis [Cook and Scherer, 2008]. Some of the more highly penetrant ASD CNVs that are transmitted from unaffected parents may be explained by a difference in gender expression through parent-of-origin effects (e.g., 15q11–13 duplications of the maternal allele), or recessive [Morrow *et al*., 2008] or X-linked transmission in males [Jamain *et al*., 2003; Noor *et al*., 2010]. However, most of the inherited CNVs (up to 40%) have a reduced penetrance as they may be observed in non-ASD family members and in population controls, or display pleiotropy in contributing to other neurodevelopmental disorders. In particular, common CNVs, that have been found at a frequency significantly higher in ASD patients compared to the control population, could act as susceptibility factors for the onset of ASD, modulating the "disease" phenotype [Abrahams and Geschwind, 2008, Cuscò *et al*., 2009, Bremer *et al*., 2011; Pinto *et al*., 2010].

CNV analysis focused on rare variants, both *de novo* and inherited, has led to the discovery of dozens of ASD susceptibility loci. The involvement of individually rare variants overlapping genes important for development and function of neuronal circuits has been reported across multiple CNV screening studies. In detail:

- genes implicated in synaptic complex. At the synaptic membranes neurexins (NRXNs) bind with neuroligins (NLGNs) and together act as organizers of excitatory glutamatergic synapses. All the three members of NRXN family have been implicated in ASD, as recent CNVs [Vaags *et al*., 2012] and, in particular, rare exonic *NXRN1* deletions have been consistently found in ASD CNV screens [Reichelt *et al*., 2011];

- genes expressed at the postsynaptic density. Several CNVs affect genes for scaffolding proteins such as *SHANK2* [Berkel *et al*., 2010; 2012; Leblond *et al*., 2012], *SHANK3* [Durand *et al*., 2007; Moessner *et al*., 2007], and *DLGAP2* [Pinto *et al*., 2010]. The SHANK proteins are crucial components of the postsynaptic density and complex with the NLGNs which, in turn, bind with the trans-synaptic NRXNs;
- genes important for axonal growth and guidance. Several CNVs affecting genes encoding cell-adhesion molecules have been reported, such as *CNTN4* (contactin 4) [Cottrell *et al*., 2011; Roohi *et al*., 2009] and *CNTNAP2* (contactin-associated protein) [Nord *et al*., 2011] as well as a few members of the cadherin and protocadherin families, namely *CDH8* [Pagnamenta *et al*., 2011], *CDH13* [Sanders *et al*., 2011], *PCDH9* [Marshall *et al*., 2008], and *PCDH10* [Morrow *et al*., 2008].
In addition, pathway analysis shows enrichment of particular gene sets including GTPase/Ras [Pinto *et al*., 2010], ubiquitin degradation genes [Glessner *et al*., 2009], axon targeting, and neuron motility [Gilman *et al*., 2011], and genes in the TSC/SHANK network [Sakai *et al*., 2011].

A crucial point is understanding how a CNV may contribute to the pathogenesis of autism. Different mechanisms have been proposed:
1) changing in gene dosage which may affect the dosage-sensitive genes involved in the CNV. For example, the SHANK proteins that participate in the formation of the postsynaptic scaffold are particularly sensitive to the dosage whose variations may alter the stoichiometry of SHANK multi-protein complex (**Fig. 7d**);
2) breaking of a gene that maps within the breakpoint region;
3) physical separation of a gene from its regulatory sequences with consequent alteration of transcription (position effect);
4) in case of deletions, unmasking of point mutations [Toro *et al*., 2010]. In particular:
4a) the CNV could reveal the presence of a mutation on the second allele (**Fig. 7a**), as it has been recently demonstrated for the *NRXN1* and *CNTNAP2* genes, both responsible for the Pitt-Hopkins-like syndrome. The patients are generally carriers of a CNV that affects one of the disease-genes, together with a mutation on the other allele, with a recessive mode of inheritance [Zweier *et al*., 2009]. Alternatively, the silencing of the second allele could be due to epigenetic modifications, as demonstrated in diseases related to imprinting disorders such as Prader-Willi and Angelman syndromes [Schanen, 2006];
4b) the second mutation may be present only in certain tissues, for example specific regions of the brain (**Fig. 7b**). This is well known in some familiar neoplasias where a germline mutation together with a somatic mutation are required for the development of the disease ("two-hits" model). This model has never been reported in ASD;

4c) the allelic exclusion (i.e. the expression of a single allele per cell) may silence the "healthy" allele in all cells or in certain cell populations (**Fig. 7c**). This mechanism is well documented for genes encoding immunoglobulins and olfactory receptors [Serizawa *et al*., 2004]. In the brain, allelic exclusion has been reported for adhesion molecules such as cadherins and protocadherins [Esumi *et al*., 2005], which could result in the complete lack of these proteins in neurons.

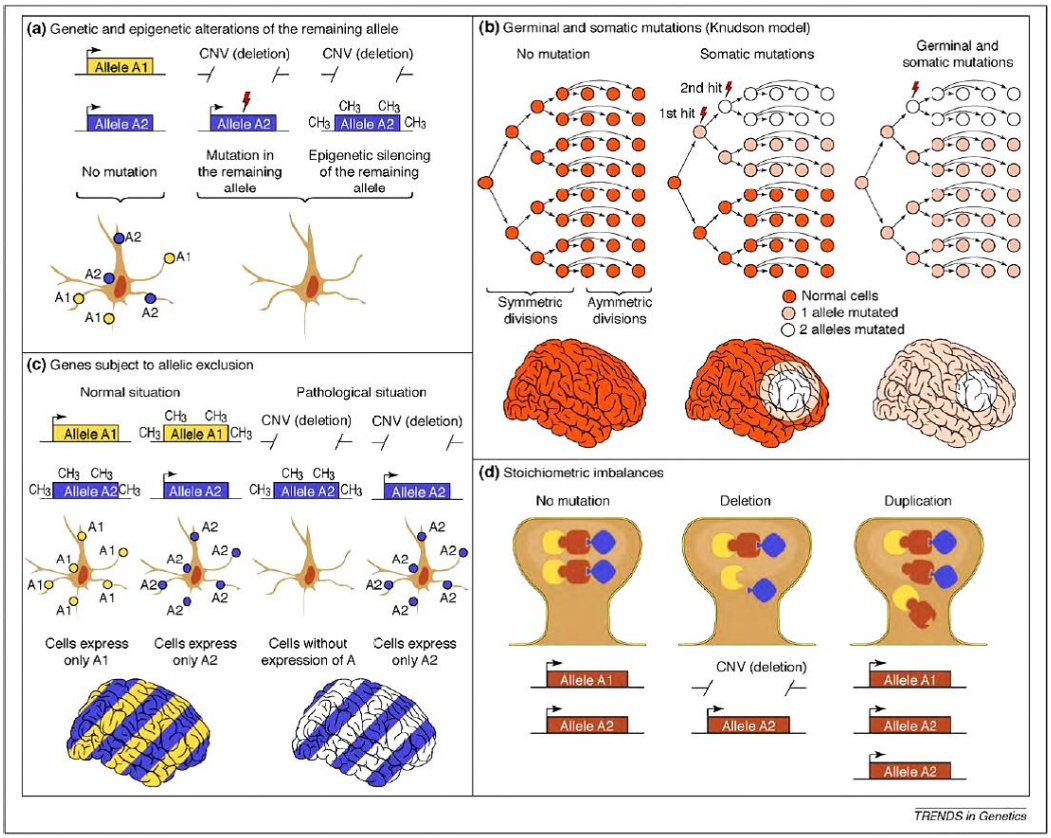

Fig. 7. Potential mechanisms that may perturb neurodevelopment: (a) genetic and epigenetic alterations; (b) two-hits model in specific areas of the brain; (c) allelic exclusion which lead to the fully gene silencing or to the expression of only one allele; (d) stoichiometric imbalances [Toro *et al*., 2010].

3. Neurobiology of Autism Spectrum Disorders

3.1 Neuroanatomical abnormalities in ASD

The neurobiology of autism has been well documented since the initial description of the disorder [Kanner, 1943]. For example, differences in gross brain morphology, such as early brain overgrowth, have been well documented [Courchesne *et al*., 1988, 2001, 2007; Lainhart *et al*., 1997]. A large epidemiological study of autism revealed an increased proportion of macrocephaly (defined as head circumference greater than the 97th percentile) in patients with autism, up to almost 15%. Although macrocephaly is common in children and adults with autism, it is not common at birth. Macrocephaly appears to develop after birth in about 80% of cases, as an aberrant early postnatal brain overgrowth [Courchesne *et al*., 1988, 2001, 2007; Lainhart *et al*., 1997], which in autism can occur as the result of three distinct developmental processes: increased neurogenesis, decreased neuronal death and/or increased production of non-neuronal brain tissues such as glial cells or blood vessels.

More recent studies investigating the nature of these gross abnormalities have produced incongruous results [Stigler *et al*., 2011]. Frontal lobe volume appears decreased in autism [Schmitz *et al*., 2007], and decreased gray matter volume in orbitofrontal cortex [Hardan *et al*., 2006a], as well as abnormally thin frontotemporal cortex [Hadjikhani *et al*., 2006] has been reported. In contrast, others have reported that gray matter volume and thickness is enlarged in these cortical regions [Hardan *et al*., 2006b; Hazlett *et al*., 2006; Schumann *et al*., 2010]. Similarly, discrepant white matter abnormalities have been reported in autism, including regional increases [Amaral *et al*., 2008; Hazlett *et al*., 2005; Herbert *et al*., 2004], as well as decreases in crosssectional area and microstructure of the corpus callosum [Alexander *et al*., 2007; Vidal *et al*., 2006]. Concomitant white matter disruptions have been reported in prefrontal, superior temporal, temporoparietal cortices, and corpus callosum [Barnea-Goraly *et al*., 2004], but increases in whole brain white matter volume have also been observed [Hazlett *et al*., 2005; Schumann *et al*., 2010].

Despite some incongruent results, the limitations of small sample sizes, bias in quantification techniques and co-morbidity that hamper neuropathological investigation, a few classic neuropathological findings from post-mortem studies are rather consistent:

- an increased cell packing density with limited dendritic arbores and reduced cell size in hippocampus, subiculum and amygdalae. This pattern resembles a pattern typical of the earlier stages of brain maturation and may therefore reflect features of an immature brain [Bauman and Kemper, 1985; Kemper and Bauman, 1993, 1998; Raymond *et al*., 1996];
- a decreased number of Purkinje cells in the cerebellar hemisphere and vermis [Bauman and Kemper, 1985; Kemper and Bauman, 1993, 1998; Ritvo *et al*., 1986];

- cerebral and cerebellar cortical dysgenesis with thickened cortices, high neuronal density, presence of neurons in the molecular layer, irregular laminar patterns and poor grey-white matter boundaries [Bailey *et al*., 1998]. These findings are consistent with a reduction in Reelin and Bcl-2 proteins in cerebellar cortex, which are involved in neuronal migration and programmed cell death, respectively [Fatemi *et al*., 2001a and b].

Moreover, magnetic resonance brain imaging has improved the classification of autistic brain anomalies, allowing the *in vivo* examination of the brain:

- abnormalities in the neocerebellum were reported, consistent with earlier data from post-mortem studies (i.e., reduction in the number of Purkinje cells), thus suggesting that these anomalies indirectly could affect, through its connections to the brain stem, hypothalamus and thalamus, the development and functioning of cognitive, sensory, autonomic and motor activities [Courchesne *et al*., 1988; Murakami *et al*., 1989; Stanfield *et al*., 2007];
- the right anterior cingulate area was found in autistic subjects significantly smaller in relative volume and also metabolically less active [Haznedar *et al*., 1997];
- the corpus callosum is generally reduced in size. This size reduction may diminish interhemispheric connectivity and may be involved in pathophysiology of cognitive impairments and clinical features of autism [Cody *et al*., 2002; Stanfield *et al*., 2007];
- at the level of the basal ganglia an enlargement of the caudate nucleus was shown. The caudate has connections to the pre-frontal cortex and is known to play an inhibitory role in behaviour. A correlation was found between ritualistic and repetitive behaviours and increased volume of the caudate nucleus [Brambilla *et al*., 2003].

Recently, the development of new quantitative structural imaging studies like voxel-based whole brain analysis, led to more standardized methods for data analysis. Localized grey matter reductions within the fronto-striatal and parietal networks were reported and additional decreases were described in the ventral and superior temporal grey matter [Boddaert *et al*., 2004; McAlonan *et al*., 2005].

Morphometric differences were also shown in key language regions. In normal individuals there exists a bias towards larger cortical language regions in the left hemisphere, whereas boys with autism showed a significant asymmetry reversal in the inferior lateral frontal language cortex, which was 27% larger on the right side. Thus, it was hypothesized that semantic encoding, normally performed by the inferior frontal gyrus, in autistic patients is performed via alternative pathways [Herbert *et al*., 2002, 2005; Just *et al*., 2004], and a PET study seemed to confirm these findings with less percentual change in blood flow in the dorsolateral pre-frontal area while listening to, repeating, and generating sentences [Muller *et al*., 1998].

3.2 ASDs represent a "synaptopathy"

In healthy brains, a balance of excitation and inhibition is essentially for nearly all functions, including representation of sensory information, cognitive processes such as decision making, sleep and motor control. At the cellular level, the number and distribution of excitatory and inhibitory inputs onto single neurons has significant impact on the integration of synaptic inputs and the output from neurons [Gulledge *et al*., 2005]. This in turn affects circuit function and plasticity, for instance by affecting long-term potentiation or the stereotypic output from central pattern generators [Alford *et al*., 2003].

Moreover, during development the balance between excitation and inhibition governs the establishment of sensory system projections, including the onset of the critical period for visual system plasticity [Fagiolini *et al*., 2004].

Currently, more than 100 genes are susceptibility candidates for ASD, indicating that ASD represents a collection of conditions with heterogeneous causation [Betancur, 2011]. These genes can be divided into two groups on the basis of the penetrance of the mutation in relation to the risk of developing the disease. **Table 1** lists the major genes whose mutations show a high penetrance; variants are usually point mutations or rare CNVs, *de novo* or inherited, and duplications/deletions detectable by conventional cytogenetics [Toro *et al*., 2010].

Gene	Chromosome	Function	Evidence	Inheritance	Diagnosis
FMR1	Xq27	Synaptic translation	Mutations	*De novo* (permutations)	ASD, Fragile X syndrome
MECP2	Xq26	Chromatin remodeling	CNV, mutations	*De novo* (rarely inherited)	ASD, Rett syndrome
TSC1	9q34.13	mTOR/PI3K pathway	CNV, mutations	*De novo*, inherited	ASD, tuberous sclerosis
TSC2	16p13.3	mTOR/PI3K pathway	CNV, mutations	*De novo*, inherited	ASD, tuberous sclerosis
NF1	17q11.2	mTOR/PI3K pathway	CNV, mutation	*De novo*, inherited	ASD, neurofibromatosis
PTEN	10q23.31	mTOR/PI3K pathway	CNV, mutations	*De novo*, inherited	ASD, Cowden syndrome
CACNA1C	12p13.33	Calcium channel	Mutation	*De novo*	ASD, Timothy syndrome
DPYD	1p21.3	Pyrimidine biosynthesis	CNV	*De novo*	ASD
RFWD2	1q25.1–q25.2	Ubiquitination	CNV	*De novo*, inherited	ASD
NRXN1	2p16.3	Synaptic CAM	CNV, mutations, SNP	*De novo*, inherited	ASD, SCZ
CNTN4	3p26.3	Synaptic CAM	CNV	Inherited	ASD, MR
MEF2C	5q14.3	Transcription factor	CNV, mutations	*De novo*	MR, seizures
SYNGAP1	6p21.3	Synaptic Ras GAP	CNV	*De novo*	ASD, MR
CNTNAP2	7q35–7q36.1	Synaptic CAM	CNV, rare variants[a]	Inherited	ASD, MR, SCZ, TS,
DPP6	7q36.2	Dipeptidyl-peptidase activity	CNV	*De novo*, inherited	ASD
DLGAP2	8p23.3	Synaptic scaffold	CNV	*De novo*	ASD
ASTN2	9q33.1	Neuron–glia interaction	CNV	Inherited	ASD, SCZ, ADHD
SHANK2	11q13	Synaptic scaffold	CNV	*De novo*	ASD
NBEA	13q13.2	Synaptic protein	Translocation	*De novo*	ASD
UBE3A	15q11–q13	Ubiquitination	CNV	*De novo*, inherited	ASD
SHANK3 (del 22q13)	22q13	Synaptic scaffold	CNV, mutations	*De novo*, inherited	ASD, MR, SCZ
NLGN3	Xq13.1	Synaptic CAM	Mutation	Inherited	ASD
IL1RAPL1	Xp21.3–p21.2	Synaptic receptor	CNV, mutations	*De novo*, inherited	ASD, MR
NLGN4	Xp22	Synaptic CAM	CNV, mutations	*De novo*, inherited	ASD, MR, TS
PTCHD1	Xp22.11	Hedgehog receptor activity	CNV	Inherited	ASD
GRIA3	Xp25	Synaptic receptor	CNV	Inherited	ASD

Table 1. Genes associated with high risk for ASD. ADHD, attention-deficit hyperactivity disorder; ASD, autism spectrum disorder; CNV, copy number variation; MR, mental retardation; SCZ, schizophrenia; SNP, single nucleotide polymorphism; TS, Tourette syndrome. [a]The pathogenetic role of these rare variants have not yet be confirmed [Toro *et al*., 2010].

Most of genes that are affected by variants at low penetrance, such as SNPs or inherited common CNVs, are listed in **Table 2** [Toro *et al*., 2010]. These variations, which seem to be responsible for increased susceptibility to the development of ASD, may be considered with caution because they have not always been replicated in the genome-wide association studies [Anney *et al*., 2010, 2012; Weiss *et al*., 2009].

Gene	Chromosome	Function	Evidence	Diagnosis
ASMT	PAR1	Melatonin pathway	Inherited CNV, SNPs, mutations	ASD
DISC1/DISC2	1q42.2	Axonal growth	Inherited CNV	ASD, SCZ
TSNAX	1q42.2	Cell differentiation	Inherited CNV	ASD, SCZ
DPP10	2q14.1	Dipeptidyl-peptidase activity	Inherited CNV	ASD
CNTN3	3p12.3	Synaptic CAM	Inherited CNV	ASD
FBXO40	3q13.3	Unknown function	Inherited CNV, $P = 3.3 \times 10^{-3}$	ASD
SLC9A9	3q24	Transporter	Inherited CNV, mutations	ASD, ADHD, MR
PCDH10	4q28	Synaptic CAM	Inherited CNV	ASD
PARK2	6q26	Ubiquitination	Inherited CNV, $P = 3.3 \times 10^{-3}$	ASD, PD
IMMP2L	7q31.1	Mitochondrial protease	Inherited CNV	ASD, TS, ADHD
PCDH9	13q21	Synaptic CAM	Inherited CNV	ASD
MDGA2	14q21.3	GPI anchor protein	Inherited CNV, $P = 1.3 \times 10^{-4}$	ASD
BZRAP1	17q22	Benzodiazepine receptor binding	Inherited CNV, $P = 2.3 \times 10^{-5}$	ASD
PLD5	1q43	Phospholipase D	SNP rs2196826, $P = 1.1 \times 10^{-8}$	ASD
SLC25A12	2q31.1	Synaptic receptor	SNP rs2056202, $P = 1 \times 10^{-3}$	ASD
CDH9/CDH10	5p14.2	Synaptic CAM	SNP rs4307059, $P = 3.4 \times 10^{-8}$	ASD
SEMA5A	5p15.2	Axonal guidance	SNP rs10513025, $P = 2 \times 10^{-7}$	ASD
TAS2R1	5p15.2	Receptor	SNP rs10513025, $P = 2 \times 10^{-7}$	ASD
GRIK2	6q16.3	Synaptic receptor	SNP rs3213607, $P = 0.02$	ASD, SCZ, OCD, MR
POU6F2	7p14.1	Transcription factor	SNP rs10258862, $P = 4.4 \times 10^{-7}$	ASD
RELN	7q22.1	Axonal guidance	GGC repeat in the 5′ UTR, $P < 0.05$	ASD, BP
NRCAM	7q31.1	Synaptic receptor	SNP rs2300045, $P = 0.017$	ASD
MET	7q31.2	Tyrosine kinase	SNP rs1858830, $P = 2 \times 10^{-3}$	ASD
EN2	7q36.3	Transcription factor	SNP rs1861973, $P = 3 \times 10^{-6}$	ASD
ST8SIA2	15q26.1	N-glycan processing	SNP rs3784730, $P = 4 \times 10^{-7}$	ASD
GRIN2A	16p13.2	Synaptic receptor	SNP rs1014531, $P = 2.9 \times 10^{-7}$	ASD, SCZ
ABAT	16p13.2	Enzyme	SNP rs1731017, $P = 1 \times 10^{-3}$	ASD, GABA aminotransferase deficiency
SLC6A4	17q11.2	Serotonin transporter	Meta analysis, $P > 0.05$	ASD, OCD
ITGB3	17q21.3	Cell-matrix adhesion	SNP Leu33Pro, $P = 8.2 \times 10^{-4}$	ASD
TLE2/TL6	19p13	WNT receptor signaling pathway	SNP rs4806893, $P = 7.8 \times 10^{-5}$	ASD, FHM2, AHC
MACROD2	20p12	Unknown function	SNP rs4141463, $P = 2 \times 10^{-8}$	ASD

Table 2. Proposed susceptibility genes for ASD. ADHD, attention-deficit hyperactivity disorder; AHC, alternating hemiplegia of childhood; ASD, autism spectrum disorder; BP, bipolar disorder; CNV, copy number variation; FHM2, familial hemiplegic migraine 2; MR, mental retardation; OCD, obsessive-compulsive disorder; PD, Parkinson disease; SCZ, schizophrenia; SNP, single nucleotide polymorphism; TS, Tourette syndrome [Toro *et al*., 2010].

Recent evidences have unveiled a remarkable convergence of several of these genes on common cellular pathways that intersect at neuronal synapses [Peça and Feng, 2012]. Indeed, the notion that some ASD and ID represent "synaptophaties" is supported by the preponderance of penetrant mutations in genes associated with synaptic structure and function. ASD and ID appear to be common consequences of disruptive mutations that cause synaptic pathophysiology at both ends of a spectrum as both "gain of function" and "loss of function" mutations can manifest in similar ways (**Fig. 8**). Furthermore, the interdependence among proteins that work as a biochemical complex suggest that even single gene disorders may perturb the full complex and the related pathways and networks [Zoghbi and Bear, 2012].

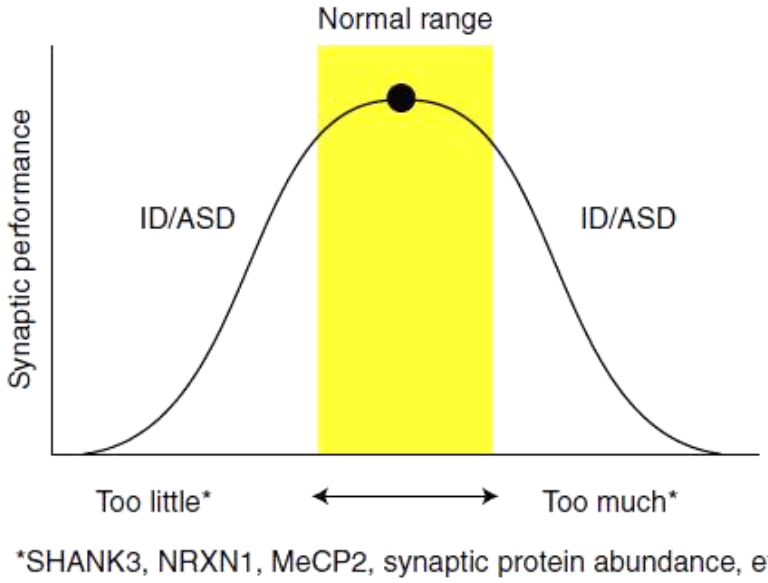

Fig. 8. Gain or loss of function of individual genes often yields an overlapping behavioral phenotype in humans

that includes ASD and ID. Optimal synaptic function may occur within a limited dynamic range, and the pathophysiology at both ends of this range can cause autistic behavior and intellectual disability [Zoghbi and Bear, 2012].

Synaptic scaffolding disorders

For ASD, the trans-synaptic complex composed of Neurexin/Neuroligin/PSD-95/SAPAP/Shank pathway is a good examples of a set of genes converging on both function, location and associated disorders. From these, Neurexin-1 [Feng *et al*., 2006; Kim *et al*., 2008; Wisniowiecka-Kowalnik *et al*., 2010; Yan *et al*., 2008], Neuroligin-3 and Neuroligin-4 [Jamain *et al*., 2003], PSD-95 [Feyder *et al*., 2010], SAP97 [Willatt *et al*., 2005], SAPAP2 [Pinto *et al*., 2010], Shank1, Shank2 and Shank3 [Berkel *et al*., 2010, 2012; Durand *et al*., 2007; Leblond *et al*., 2012; Pinto *et al*., 2010] have all been implicated in ASD and validated across multiple studies (**Fig. 9**).

All Shank proteins are expressed in the brain, especially in cortical and hippocampal neurons, and localized to the post-synaptic density (PSD) of dendritic spines. The PSD is key for organizing and maintaining proper synaptic communication, and within this structure Skanks have been hypothesized to function as master scaffolds. Shanks stabilize PSD-95/SAPAP/Shank/Homer complexes, which form a platform for the anchoring of ionotropic and metabotropic glutamate receptors at synapses [Baron *et al*., 2006; Hayashi *et al*., 2009]. In addition, they recruit inositol 1,4,5-triphosphate (IP3) and F-actin to the synapse, thereby enlarging dendritic spine heads and stabilizing them [Sala *et al*., 2001; Tu *et al*., 1998, 1999] (**Fig. 9**). For example, overexpression of *Shank1* in hippocampal neurons leads to increased maturation and size of dendritic spines, whereas deletion of *Shank1* in mice leads to smaller spines and weakened synaptic transmission.

Furthermore, knockdown of *Shank3* in hippocampal neurons cultured *in vitro* leads to reduced number and increased length of dendritic spines, whereas overexpression of *Shank3* in aspiny cerebellar granule neurons is sufficient to induce functional dendritic spines [Roussignol *et al*., 2005].

Interestingly, the four different lines of *Shank3* mutant mice characterized so far show neuronal deficits that all lead to decreased glutamatergic signaling, loss of synaptic strength and, at the behavioral level, to deficiencies pertaining social interactions and other ASD-related behavior.

Neurexins (NRXNs) and Neuroligins (NLGNs) are synaptic cell adhesion molecules whose critical role in synaptic function has been well established. Both NRXNs and NLGNs have single transmembrane domains and short cytoplasmic domains containing PDZ-binding motifs at the carboxyl terminus [Hata *et al*., 1996; Irie *et al*., 1997]. They form a trans-synaptic complex believed to organize the presynaptic and postsynaptic compartments through various interactions with proteins like CASK, MAGUK, and PSD-95 [Sudhof, 2008] (**Fig. 9**). Mice lacking *Nlgn1*, *2*, and *3* have normal synapse number and ultrastructures, but die perinatally from respiratory failure. Neurophysiologically studies revealed that glutamatergic and GABAergic synaptic transmission is impaired in the respiratory center of the triple null animals [Varoqueaux *et al*., 2006]. Interestingly, mice carrying a single deletion or double knockout of these genes are viable, that is consistent with the finding, for example, that *Nlgn1* deficiency impairs N-methyl-D-aspartate (NMDA) receptor signaling, whereas *Nlgn2* deficiency impairs inhibitory synaptic transmission [Chubykin *et al*., 2007]. Furthermore, a deletion of an *NLGN4* ortholog in mice caused impaired social interactions consistent with the loss-of-function mutation in humans that cause ASD and ID [Jamain *et al*., 2008].

In the different individual *Nrxn* knockout mice synaptic function is impaired, as evident by decrease spontaneous as well as evoked neurotransmitter release in both the neocortex and brain stem of α-NRXN deficient mice. Moreover, Ca^{2+} channel function is also compromised based on decreased presynaptic Ca^{2+} currents [Missler *et al*., 2003].

Synaptic signaling disorders

It is noteworthy that pathogenesis of Fragile X syndrome (FXS), Angelman syndrome (AS), and Tuberous Sclerosis syndrome (TSC), which all show comorbidity with ASD, involves regulation of synaptic protein abundance and turnover (**Fig. 9**). In FXS this is due to derepression of translation of FMRP-target mRNAs. Indeed FMRP, which is enriched in neuronal soma and dendrites, serves as negative regulator of translation of many mRNA transcripts [O'Donnell and Warren, 2002], and an increased rate of basal protein synthesis is observed in the hippocampus of *Fmr1* null mice [Osterweil *et al*., 2010; Qin *et al*., 2005]. Another consistent finding in animal models of fragile X

is evidence of impaired GABAergic inhibition [Levenga *et al*., 2010], which could be a consequence of excessive protein synthesis during development.

Reduced proteolysis of UBE3a-target proteins is the AS underlying pathogenic mechanism. Several synaptic deficits have been described in the *UBE3a* knockout mouse model of AS, including reduced density and strength of excitatory synapses and, at a later developmental stage, reduced functional inhibition [Philpot *et al*., 2010]. In addition to UBE3A, other proteins involved in CNVs affecting other ubiquitin genes have been collected in patients with ASD, such as *PARK2*, *RFWD2*, and *FBXO40* [Glessner *et al*., 2009]. Interestingly, changing neuronal activity levels produces a rapid bidirectional change in the composition of PSD proteins, an effect that is mediated by the proteasome system [Ehlers, 2003], thus supporting the importance of ubiquitin ligases to the regulation of excitatory synapses (**Fig. 9**) [Peça and Feng, 2012].

The proteins encoded by *TSC1* and *TSC2*, harmartin (TSC1) and tuberin (TSC2), form a heterodimeric complex that responds to numerous intracellular signals to negatively regulate the protein kinase mTOR (mammalian target of rapamycin) residing in the protein complex mTORC1. Relief from TSC1/2 repression of mTOR by upstream signaling (e.g., PI3 kinase acting through PDK1 and AKT) stimulates cell growth and proliferation (**Fig. 9**). Homozygous silencing mutations of either *TSC1* or *TSC2* are embryonic lethal. Humans born with the disease typically have heterozygous truncating germline mutations in either *TSC1* or *TSC2* [Han and Sahin 2011; Orlova and Crino 2010]. Heterozygous null mutations of *TSC1* or *TSC2* were both shown to cause cognitive and synaptic impairments in the absence of gross neuropathology or seizures [Ehninger *et al*., 2008; Goorden *et al*., 2007; Nie *et al*., 2010; von der Brelie *et al*., 2006]. One particularly interesting phenotype reported in the Tsc2+/- mouse is an enhancement of late-phase LTP [Ehninger *et al*., 2008]. Persistent LTP requires synthesis of synaptic proteins that might be increased in abundance owing to excess mTOR activity.

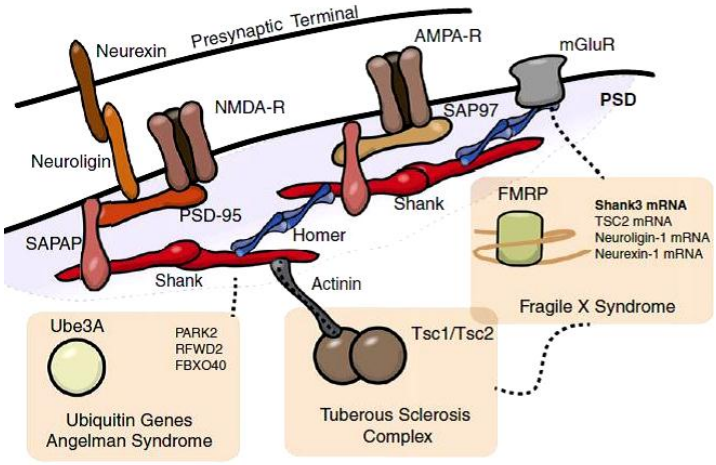

Fig. 9. Shank proteins at the center of an ASD disease-module. Neurexin and Neuroligins are trans-synaptic partners which in the postsynaptic density bind to the SAPAP family of proteins, PSD-95, SAP97, Shank2 and Shank3. Shank dimers are thought to organize a molecular platform in concert with Homer tetramers to stabilize the larger PSD, connecting AMPAR, NMDAR and mGluR into one protein hub. In the deeper synaptic compartment the control of PSD protein levels may be tightly controlled by independent complexes such as TSC1/2 through mTOR, or via FMRP regulation of synaptic transcripts, and most likely also through synaptic ubiquitin ligases [Peça and Feng, 2012].

Synaptic transcriptional dysregulation

MeCP2, whose mutations cause Rett syndrome (RTT), is a nuclear protein that binds to methylated cytosines [Lewis *et al*., 1992] and is a member of a methyl-CpG-binding protein family [Hendrich and Bird, 1998]. MeCP2 interacts with histone deacetylase–containing complexes and represses transcription [Jones *et al*., 1998; Nan *et al*., 1998]. Surprisingly, in mouse models of RTT several genes are down-regulated upon loss of MeCP2 but are increased upon its overexpression, suggesting that this protein is not a classical transcriptional repressor [Chahrour *et al*., 2008; Jordan *et al*., 2007; Nuber *et al*., 2005; Tudor *et al*., 2002]. Among these genes there are brain-derived neurotrophic factor (*BDNF*) and several other neuronal genes [Chahrour *et al*., 2008; Chen *et al*., 2003; Martinowich *et al*., 2003; Yasui *et al*., 2007].

MeCP2 is abundant in neurons, and its levels increase postnatally as neurons mature [Balmer *et al*., 2003; Kishi and Macklis 2004; Shahbazian *et al*., 2002b]. Recent studies revealed that MeCP2 is also expressed in glia, albeit at lower levels than neurons, and that glia lacking MeCP2 fail to support dendritic morphology of either wild-type or *Mecp2*-null neurons [Ballas *et al*., 2009; Kifayathullah *et al*., 2010; Maezawa and Jin 2010; Maezawa *et al*., 2009]. Mice lacking functional MeCP2 reproduce features of RTT [Chen *et al*., 2001; Guy *et al*., 2001; Pelka *et al*., 2006; Shahbazian *et al*., 2002a]. Despite the devastating neurological phenotypes, the brain appears normal, with the exception of microcephaly, decrease in dendritic spine density, and dendritic

swelling [Belichenko *et al*., 2009]. Furthermore, deletion of MeCP2 from various neuronal types revealed that this protein is critical for the functional integrity of a diverse set of neurons. Loss of *Mecp2* from forebrain glutamatergic neurons causes motor abnormalities, anxiety-like behavior, social abnormalities, and impaired learning [Gemelli *et al*., 2006]. A more recent study revealed that deletion of *Mecp2* in GABAergic neurons reproduced most of the features of RTT (including the stereotyped behavior and premature lethality) and resulted in reduced GABA signaling [Chao *et al*., 2010]. Interestingly, the RTT model shows a decrease in AKT/mTOR signaling in contrast with models of TSC and fragile X, in which AKT/mTOR activity is increased [Zoghbi and Bear, 2012].

To date, the role of neurotransmitters transporters and synaptic glutamatergic receptors in susceptibility to ASD must be still clarified. Since abnormal levels of serotonin have been found in patients with ASD [Cook and Leventhal, 1996], the *SLC6A4* gene encoding the serotonin transporter has been extensively studied, although only a weak association with ASD has been highlighted as in the case of the *GRIK* gene, which encodes a receptor for glutamate [Jamain *et al*., 2002].

Moreover, it is likely that many proteins involved in axonal growth and synaptic identity have a role in the pathogenesis of ASD. The semaphorins, for example, are involved in axonal growth and maturation of dendritic spines and SNPs in the *SEMA5A* gene have been associated with ASD in a large cohort of patients [Weiss *et al*., 2009]. In addition, deletions in the genes encoding the contactins proteins (*CNTN3* and *CNTN4*), which are also involved in axonal growth and in the mediation of the connections between axon and glial cells, have been identified in patients with ASD [Morrow *et al*., 2008].

Finally, many pathways are involved in the intracellular signaling from the synapse to the neuronal soma, whose perturbation may be potentially related to ASD development (**Fig. 10**).

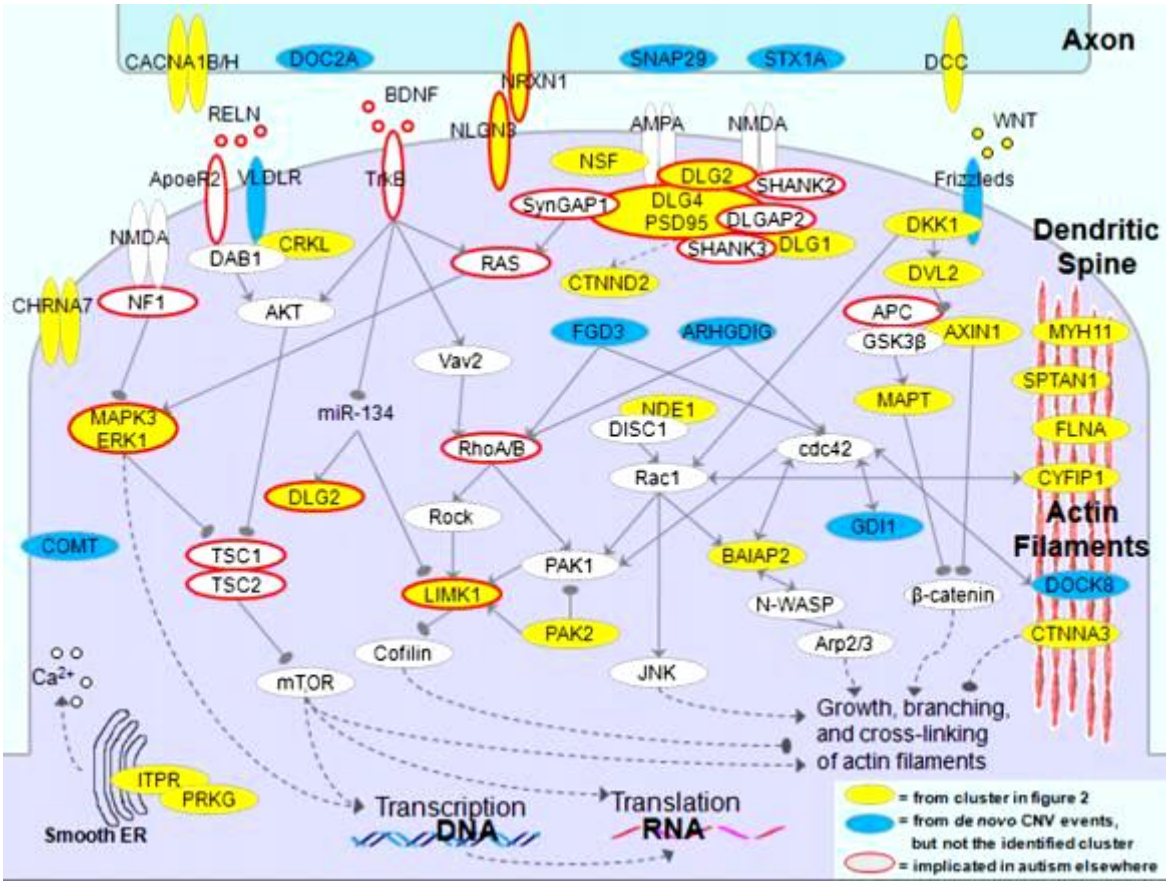

Fig. 10. Schematic view of the synaptic genes that are involved in the pre-postsynaptic connection and in the signal transduction from the synapse to the neuronal soma, most of which have been already implicated in ASD.

In detail, genes working in the same network are depicted in yellow, genes potentially perturbed by rare *de novo* CNVs reported by Levy *et al*. [Levy *et al*., 2011] in blue, and genes associated with ASD in previous studies in red [Gilman *et al*., 2011].

In detail:

- remodeling of actin cytoskeleton. The information for the regulation of the morphology of dendritic spine are transmitted through the GTPase Rho family of proteins, such as RhoA/B, Cdc42, and Rac1 [Linseman and Loucks, 2008] to downstream targets, for example LIMK1 and PAK1/2/3, that are linked to proteins which are able to modify the morphology of the actin cytoskeleton (cofilin and Arp2/3) [Blanchoin *et al*., 2000]. The GTPase activity is regulated pre- and post-synaptically by different GEF (guanine nucleotide exchange factor), GDI (GDP dissociaton inhibitors) and GAP (GTP-activating proteins) proteins (**Fig. 10**);
- Wnt/β-catenin pathway, that plays a crucial role in the formation of neuronal circuits [Salinas and Zou, 2008] and is directly involved in the reorganization of actin filaments (**Fig. 10**) [Rosso *et al*., 2005; Salinas *et al*., 1994];

- reelin-mediated signaling. Reelin is secreted at the synaptic space and regulates the mTOR pathway acting on TSC1/TSC2 by the AKT-mediated signaling (**Fig. 10**) [Fatemi *et al*., 2005; Jossin and Goffinet, 2007; Kumar *et al*., 2005; Niu *et al*., 2008; Shaw and Cantley, 2006];
- MAPK3/ERK1 signaling pathway, that is activated by both NF1 and Ras and represents another way of regulation of mTOR. Indeed, mTOR is able to integrate the stimuli that arrive from the upstream pathways involved in the regulation of cell growth and to mediate the morphogenesis of the dendrite (**Fig. 10**) [Tavazoie *et al*., 2005].

PATIENTS AND METHODS

4.1 Patients

We collected a series of 115 patients (92 males and 23 females, M:F sex ratio 4:1) who were diagnosed with ASD as a result of evaluation by geneticists and psychiatrics in agreement with the international criteria of DSM-IV Text Revised [Task Force on DSM-IV, 2000]. The overall phenotypic picture was therefore characterized as: idiopathic Autism (AU), which always involves intellectual disability (ID); Pervasive Development Disorder Not Otherwise Specified (PDD-NOS), which may or may not involve ID; High-Functioning Autism (HF-AU); Asperger syndrome (AS); and Syndromic Autism (S-AU), where the autistic phenotype is part of a syndromic picture characterized by dysmorphism and/or major malformations. One patient with S-AU suffered from Tourette syndrome. Four of the 115 patients demonstrated epilepsy (~3.5%).

The detailed clinical diagnoses were as follows (**Fig. 11**):

- 46 patients with PDD-NOS;
- 41 with AU-ID (notably, only one female patient suffered from a severe ID);
- 15 with S-AU;
- 8 with HF-AU;
- 5 with AS.

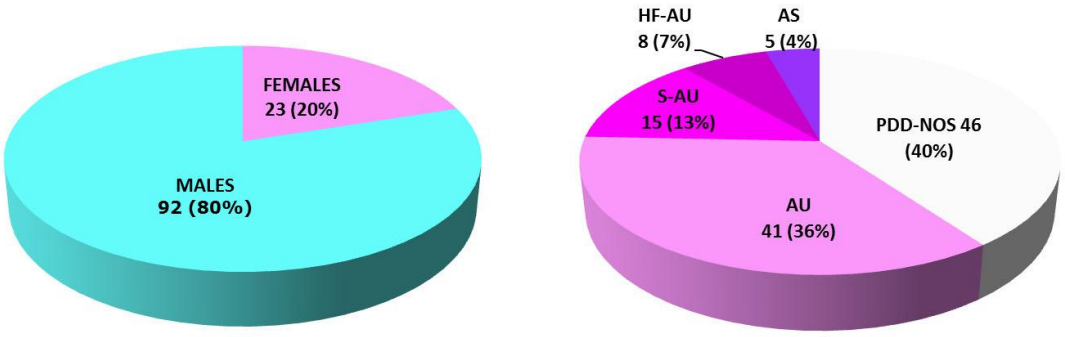

Fig. 11. Distribution of the sample by sex (left) and phenotype (right). The number and percentage of patients are shown for each category.

Genomic DNA from all patients, which was extracted from peripheral blood samples collected in tubes containing EDTA, was used to perform array CGH analysis (Agilent Technology) in order to detect copy number variants (CNVs). Three kits with different resolutions were used, specifically:

- 6 patients were analyzed with the 60K SurePrint G3 Human CGH Microarray, which includes approximately 55,077 oligonucleotide probes at an average spatial resolution of 41 kb (33 kb in gene-enriched genomic regions);

- 12 patients with 44K Human Genome CGH Microarray, which includes approximately l42,494 probes at an average spatial resolution of 43 kb (24 kb in gene-enriched genomic regions);
- 97 patients with 244K Human Genome CGH Microarray, which includes approximately 236,381 probes at an average spatial resolution of 8.9 kb (7.4 kb in gene-enriched genomic regions).

In the event of detection of rare CNVs, if possible, patients' parents were analyzed to characterize the origin of the unbalanced microrearrangement (*de novo* or inherited).

4.2 Molecular karyotyping by means of array-based Comparative Genomic Hybridization (array CGH) analysis

4.2.1 Extraction of genomic DNA (gDNA) from peripheral blood

For gDNA extraction from fresh or frozen (-80°C) peripheral blood collected in anticoagulant (EDTA) tubes, the GenElute Blood Genomic DNA Kit (Sigma) was used following the manufacturer's instructions. Briefly:

- add 500 µl of whole blood to a 2 ml tube containing 50 µl of proteinase K and 40 µl of RNAseA;
- add 550 µl of Lysis Buffer C Solution, then vortex thoroughly for 15 seconds and incubate at 55 °C for 10 minutes;
- add 550 µl of 100% ethanol and vortex the sample again for 15 seconds;
- in the meantime, for gDNA recovery moisturize the pre-assembled GenElute Miniprep Binding Columns by washing them with 500 µl of the Column Preparation Solution, then centrifuge at 7000 rpm for 1 minute and discard the flow-through liquid;
- load 500 µl of the sample into the column, centrifuge at 7000 rpm for 1 minute and discard the collection tube containing the flow-through liquid;
- repeat this step until the entire sample was loaded into the column and place the binding column to a new 2 ml tube;
- add 500 µl of Prewash Solution, centrifuge at 7000 rpm for 1 minute and discard the collection tube containing the flow-through liquid. Place the binding column to a new 2 ml tube;
- add 500 µl of Wash Buffer, centrifuge at 13000 rpm for 3 minutes and discard the collection tube containing the flow-through liquid;
- place the binding column to a new 2 ml tube and let it dry under a safety hood for 10 minutes;
- add 50 µl of Elution Buffer directly into the centre of the binding column and wait for 5 minutes, then centrifuge at 7000 rpm for 2 minutes;
- the concentration and quality of the gDNA (it should be free of contaminants such as carbohydrates, proteins, and traces of organic solvents) are determined by a spectrophotometric analysis by using the NanoDrop ND-1000 UV-VIS Spectrophotometer. Moreover, by means of agarose gel electrophoresis it could be verified if the gDNA is intact or degradated.

4.2.2 gDNA enzymatic restriction digestion

Test and reference DNAs must be processed separately. The amount of DNA required, which must necessarily be the same for test and reference, depends on the slide used as indicated in the table below.

	44K array	60K array	array 244K
DNA (µg)	0.5-1.5 µg	0.2-0.5µg	0.5-3 µg

For 44K and 244K arrays:

- add to DNA Nuclease-free water up to volume 20.2 µl;
- preparation of Digestion Master Mix, component per reaction:

2 µl of nuclease-free water

2.6 µl of Buffer C

0.2 µl of BSA (10 µg/µl)

0.5 µl of AluI (10 U/µl)

0.5 µl of RsaI (10 U/µl)

5.8 µl final volume

- add 5.8 µl of Digestion Master Mix to the genomic DNA;
- make a total volume of 26 µl;
- mix well by pipetting up and down;
- incubate at 37°C for 2 hours;
- incubate at 65°C for 20 minutes to inactivate the enzymes;
- move the sample tubes to ice;
- verify the digestion reaction by means of agarose gel electrophoresis run (Agarose 0.8% in TAE buffer; ethidium bromide 0.01 mg/ml) of 2 µl of digested DNA.

For 60K array:

- add to DNA Nuclease-free water up to volume 10.1 µl;
- preparation of Digestion Master Mix, component per reaction:

1 µl of nuclease-free water

1.3 µl of Buffer C

0.1 µl of BSA (10 µg/µl)

0.25 µl of AluI (10 U/µl)

0.25 µl of RsaI (10 U/µl)

2.9 µl final volume

- add 2.9 µl of Digestion Master Mix to the genomic DNA;
- make a total volume of 13 µl;
- mix well by pipetting up and down;

- incubate at 37°C for 2 hours;
- incubate at 65°C for 20 minutes to inactivate the enzymes;
- move the sample tubes to ice;
- verify the digestion reaction by means of agarose gel electrophoresis run (Agarose 0.8% in TAE buffer; ethidium bromide 0.01 mg/ml) of 2 µl of digested DNA.

4.2.3 Fluorescent Labeling of DNA by Agilent Genomic DNA Labeling Kit PLUS

For labeling reaction fluorescent cyanines (C_Y3 and C_Y5) are used so the reaction must be performed in the dark.

<u>For 44K and 244K arrays</u>:
- add 5 µl of Random Primers to each reaction tube containing 24 µl of digested gDNA to make a total volume of 29 µl;
- mix well by pipetting up and down gently;
- incubate at 95-100°C for 5 minutes;
- move to ice and incubate on ice for 5 minutes;
- preparation of Labeling Master Mix, component per reaction:

2 µl of Nuclease-free water
10 µl of 5X Buffer
5 µl of 10X dNTP
3 µl of Cyanine 3-dUTP (1.0 mM) or Cyanine 5-dUTP (1.0 mM)
1 µl of Exo-Klenov fragment
21 µl final volume

- add 21 µl of Labeling Master Mix to 29 µl of digested gDNA;
- make a total volume of 50 µl;
- mix well by gently pipetting up and down;
- incubate at 37°C for 2 hours;
- incubate at 65°C for 10 minutes to inactivate the enzyme, then move to ice.

<u>For 60K array</u>:
- add 2.5 µl of Random Primers to each reaction tube containing 11 µl of digested gDNA to make a total volume of 13.5 µl;
- mix well by pipetting up and down gently;
- incubate at 95-100°C for 5 minutes;
- move to ice and incubate on ice for 5 minutes;
- preparation of Labeling Master Mix, component per reaction:

2 µl of Nuclease-free water

5 µl of 5X Buffer

2.5 µl of 10X dNTP

1.5 µl of Cyanine 3-dUTP (1.0 mM) or Cyanine 5-dUTP (1.0 mM)

0.5 µl of Exo-Klenov fragment

11.5 µl final volume

- add 11.5 µl of Labeling Master Mix to 13.5 µl of digested gDNA;
- make a total volume of 25 µl;
- mix well by gently pipetting up and down;
- incubate at 37°C for 2 hours;
- incubate at 65°C for 10 minutes to inactivate the enzyme, then move to ice.

4.2.4 Clean-up of Labeled Genomic DNA

- Add 430 µL of 1X TE (pH 8.0, Promega) to a Microcon YM30 (Millipore) filter (the filter is into a 1.5-ml microfuge tube);
- load each labeled gDNA into the filter, mix well;
- spin 10 minutes at 14,000 × g in a microcentrifuge at room temperature. Discard the flow-through;
- add 480µl of 1X TE (pH 8.0) to each filter. Spin for 10 minutes at 14,000 × g in a microcentrifuge at room temperature. Discard the flow-through;
- invert the filter into a fresh 1.5-mL microfuge tube;
- spin for 1minute at 1,000 × g in a microcentrifuge at room temperature to collect purified sample;
- measure and record volume (µl) of each eluate and repeat the last steps until the volume is <9.5 µl (60K), 21 µl (44K), or 80.5 µl (244K). Bring total sample volume to the final volume with 1X TE Buffer (pH 8.0);

-Take 1.5µl of each sample to determine the yield and specific activity by using the NanoDrop ND-1000 UV-VIS Spectrophotometer.

-Labeled DNA can be stored overnight at -20°C in the dark.

4.2.5 Preparation of Labeled Genomic DNA for Hybridization

The hybridization reaction must be performed in the dark:

- mix the labeled reference and test DNAs;
- add the reagents as described in the table below:

	60K	44K	244K
Labeled DNAs	16 µl	39 µl	158 µl
Human Cot-1 DNA (1mg/ml)	2 µl	5 µl	50 µl
Agilent 10X Blocking agent	4.5 µl	11 µl	52 µl
Agilent 2X Hybridization buffer	22.5 µl	55 µl	260 µl
Finale volume	45 µl	110 µl	520

- mix the sample by pipetting up and down, then quickly spin in a microcentrifuge to drive contents to the bottom of the reaction tube;
- incubate at 95°C for 5 minutes;
- immediately incubate at 37°C for 30 minutes;
- spin 1 minute at 13000 rpm in a microcentrifuge to collect the sample at the bottom of the tube;
- put the full volume on the cover-slip using the Agilent microarray 8/slide gasket for 60K array, the Agilent microarray 4/slide gasket for 44K arrays and the Agilent microarray 1/slide gasket for 244K array, previously lying on the bottom of the Agilent Microarray Hybridization Chamber;
- place the slide with the up-face toward the sample and close the Agilent Hybridization Chamber;
- place the slide into a preheated oven at 65°C and leave for 24-48 hours.

4.3 Copy Number Variant analysis

The evaluation of the possible pathogenicity of all the identified CNVs was performed based on international guidelines, such as those described by Miller in 2010 [Miller *et al*., 2010] and Marshall *et al.* in 2012, who focused on the prioritization criteria [Marshall *et al*., 2012] (**Fig. 12**).

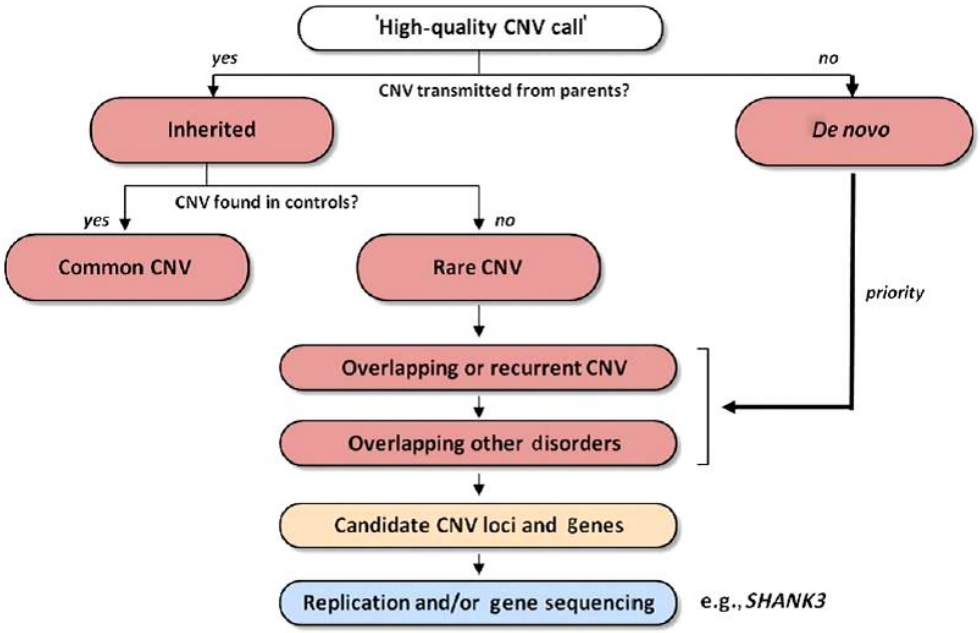

Fig. 12. General analysis and prioritization workflow (pink boxes) for discovery of rare CNVs associated with ASD.

Once a CNV was detected, the first target was to understand whether it was a rare variant or a CNV already reported in healthy controls according to the Database of Genomic Variants (http://projects.tcag.ca/variation/). In the case of rare CNVs, array CGH analysis was performed on the parents' DNA to determine whether the rearrangement is *de novo* or inherited. Although in terms of follow-up the *de novo* CNVs have the highest priority (**Fig. 12**, pink boxes), parameters such as recurrence in ASD cases and overlap with other neurodevelopmental disorders, together with a precise analysis of the CNV gene content, may be of help in identifying possible ASD candidate loci (**Fig. 12**, light pink box) affected by both *de novo* and inherited CNVs.

We performed *ad hoc* analysis of the identified CNV gene content using public databases, specifically:

- the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) and NCBI Entrez Gene (http://www.ncbi.nlm.nih.gov/gene), which are both useful to collect information about the genes involved in the imbalance (i.e. gene function, molecular structure, presence of different isoforms, etc.), their expression in different tissues, and the pathways in which they are involved;
- the Decipher database (http://decipher.sanger.ac.uk/), which collects all pathogenetic CNVs reported to date as well as the clinical description of the related patients;

- the OMIM database (Online Mendelian Inheritance in Man) (http://www.ncbi.nlm.nih.gov/omim), which collects information about all Mendelian disorders described to date, focusing particularly on genotype-phenotype correlation;
- PubMed (http://www.ncbi.nlm.nih.gov/pubmed), which provides updated international medical literature.

Furthermore, two databases specific for ASD were consulted, namely the Autism Database (http://www.mindspec.org/autdb.html) and the SFARI Database (Simmons Foundation Autism Research Initiative) (http://sfari.org/resources/sfari-gene), which collect the genes previously reported as mutated or affected by CNVs in ASD patients, as well as genes associated with ASD by association studies.

A possible replication of the findings in other cohorts, as well as gene sequencing in the case of CNV affecting single genes, may be good approaches to validate the collected data (**Fig. 11**, blue box).

Of note, considering the autism disease, which shows an oligo-/polygenic genetic aetiology, it cannot be assumed *a priori* that an inherited CNV is benign *per se*. Indeed, the combination of several variants in different loci, both *de novo* and/or inherited, often leads to the manifestation of the disease, which is not present in the parents as they do not share the same combination of variants identified in the children. Although we cannot exclude the possibility that any of the "common" CNVs detected in our series may have contributed to ASD susceptibility, the assessment of this susceptibility would have required a time-expensive association study that did not fit appropriately with the goals of the present work.

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RESULTS

5.1 Identification and classification of rare CNVs

A cohort of 115 patients with ASD was analyzed by high-resolution array CGH analysis to identify CNVs possibly implicated in ASD pathogenesis. In 63 of 115 patients (55%), rare CNVs (one or more) were detected that were not already reported in healthy subjects according to the DGV. This group comprised:

- 52 males and 11 females;
- 51 sporadic and 12 familial cases;
- 49 patients analyzed by the Agilent 244K Human Genome CGH Microarray Kit and 14 by the 44K or the 60K Kits.

The phenotype distribution of the 63 patients is shown in **Fig. 13**.

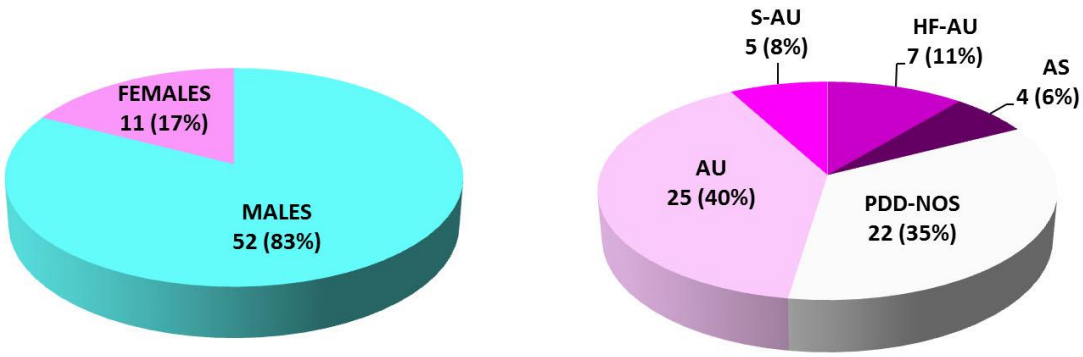

Fig. 13. Distribution of the group of 63 patients with at least one rare CNV by sex (left) and phenotype (right). For each subgroup, the number and percentage of patients are shown.

The detection rate for rare CNVs using the high-resolution array CGH analysis was approximately 50.5% (49 of 97 patients analyzed by means of the Agilent 244K Kit) vs. 77.8% using the array CGH analysis at a lower resolution (14 of 18 patients analyzed by means of the Agilent 44K or 60K Kits). Of note, 6 of 97 patients analyzed with the Agilent 244K Kit were negative using a previous array CGH analysis at a lower resolution, and in 4 of these patients, at least one rare CNV was subsequently identified. Overall, 120 rare CNVs were detected, 73 gains (60.8%) and 47 losses (39.2%), ranging from 10 kb to 11 Mb in size (**Fig. 14**). Furthermore, inheritance is unknown for 13 CNVs (10.8%). Twenty of the remaining 107 CNVs were de novo (16.7%), and 87 were inherited (72.5%), 50 from the mother (57.5%) and 37 from the father (42.5%). All these data are summarized in **Fig. 14**.

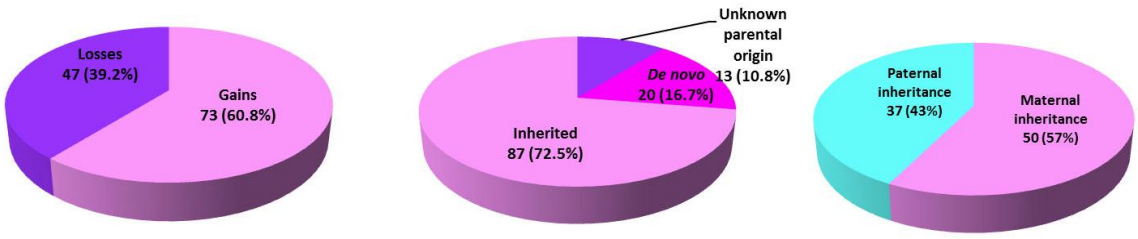

Fig. 14. Schematic view of the 120 rare CNVs identified, which have been subdivided based on the type of rearrangement (left), origin (middle) and parental inheritance (right). For each subgroup the number of patients and the percentage are shown.

Exclusively rare *de novo* CNVs (one or more) were found in only 10 of 63 patients (15.9%), whereas in 15 of 63 patients (23.8%), only one inherited CNV was detected. In the remaining 38 patients, more than one CNV was identified (60.3%), in different combinations. Specifically:

- in 7 of 63 patients (11.1%), a single rare *de novo* CNV together with one or more inherited CNV were detected (7 CNVs were inherited from the mother and one from the father), with only one patient who inherited the CNVs from both parents;
- in 25 of 63 patients (39.7%), more than one rare inherited CNV was detected, 18 of whom inherited the CNVs from both parents, 7 from the same parent;
- in a single patient (1.6%), two CNVs were found, one inherited and one with an unknown origin;
- 5 of 63 patients (7.9%) were found to carry rare CNVs with an unknown origin (**Fig. 15**).

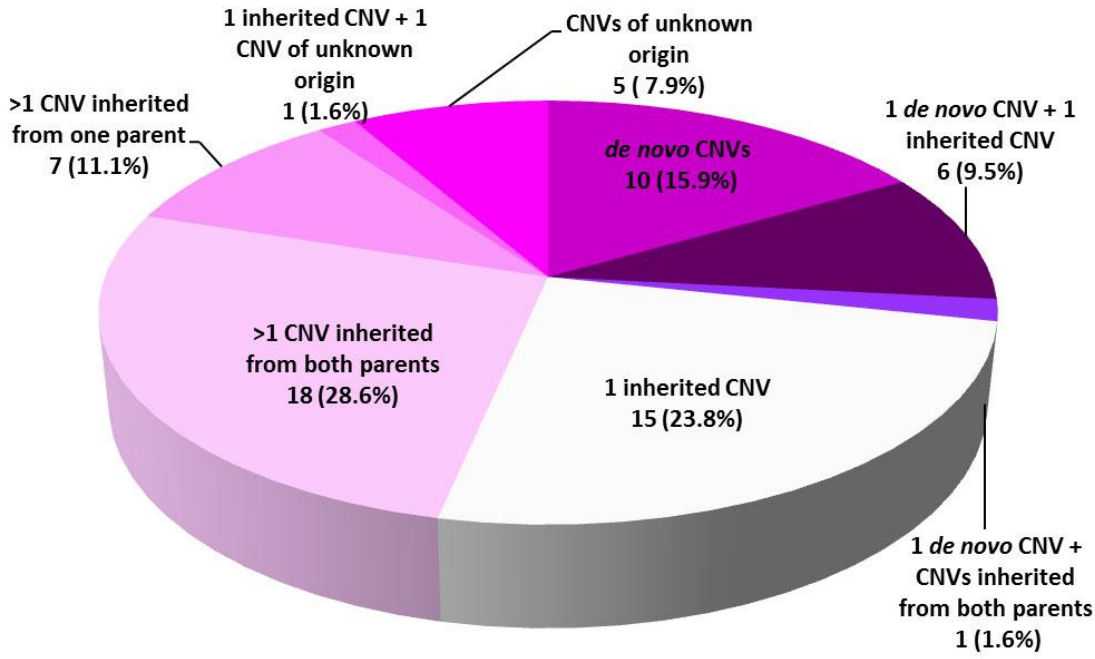

Fig. 15. Schematic view of the 63 patients who were found to be carriers of rare CNVs, based on the origin of the identified CNVs. For each subgroup the number of patients and the percentage are shown.

A detailed list of the identified rare CNVs is shown in **Table 3**. Furthermore, high-resolution array CGH analysis allowed the identification of a few CNVs already reported in the DGV in a subset of the ASD cohort. These variations were very heterogeneous in terms of size and physical localization, spread throughout the genome, and usually recurrent in our cohort. Among these "common" CNVs, which have been selected for their possible role as susceptibility loci for ASD pathogenesis, two variants appeared to be more significant. They both involve genes highly expressed in the CNS and affect the Protocadherin gene cluster and the *KIAA1267* gene, respectively (data not shown).

Tab. 3. Rare CNVs (*de novo* or inherited) identified in the ASD cohort by means of array CGH analysis.

ID	Sex	Phenotype	Chromosome band	Gain/ Loss	Size	Genes	Total	Physical position[#]	Inheritance
Array CGH 244K									
1	F	AU, severe ID, EP	Xp22.11	Gain	99 kb	***EIF2S3, ZFX***	2	chrX:24091852-24190826	pat
2	M	AU, ID	1p34.1	Loss	108 kb	***JMJD2A***	3	chr1:44149337-44257788	pat
			5q23.1	Loss	222 kb		/	chr5:119532516-119755412	pat
3	M	HF-AU	3p21.31	Gain	173 kb	***CSPG5, SMARCC1***	2	chr3:47578922-47752329	pat
			20p12.1	Loss	85 kb	***MACROD2***	1	chr20:15055853-15140973	mat
4	M	AU, ID	10p12.31	Gain	106 kb		/	chr10:22394622-22500888	pat
5	F	PDD-NOS	18q21.1	Gain	51 kb		1	chr18:47902251-47953250	mat
			21q21.3	Gain	52 kb	***PDE9A***	1	chr21:44168808-44220396	mat
6	M	AU, ID	4q23	Gain	81 kb	***TSPAN5***	1	chr4:99393391-99474056	*de novo*
7	M	PDD-NOS	2p23.1	Loss	34 kb	***LCLAT1***	1	chr2:30814684-30848349	mat
			8q12.1	Gain	45.5 kb	***PLAG1***	1	chr8:57052812-57098333	pat
8	M	HF-AU	4p15.2	Loss	54 kb		/	chr4:28107488-28161143	mat
			7q11.23	Gain	51 kb		1	chr7:72745047-72795632	mat
			18q22.1	Gain	139 kb		/	chr18:61838447-61977366	mat
9	M	AU, ID, EP, BM	5q14.3	Loss	25 kb	***GPR98***	1	chr5:90287474-90312790	mat
			5q23.1	Gain	23 kb	***PRR16***	1	chr5:119982811-120005757	pat
10	M	AU, ID	2q14.2q14.3	Gain	3.9 Mb	***CLASP1, C1QL2, DBI, EN1, EPB41L5, GLI2, MARCO, PCDP1, PTPN4, RALB, RNU4ATAC, SCTR***	20	chr2:119130298-123004562	pat
			3q26.1	Gain	185 kb		/	chr3:165221892-165406558	mat
11	M	PDD-NOS	4q23	Gain	35 kb	***RAP1GDS1***	1	chr4:99227050-99262338	pat

Tab 3. Continued.

12	M	AU, ID	11q14.2	Gain	36 kb	***ME3***	1	chr11:86370779-86407258	mat
13	M	HF-AU	13q21.1	Loss	218 kb		/	chr13:56137534-56355454	pat
			13q31.1	Loss	77 kb		/	chr13:84541850-84619100	mat
14	M	S-AU	9q34.3	Gain	442.5 kb	***CACNA1B, EHMT1***	2	chr9:140527202-140969676	*de novo*
			Xq22.3	Gain	24 kb	***IL1RAPL2***	1	chrX:104155507-104179536	mat
15	M	AU, ID	15q26.2	Loss	38 kb	***MCTP2***	1	chr15:94783059-94819771	mat
16	M	PDD-NOS	17q23.1	Gain	44 kb	***CA4***	1	chr17:58206236-58249853	*de novo*
17	M	PDD-NOS	12p13.1	Gain	69 kb		/	chr12:14400276-14468981	pat
			12p12.2p12.1	Loss	387 kb		3	chr12:21017576-21404166	mat
			14q13.1	Gain	131 kb	***CFL2, SNX6***	2	chr14:35062258-35193276	pat
18	M	PDD-NOS	6q21	Loss	47 kb	***PREP***	1	chr6:105824080-105871245	*de novo*
19	M	AS	Yq11.21	Gain	449 kb	***USP9Y***	1	chrY:14492654-14941561	pat
20[§*]	M	HF-AU	8q12.1	Gain	45.5 kb	***PLAG1***	1	chr8:57052812-57098274	mat
21	M	HF-AU	5q31.3	Gain	76 kb	***ARHGAP26***	1	chr5:142148254-142224066	mat
22	M	AU, ID	3p14.1	Gain	94 kb	***ADAMTS9***	1	chr3:64408148-64502388	mat
			6q25.2	Loss	15 kb	***IPCEF1***	2	chr6:154614264-154628947	pat
23	M	PDD-NOS	17q21.31	Loss	748 kb	***ACBD4, CRHR1, FMNL1***	10	chr17:43193251-43941693	*de novo*
			17q24.2	Loss	93 kb	***PRKCA***	1	chr17:64341093-64433941	mat
24	M	AU, ID	9p24.2	Loss	191 kb	***RFX3***	1	chr9:3454648-3645936	*de novo*
			13q12.11	Gain	363 kb	***MPHOSPH8, PSPC1, ZMYM5***	4	chr13:20181070-20544241	mat
25	M	HF-AU	10p11.21	Gain	61 kb	***PARD3***	1	chr10:35044586-35105887	mat
			10p11.21	Gain	223 kb	***CCNY, CREM***	2	chr10:35485580-35708979	mat
			15q13.3	Gain	377 kb	***CHRNA7***	2	chr15:32085731-32462701	mat
			16p11.2	Gain	538 kb	***ALDOA, ASPHD1, CDIPT, C16orf53, DOC2A, FAM57B, GDPD3, HIRIP3, INO80E, KCTD13, KIF22, MAPK3, MAZ, MVP, QPRT, PPP4C, PRRT2, SEZ6L2, SPN, TAOK2, YPEL3***	27	chr16:29652999-30190568	pat
			17q21.31	Loss	8 kb	***VAT1***	2	chr17:41159926-41167557	pat

Tab 3. Continued.

26	F	PDD-NOS, severe ID	6p22.3	Loss	67 kb		/	chr6:22696464-22763720	*de novo*
			11q14.1	Loss	46 kb	***DLG2***	1	chr11:84239601-84285477	mat
27	M	AU, ID	1q44	Loss	242 kb	***SMYD3***	1	chr1:246143162-246385329	pat
			2p11.2	Gain	217 kb	***POLR1A, REEP1***	5	chr2:86289130-86506034	mat
			22q11.21	Gain	2.6 Mb	***AIFM3, ARVCF, CDC45, CLDN5, CLTCL1, COMT, CRKL, DGCR2, DGCR6, DGCR6L, DGCR8, DGCR14, GNB1L, GP1BB, GSC2, HIRA, KLHL22, LZTR1, MED15, MRPL40, PI4KA, PRODH, RANBP1, RTN4R, SEPT5, SLC7A4, SLC25A1, SNAP29, TBX1, TRMT2A, TXNRD2, UFD1L, ZDHHC8, ZNF74***	44	chr22:18894835-21505417	*de novo*
28	M	AU, ID	21q22.3	Gain	40 kb	***DIP2A, PCNT***	2	chr21:47864658-47904775	mat
29	M	AU, ID	4p15.1	Gain	1.1 Mb	***PCDH7***	1	chr4:30096956-31196169	mat
			15q11.1q13.1	Gain	8.0 Mb	***ATP10A, C15orf2, CYFIP1, GABRA5, GABRB3, GABRG3, MAGEL2, MKRN3, NDN, NIPA1, NIPA2, SNRPN, SNURF, TUBGCP5, cluster snoRNAs, UBE3A***	~80 a	chr15:20575646-28535051	*de novo*
30[§]	M	AU, ID	3p35.3-p25.2	Gain	145 kb		2	chr3:11732027-11876792	no mat
			7q11.23	Gain	95 kb	***PTPN12***	1	chr7:77126596-77221182	no mat
			16p13.11	Gain	800 kb	***NDE1***	8	chr16:15492317-16292235	no mat
			22q11.22	Loss	200 kb		1	chr22:23046186-23245888	mat
31[§]	F	PDD-NOS	7q22.1	Loss	260 kb	***CUX1***	1	chr7:101463620-101723676	NA
			22q11.22	Loss	524 kb	***ZNF280A***	5	chr22:22721907-23245888	NA
32	F	AU, ID	10p14	Gain	349 kb		/	chr10:10587763-10936503	mat
			10p12.31	Gain	22 kb		/	chr10:20790681-20812664	pat
			22q11.21	Loss	120 kb	***DGCR6, PRODH***	2	chr22:18890271-19010508	mat

Tab 3. Continued.

33	M	AU, ID	2p23.1	Loss	34 kb	***LCLAT1***	1	chr2:30814684-30848349	mat
			9p24.3	Gain	107 kb	***DOCK8***	1	chr9:254654-361777	pat
			9p24.3	Gain	78 kb	***KANK1***	1	chr9:509521-587418	pat
			20p12.1	Loss	103 kb	***MACROD2***	1	chr20:14653662-14756452	pat
34[§]	M	S-PDD	13q14.3	Loss	68 kb	***RNASEH2B***	2	chr13:51530516-51598396	mat
35[§]	M	PDD-NOS, language delay	12q24.31	Loss	93 kb	***MPHOSPH9***	2	chr12:123585931-123678954	pat
			Xp22.31	Gain	414 kb	***NLGN4X***	1	chrX:6,031,746-6,445,321	mat
36	M	AU, ID	7q11.23	Loss	26 kb	***HSPB1***	2	chr7:75913642-75939538	*de novo*
			10q22.2	Loss	24 kb	***CAMK2G, NDST2***	3	chr10:75559706-75583870	*de novo*
			15q22.2	Loss	48 kb		1	chr15:59776382-59824713	*de novo*
37	M	AU, ID	6q12	Loss	260 kb		1	chr6:66158720-66418279	pat
			8q24.3	Loss	33 kb	***MAPK15***	3	chr8:144766624-144799957	mat
38[§]	M	PDD-NOS	9p24.1	Gain	102 kb	***GLDC, JMJD2C***	2	chr9:6641759-6743452	mat
			12p13.33	Gain	262 kb	***CACNA1C***	1	chr12:2205044-2467239	pat
			12p13.33p13.32	Gain	489 kb	***PRMT8***	2	chr12:3169239-3658542	pat
			16p11.2	Gain	659 kb	***ALDOA, ASPHD1, CDIPT, CORO1A, C16orf53, DOC2A, FAM57B, GDPD3, HIRIP3, INO80E, KCTD13, KIF22, MAPK3, MAZ, MVP, QPRT, PPP4C, PRRT2, SEZ6L2, SULT1A3, SPN, TAOK2, YPEL3***	31	chr16:29673954-30332581	mat
39[§]	F	PDD-NOS	9p24.1	Gain	102 kb	***GLDC, JMJD2C***	2	chr9:6641759-6743452	mat
			12p13.33	Gain	262 kb	***CACNA1C***	1	chr12:2205044-2467239	pat
			12p13.33p13.32	Gain	489 kb	***PRMT8***	2	chr12:3169239-3658542	pat
			16p11.2	Gain	659 kb	***ALDOA, ASPHD1, CDIPT, CORO1A, C16orf53, DOC2A, FAM57B, GDPD3, HIRIP3, INO80E, KCTD13, KIF22, MAPK3, MAZ, MVP, QPRT, PPP4C, PRRT2, SEZ6L2, SULT1A3, SPN, TAOK2, YPEL3***	31	chr16:29673954-30332581	mat

Tab 3. Continued.

40[§]	M	AS	9p22.3p22.2	Loss	119 kb		1	chr9:16591045-16710334	mat
			20p12.1	Loss	78 kb	***MACROD2***	1	chr20:14685390-14763042	pat
41[§]	M	TS	6p12.1	Gain	31 kb		1	chr6:56917608-56948694	pat
			18q23	Loss	28 kb	***PARD6G***	1	chr18:77982067-78010032	mat
			20p12.1	Loss	78 kb	***MACROD2***	1	chr20:14685390-14763042	pat
42	M	AU, ID	4q22.1	Loss	25 kb	***GRID2***	1	chr4:93565167-93590451	NA
			18q23	Gain	55 kb	***MBP, ZNF236***	2	chr18:74671482-74726694	NA
43	M	AS	3p22.3	Gain	63 kb	***ARPP21***	1	chr3:35807767-35870363	pat
			3q23	Gain	245 kb	***XRN1***	3	chr3:141839309-142083916	pat
			16q23.1	Loss	78 kb	***CFDP1***	1	chr16:75343357-75421614	mat
44	M	AU, ID	3p26.1	Gain	287 kb	***GRM7***	1	chr3:7474361-7761159	mat
			18q22.1	Gain	139 kb		/	chr18:61838447-61977366	mat
45[§]	M	AU, ID	17q23.3	Loss	23 kb	***CSH1, GH2***	2	chr17:61954172-61977250	mat
46	F	AU, ID	1q43	Loss	78 kb	***PLD5***	1	chr1:242364270-242442157	pat
47	M	AS	4p15.31	Gain	139 kb		/	chr4:19301430-19440122	mat
			7q11.21	Loss	74 kb	***ZNF138***	1	chr7:64204753-64278830	pat
48	M	AU, ID	3q27.1	Gain	46 kb	***AP2M1, DVL3***	3	chr3:183872255-183917986	pat
49	M	AU, ID	5q12.1	Gain	242 kb	***KIF2A, IPO11***	3	chr5:61618444-61860338	pat
			18q22.1	Gain	127 kb		/	chr18:62131257-62258013	mat
	Array CGH 44K								
50	M	PDD-NOS	15q11.2	Loss	211 kb	***CYFIP1, NIPA1, NIPA2, TUBGCP5***	4	chr15:22873688-23085096	*de novo*
51	F	PDD-NOS	2q14.3q21.3	Loss	8.8 Mb	***ACMSD, ARHGEF4, BIN1, CCDC115, ERCC3, FAM123C, FAM168B, GPR17, GPR39, GPR148, HS6ST1, LYPD1, MAP3K2, MGAT5, NCKAP5, PLEKHB2, RAB6C, RAB3GAP1, TUBA3D, TUBA3E***	50	chr2:127083045-135910585	*de novo*
52[§*]	F	PDD-NOS	7q11.23	Gain	1.4 Mb	***BAZ1B, BCL7B, CLDN3, CLDN4, CLIP2, EIF4H, FZD9, GTF2I, GTF2IRD1, LAT2, LIMK1, STX1A, TRIM50***	24	chr7:72726578-74139390	*de novo*

Tab 3. Continued.

53	M	PDD-NOS	2q31.3q32.3	Loss	11.1 Mb	***FRZB, GLS, GULP1, HIBCH, INPP1, ITGA4, NAB1, NCKAP1, NEUROD1, ORMDL1, PDE1A, STAT1, STAT4, TMEFF2, UBE2E3, ZNF804A***	54	chr2:181882353-193007633	*de novo*
			Xp11.4	Gain	103 kb		2	chrX:37850095-37953580	mat
54	M	AU, ID	1p36.32	Gain	304 kb		2	chr1:2857518-3161082	pat
55	F	S-AU, ID	1q24.2	Gain	399 kb	***SELE, SELL***	8	chr1:169413880-169812887	mat
			15q11.2	Gain	203 kb	***CYFIP1, NIPA1, NIPA2, TUBGCP5***	4	chr15:22873688-23076420	mat
56	M	PDD-NOS	16p11.2	Loss	162 kb	***SH2B1***	9	chr16:28837450-28998957	*de novo*
57	F	PDD-NOS	5q31.1q31.2	Gain	3.8 Mb	***CAMLG, C5orf20, CDC23, CDC25C, CXCL14, DDX46, FAM13B, FAM53C, FBXL21, H2AFY, IL9, KIF20A, KLHL3, LECT2, NEUROG1, PITX1, SAR1B, SEC24A, SLC25A48, SPOCK1, WNT8A***	31	chr5:133871536-137708167	*de novo*
58	M	PDD-NOS	15q23	Loss	85 kb		2	chr15:69192894-69277766	mat
59	M	AU, ID	8p23.3	Gain	654 kb	***ARHGEF10, DLGAP2***	5	chr8:1434838-2088785	mat
			19q13.2	Gain	94 kb	***B9D2, BCKDHA***	5	chr19:41836441-41930226	NA
60	M	AU, ID	5p15.33p15.31	Loss	7.8 Mb	***ADCY2, AHRR, CEP72, C5orf38, EXOC3, IRX1, IRX2, IRX4, LPCAT1, NSUN2, PDCD6, SDHA, SLC6A3, SRD5A1, TPPP, ZDHHC11***	36	chr5:95243-7859564	*de novo*
			18p11.32p11.22	Gain	9.1 Mb	***ADCYAP1, ARHGAP28, DLGAP1, EPB41L3, LAMA1, NDUFV2, PTPRM, RAB12, TGIF1, THOC1, USP14***	34	chr18:180229-9281969	*de novo*
			Xq22.2	Gain	175 kb	***TMSB15B***	2	chrX:103094005-103269195	NA

Tab 3. Continued.

	Array CGH 8x60K								
61	M	PDD-NOS	Xq28	Gain	246 kb	***ATP6AP1, FLNA, IKBKG, PLXNA3, RPL10***	16	chrX:153576890-153822717	NA
62[§*]	M	S-AU	Xp22.31	Gain	1.5 Mb	***STS, PNPLA4***	4	chrX:6551155-8032120	NA
63	M	PDD-NOS	Xp22.31	Gain	551 kb	***STS***	2	chrX:7269569-7820659	NA
			Yp11.2	Gain	878.5 kb		/	chrY:8028838-8907306	NA

[#]Physical position of the identified CNVs based on UCGC Genome Browser, hg19, released February 2009; [§]familiar cases: patients 30 and 31, son and mother, patients 34 and 35 first cousins, patients 38-39 and 40-41 siblings, the mother of patient 45 shows autistic traits but she has not been included in the analyzed ASD cohort; [§*]sibling with a similar phenotype who does not carry the same genetic anomaly.
AS, Asperger syndrome; AU, full autism; BM, brain malformations; EP, epilepsy; HF-AU, high-functioning autism; ID, intellectual disability; NA, not available; PDD-NOS, pervasive developmental disorder not otherwise specified; S-AU, syndromic autism; S-PDD-NOS, syndromic PDD-NOS; TS, Tourette syndrome.

5.2. Analysis of the gene content of the identified rare CNVs

The ad hoc analysis of the rare CNV gene content using databases revealed a total of 276 genes that were considered good candidates for ASD. A detailed list of the selected genes is shown in **Table 4**, which reports the genes affected by rare CNVs localized to genomic regions that are not involved in recurrent genomic rearrangements, and in **Table 4.1**, which conversely reports those genes affected by rare CNVs localized to genomic regions that are either involved in recurrent rearrangements responsible for some microdeletion/microduplication syndromes that are comorbid with ASD or have been proposed by linkage studies as candidate loci for ASD or other neuropsychiatric disorders.

A small percentage of the selected genes (~11%) (depicted in red in **Tabs. 4** and **4.1**) have been previously reported as causative genes based on mutations and/or CNVs, often *de novo*, that have been described in autistic patients. Moreover, SNPs in a very few genes (~5%) (depicted in purple **in Tabs. 4** and **4.1**) have been significantly associated with ASD. However, most of the proposed candidate genes (54%) have not been previously reported in association with ASD, and a significant percentage (30.5%) have been involved in some microdeletion/microduplication syndromes that are comorbid with ASD (depicted in dark red in **Tabs. 4** and **4.1**) (**Fig. 16**).

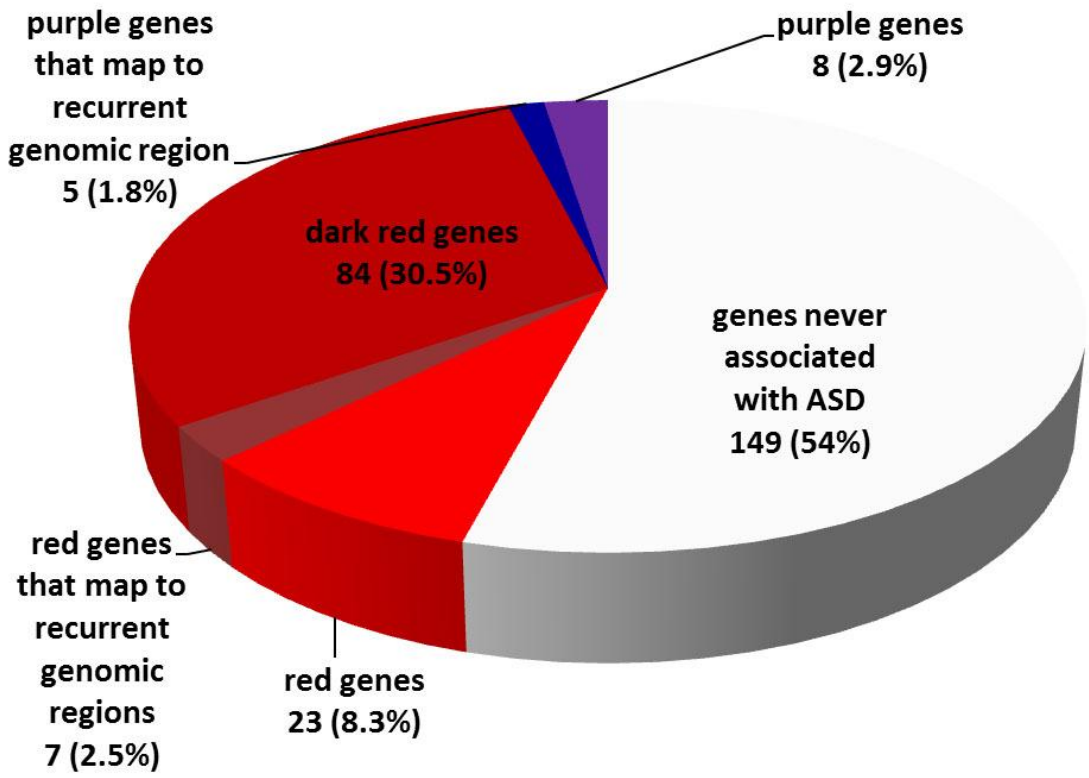

Fig. 16. Schematic view of the classification of the collected genes, based on their previous implication in ASD.

On the basis of gene expression data, function, and pathway of action, all the selected genes contribute to CNS neurodevelopment and maintenance, acting during embryonic and foetal development as well as in the early postnatal period and, in some cases, in adult life. Sixty-six of the 276 selected genes are implicated in neurogenesis and neurodevelopment (24%), 27 in CNS metabolism (10%), 29 in synaptogenesis and synaptic plasticity (10.5%), 19 in CNS development, homeostasis, and immunosurveillance mediated by the immune system (7%), 81 in intracellular signaling and trafficking (29%), and 51 in transcriptional and translational regulation and chromatin remodeling (18.5s%), as shown in **Fig. 17**. For 3 of the 276 genes, the function is still unknown (1%).

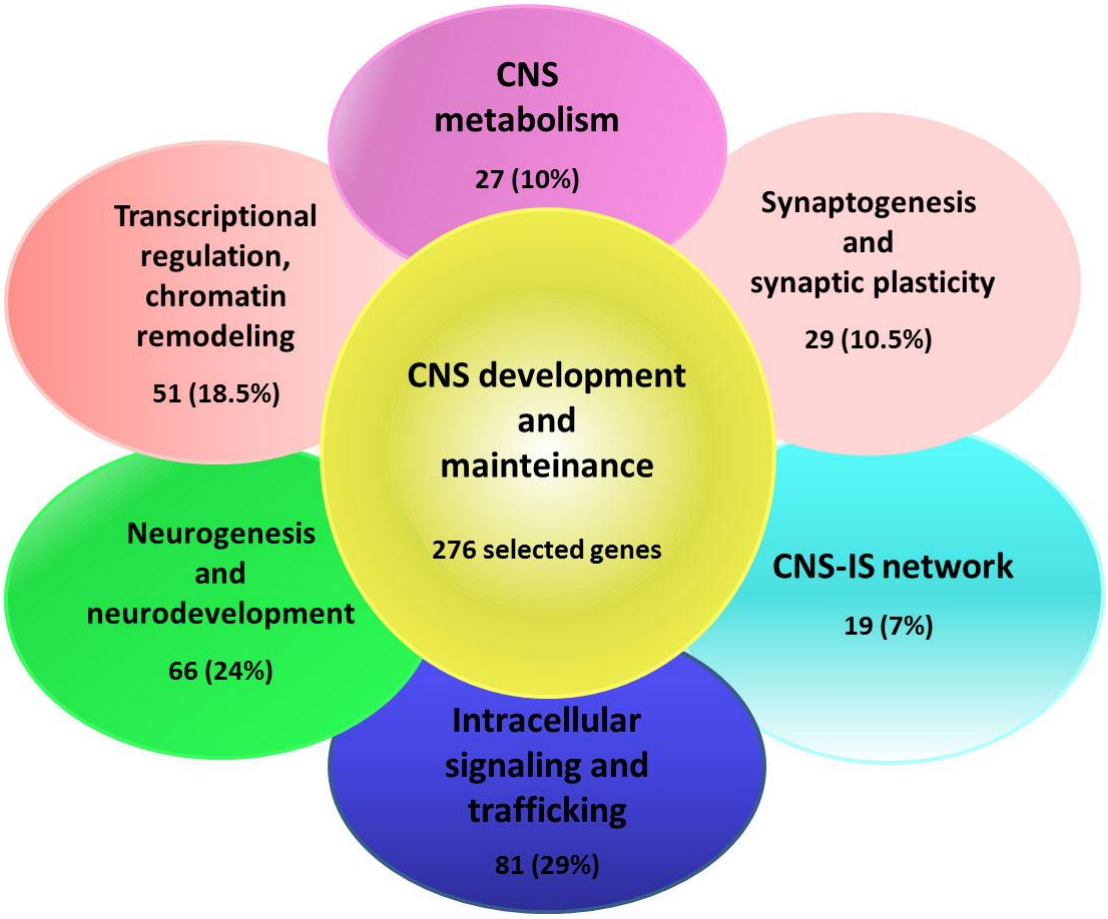

Fig. 17. Schematic view of the different pathways of action that contribute to neurodevelopment and maintenance. CNS, central nervous system; IS, immune system.

A detailed analysis of the 276 selected genes is reported in **Tabs. 4** and **4.1**, where each gene box is depicted with a different color to indicate the specific gene function. Of note, 89 of 276 selected genes, which are indicated in the following tables with light blue boxes or with a star shape, contribute to CNS development and maintenance acting in concert with the immune system.

Tab. 4. Detailed list of the genes potentially perturbed by the identified rare CNVs and possible implicated in ASD pathogenesis (UCSC Genome Browser, hg19, release February 2009)[§].

Gene name	Function and expression	Interactors and possible role in brain	Findings in ASDs o rin other neuropsychiatric disorders	References
ADAMTS9(-)* Organogenesis	This gene encodes the ADAM metallopeptidase 9 with thrombospondin type 1 motif protein. Members of the ADAMTS family have been implicated in the cleavage of proteoglycans and the control of organ shape during development. Expression detected in all fetal tissues.	The ADAM and the related ADAMTS metalloproteinases are membrane-anchored and secreted proteins exhibiting key roles in mediating cell adhesion, proteolytic shedding, and cell signaling. Dysregulation of these proteins has been observed in some pathologic states, including cancers. Indeed, ADAMTS9 was aberrantly expressed by primary malignant pl ma cells. During mouse development ADAMTS9 expression in the CNS is limited to the floor plate of the diencephalon, to the ventricular zone of the cerebral cortex and to the choroid plexus.	Mutations and/or CNVs affecting *ADAMTS9* have never been reported in patients with ASD.	Bret *et al.*, 2011 Jungers *et al.*, 2005
AP2M1(+) Intracellular membrane trafficking: clathrin-mediated endocytosis	This gene encodes the adaptor-related protein complex 2, mu 1 subunit, which is a subunit of the heterotetrameric coat assembly protein complex 2 (AP2). The encoded protein is required for the activity of a vacuolar ATPase, which is responsible for proton pumping occurring in the acidification of endosomes and lysosomes. The encoded protein may also play an important role in regulating the intracellular trafficking and function of CTLA-4 protein. Moderate expression in fetal brain and good expression in postnatal CNS.	It is well known that clathrin-mediated endocytosis is crucial for the normal functioning and integrity of neurons in the CNS. Recently, it has been demonstrated that expression of coat proteins changes during postnatal development in selected areas of the rat brain. This finding supports the hypothesis that proteins that conform the intracellular transport machinery in the brain cells seems to accompany development, according to the maturation of the different brain areas.	Mutations and/or CNVs affecting *AP2M1* have never been reported in patients with ASD. Mutations in the *AP1S2* gene, encoding the sigma1B subunit of the clathrin-associated adaptor protein complex (AP)-1, are responsible for a clinically recognizable XLMR and autism syndrome associating hypotonia, delayed walking, speech delay, aggressive behavior, brain calcifications, and elevated CSF protein levels.	Borck *et al.*, 2008 Borgonovo *et al.*, 2012
ARHGAP26(-)* Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encodes the Rho GTPase activating protein 26 which binds to focal adhesion kinase and mediates the activity of the GTP binding proteins RhoA and Cdc42. Focal adhesion kinase is one of the protein involved in the signaling cascades that regulate the organization of the actin-cytoskeleton which mediate the interaction of a cell with the extracellular matrix. Quite high expression in postnatal parietal and occipital lobes, cerebellum peduncles, and hypothalamus. Moderate expression in immune cell types.	The Rho GTPases, RhoA and Cdc42, are involved in neuronal morphogenesis, axonal guidance and synaptic plasticity by modulating the organization of actin cytoskeleton. The same pathway is involved in T-cells activation, migration, and cell-cell adhesion.	Mutations and/or CNVs affecting *ARHGAP26* have never been reported in patients with ASD. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD and Tuberous Sclerosis 1 or 2. Both the TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates CdC42, thus regulating cell adhesion and migration.	Fombonne *et al.*, 1997 Lewis *et al.*, 2004 Muzykewicz *et al.*, 2007 Wiznitzer, 2004 Wong, 2006

Tab. 4. Continued.

★ ***ARHGEF10(+)*** Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encodes the Rho guanine nucleotide exchange factor (GEF) 10 protein. Rho GTPases play a fundamental role in numerous cellular processes that are initiated by extracellular stimuli that work through G protein coupled receptors. The encoded protein may form complex with G proteins and stimulate Rho-dependent signals. Moderate expression in fetal brain and good expression in postnatal brain, in particolar in prefrontal cotex, amygdalaee and hypothalamus. High expression in immune cell types.	RhoGTPases play a pivotal role in regulating the actin cytoskeleton and influence cell polarity, microtubule dynamics, membrane-transport pathways, and transcription-factor activity. Numerous evidence has implicated RhoGTPases in neuronal morphogenesis, including cell migration, axonal growth and guidance, dendrite elaboration and plasticity, and synapse formation. RhoGEFs activate RhoGTPases by catalyzing the exchange of bound GDP for GTP, which induces a conformational change in the GTP-bound GTPase that allows its interaction with downstream effector proteins, thus playing a central role in defining the temporal and spatial activation of the corresponding GTPase within neuronal cells. In particular, ARHGEF10 is mostly involved in peripheral nerve development.	Mutations and/or CNVs affecting *ARHGEF10* have never been reported in patients with ASD. However, mutations in *ARHGEF10* have been aasociated to slowed nerve-conduction velocities, a biological endophenotype in the majority of the hereditary motor and sensory neuropathies. In addition, a weak association of SNPs in *ARHGEF10* with SCZ has been reported. RhoGEFs have been previously implicated in human genetic disorders: - a mutation in the DH domain of *FGD1* *GEF* cosegregates with faciogenital dysplasia, a developmental disorder; - mutations in *ARHGEF6* are associated with X-linked nonsyndromic MR; - aberrant EphB/Ephexin5 signaling during the development of synapses may contribute to the abnormal cognitive function that occurs in AS and, possibly, ASD.	Boguski and McCormick 1993 Bourne *et al.*, 1990 Etienne-Manneville and Hall 2002 Hart *et al.*, 1994 Kutsche *et al.*, 2000 Margolis *et al.*, 2010 Pasteris *et al.*, 1994 Verhoeven *et al.*, 2003
ARPP21(-)* Intracellular signaling: regulation of CaM-dependent signaling	This gene encodes the cAMP-regulated phosphoprotein, 21kDa. The encoded protein is enriched in the caudate nucleus and cerebellar cortex. Very high expression in fetal brain and in postnatal CNS.	ARPP21 may act as a competitive inhibitor of calmodulin-dependent enzymes such as calcineurin in neurons. Indeed, ARPP21, also known as regulator of calmodulin (CaM) signaling (RCS), when phosphorylated by protein kinase A binds to CaM and inhibits CaM-dependent signaling. RCS expression is high in the dorsal striatum, nucleus accumbens and amygdalaee, suggesting that the protein is involved in limbic-striatal function. In mouse a similar protein is enriched in the central extended amygdalaee. Moreover, it may be involved in regulating the effects of dopamine in the basal ganglia. Recently, it has been demonstrated that the regulator of calmodulin signaling knockout mice display anxiety-like behavior and motivational deficits.	Mutations and/or CNVs affecting *ARPP21* have never been reported in patients with ASD.	Becker *et al.*, 2008 Davis *et al.*, 2012
ATP6AP1(+) Intracellular membrane trafficking: neurotransmitter uptake	This gene encodes the ATPase, H+ transporting, lysosomal accessory protein 1, which is a component of a multisubunit enzyme that mediates acidification of eukaryotic intracellular organelles. The encoded protein is approximately 45 kD and may assist in the V-ATPase-mediated acidification of neuroendocrine secretory granules. Good-high expression in fetal brain and in postnatal CNS.	The vacuolar (H+)-ATPase (V-ATPase) is a universal proton pump and its activity is required for a variety of cell-biological processes such as membrane trafficking, receptor-mediated endocytosis, lysosomal protein degradation, osteoclastic bone resorption and maintenance of acid-base homeostasis by renal intercalated cells. In neuronal and neuroendocrine cells, the V-ATPase is the major regulator of intragranular acidification which is indispensable for correct prohormone processing and neurotransmitter uptake.	Mutations and/or CNVs affecting *ATP6AP1* have never been reported in patients with ASD. Recurrent Copy Number gains at Xq28 including *ATP6AP1* have been reported in mentally retarded patients.	Jansen and Martens, 2012 Vandewalle *et al.*, 2009

Tab. 4. Continued.

BCKDHA(-)* Branched-chain amino acid catabolism	This nuclear gene encodes the branched chain keto acid dehydrogenase E1, alpha polypeptide, which is a subunit of an innter mitochondrial enzyme complex that catalyzes the second major step in the catabolism of the branched-chain amino acids leucine, isoleucine, and valine. Moderate expression in postnatal whole brain.	In the brain, metabolism of the essential branched chain aminoacids (BCAAs) leucine, isoleucine, and valine, is regulated in part by protein synthesis requirements. Excess BCAAs are catabolized or excreted. The first step in BCAA catabolism is catalyzed by the branched chain aminotransferase (BCAT) isozymes. A product of this reaction, glutamate, is the major excitatory neurotransmitter and precursor of the major inhibitory neurotransmitter GABA. The BCATs are thought to participate in a α-keto-acid nitrogen shuttle that provides nitrogen for synthesis of glutamate from α-ketoglutarate. The branched-chain α-ketoacid dehydrogenase enzyme complex (BCKDC) catalyzes the second, irreversible step in BCAA metabolism, which is oxidative decarboxylation of the branched-chain α-ketoacid (BCKA) products of the BCAT reaction. Defects in *BCKDHA* are a cause of maple syrup urine disease type IA. MSUD is an autosomal recessive disorder characterized by mental and physical retardation, feeding problems, and a maple syrup odor to the urine. In individuals with MSUD, the oxidation of BCAAs is inhibited and, therefore, intake of BCAAs above the daily requirement for protein synthesis causes accumulation of BCAAs and their BCKAs to toxic level. If left untreated, most patients experience seizures, changes in muscletone, and coma due to brain swelling. Analysis of MSUD brains by magnetic resonance diffusion imaging spectroscopy suggests impaired brain energy metabolism. Neurological disorders frequently involve disruption of the proper balance of these excitatory (glutamate) and inhibitory (GABA) neurotransmitters, which result in altered excitability.	Mutations or CNVs affecting *BCKDHA* have never been reported in patients with ASD. Inactivating mutations affecting *BCKDK* (Branched Chain Ketoacid Dehydrogenase Kinase) have been reported in consanguineous families with autism, EP, and ID. The encoded protein is responsible for phosphorylation-mediated inactivation of the E1-α subunit of branched chain ketoacid dehydrogenase (BCKDH).	Cole *et al.*, 2012 Novarino *et al.*, 2012
B9D2(+) Ciliogenesis: regulation of microtubule cytoskeleton dynamics	This gene encodes the B9 protein domain 2, which is exclusively found in ciliated organisms. The gene is upregulated during mucociliary differentiation, and the encoded protein localizes to basal bodies and cilia. Disrupting expression of this gene results in ciliogenesis defects. Good expression in fetal brain and moderate expression in postnatal parietal and temporal lobes, and in thalamus.	B9D2, as well as KIF2A, belongs to kinetochore and is involved in the microtubule-bound to the kinetochore. KIF2A, the kinesin heavy chain member 2A protein, may regulate microtubule dynamics during axonal growth. By analogy, also B9D2 is probably involved in brain development.	Mutations and/or CNVs affecting *B9D2* have never been reported in patients with ASD. Mutations in human genes encoding the B9 domain-containing proteins (MKS1, B9D1, and B9D2) cause Meckel syndrome, a severe ciliopathy characterized by occipital encephalocele, liver ductal plate malformations, polydactyly, and kidney cysts.	Dowdle *et al.*, 2011

Tab. 4. Continued.

CA4(+) ***de novo*** Ecitatory synaptic plasticity	This gene encodes the carbonic anhydrase IV, which belongs to a large family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide. They participate in a variety of biological processes, including respiration, calcification, acid-base balance, bone resorption, and the formation of aqueous humor, cerebrospinal fluid, saliva, and gastric acid. The protein CA4 has been found in the endothelium of the choriocapillaris in eyes and no detectable levels are found in renal capillaries. Good expression in postnatal brain and cerebellum.	The presence of extracellular carbonic anhydrases in the CNS was detected in physiological studies of rat hippocampal slices. The enzymes are indirectly implicated in regulation of excitatory synaptic transmission, because the curtailment of extracellular alkaline shifts by extracellular carbonic anhydrases was shown to limit postsynaptic NMDA receptor activation during synchronous neural activity. The principal isoforms of carbonic anhydrases in the brain are CA4 and CA14, and both enzymes catalyze the buffering of activity-dependent pHe transients. Defects in *CA4* are the cause of retinitis pigmentosa type 17.	Mutations and/or CNVs affecting *CA4* have never been reported in patients with ASD. Carbonic anhydrase II (CA II) deficiency in man is an autosomal recessive disorder manifest by osteopetrosis, renal tubular acidosis, cerebral calcification, growth retardation and MR. Rare single gene mutations affecting *CA6* has been reported in a few autistic patients.	Bucan *et al.*, 2009 Fedirko *et al.*, 2007 Parkkila *et al.*, 2001 Shah *et al.*, 2005 Sly *et al.*, 1991 Tong *et al.*, 2000
CACNA1C(-)* Synaptogenesis and synaptic plasticity	This gene encodes the calcium channel, voltage-dependent, L type, alpha 1C subunit. Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization, and they are involved in a variety of calcium-dependent processes, including muscle contraction, hormone or neurotransmitter release, gene expression, cell motility, cell division and cell death. Good-high expression in fetal brain and in postnatal temporal lobe, prefrontal and cingulate cortex, and amygdalaee.	CACNA1C is most frequently implicated in coupling of cell membrane depolarization to transient increase of the membrane permeability for calcium, leading to activation and, potentially, changes in intracellular signaling pathway activity, gene transcription, and synaptic plasticity. It is involved in the proper function of numerous neurological circuits including those involving the hippocampus, amygdalaee, and mesolimbic reward system, which are strongly implicated in psychiatric disease pathophysiology. In particular, it has been reported that a gain of function mutation in *CACNA1C* may result in an inappropriate activation of the ERK cascade as seen in a subset of autistic patients.	Defects in *CACNA1C* are the cause of Timothy syndrome, which is comorbid with ASD. Moreover, mutations in *CACNA1C* have been associated to depression and SCZ. Mutations affecting different CACNA genes, such as *CACNA1F* and *CACNA1H*, have been previously reported in idiopathic or syndromic patients with ASD.	Bhat *et al.*, 2012 Hemara-Wahanui *et al.*, 2005 Kalkman, 2012 Splawski *et al.*, 2004, 2006
CAMK2G(-) ***de novo*** Intracellular Wnt/Ca^{2+} signaling pathway	This gene encodes the calcium/calmodulin-dependent protein kinase II gamma, which is one of the four subunits of an enzyme which belongs to the serine/threonine protein kinase family, and to the Ca(2+)/calmodulin-dependent protein kinase subfamily. Calcium signaling is crucial for several aspects of plasticity at glutamatergic synapses. High expression in fetal brain and in postnatal CNS.	Calcium/calmodulin-dependent protein kinase type II (CaMKII) is a highly abundant serine/threonine kinase comprising a significant fraction of total protein in mammalian forebrain and forming a major component of the postsynaptic density. CaMKII is essential for certain forms of synaptic plasticity and memory consolidation and this is mediated through substrate binding and intramolecular phosphorylation of holoenzyme subunits. It has been suggested that cellular specific pattern of the different isoforms of the holoenzyme subunits might play a role in propagating the type of recurrent neuronal activity associated with disorders such as temporal lobe EP. CAMK2G is involved in the Wnt/Ca^{2+} signaling which is mediated through G proteins and phospholipases and leads to transient increases in cytoplasmic free calcium that subsequently activate the kinase PKC (protein kinase C) and CAMKII (calcium calmodulin mediated kinase II) and the phosphatase calcineurin.	Mutations and/or CNVs affecting *CAMK2G* have never been reported in patients with ASD. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Chung *et al.*, 2011 De Ferrari and Moon, 2006 Liu and Murray, 2012 Okerlund and Cheyette, 2011 Wang *et al.*, 2010 Zhang *et al.*, 2012

Tab. 4. Continued.

CCNY(-)* Intracellular Wnt signaling pathway	This gene encodes the cyclin Y protein. Cyclins, such as CCNY, control cell division cycles and regulate cyclin-dependent kinases (e.g., CDC2). CCNY acts as a cell-cycle regulator of Wnt signaling pathway during G2/M phase by recruiting CDK14/PFTK1 to the plasma membrane and promoting phosphorylation of LRP6, leading to the activation of the Wnt signaling pathway. Moderate expression in postnatal CNS.	CCNY directly interacts with CDK14, the cyclin-dependent kinase 14, which plays a role in neuron differentiation and/or function. CDK14/cyclin Y complex promotes Wnt signaling through phosphorylation of the LRP6 co-receptor, a key regulatory nexus in the Wnt/beta-catenin pathway, thus suggesting that this pathway might orchestrate mitotic processes.	Mutations and/or CNVs affecting *CCNY* have never been reported in patients with ASD. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Chung *et al.*, 2011 Davidson and Niehrs, 2010 De Ferrari and Moon, 2006 Jiang *et al.*, 2009 Okerlund and Cheyette, 2011 Wang *et al.*, 2010 Zhang *et al.*, 2012
★ ***CFDP1(-)*** Embryonal development	This gene encodes the craniofacial development protein 1, which may play a role during embryogenesis. Good expressione in fetal brain and in postnatal CNS, in particolar in temporal lobe, prefrontal cortex, amygdalaee, thalamus, and hypothalamus. Good expression in immune cell types.	It has been suggested that CP27, a mouse homologous of human CFDP1, has a role in organogenesis. CFDP1 is a substrate of CSNK2A1, the casein-kinase 2 alpha which is ubiquitously expressed in different brain regions and phosphorylates several proteins with a known role in brain development (e.g. STX1A, L1CAM).	Mutations and/or CNVs affecting *CFDP1* have never been reported in patients with ASD. SNPs in *STX1A* showed nominal associations with HF-AU. A missense mutation in *L1CAM* has been reported in an adult male patient with L1 disease and autism.	Iwashita *et al*, 1999 Luan and Diehwisch, 2002 Nakamura *et al.*, 2008, 2011 Risinger and Bennett, 1999 Simonati *et al.*, 2006 Wong *et al.*, 1996
CFL2(+) Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encode the cofilin 2 protein, an intracellular protein that is involved in the regulation of actin-filament dynamics in a pH-sensitive manner. Low expression in fetal brain and high expression in postnatal CNS.	The activity of CFL2 is directly regulated by the opposite functions of LIMK1, a protein kinase, and SSH1 (slingshot homolog 1 of Drosophila), a protein phosphatase, both involved in brain development. Indeed, the activity of cofilin is repressed by phosphorilation by LIM kinase and is reactived by dephosphorilation by SSH1. LIMKs are activated by Rho family GTPases via actions of their downstream effectors, such as Rho-associated kinase (ROCK) and p21-activated kinase (PAK). Thus, LIMKs seem to play a critical role in stimulus-induced actin cytoskeletal remodeling by linking the signal from Rho family GTPases to the change in cofilin activity. This regulation is essential in controlling growth cone motility and morphology and neurite extension. Mutations in *CFL2* cause nemaline myopathy type 7, a form of congenital myopathy.	Mutations and/or CNVs affecting *CFL2* have never been reported in patients with ASD. Rho GTPases, RhoA and Cdc42, are involved in neuronal morphogenesis, axonal guidance and synaptic plasticity by modulating the organization of actin cytoskeleton. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD and Tuberous Sclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates CdC42, thus regulating cell adhesion and migration.	Agrawal *et al.*, 2007 Endo *et al.*, 2003 Fombonne *et al.*, 1997 Galkin *et al.*, 2011 Lewis *et al.*, 2004 Meyer and Feldman, 2002 Muzykewicz *et al.*, 2007 Samiere and Bamburg, 2004 Wiznitzer, 2004 Wong, 2006
★ ***CREM(-)**** Trascriptional regulation	This gene encodes the cAMP responsive element modulator protein, which is a transcription factor that binds to the cAMP responsive element found in many viral and cellular promoters. It is an important component of cAMP-mediated signal transduction during the spermatogenetic cycle, as well as other complex processes. Alternative promoter and translation initiation site usage allows this gene to exert spatial and temporal specificity to cAMP responsiveness. Moderate expression in postnatal whole brain and good expression in amygdalaee.	The family of CREB (cAMP response element-binding protein) transcription factors are involved in a variety of biological processes including the development and plasticity of the nervous system. In the maturing and adult brain, CREB genes are required for activity-dependent processes, including synaptogenesis, refinement of connections and long-term potentiation. By examining CREB1-CREM(-/-) mouse mutants, it has been demonstrated that the lack of CREB/CREM genes, specifically in neural and glial progenitors, leads to migration abnormalities during brain development and that CREB/CREM transcription factors negatively regulate early synaptogenesis and spontaneous network activity.	A rare inherited CNV involving *CREM* has been reported in a patient with ASD and ID.	Aguado *et al.*, 2009 Diaz-Ruiz *et al.*, 2008

Tab. 4. Continued.

CSH1(-) Organogenesis, intrauterine growth	This gene encodes the chorionic somatomammotropin hormone 1, which is a member of the somatotropin/prolactin family of hormones and plays an important role in growth control. The gene is located at the growth hormone locus on chromosome 17 along with four other related genes. It is produced only during pregnancy and is involved in stimulating lactation, fetal growth and metabolism. Good expression in fetal brain and in postnatal temporal, parietal and occipital lobes, and cerebellum.	Pituitary GH1/IGF-I axis may play an important role in CNS functions, including those associated with neuronal growth, development, and protection. Furthermore, the GH1/IGF axis may play a role in influencing aspects of mood and cognition. GH-binding sites have been identified in several areas of the brain, including the choroid plexus, putamen, thalamus, pituitary, hippocampus, and cortex.. It has been demonstrated that GH modulates synaptic efficacy of hippocampal neurons and itself is regulated during memory formation, learning processes, and emotional experiences. Somatotropin/prolactin hormonedeficiency may cause intrauterine growth restriction (IUGR), which has an effect on the hippocampus structure that correlates with behavioural problems in preterm infants.	Mutations and/or CNVs affecting *CSH1* have never been reported in patients with ASD. A few autistic patients showing growth hormone deficiency have been reported.	Devillard *et al.*, 2010 Donahue et al., 2006 Gingell *et al.*, 1996 Lodygensky *et al.*, 2008 Ragusa *et al.*, 1993 Zearfoss et al., 2008
CSPG5(+) Neuronal growth	This gene encodes the chondroitin sulfate proteoglycan 5, also known as neuroglycan C, which is a proteoglycan that functions as a neural growth and differentiation factor. Very high and specific expression in fetal brain and in postnatal CNS.	Neuroglycan C (NGC) is a transmembrane-type chondroitin sulfate proteoglycan that is exclusively expressed in the CNS. Both protein kinase C (PKC) inhibitors and phosphatidylinositol 3-kinase (PI3K) inhibitors attenuated the NGC-mediated neurite outgrowth in a dose-dependent manner, suggesting that NGC promotes neurite outgrowth via PI3K and PKC pathways. NGC directly interacts with GOLPH3, the golgi phosphoprotein 3, which is involved in modulation of mTOR signaling, that is in turn regulated by both TSC1 and TSC2.	Mutations and/or CNVs affecting *CSPG5* have never been reported in patients with ASD. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD and Tuberous Sclerosis 1 or 2.	Fombonne *et al.*, 1997 Hassel *et al.*, 2003 Lewis *et al.*, 2004 Muzykewicz *et al.*, 2007 Nakanishi *et al.*, 2006 Wiznitzer, 2004 Wong, 2006
CUX1(-) Transcriptional regulation	This gene encodes the cut-like homeobox 1 protein, which is a member of the homeodomain family of DNA binding proteins. It may regulate gene expression, morphogenesis, and differentiation and it also has a role in the cell cycle progession. Low expression in fetal brain, moderate expression in postnatal CNS, in particolar in prefrontal cortex, cerebellum and cerebellum peduncles. Good-high expression in immune cell types.	CUX1 is a regulator of brain development. It is co-expressed with *EN2* during CNS development and throughout the postnatalhood. Mouse Cux1 regulates dendritic branching, spine morphology and synapse formation in cerebral cortex, which contributes to cognitive circuitry. CUX1 mediates changes in the chromatin conformation and influences V(D)J recombination in B-cells.	Mutations and/or CNVs affecting *CUX1* have never been reported in patients with ASD. SNPs in *EN2* have previously been associated with ASD.	Benayed *et al.*, 2005 Choi *et al.*, 2012 Cubelos *et al.*, 2010 Gharani *et al.*, 2004 Goebel *et al.*, 2002 Hulea and Nepveu, 2012 Li *et al.*, 2010
DIP2A(-)* Neurogenesis: axon patterning	This gene encodes the DIP2 disco-interacting protein 2, homolog A (Drosophila), which is involved in axon patterning in the CNS. Low expression in fetal brain, and in postnatal CNS, escept for prefrontal cortex and hypothalamus where expression is good. High expression in immune cell types.	DIP2A is the plasma membrane receptor for follistatin-like 1 protein, FSTL1, which has been implicated in diverse disease processes as a regulator of inflammatory cytokine expression.	Mutations and/or CNVs affecting *DIP2A* have never been reported in patients with ASD. *DIP2A* has been proposed as a candidate gene for dyslexia.	Adams *et al.*, 2010 Poelmans *et al.*, 2009

Tab. 4. Continued.

DLGAP2(SAPAP2) (+) Synaptogenesis and synaptic plasticity	This gene encodes the discs, large (Drosophila) homolog-associated protein 2, which is one of the membrane-associated guanylate kinases localized at postsynaptic density in neuronal cells. This protein is an adapter protein linking ion channel to the subsynaptic cytoskeleton and plays a role in the molecular organization of synapses and in neuronal cell signaling. High expression in fetal brain and in postnatal CNS, in particolar in thalamus and amygdalaee.	NMDA neurotransmitter receptors and SAPAP2 (DLGAP2) are integral components of post-synaptic macromolecular signaling complexes that serve to propagate glutamate responses intracellularly. Recently, NMDA receptor subtype-specific binding sites, that mediate direct interactions with scaffold protein SAPAP2, have been identified. Furthermore, DLGAP2 binds to SHANK2, which acts as a link between the post-synaptic receptor on the plasma membrane and the cytoskeleton, and directly interacts with NLGN4X.	A *de novo* CNV involving *DLGAP2* has been reported in a patient with ASD. Mutations and/or CNVs affecting *SHANK2* and *NLGN4X* have been previously reported in autistic patients.	Baris *et al.*, 2007 Berkel *et al.*, 2010, 2012 Bolliger *et al.*, 2001 Cousins and Stephenson, 2012 Jamain *et al.*, 2003 Kent *et al.*, 2008b Laumonnier *et al.*, 2004 Lawson-Yuen *et al.*, 2008 Leblond *et al.*, 2012 Marshall *et al.*, 2008 Pinto *et al.*, 2010
DLG2(-) Synaptogenesis and synaptic plasticity	This gene encodes the discs, large homolog 2 (Drosophila) protein, which is a member of the membrane-associated guanylate kinase (MAGUK) family. The encoded protein forms a heterodimer with a related family member that may interact at postsynaptic sites to form a multimeric scaffold for the clustering of receptors, ion channels, and associated signaling proteins. Good expression in fetal brain, very high expression in postnatal CNS.	DLG2 is part of the postsynaptic protein scaffold of excitatory synapses; it is required for perception of chronic pain through NMDA receptor signaling and is involved in regulation of synaptic stability at cholinergic synapses. DLG2 interacts with NMDA glutamate receptors GRIN2A and GRIN2B, as well as with other proteins of the postsynaptic scaffold such as DLGAP1 and DLGAP4 and cytoskeleton proteins such as MAP1A.	Mutations and/or CNVs affecting *DLG2* have never been reported in patients with ASD. *DLG2* is deleted in SCZ in a study of Genome-Wide Copy Number Variation and shows a reduction in protein expression in post-mortem brain samples from schizophrenics. Moreover, mouse knockouts of *DLG2* show hypofunction of NMDA receptor signaling, a process implicated in SCZ. It has been reported that *DLG4* gene disruption in mice produces a complex range of behavioral and molecular abnormalities relevant to autism spectrum disorders and Williams' syndrome. A SNP in *GRIN2B* has been associated with ASD in Korean patients. A *de novo GRIN2B* mutation has been reported in an autistic patient. SNPs in *GRIN2A* have been associated with SCZ. Two *de novo GRIN2A* mutations has been reported in SCZ patients.	Barnby *et al.*, 2005 Feyder *et al.*, 2010 MacLaren *et al.*, 2011 Tarabeux *et al.*, 2011 Walsh *et al.*, 2008 Yoo *et al.*, 2012

Tab. 4. Continued.

★ ***DOCK8(-)**** Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encodes the dedicator of cytokinesis 8 protein, which is a member of the DOCK180 family of guanine nucleotide exchange factors. GEFs proteins interact with Rho GTPases and are components of intracellular signaling networks. It is expressed at low levels in brain tissue.	In mammalian cells, the DOCK family of proteins have roles in regulating cytoskeletal reorganization, which is important for neuronal and immune function. DOCK8 is involved in the reorganization of the actin filament system through its direct interaction with CDC42, RhoJ, and RhoK. The interaction between DOCK8 and Cdc42-is critical for interstitial dendritic cell migration through the interstitium and for the polarity changes necessary for T-cell activation and function. Indeed, both B and T-cells from DOCK8 mutant mice form defective immunological synapses and have abnormal functions, in addition to impaired immune memory development. In humans, mutations in *DOCK8* result in the autosomal recessive form of the hyper-IgE syndrome. It is a rare disorder of immunity characterized by immunodeficiency, recurrent infections, eczema, increased serum IgE, eosinophilia and lack of connective tissue and skeletal involvement.	Two *de novo* 9p24 terminal deletion including *DOCK8* and *ANKRD15* have been reported in a female and a male patient with ASD. The male patient presented also a gonadal dysgenesia. *DOCK8* disrupton has been reported in two patients with MR and developmental disabilities. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD and Tuberous Sclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates CdC42, thus regulating cell adhesion and migration.	Fombonne *et al.*, 1997 Griggs *et al.*, 2008 Harada *et al.*, 2012 Jabara *et al.*, 2012 Lewis *et al.*, 2004 Muzykewicz *et al.*, 2007 Ounap *et al.*, 2004 Ruusala and Aspenstrom, 2004 Vinci *et al.*, 2007 Wiznitzer, 2004 Wong, 2006
DVL3(+) Intracellular Wnt signaling pathway	This gene encodes the dishevelled, dsh homolog 3 protein, which is a member of a multi-gene family that shares strong similarity with the Drosophila dishevelled gene, dsh. The Drosophila dishevelled gene encodes a cytoplasmic phosphoprotein that regulates cell proliferation. Moderate expression in fetal brain and in postnatal CNS.	DVL3 plays a role in the signal transduction pathway mediated by multiple Wnt genes. In a recent genome-wide analysis of repressive histone methylation in nucleus accumbens of mice subjected to chronic social defeat stress, numerous genes were identified where stress induced changes in histone methylation in susceptible but not resilient mice. A prominent gene was dishevelled (DVL)-2, a key step in the WNT-Frizzled signaling cascade. Indeed, under basal conditions, DVL is maintained in the cytoplasm in an inactive, depolymerized form. WNT, secreted from afferent cells, activates Frizzled, a plasma membrane receptor, which then triggers the binding and polymerization of DVL. DVL activation leads to its binding of Axin, phosphorylation and inhibition of glycogen synthase kinase-3β (GSK3β), and the regulation of several downstream targets, including β-catenin. More recently, a concerted regulation of multiple proteins in this pathway, including all three isoforms of DVL (DVL1–3) and GSK3β, has been demonstrated in susceptible but not resilient mice, and provide direct, causal evidence that such regulation represents a prodepression-like maladaptation that promotes susceptibility to chronic stress.	Mutations and/or CNVs affecting *DVL3* have never been reported in patients with ASD. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Chung *et al.*, 2011 De Ferrari and Moon, 2006 Gao and Chen, 2010 Okerlund and Cheyette, 2011 Wang *et al.*, 2010 Wilkinson *et al.*, 2009, 2011 Zhang *et al.*, 2012

Tab. 4. Continued.

EIF2S3(-)* Translational regulation	This gene encodes the eukaryotic translation initiation factor 2 protein, which functions in the early steps of protein synthesis by forming a ternary complex with GTP and initiator tRNA. Moderate expression in fetal brain and in postnatal amygdalaee and hypothalamus.	Another member of the eukaryotic translation initiation factor proteins, EIF4E, plays a key role in learning and memory through its control of translation within the synapse. EIF4E mediated translation is the final common process modulated by mTOR (upstream regulator of EIF4E), PTEN and FRMP pathways, which are implicated in autism.	Mutations and/or CNVs affecting *EIF2S3* have never been reported in patients with ASD. A chromosome translocation t(4;5) which likely interrupted an alternative transcript of *EIF4E*, has been identified in a boy with a classical autism. Mutations in *EIF4E* promoter harboured by two autistic siblings and one of the parents have been reported in two unrelated autism families.	Neves-Pereira *et al.*, 2009
★ ***FLNA*(-)*** Neurodevelopment: actin cytoskeleton organization	This gene encodes filamin A, alpha protein which is an actin-binding protein that crosslinks actin filaments and links actin filaments to membrane glycoproteins. FLNA is involved in remodeling the cytoskeleton to effect changes in cell shape and migration. Expressed at moderate levels in brain. Very high expression in immune cell types.	*FLNA* is associated with a broad range of congenital disorders affecting multiple organs. Loss-of-function mutations, although lethal in males, result in defective neuronal migration leading to periventricular nodular heterotopia (PVNH) in females. On the contrary, clustered missense mutations are associated with a diverse spectrum of congenital malformations in males and females, referred to as otopalatodigital spectrum disorders. It has recently been reported a direct interaction between FLNA and SHANK3 (whose mutations cause syndromic ASD) in mouse brain extracts.	One patient with ASD, carrying a duplication at Xq28 which encompasses the entire *FLNA* gene, has been recently reported. Recurrent Copy Number gains at Xq28 including *FLNA* have been reported in mentally retarded patients.	Lian *et al.*, 2012 Robertson, 2005 Sakai *et al.*, 2011 Vandewalle *et al.*, 2009
GH2(-) Organogenesis, intrauterine growth	This gene encodes the growth hormone 2, which is a member of the somatotropin/prolactin family of hormones that play an important role in growth control. The gene, along with four other related genes, is located at the growth hormone locus on chromosome 17 where they are interspersed in the same transcriptional orientation; an arrangement which is thought to have evolved by a series of gene duplications. The five genes share a remarkably high degree of sequence identity. Good expression in fetal brain and low expression in postnatal CNS.	Pituitary GH1/IGF-I axis may play an important role in CNS functions, including those associated with neuronal growth, development, and protection. Furthermore, the GH1/IGF axis may play a role in influencing aspects of mood and cognition. GH-binding sites have been identified in several areas of the brain, including the choroid plexus, putamen, thalamus, pituitary, hippocampus, and cortex.. It has been demonstrated that GH modulates synaptic efficacy of hippocampal neurons and itself is regulated during memory formation, learning processes, and emotional experiences. Somatotropin/prolactin hormonedeficiency may cause intrauterine growth restriction (IUGR), which has an effect on the hippocampus structure that correlates with behavioural problems in preterm infants.	Mutations and/or CNVs affecting *GH2* have never been reported in patients with ASD. A few autistic patients showing growth hormone deficiency have been reported.	Devillard *et al.*, 2010 Donahue *et al.*, 2006 Gingell *et al.*, 1996 Lodygensky *et al.*, 2008 Ragusa *et al.*, 1993 Zearfoss *et al.*, 2008

Tab. 4. Continued.

GLDC(+)* Glycine metabolism	This nuclear gene encodes the mitochondrial glycine dehydrogenase protein. Degradation of glycine is brought about by the glycine cleavage system, which is composed of four mitochondrial protein components: P protein (a pyridoxal phosphate-dependent glycine decarboxylase), H protein (a lipoic acid-containing protein), T protein (a tetrahydrofolate-requiring enzyme), and L protein (a lipoamide dehydrogenase). The protein encoded by this gene is the P protein, which binds to glycine and enables the methylamine group from glycine to be transferred to the T protein. Good-high expression in fetal brain and in postnatal whole brain, in particular in prefrontal cortex, cerebellum, amygdalaee and hypothalamus.	Defects in GLDC are a cause of non-ketotic hyperglycinemia (NKH), also known as glycine encephalopathy (GCE). NKH is an autosomal recessive disease characterized by accumulation of a large amount of glycine in body fluid and by severe neurological symptoms. The majority of glycine encephalopathy presents in the neonatal period. The neonatal form manifests in the first hours to days of life with progressive lethargy, hypotonia, and myoclonic jerks leading to apnea and often death. Surviving infants have profound ID and intractable seizures. The infantile form is characterized by hypotonia, developmental delay, and seizures. The atypical forms range from milder disease, with onset from late infancy to adulthood, to rapidly progressing and severe disease with late onset.	Mutations and/or CNVs affecting *GLDC* have never been reported in patients with ASD.	Hamosh *et al.*, 2009
GPR98(-) CNS development	This gene encodes the G protein-coupled receptor 98, which contains a 7-transmembrane receptor domain and binds calcium. Good expression in fetal brain, and generally low expression in postnatal tissues except for postnatal CNS, where the expression is .good	*In situ* hybridization studies with mouse embryo sections have shown that high level expression of *GPR98* is restricted to the developing CNS and eye and strong expression in the ventricular zone, home of neural progenitor cells during embryonal neurogenesis, have suggested a fundamental role for GPR98 in the development of the CNS. Mutations in *GPR98* are associated with Usher syndrome 2 and familial febrile seizures. Indeed, *GPR98* is one of the disease-causing genes for EP, which include *SCN1A*.	Mutations and/or CNVs affecting *GPR98* have never been reported in patients with ASD. Rare point mutations affecting *SCN1A* were found in sporadic and familial cases of ASD.	McMillan *et al.*, 2002 Munoz-Yunta *et al.*, 2008 O'Roak *et al.*, 2011 Wang *et al.*, 2005 Weiss *et al.*, 2003
GRID2(-) Synaptogenesis and synaptic plasticity	This gene encodes the ionotropic glutamate receptor delta 2, a relatively new member of the family of ionotropic glutamate receptors which are the predominant excitatory neurotransmitter receptors in the mammalian brain. GRID2 is selectively expressed in cerebellar Purkinje cells. Good expression in postnatal cerebellum and cerebellum peduncles.	GRID2 directly interacts with DLG2 and SHANK2 which act as adapter proteins in the postsynaptic density of excitatory synapses, interconnecting receptors of the postsynaptic membrane and the actin-based cytoskeleton.	Rare single gene mutations affecting *GRID2* has been reported in two autistic patients. Moreover, a *de novo* CNV at 4q22.2 including *GRID2* has recently been reported in an autistic patient. Mutations and CNVs (*de novo* and inherited) affecting *SHANK2* have been reported in patients with ASD.	Berkel *et al.*, 2010, 2012 He *et al.*, 2012 Leblond *et al.*, 2012 Pinto *et al.*, 2010 Schaaf *et al.*, 2011 Uemura *et al.*, 2004

Tab. 4. Continued.

GRM7*(-) Synaptogenesis and synaptic plasticity	This gene encodes the glutamate receptor, metabotropic 7. The metabotropic glutamate receptors are a family of G protein-coupled receptors that have been divided into three groups on the basis of sequence homology, putative signal transduction mechanisms, and pharmacologic properties. Group I includes GRM1 and GRM5, and these receptors have been shown to activate phospholipase C. Group II includes GRM2 and GRM3, while Group III includes GRM4, GRM6, GRM7 and GRM8. Group II and III receptors are linked to the inhibition of the cyclic AMP cascade but differ in their agonist selectivities. It is expressed at a very high level in fetal brain and in many areas of the postnatal brain, especially in cerebral cortex, hippocampus, and cerebellum.	L-glutamate is the major excitatory neurotransmitter in the central nervous system, and it activates both ionotropic and metabotropic glutamate receptors. Glutamatergic neurotransmission is involved in most aspects of normal brain function and can be perturbed in many neuropathologic conditions, including SCZ, alcohol and drug addiction.	*De novo* CNVs affecting *GRM7* have been recently reported in patients with ASD. Mutations and CNVs affecting *GRM5* and *GRM8*, respectively, have been reported in a few autistic patients.	Choi *et al.*, 2009 He *et al.*, 2012 Iossifov *et al.*, 2012 Serajee *et al.*, 2003 Vadasz *et al.*, 2007
HSPB1(-) ***de novo*** Intracellular signaling: neuropreotection during stress response mediated by activation of mTOR pathway	This gene encodes the heat shock 27kDa protein 1, which is induced by environmental stress and developmental changes. The encoded protein is involved in stress resistance and actin organization and translocates from the cytoplasm to the nucleus upon stress induction. Low expression in fetal brain and in postnatal CNS, except for cortex, thalamus and hypoyhalamus where the expression is good.	The stress response involving up-regulation of heat shock proteins (Hsps) is a powerful mechanism of cells to deal with harmful conditions to which they are exposed throughout life, such as hyperthermia, hypoxia, or oxidative stress. A direct interaction of HSPB1 with Akt1 (mTOR pathway) during stress response has been reported, which results in the activation of Akt signal transduction pathway during various forms of stress. In cultured murine hippocampal neurons it has been demonstrated that stress-induced phosphorylation of HspB1 and B5 may lead to the translocation from the nucleus to neuronal processes and to the binding of these Hsps to their targets at synapses and neuronal processes which might provide one important mechanism of how they exert their neuroprotective effect. Defects in *HSPB*1 are a cause of Charcot-Marie-Tooth disease type 2F and distal hereditary motor neuropathy.	Mutations and/or CNVs affecting *HSPB1* have never been reported in patients with ASD. Point mutations and CNVs affecting *TSC1* and *TSC2* (mTOR pathway) have been reported in patients with ASD and Tuberous Sclerosis 1 or 2.	Capponi *et al.*, 2011 Fombonne *et al.*, 1997 Kirbach and Golenhofen, 2011 Konishi *et al.*, 1997 Lewis *et al.*, 2004 Muzykewicz *et al.*, 2007 Schmidt *et al.*, 2012 Wiznitzer, 2004 Wong, 2006
IKBKG(+) CNS development: myelin formation	This gene encodes the inhibitor of kappa light polypeptide, which is the regulatory subunit of the inhibitor of kappaB kinase (IKK) complex, which activates NF-kappaB resulting in activation of genes involved in inflammation, immunity, cell survival, and other pathways. Moderate expression in fetal brain and good expression in postnatal CNS, in particular in amygdalaee and thalamus. Very high expression in immune cell types.	Mutations in *IKBKG* result in incontinentia pigmenti, hypohidrotic ectodermal dysplasia, and several other types of immunodeficiencies. Recently, it has been reported that brain abnormalities correlate with additional copies of the IKBKG. Indeed, IKBKG overexpression causes impaired NF-κB signaling in skin fibroblasts derived from patients with white matter anomalies, thus further supporting the role of NF-κB signaling in astroglial cells for normal myelin formation of the CNS.	Mutations and/or CNVs affecting *IKBKG* have never been reported in patients with ASD. However, *IKBKG* maps in the genomic region involved in Xq28 duplication syndrome (*MECP2*), which is comorbid with ASD. NF-κB is an important gene transcriptional factor that mediates cellular responses in inflammation, immunity, development, cell proliferation and apoptosis. Elevated levels of NF-κB have been reported in autistic patients vs. controls.	Malik *et al.*, 2011 Naik *et al.*, 2011 Philippe *et al.*, 2012 Ramocki *et al.*, 2009, 2010

Tab. 4. Continued.

IL1RAPL2(-)* Synaptogenesis and synaptic plasticity	This gene encodes the interleukin 1 receptor accessory protein-like 2, which is a member of the interleukin 1 receptor family. Expressed at low levels in fetal and postnatal brain, in particular in frontal and temporal lobes and cerebellum.	A member of the same family, *IL1RAPL1*, encodes a transmembrane protein that does not seem to be involved in interleukin-1 pathway. It interacts with the neuronal calcium sensor 1 protein, thus playing a role in the down-regulation of voltage-dependent calcium channels activity, in calcium-dependent exocytosis in excitatory synapses and NGF-induced neurite outgrowth. Both *IL1RAPL1* and *IL1RAPL2* are located at a genomic region on chromosome X previously associated with X-linked non-syndromic MR. Recently, it has been reported that both IL1RAPL1 and IL1RAPL2 can induce excitatory pre-synapse differentiation and dendritic spine formation.	SNPs affecting *IL1RAPL2* have been recently associated with ASD. Mutations, CNVs and chromosomal rearrangements involving *IL1RAPL1* have been reported in patients with ASD associated or not with XLMR.	Bhat *et al*., 2008 Chung *et al*., 2011 Kantojärvi *et al*., 2011 Piton *et al*., 2008 Pinto *et al*., 2010 Valnegri *et al*., 2011 Yoshida *et al*., 2011
★ ***IPCEF1(-)*** Intracellular ARF6 signaling: regulation of cytoskeleton dynamics and membrane trafficking	This gene encodes the interaction protein for cytohesin exchange factors 1. High expression in fetal brain and very high expression in postnatal CNS. Very high expression in immune cell types.	IPCEF1 has been reported to interact with the low weight ARF GEF (ADP-ribosylation factor GTP exchange factors) proteins of the cytohesin family (in particular cytohesin 2/ARNO) and function by modulating the cytohesin activity by stimulating the formation of ARF_{GTP} (including $ARF6_{GTP}$ which is particularly active in brain). The interaction of cytohesin 2 and IPCEF1 in mammalian cells was demonstrated by immunoprecipitation. A regulatory role for cytohesin 2 in dendritic branching and axonal elongation and branching during neuritogenesis, particularly with respect to cytoskeletal dynamics, has been demonstrated as well as a role in endosomal dynamics during neurite elongation in hippocampal neurons has been recently reported. Cytohesin regulates the activation of RhoA in primary dendritic cells (DCs). Cytohesin-1 and RhoA are both required for the induction of chemokine-dependent conformational changes of the integrin beta-2 subunit of DCs during adhesion.	Mutations and/or CNVs affecting *IPCEF1* have never been reported in patients with ASD.	Hernández-Deviez *et al*., 2004, 2007 Hernández-Deviez and Wilson, 2005 Jaworski, 2007 Quast *et al*., 2009 Venkateswarlu, 2003
IPO11(-)* Synapto-nuclear trafficking	This gene encodes the importin 11 protein. Importins, including IPO11, are a members of the karyopherin/importin-beta family of transport receptors that mediate nucleocytoplasmic transport of protein and RNA cargoes. Good expression in fetal brain and in postnatal CNS, in particular in prefrontal and cingulated cortex.	It is known that retrograde axonal injury signaling stimulates cell body responses in lesioned peripheral neurons and that importins are involved in retrograde transport. Recently, it has been reported that multiple transcription factors are found in axons and associate with dynein in axoplasm from injured nerve. For example, axonal STAT3 is locally translated and activated upon injury, and is transported retrogradely with dynein and importin α5 to modulate survival of peripheral sensory neurons after injury.	Mutations and/or CNVs affecting *IPO11* have never been reported in patients with ASD.	Ben-Yaakov *et al*., 2012

Tab. 4. Continued.

JMJD2A(-) (KDM4A) Chromatin remodeling	This gene encodes the lysine (K)-specific demethylase 4A protein, which functions as a trimethylation-specific demethylase, converting specific trimethylated histone residues to the dimethylated form, and acts as a transcriptional repressor. Good expression in fetal cerebellum, postnatal cortex and cerebellum.	JMJD2A interacts with NCOR1, the nuclear receptor co-repressor 1, which mediates transcriptional repression by certain nuclear receptors. Together with SIN3A and MECP2, it is part of a complex which promotes histone deacetylation and the formation of repressive chromatin structures which may impede the access of basal transcription.	Mutations and/or CNVs affecting *JMJD2A* have never been reported in patients with ASD. A SNP in *JMJD2C* has been associated with ASD in Finnish samples. *MECP2* mutations or deletions cause Rett syndrome (comorbidity with autism) in female, and congenital encephalopathy or non syndromic ID in males.	Abdul-Rahman and Hudgins, 2006 Auger *et al.*, 2011 Carney *et al.*, 2003 Kantojärvi *et al.*, 2010 Mount *et al.*, 2003 Schaefer and Lutz, 2006 Zappella *et al.*, 2003 Zhang *et al.*, 2005
JMJD2C(-)* (KDM4C) Chromatin remodeling	This gene encodes the lysine (K)-specific demethylase 4C protein, which is a member of the Jumonji domain 2 (JMJD2) family. This nuclear protein functions as a trimethylation-specific demethylase, converting specific trimethylated histone residues to the dimethylated form. Good-high expression in fetal brain and in postnatal prefrontal cortex, cerebellum, cerebellum peduncles, and amygdalaee.	JMJD2C interacts with androgen receptor *in vitro* and *in vivo*. Assembly of ligand-bound androgen receptor and JMJD2C on androgen receptor-target genes results in demethylation of trimethyl H3K9 and in stimulation of androgen receptor-dependent transcription.	A SNP in *JMJD2C* has been associated with ASD in Finnish samples. The GGN polymorphism in exon 1 of the *AR* (androgen receptor) gene has been associated with ASD susceptibility with gender specificity: the rare 20-repeat allele has been reported undertransmitted to male cases and the 23-repeat allele overtransmitted to female cases.	Henningsson *et al.*, 2009 Kantojärvi *et al.*, 2010 Wissmann *et al.*, 2007
KANK1(-)* Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encodes the KN motif and ankyrin repeat domains 1 protein, which belongs to the Kank family of proteins that contain multiple ankyrin repeat domains. KANK1 functions in cytoskeleton formation in a RhoA-dipendent manner by regulating actin polymerization and is a candidate tumor suppressor for renal cell carcinoma. Mild expression in fetal brain, good expression in postnatal CNS, in particolar in prefrontal cortex, amygdalaee, thalamus and hypothalamus.	Defects in *KANK1* (*ANKRD15*) are the cause of cerebral palsy spastic quadriplegic type 2 which is a non-progressive disorder of movement and/or posture resulting from defects in the developing CNS. Affected individuals manifest congenital hypotonia evolving over the first year to spastic quadriplegia with accompanying transient nystagmus and varying degrees of MR. Neuroimaging shows brain atrophy and ventriculomegaly. Recently, it has been demonstrated that nucleo-cytoplasmic shuttling of human Kank protein accompanies intracellular translocation of beta-catenin and, therefore, beta-catenin-dependent transcription.	Two *de novo* 9p24 terminal deletion including *DOCK8* and *ANKRD15* have been reported in a female and a male patient with ASD. The male patient presented also a gonadal dysgenesia. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD and Tuberous Sclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates CdC42, thus regulating cell adhesion and migration.	Chung *et al.*, 2011 De Ferrari and Moon, 2006 Fombonne *et al.*, 1997 Kakinuma *et al.*, 2008 Lewis *et al.*, 2004 Lerer *et al.*, 2005 Muzykewicz *et al.*, 2007 Okerlund and Cheyette, 2011 Ounap *et al.*, 2004, Vinci *et al.*, 2007 Wang *et al.*, 2006 Wang *et al.*, 2010 Wiznitzer, 2004 Wong, 2006 Zhang *et al.*, 2012
★ ***KIF2A(-)**** Intracellular trafficking: regulation of microtubule cytoskeleton dynamics	This gene encodes the kinesin heavy chain member 2A, which is a plus end-directed motor required for normal mitotic progression. The encoded protein is required for normal spindle activity during mitosis and is necessary for normal brain development. High expression in fetal brain and in postnatal CNS. High expression in immune cell types.	Kinesin superfamily proteins (KIFs) are motor proteins that transport membranous organelles and macromolecules fundamental for cellular functions along microtubules. Their roles in transport in axons and dendrites have been studied extensively, but KIFs are also used in intracellular transport in general. KIFs play important roles in higher order neuronal activity; transgenic mice overexpressing KIF17, which transports N-methyl-d-asp (NMDA) receptors to dendrites, show enhanced memory and learning. KIFs also play significant roles in neuronal development and brain wiring: KIF2A suppresses elongation of axon collaterals by its unique microtubule-depolymerizing activity.	Mutations and/or CNVs affecting *KIF2A* have never been reported in patients with ASD. A recurrent 5q12.1 deletion, encompassing *KIF2A*, has been recently reported in four patients in association with a phenotype including MR and ocular defects. A de novo mutation affecting *KIF5C* has been reported in a patient with ASD.	Awadalla *et al.*, 2010 Hirokawa and Takemura, 2004 Jaillard *et al.*, 2011

Tab. 4. Continued.

LCLAT1(-) Phospholipid metabolism	This gene encodes the lysocardiolipin acyltransferase 1, which has a role in phospholipid metabolism, in particular in cardiolipin metabolism. It is required for establishment of the hematopoietic and endothelial lineages. Good expression in fetal brain and postnatal hypothalamus.	Recently it has been demonstrated that LCLAT1 controls mitochondrial DNA fidelity and biogenesis through cardiolipin remodeling in response to oxidative stress. LCLAT1 interacts with LPPR4, the lipid phosphate phosphatase-related protein type 4, a protein specifically expressed in neurons. It is located in the membranes of outgrowing axons and has been shown to be important for axonal outgrowth during development and regenerative sprouting.	Mutations and/or CNVs affecting *LCLAT1* have never been reported in patients with ASD although it is well known the involvement of mithocondrial oxidative stress response dysfunctions in ASD pathogenesis. Anti-cardiolipin antibodies have been associated to psychiatric disorders such as SCZ, developmental delay and autism. *LPPR4* haploinsufficiency has been found in patients affected by severe MR and psychomotor development delay.	Chang *et al.*, 2011 Christie *et al.*, 2011 Dhillon *et al.*, 2011 Lehtimaki *et al.*, 2011 Li *et al.*, 2012 Mekinian *et al.*, 2012 van Kuilenburg *et al.*, 2009
MACROD2(-) CNS development?	This gene encodes the MACRO domain containing 2 protein, which has an unknown function. Good expression in postnatal parietal and occipital lobes, cingulate cortex, subthalamic nucleus, globus pallidus, ciliary ganglion and spinal cord.	The *MACROD2* haploinsuffciency may be the cause of Kabuki syndrome in a minority of patients.	A *MACROD2* SNP has been associated with ASD (datum not replicated in two independent studies). A *de novo* CNV affecting *MACROD2* has been reported in a patient with ADHD.	Anney *et al.*, 2010 Curran *et al.*, 2011 Lionel *et al.*, 2011 Maas *et al.*, 2007 Prandini *et al.*, 2012
MAPK15-ERK8(-) Intracellular MAPK signaling: regulation of cell growth and differentiation	This gene encodes the mitogen-activated protein kinase 15 (ERK8), which is involved in cell growth and differentiation. The protein is ubiquitously expressed.	Different findings support a role for ERK8 in mainting genomic integrity through DNA repair. Moreover, it has been reported by an *in vitro* assay a direct interaction between ERK8 and MBP, the myelin basic protein, one of the most abundant protein components of the myelin membrane in the CNS, with a role in both its formation and stabilization.The major residue in MBP phosphorylated by ERK8 (Ser-126) is distinct from that phosphorylated by ERK2 (Thr-97), demonstrating that, although ERK8 is a proline-directed protein kinase, its specificity is distinct from ERK1/ERK2.	Mutations and/or CNVs affecting *MAPK15* have never been reported in patients with ASD.	Abe *et al.*, 2002 Groehler and Lannigan, 2010 Klevernic *et al.*, 2006, 2009
★ ***MBP(-)**** Intracellular Ras/Raf/ERK1/2 signaling	This gene encodes the myelin basic protein, which is a major constituent of the myelin sheath of oligodendrocytes and Schwann cells in the nervous system. However, MBP-related transcripts are also present in bone marrow and immune cell types. Good expression in fetal brain and very high expression in postnatal CNS.	MBP directly interacts with ERK1 (extracellular signal-regulated kinase 1) and ERK2, which encode the mitogen-activated protein kinases 3 and 1, respectively. It is known that the Ras/Raf/ERK1/2 signaling pathway plays important roles in the genesis of neural progenitors, learning and memory as well as in death-promoting apoptotic roles in neural cells.	Mutations and/or CNVs affecting *MBP* have never been reported in patients with ASD. Auto-antibodies anti-MBP were found enriched in ASD patient sera, thus implicating an autoimmune reaction in ASD pathogenesis. More recently, this datum has not been replicated. Up-regulation of the Ras/Raf/ERK1/2 signaling pathway has been found in the brain of autistic subjects and mouse animal model. In addition, two studies reported that a recurrent deletion on chromosome 16p11.2, which includes the *MAPK3 (ERK1)* gene, is associated with autism.	Libbey *et al.*, 2008 Mostafa and Al-Ayadhi, 2011 Singh, 2009 Stephenson *et al.*, 2011 Vojdani *et al.*, 2002 Yang *et al.*, 2011, 2012 Zimmerman *et al.*, 2007 Zou *et al.*, 2011

Tab. 4. Continued.

MCTP2(-) Intracellular membrane trafficking	This gene encodes the multiple C2 domains, transmembrane 2 protein. Mild expression in postnatal temporal lobe and amygdalaee.	MCTP2 may be involved in intercellular signal transduction and synapse function. A possible involvement of *MCTP2* as a potential novel susceptibility gene for SCZ has been reported in genotyping studies (37 SNPs across *MCTP2*) in three independent Scandinavian samples.	Mutations and/or CNVs affecting *MCTP2* have never been reported in patients with ASD.	Djurovic *et al.*, 2009 Shin *et al.*, 2005
ME3(-)* Pyruvate metabolism-glycolysis	This nuclear gene encodes the mitochondrial protein malic enzyme 3, NADP(+)-dependent. Malic enzyme catalyzes the oxidative decarboxylation of malate to pyruvate using either NAD^+ or $NADP^+$ as a cofactor. High expression in fetal brain and in postnatal CNS, in particular in prefrontal cortex, thalamus, hypothalamus, amygdalaee, caudate nucleus, and amygdalaee.	ME3 is involved in the metabolic pathway of glycolysis. Energy metabolism is essential for neuronal growth and function. Proteome analysis of the thalamus and cerebrospinal fluid performed post-mortem in patients affected by SCZ reveals glycolysis dysfunction.	Mutations and/or CNVs affecting *ME3* have never been reported in patients with ASD.	Martins-de-Souza *et al.*, 2010
MPHOSPH8(+) Transcriptional regulation	This gene encodes the M-phase phosphoprotein 8, which has a role in negative regulation of transcription through methylated DNA binding and in cell cycle regulation. Good-high expression in fetal brain and in postnatal CNS, in particular in occipital and parietal lobes, prefrontal cortex, amygdalaee, thalamus and hypothalamus. High expression in immune cell types.		Mutations and/or CNVs affecting *MPHOSPH8* have never been reported in patients with ASD.	Matsumoto-Taniura *et al.*, 1996
MPHOSPH9(-) Cell cycle regulation	This gene encodes the M-phase phosphoprotein 9, which is involved in cell cycle regulation. High expression in fetal brain and in postnatal CNS, in particular in cortex, parietal lobe, thalamus and hyphotalamus. Very high expression in immune cell types.	A SNP in the *MPHOSPH9* locus has recently been associated to multiple sclerosis. Indeed, it has been demonstrated that the risk allele correlate with diminished *MPHOSPH9* RNA expression in both lymphoblastic cell lines and in peripheral blood mononuclear cells from subjects with multiple sclerosis. Thus, *MPHOSPH9* might represent a novel inflammatory disease locus that could affect autoreactive cell proliferation.	Mutations and/or CNVs affecting *MPHOSPH9* have never been reported in patients with ASD.	IMSGC, 2010
NDST2(-) ***de novo*** Heparan sulfate and heparin biosynthesis	This gene encodes the N-deacetylase/N-sulfotransferase 2 protein, which is a member of the N-deacetylase/N-sulfotransferase subfamily of the sulfotransferase 1 proteins. The encoded enzyme has dual functions in processing glucosamine and heparin polymers, including N-deacetylation and N-sulfation. Moderate expression in fetal brain and good expression in postnatal cortex and thalamus. High expression in immune cell types.	Roles of heparan sulfate (HS) in neural development have been well established by using animal models that carry mutations in genes encoding enzymes involved in HS synthesis, thus revealing that HS is necessary for the specification of certain brain structures, such as the cerebellum and the olfactory bulbs, cortical neurogenesis, and a variety of axon path-finding processes. However, a key unresolved issue concerning is the role of HS in the adult brain and its possible relevance to human neurological and mental disorders. Several pieces of evidence suggest a role for HS in synaptic function as well as in higher cognitive function. For example, in adult neurons HS is enriched in synapses, especially in the postsynaptic membrane of dendritic spines.	Mutations and/or CNVs affecting *NDST2* have never been reported in patients with ASD. The association of autism and other symptoms of mental impairment with multiple exostoses in patients carrying mutations in HS/HSPG genes has been reported. More recently, genetic association has been found between autism and the *HS3ST5* gene encoding one of the HS 3-*O* sulfotransferases in two large cohorts of European ancestry. Furthermore, a genome-wide scan for rare CNVs in 996 autism cases has identified four independent CNVs in the *GPC5/GPC6* gene cluster, which encodes the glypican-5 and glypican-6 HSPGs in tandem array, on chromosome 13q22.	Bolton *et al.*,1995 Conway *et al.*, 2011 Ethell and Yamaguchi, 1999 Hsueh and Sheng 1999 Inatani *et al.*, 2003 Irie *et al.*, 2012 Ishikawa-Brush *et al.*, 1997 Kantor *et al.*, 2004 Li *et al.*, 2002 Matsumoto *et al.*, 2007 Pinto *et al.*, 2010 Pratt *et al.*, 2006 Swarr *et al.*, 2010 Wang *et al.*, 2009 Wuyts *et al.*, 2002

Tab. 4. Continued.

NLGN4X*(-) Synaptogenesis and synaptic plasticity	This gene encodes the neuroligin 4, X-linked protein, which is a member of a family of neuronal cell surface proteins. Members of this family act as splice site-specific ligands for beta-neurexins and are involved in the formation and remodeling of the CNS synapses. Very high expression in the fetal brain and postnatal CNS.	NLGNX4 directly interacts with neurexinn-1β through Ca^{2+} to form *trans*-synaptic associations which are required for the maturation of glutamatergic excitatory and GABAergic inhibitory synapses.	Mutations and/or CNVs affecting *NLGN4X* have been reported in patients affected by non syndromic ASD, ID, Asperger and Tourette syndromes.	Baris *et al*., 2007 Jamain *et al*., 2003 Kent *et al*., 2008b Laumonnier *et al*., 2004 Lawson-Yuen *et al*., 2008 Marshall *et al*., 2008 Pampanos *et al*., 2009 Zhang *et al*., 2009
PARD3*(-) Intracellular signaling: regulation of actin cytoskeleton dynamics for asymmetric cell division and polarized neuronal growth	This gene encodes the par-3 partitioning defective 3, homolog (C. elegans) protein, which is a member of the PARD protein family. PARD family members affect asymmetrical cell division and direct polarized cell growth. Good expression in fetal brain and in postnatal CNS, in particular in prefrontal cortex, thalamus and hypothalamus.	It has been demonstrated that PARD3, a key cell polarity determinant, exhibits dynamic distribution in radial glial progenitors, thus contributing in asymmetrically dividing of these cells which is essential for embryonic neocortex development. Indeed, radial glial cells constitute a major population of neural progenitor cells in mammals. The division of radial glial progenitors can be either symmetrical or asymmetrical, which is reflected by the fate of the two daughter cells. During the peak phase of neurogenesis, they predominantly divide asymmetrically to both self-renew, and to produce either a neuron or an intermediate progenitor cell. Furthermore, Par3 was shown to play a role in Schwann cell myelination. It is recruited by the brain-derived neurotrophic factor (BDNF) to the axon-glial interface and regulates Rac1 activation. During development, active Rac1 signaling is localized to the axon-glial interface in Schwann cells by a Par3-dependent polarization mechanism.	Mutations and/or CNVs affecting *PARD3* have never been reported in patients with ASD. Recently, SNPs in *PARD3* have been associated to SCZ in Korean patients and to neural tube defects in a Chinese Han population. SNPs in *PARD3B* have been associated to ASD.	Anney *et al*., 2012 Bultje *et al*., 2009 Gao *et al*., 2012 Kim *et al*., 2012 Tep *et al*., 2012
★ ***PARD6G(-)*** Intracellular signaling: regulation of actin cytoskeleton dynamics for asymmetric cell division and polarized neuronal growth	This gene encodes the par-6 partitioning defective 6 gamma protein, which is an adapter protein involved in asymmetrical cell division and cell polarization processes through the formation of tight junction. The PARD6-PARD3 complex links GTP-bound Rho small GTPases to atypical protein kinase C proteins. Moderate expression in fetal brain and good expression in postnatal CNS, in particular in prefrontal cortex, thalamus, hypothalamus, caudate nucleus and spinal corde. Good expression in immune cell types.	PARD6G directly interacts with CDC42 and is involved in cell-cell adhesion process and polarization through the formation of tight junction, which are important processes during neuritogenesis. Moreover, PARD6G is involved in T cell polarity, motility, and ability to scan dendritic cells.	Mutations and/or CNVs affecting *PARD6G* have never been reported in patients with ASD. SNPs in *PARD3B* have been associated to ASD. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD and Tuberous Sclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates CdC42, thus regulating cell adhesion and migration.	Anney *et al*., 2012 Da Silva *et al*., 2003 Fombonne *et al*., 1997 Lewis *et al*., 2004 Lisik *et al*., 2010 Muzykewicz *et al*., 2007 Pertz *et al*., 2008 Wiznitzer, 2004 Wong, 2006

Tab. 4. Continued.

PCDH7(+) Synaptogenesis and synaptic plasticity	This gene encodes the protocadherin 7 protein. It belongs to the protocadherin gene family, a subfamily of the cadherin superfamily. PCDH7 is an integral membrane protein that is thought to function in cell-cell recognition and adhesion. Moderate expression in fetal brain and good expression in postnatal whole brain, in particular in occipital and parietal lobes, and in prefrontal cortex.	In rat brain using *in situ* hybridization the spatiotemporal distribution of mRNAs for 12 non-clustered *PCDHs* has been examined. Some of them (*PCDH1*, *PCDH7*, *PCDH9*, *PCDH10*, *PCDH11*, *PCDH17*, and *PCDH20*) exhibited region-dependent expression pattern in the cerebral cortex during the early postnatal stage (P3), which is a critical period for the establishment of specific synaptic connections, and were also expressed in the specific regions of the connecting thalamic nuclei. In particular, *PCDH7* and *PCDH20* mRNAs were predominantly expressed in the somatosensory (parietal) and visual (occipital) cortices. PCDH7 binds to PPP1CA, the protein phosphatase 1, catalytic subunit, alpha isoform, which is involved in regulation of ionic conductances and long-term synaptic plasticity. PPP1CA may play an important role in dephosphorylating substrates such as the postsynaptic density-associated Ca(2+)/calmodulin dependent protein kinase II.	Mutations and/or CNVs affecting *PCDH7* have never been reported in patients with ASD. Homozygous deletion within a protocadherin cluster proximal to *PCDH10* has been shown to be associated significantly with the pathophysiology of cognitive impairment such as autism, and recurrent and overlapping CNVs, including *PCDH9* loci, have been identified in autism patients. Another delta protocadherin PCDH17 is involved in the pathogenesis of SCZ.	Dean *et al.*, 2007 Kim *et al.*, 2007, 2011 Marshall *et al* 2008 Morrow *et al.*, 2008 Yoshida *et al.*, 1999
★ ***PCNT(-)**** Neurogenesis: regulation of microtubule cytoskeleton dynamics	This gene encodes the pericentrin protein, which binds to calmodulin and is expressed in the centrosome. PCNT is an integral component of the filamentous matrix of the centrosome involved in the initial establishment of organized microtubule arrays in both mitosis and meiosis. It plays a role, together with DISC1, in the microtubule network formation, and prevents premature centrosome splitting during interphase by inhibiting NEK2 kinase activity at the centrosome. High expression in fetal brain and in postnatal prefrontal cortex, moderate expression in amygdalaee and thalamus. High expression in immune cell types.	PCNT binds to DCTN2, the dynactin 2 protein, which modulates cytoplasmic dynein binding to an organelle, and plays a role in prometaphase chromosome alignment and spindle organization during mitosis. It is involved in anchoring microtubules to centrosomes and may play a role in synapse formation during brain development. Moreover, DISC1 localizes to the centrosome by binding to PCNT, and PCNT anchors the γ-tubulin complex to the centrosome, providing microtubule nucleation sites. Thus DISC1–PCNT interaction might be involved in the pathophysiology of mental disorders owing to their putative effect on centrosomal function.	Mutations and/or CNVs affecting *PCNT* have never been reported in patients with ASD. Mutations affecting *PCNT* cause Seckel syndrome-4 and microcephalic osteodysplastic primordial dwarfism type II, both characterized by severe microcephaly, thus involving the gene in modulating brain size. Three SNPs in *PCNT* have been associated with MDD in the Japanese population.	Griffith *et al.*, 2008 Numata *et al.*, 2009 Purohit *et al.*, 1999 Rauch *et al.*, 2008
PDE9A(-)* Intracellular cAMP and cGMP signaling	This gene encodes the phosphodiesterase 9A protein, which catalyzes the hydrolysis of cAMP and cGMP to their corresponding monophosphates and plays a role in signal transduction by regulating the intracellular concentration of these cyclic nucleotides. Vey high expression in fetal brain, postnatal cerebellum and spinal cord. Good expression in postnatal whole brain.	In rodent CNS PDE9A activity regulates neuronal cGMP signaling downstream of multiple neurotransmitter systems (dopaminergic, cholinergic, and serotonergic neurotransmission), with a possible role in sensory processing and memory. Thus, inhibition of PDE9A may provide therapeutic benefits in psychiatric and neurodegenerative diseases promoted by the dysfunction of these diverse neurotransmitter systems.	Mutations and/or CNVs affecting *PDE9A* have never been reported in patients with ASD. SNPs in *PDE9A* have been associated with a susceptibility to MDD. A lower expression of *PDE4A* and *PDE4B* in the cerebella of subjects with autism compared with matched controls has been reported.	Braun *et al.*, 2007 Fisher *et al.*, 1998 Kleiman *et al.*, 2012 Wong *et al.*, 2006

Tab. 4. Continued.

PLAG1(-)* Transcriptional regulation: control of cell fate and proliferation	This gene encodes the pleomorphic adenoma gene 1 protein, which is a developmentally regulated zinc finger protein, consistently rearranged in pleomorphic adenomas of the salivary glands. PLAG1 acts as a transcription factor whose activation results in up- regulation of target genes, such as IGFII, leading to uncontrolled cell proliferation and, possibly, transformation. Mild expression in postnatal thalamus and hypoyhalamus.	PLAG1 activation in salivary gland tumors induces a transcriptional up-regulation of *IGF2* (murine and human protein) and beta-catenin (murine protein). Moreover, it has been demonstrated in mice that *Plag1* controls cell fate and proliferation decisions in the developing nervous system.	Mutations and/or CNVs affecting *PlAG1* have never been reported in patients with ASD. Aberrant imprinting of *IGF2* is associated with BWS and SRS, characterized by growth anomalies. Both disorder have been reported in patients with ASD.	Alam *et al.*, 2005 Kent *et al.*, 2008 Zhao *et al.*, 2006
PLD5(-) Neurodevelopment	This gene encodes the phospholipase D family, member 5 protein. Good expression in fetal brain.	PLDs are known to play a key role in neurite outgrowth, especially axon outgrowth, in neuronal cells. In particular, PLD2 has been shown to regulate metabotropic glutamate receptor signaling.	SNPs affecting *PLD5* and *PLD2* have been associated with a higher risk to develop ASD.	Anney *et al.*, 2010 Dhami and Ferguson, 2006 Kanaho *et al.*, 2009
PLXNA3(+) Intracellular semaphorin signaling: regulation of actin cytoskeleton dynamics	This gene encodes the plexin A3 protein, which is a member of the plexin class of proteins. It is a coreceptor for SEMA3A and SEMA3F and is necessary for signaling by class 3 semaphorins and subsequent remodeling of the cytoskeleton. PLXNA3 plays a role in axon guidance in the developing nervous system and it is required for normal dendrite spine morphology in pyramidal neurons. Moderate-good expression in fetal brain and in postnatal prefrontal cortex, cerebellum, amygdalaee, thalamus, and hypothalamus.	PLXNA3 directly binds to SEMA3A, a candidate for SCZ, and SEMA3F, thus having a role in semaphoring signaling whose alterations may have important implications for autism pathogenesis. SEMA3F is expressed during development along the cortical/hippocampal GABAergic neuron migratory pathway. Further, its ectopic expression can alter GABAergic neuron migration. Genetic lesioning studies knocking out different components of this signaling system (NPN2, Sema3F, or plexin A3) have resulted in animals with extended infrapyramidal mossy fiber axonal pathways and spontaneous seizures, suggesting that semaphorin signaling is normally associated with experience-dependent neuronal activity and that experimental manipulations decreasing this signaling pathway function are closely allied with hyperexcitability and abnormal neuritic outgrowth in the hippocampus.	Mutations and/or CNVs affecting *PLXNA3* have never been reported in patients with ASD. Recently, it has been reported a decreased expression of axon-guidance receptors suc as PLXNA4 in the anterior cingulate cortex in autism.	Gant *et al.*, 2009 Giger *et al.*, 2000 Sahay *et al.*, 2005 Suda *et al.*, 2011 Tamamaki *et al.*, 2003 Tran *et al.*, 2009
POLR1A(-)* Transcriptional regulation	This gene encodes the RNA polymerase I polypeptide A, which is the largest and catalytic core component of RNA polymerase I that synthesizes ribosomal RNA precursors. Moderate expression in fetal brain and good expression in postnatal CNS, in particular in prefrontal cortex and cerebellum.	POLR1A is involved in cell growth and cell cycle progression.	A duplication involving *REEP1-POLR1A* has been recently detected in three autistic subjects.	Donati *et al.*, 2011 Holt *et al.*, 2012

Tab. 4. Continued.

★ ***PREP(-) de novo*** CNS metabolism: neuropeptide maturation and degradation	This gene encodes the prolyl endopeptidase protein, which is a cytosolic prolyl endopeptidase that cleaves peptide bonds on the C-terminal side of prolyl residues within peptides that are up to approximately 30 amino acids long. Prolyl endopeptidases have been reported to be involved in the maturation and degradation of peptide hormones and neuropeptides. Good expression in fetal brain and in postnatal whole brain, in particular in cortex, cerebellum, amygdalaee and thalamus.	The prolyl endopeptidase is a phylogenetically conserved serine protease and, in humans and rodents, is highly expressed in the brain. Several neuropeptides associated with learning and memory and neurodegenerative disorders have been proposed to be the substrates for PREP, suggesting a possible role for PREP in these processes. Indeed, PREP genetrap mice have decreased synaptic spine density in the hippocampus, reduced hippocampal long-term potentiation, and impaired hippocampal-mediated learning and memory, thus revealing a possible role for PREP in mediating hippocampal plasticity and spatial memory formation. PREP directly binds to TAC1, the tachykinin, precursor 1. Tachykinins are active peptides which excite neurons and evoke behavioral responses.	Mutations and/or CNVs affecting *PREP* have never been reported in patients with ASD. Recently, elevated serum levels of a pro-inflammatory neuropeptide, the neurokinin A, which is a member of the tachykinin family, have been reported in some autistic children. Interestingly, levels of neurokinin A correlated to the severity of autism and to serum levels of anti-ribosomal P protein antibodies, thus supporting the pathogenic role of neurokinin A and its possible link to autoimmunity in autism.	D'Agostino *et al.*, 2012 Mostafa and AL-Ayadhi, 2011
★ ***PRKCA(-)*** Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encoder the protein kinase C-alpha, which is a member of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. PKC proteins phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signaling pathways. Moderate expression in postnatal parietal lobe, thalamus, subtalamic nucleus, and spinal cord. Moderate expression in immune cell types.	PKCα directly interacts with RhoA and Cdc42 and is involved in different pathways such as focal adhesion, the formation of tight junctions, the Wnt signaling pathway, and leukocyte transendothelial migration, which are known to be involved in neurogenesis as well as in immune response. Recently, it has been demonstrated that Erk1/PKCα pathway in mice hippocampal neurons is involved in the signaling activity mediated by the brain serotonin 1A receptor (5–HT1A-R), which has been implicated in a large number of behavioural abnormalities. Indeed, both decreased as well as increased 5–HT1A-R signaling in the brain have been linked to a number of affective disorders. Moreover, PKCα has an important role in T-cell function.	Mutations and/or CNVs affecting *PRKCA* have never been reported in patients with ASD. SNPs in *PRKCB1* have been strongly associated with autism. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD and Tuberous Sclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA, thus regulating cell adhesion and migration.	Astrinidis *et al.*, 2002 Fombonne *et al.*, 1997 Gillberg *et al.*, 1994 Lewis *et al.*, 2004 Mogha *et al.*, 2012 Muzykewicz *et al.*, 2007 Pfeifhofer-Obermair *et al.*, 2012 Philippi *et al.*, 2005 Slater *et al.*, 2001 Wiznitzer, 2004 Wong, 2006
PRMT8(-)* Post-translation modifications: arginine methylation	This gene encodes the protein arginine methyltransferase 8. Arginine methylation is a widespread post-translational modification mediated by arginine methyltransferases, such as PRMT8. Arginine methylation is involved in a number of cellular processes, including DNA repair, RNA transcription, signal transduction, protein compartmentalization, and possibly protein translation High expression in fetal brain and in postnatal CNS.	PRMT8 expression was firstly studied in mouse CNS where it was demonstrated a broadly distribution in the CNS neurons with markedly intense signals in the cerebellum, hippocampal formation, and cortex. More recently, a high PRMT8 expression was identified in human brain cortex where PRMT8 is a marker of post-mitotic neurons.	Mutations and/or CNVs affecting *PRMT8* have never been reported in patients with ASD.	Kousaka *et al.*, 2009 Lee *et al.*, 2005 Weng *et al.*, 2012
PRR16(-)* Unknown function	This gene encodes for the proline rich 16 protein with unknown function. High expression in fetal and postnatal brain, particularly in postnatal parietal lobe, cerebellum, thalamus, and caudate nucleus.	No interacting proteins have been reported.	Mutations and/or CNVs affecting *PRR16* have never been reported in patients with ASD.	

Tab. 4. Continued.

PSPC1(+) Transcriptional regulation	This gene encodes the paraspeckle component 1 protein, a nucleolar protein which localizes to punctate subnuclear structures that occur close to splicing speckles, known as paraspeckles. Paraspeckles may function in the control of gene expression via an RNA nuclear retention mechanism. Moderate-good expression in fetal brain, prefrontal cortex and hypothalamus.	PSPC1 directly interacts with TLE3, the transducin-like enhancer of split 3 protein, a transcriptional co-repressor that inhibits the transcriptional activation mediated by CTNNB1 and TCF family members in Wnt signaling. A role of the repressor TLE1 in neurogenic switching from transcription repression to activation, which is needed for brain development, has been reported.	Mutations and/or CNVs affecting *PSPC1* have never been reported in patients with ASD. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Chung *et al.*, 2011 De Ferrari and Moon, 2006 Ju *et al.*, 2004 Okerlund and Cheyette, 2011 Wang *et al.*, 2010 Zhang *et al.*, 2012
★ ***PTPN12(-)**** Intracellular FAK signaling: regulation of actin cytoskeleton dynamics	This gene encodes the protein tyrosine phosphatase, non-receptor type 12, which is a member of the protein tyrosine phosphatase (PTP) family. PTPs are signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. It may also have a regulatory role in controlling cell shape and mobility. Moderate expression in fetal brain and in postnatal CNS, in particolar in prefrontal cortex, amygdalaee, and hypothalamus. High expression in immune cell types.	It has been reported that the focal adhesion kinase (FAK) functions in regulating tyrosine phosphorylation of several of focal adhesion proteins, including paxillin, which may be involved in the regulation of the cytoskeleton and in the control of signals for growth and survival. Protein tyrosine phosphatases, such as PTPN12, the counterparts of protein-tyrosine kinases, also presumably regulate phosphorylation of these proteins. The association of both FAK and PTPN12 with paxillin suggests that these protein may play a critical role in the regulation of the phosphotyrosine content of proteins in focal adhesions. Recent studies suggest that one of the major pathways to the pathogenesis of autism is reduced cell migration due to an abnormal FAK signaling. Indeed, FAK has an important role in neural migration, dendritic morphological characteristics, axonal branching, and synapse formation.	Mutations and/or CNVs affecting *PTPN12* have never been reported in patients with ASD. Using B lymphoblasts as a model, it has been demonstrated that FAK-Src signaling is abnormally regulated in autism, due to a reduced expression of the *paxillin* gene and that FAK-Src signaling leads to defects in B-lymphoblast adhesion, migration, proliferation, and IgG production.	Shen *et al.*, 1998 Wei *et al.*, 2011
★ ***RAP1GDS1(-)**** Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encodes the RAP1, GTP-GDP dissociation stimulator 1 protein, which stimulates GDP/GTP exchange reaction of a group of small GTP-binding proteins (G proteins) including Rap1a/Rap1b, RhoA, RhoB and KRas, by stimulating the dissociation of GDP and the subsequent binding of GTP to each small G protein. RAP1GDS1 is involved in signal transduction. Very high expression in fetal brain and postnatal CNS. Very high expression in immune cell types.	One of the main interacting protein is RhoA which is involved in different pathways such as endocytosis, Wnt signaling pathway, TGF-beta signaling pathway, axon guidance, focal adhesion, regulation of actin cytoskeleton, chemokine signaling pathway, T cell receptor signaling pathway, leukocyte transendothelial migration.	Mutations and/or CNVs affecting *RAP1GDS1* have never been reported in patients with ASD. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD with Tuberous Sclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates CdC42, thus regulating cell adhesion and migration. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Chung *et al.*, 2011 De Ferrari and Moon, 2006 Fombonne *et al.*, 1997 Ghandour *et al.*, 2007 Hamel *et al.*, 2011 Lewis *et al.*, 2004 Muzykewicz *et al.*, 2007 Okerlund and Cheyette, 2011 Wang *et al.*, 2010 Wiznitzer, 2004 Wong, 2006 Zhang *et al.*, 2012

Tab. 4. Continued.

REEP1*(-) Intracellular membrane trafficking : ER-shaping	This gene encodes the receptor accessory protein 1, which encodes a mitochondrial protein that functions to enhance the cell surface expression of odorant receptors. High expression in fetal brain and in postnatal CNS.	Mutations in *REEP1* cause spastic paraplegia autosomal dominant type 31, a neurodegenerative disorder. Pathological mechanisms for hereditary spastic paraplegias (HSPs) include mitochondrial dysfunction, abnormalities in axonal pathfinding or myelination, and intracellular trafficking defects. A majority of HSP gene products have been implicated generally in intracellular membrane and protein trafficking. In particular, REEP1 is an ER-shaping proteins and localizes to the ER in cultured rat cerebral cortical neurons, where it colocalizes with other two HSP proteins, spastin and atlastin-1. REEP proteins are required for ER network formation in vitro, and REEP1 also bound microtubules and promoted ER alignment along the microtubule cytoskeleton.	CNVs affecting *REEP1* have recently been reported in patients with ASD.	Holt *et al.*, 2012 Park *et al.*, 2010
RFX3(-)* *de novo Transcriptional regulation in ciliogenesis	This gene encodes the regulatory factor X 3, which is a member of the regulatory factor X gene family that encodes transcription factors. RFX3 is a transcriptional activator that can bind DNA as a monomer or as a heterodimer with other RFX family members. Moderate expression in fetal brain and in postnatal parietal and temporal lobes, and thalamus.	RFX3 acts as a transcription factor required for ciliogenesis and islet cell differentiation during endocrine pancreas development. It regulates the expression of genes involved in ciliary assembly (*DYNC2LI1*, *FOXJ1* and *BBS4*) and genes involved in ciliary motility (*DNAH11*, *DNAH9* and *DNAH5*). It has been reported that in mouse RFX3 is expressed strongly in the ciliated ependymal cells of the subcommissural organ, choroid plexuses and ventricular walls during embryonic and postnatal development. *Rfx3*-/-deficient mice show several hallmarks of ciliopathies including left–right asymmetry defects and hydrocephalus. Moreover, these mice suffer from corpus callosum agenesis associated with a marked disorganisation of guidepost neurons required for axon pathfinding across the midline.	Mutations and/or CNVs affecting *RFX3* have never been reported in patients with ASD.	Baas *et al.*, 2006 Benadiba *et al.*, 2012 El-Zein *et al.*, 2009
RNASEH2B(-) DNA replication	This gene encodes the ribonuclease H2, subunit B, protein. RNase H2 is composed of a single catalytic subunit (A) and two non-catalytic subunits (B and C) and specifically degrades the RNA of RNA:DNA hybrids. Moderate expression in fetal brain and amygdalaee. High expression in immune cell types.	Defects in *RNASEH2B* are a cause of Aicardi-Goutieres syndrome type 2 (autosomal recessive inheritance and, rarely, autosomal dominant) which is a genetically heterogeneous disease characterized by cerebral atrophy, leukoencephalopathy, intracranial calcifications, chronic cerebrospinal fluid (CSF) lymphocytosis, increased CSF alpha-interferon, and negative serologic investigations for common prenatal infection. Severe neurological dysfunctions manifest in infancy as progressive microcephaly, spasticity, dystonic posturing and profound psychomotor retardation.	Mutations and/or CNVs affecting *RNASEHB* have never been reported in patients with ASD.	Crow and Livingston, 2008
***RPL10*(+)** Protein synthesis	This gene encodes the ribosomal protein L10 that is a component of the ribosomal 60S subunit. High expression in brain, in particular in hippocampus.	High expression of *RPL10* has been detected by RNA *in situ* hybridization in mouse hippocampus, a constituent of the brain limbic system known to be afflicted in autism.	Two missense mutations affecting *RPL10* have been reported in two autistic patients. Recurrent Copy Number gains at Xq28 including *RPL10* have been reported in mentally retarded patients.	Klauck *et al.*, 2006 Vandewalle *et al.*, 2009 Chiocchetti *et al.*, 2011

Tab. 4. Continued.

SELE(+) CNS immunosurveillance	This gene encodes the selectin E protein, which is found in cytokine-stimulated endothelial cells and is thought to be responsible for the accumulation of blood leukocytes at sites of inflammation by mediating the adhesion of cells to the vascular lining. It is part of the selectin family of cell adhesion molecules. Good expression in fetal brain and low expression in postnatal CNS. Good expression in whole blood, in particular in monocytes.	P-selectin, L-selectin and E-selectin are involved in the capture and rolling of lymphocytes along the endothelial cell surface at the first step of lymphocyte migration. In lupus-prone mice it has been recently demonstrated an up-regulation of the adhesion molecule expression, such as ICAM-1 and E-selectin, that precedes brain damages of and correlates with kidney pathology. Immunofluorescence studies revealed that ICAM-1 and E-selectin upregulation localizes to blood vessel walls, astrocytes related to the blood-brain barrier, and microglial cells. The collected data indicated that brain involvement, even subclinical, should be presumed when peripheral organs are inflamed.	Mutations and/or CNVs affecting *SELE* have never been reported in patients with ASD. No differences between serum levels of E-selectin in autistic or SCZ patients vs. controls have been so far reported.	Engelhardt and Ransohoff , 2005 Iwata et al., 2007, 2008 Onore *et al*., 2012 Stielke *et al*., 2012
SELL(+) CNS immunosurveillance	This gene encodes the selectin L protein, which is a cell surface adhesion molecule that belongs to a family of adhesion/homing receptors. The encoded protein contains a C-type lectin-like domain, a calcium-binding epidermal growth factor-like domain, and two short complement-like repeats. It is required for binding and subsequent rolling of leucocytes on endothelial cells, facilitating their migration into secondary lymphoid organs and inflammation sites. Low expresiion in fetal brain and in postnatal CNS, except for corpus callosum where the expression is moderate. Very high expression in immune cell types, in particular in B and T lymphocytes.	As known, P-selectin is expressed on the endothelium of the blood–CNS barrier and soluble L-selectin has been found in cerebrospinal fluid. Moreover, both P and L-selectin play important roles in the entry of circulating T-lymphocytes into the CNS. It is possible that molecules not expressed in the brain may alter CNS function. Indeed, aberrant immune activity during brain development might play a role in the neural basis of neurodevelopment disorders such as ASD. Diminished expression of P-selectin has been associated with delayed neutrophil transmigration in neonatal rats. Therefore, decreased expression of P-selectin in individuals early in life may contribute to delayed leukocyte transmigration and increased susceptibility to infection, which may in turn damage neural tissues during CNS development. In fact, there is evidence that maternal viral infection in the first trimester can increase the risk of offspring developing an autistic-spectrum disorder.	Mutations and/or CNVs affecting *SELL* have never been reported in patients with ASD. A decreased serum level of P- and L-selectin has been recently observed in a group of autistic subjects vs. controls, confirming a previous finding in a cohort of HF-AU patients, thus indicating an involvement of hypoactivity of T-lymphocytes in the pathophysiology of autistic-spectrum disorders. Conversely, it has been found that the serum level of L-selectin in patients with SCZ was significantly higher than that in controls, whereas the level of P-selectin was not altered, suggesting distinct patterns of alterations for the two disorders.	Engelhardt and Ransohoff , 2005 Iwata et al., 2007, 2008 Onore *et al*., 2012
★ ***SH2B1(-)*** ***de novo*** Intracellular signaling	This gene encodes the SH2B adaptor protein 1,which is a member of the SH2-domain containing mediators family. The encoded protein mediates activation of various kinases and may function in cytokine and growth factor receptor signaling and cellular transformation.	SH2B adaptor protein family members (SH2B1-3) regulate various physiological responses through affecting signaling, gene expression, and cell adhesion. For example, SH2B1 and SH2B2 were reported to enhance nerve growth factor (NGF)-induced neuronal differentiation in PC12 cells, a well-established neuronal model system.	An autism multiplex family with 16p12.2p11.2 microduplication syndrome in monozygotic twins and distal 16p11.2 deletion in their brother, both encompassing *SH2B1*, has been recently reported. Recurrent 200-kb deletions (*de novo* or inherited) of 16p11.2 that include the *SH2B1* gene, have been associated with developmental delay and obesity.	Bachmann-Gagescu *et al*., 2010 Tabet *et al*., 2012 Wang *et al*., 2011

Tab. 4. Continued.

★ ***SMARCC1(-)**** Chromatin remodeling	This gene encodes the SWI/SNF related, matrix associated, actin dependent regulator of chromatin protein, which is a member of the SWI/SNF family of proteins, that display helicase and ATPase activities and which are thought to regulate transcription of certain genes by altering the chromatin structure around those genes. Moderate expression in fetal brain and in postnatal prefrontal cortex. Very high expression in immune cell types.	SMARCC1 belongs to the neural progenitors-specific chromatin remodeling complex (npBAF complex) and the neuron-specific chromatin remodeling complex (nBAF complex). During neural development a switch from a stem/progenitor to a post-mitotic chromatin remodeling mechanism occurs as neurons exit the cell cycle and become committed to their adult state. Furthermore, in mouse embryonic stem cells (mESCs) it has been demonstrated that Smarcc1 is necessary for heterochromatin formation and chromatin compaction during differentiation, and that it plays important roles in facilitating mESCs differentiation by coupling gene repression with global and local changes in chromatin structure. SMARCC1 is also implicated in B and T-cell development.	Mutations and/or CNVs affecting *SMARCC1* have never been reported in patients with ASD.	Choi *et al.*, 2012 Marei *et al.*, 2012 Schaniel *et al.*, 2009
SMYD3(-) Chromatin remodeling	This gene encodes the SET and MYND domain containing 3 protein, which is a histone methyltransferase that functions in RNA polymerase II complexes by an interaction with a specific RNA helicase. Moderate expression in fetal brain and good expression in postnatal whole brain, in particolar in hypothalamus.	Members of the SET and MYND domain containing (Smyd) family of proteins possess SET-dependent methyltransferase capacity and have been shown to be involved in the transcriptional control of cell differentiation and cell proliferation. With the exception of Smyd1, little is known about the distinct functional relevance of Smyd family proteins during vertebrate development, although it has been proposed a role of Smyd1 and 2 in cardiac development and in brain development for the only Smyd2, which is similar to Smyd3.	Mutations and/or CNVs affecting *SMYD3* have never been reported in patients with ASD.	Brown *et al.*, 2006 Diehl *et al.*, 2010 Hamamoto *et al.*, 2004 Kwon *et al.*, 2009
SNX6(-)* Intracellular membrane trafficking	This gene encodes the sorting nexin 6 protein, a member of the sorting nexin family which is involved in intracellular trafficking. This protein may form oligomeric complexes with family member proteins through interactions of both the PX domain and the coiled coil regions of the molecules. It plays a role in retrograde protein transport from endosomes to the trans-Golgi network. SNX6 has been detected in fetal brain and shows moderate-good expression in postnatal prefrontal cortex and hypoyhalamus.	SNX6 is expressed in fetal brain and, recently, it has been demonstrated a role in olygodendrocyte differentation.	Mutations and/or CNVs affecting *SNX6* have never beeen reported in patients with ASD. *SNX6* is a candidate locus for holoprosencephaly.	Kamnasaran *et al.*, 2005 Schimdt *et al.*, 2012
TMSB15B(+) Neurodevelopment: cytoskeleton organization	This gene encodes the thymosin beta 15B protein, which plays an important role in the organization of the cytoskeleton. It binds to and sequesters actin monomers (G actin) and therefore inhibits actin polymerization. Good expression in fetal brain.	Thymosin beta15 (Tbeta15) is a pleiotropic factor which exerts multiple roles in the development of nervous system and brain diseases. It has been demonstrated that the expressions of Tbeta15 mRNA and protein were increased in several brain regions including hippocampal formation and cerebral cortex, following kainic acid (KA)-evoked seizures in rat.	Mutations and/or CNVs affecting *TMSB15B* have never been reported in patients with ASD.	Kim *et al.*, 2008

Tab. 4. Continued.

TSPAN5(-)* ***de novo*** Neurodevelopment and IS development	This gene encodes the tetraspanin 5 protein, which mediates signal transduction events that play a role in the regulation of cell development, activation, growth and motility. Very high expression in fetal brain and in postnatal CNS.	One of the first interacting protein is NOTCH2 which is involved in differentiation, proliferation and apoptotic programs, and shows a well-known role in IS development. NOTCH2 interacts with CNTN1, contactin 1, involved in brain development, specifically in synapse formation. In mouse brain development a specific pattern of *TSPAN5* expression has been observed, particularly in cerebellar Purkinje cells.	Mutations and/or CNVs affecting *TSPAN5* have never been reported in patients with ASD. Rare single gene mutations affecting *TSPAN7* have been reported in a few autistic patients. Expression profiling of the superior temporal gyrus of six autistic subjects and matched controls revealed increased transcript levels of many immune system related genes such as *NOTCH2*. These expression patterns appear to be more associated with the late recovery phase of autoimmune brain disorders, than with the innate immune response characteristic of neurodegenerative diseases. CNVs affecting *CNTN4* have been observed in patients with ASD.	Cottrell *et al.*, 2011 Fernandez *et al.*, 2008 Garbett *et al.*, 2008 Glessner *et al.*, 2009 Juenger *et al.*, 2005 Lamprianou *et al.*, 2011 Morrow *et al.*, 2008 Piton *et al.*, 2011 Roohi *et al.*, 2009
USP9Y(-)* Intracellular signaling: protein deubiquitination pathway	This gene encodes an ubiquitin specific peptidase 9, Y-linked, which is a member of the peptidase C19 family that is similar to ubiquitin-specific proteases, which cleave the ubiquitin moiety from ubiquitin-fused precursors and ubiquitinylated proteins. It may therefore play an important regulatory role at the level of protein turnover by preventing degradation of proteins through the removal of conjugated ubiquitin. Essential component of TGF-beta/BMP signaling cascade. Quite high expression in fetal brain and postnatal CNS, in particular in parietal and temporal lobes, amygdalaee and thalamus.	It has been reported that *USP9Y*, previously considered as testis-specific, is highly expressed in developing mouse brain whereas expression in adult brain is low and probably inhibited by androgens. Furthermore, a distinctive pattern of cerebral expression of different sex chromosome genes, including *USP9Y*, has been reported in mouse and human brain in specific neuronal subpopulations, that may possibly contribute to gender differences in prevalence noted for some neuropsychiatric disorders.	A significant association of SNPs in *USP9Y* with susceptibility to ASD has been reported The protein ubiquitination pathway has been previously implicated in ASD.	Glessner *et al.*, 2009 Kishino *et al.*, 1997 Matsuura *et al.*, 1997 Vawter *et al.*, 2004 Wang *et al.*, 2009 Xu *et al.*, 2002
VAT1(-) Synaptic plasticity: cholinergic system	This gene encodes the vesicle amine transport protein 1 homolog (T. californica). Synaptic vesicles are responsible for regulating the storage and release of neurotransmitters in the nerve terminal. The protein encoded by this gene is an abundant integral membrane protein of cholinergic synaptic vesicles and is thought to be involved in vesicular transport. Moderate expression in fetal brain and in postnatal CNS.	In the CNS the cholinergic system, that uses acetylcholine (ACh) as neurotransmitter, has a variety of neuromodulation effects upon plasticity, arousal and reward. ACh has an important role in sustaining attention, learning and short-term memory. Damage to the cholinergic system in the brain has been shown to be plausibly associated with the memory deficits associated with Alzheimer's disease.	Mutations and/or CNVs affecting *VAT1* have never been reported in patients with ASD. The cholinergic system is known to regulate the function of the visual pathway, including the fusiform gyrus which is the key structure in face perception. In adults with ASD, showing deficiencies in face perception, a deficit in cholinergic innervations of the fusiform gyrus has been observed. Furthermore, the cholinergic system has been implicated in the development of autism on the basis of neuronal nicotinic acetylcholine receptor losses in cerebral and cerebellar cortex, and in thalamus, thus contributing to sensory or attentional deficits.	Himmelheber *et al.*, 2000 Ray *et al.*, 2005 Suzuki *et al.*, 2011

Tab. 4. Continued.

XRN1(-)* RNA degradation	This gene encodes the 5'-3' exoribonuclease 1, which localizes to cytoplasmic foci containing a complex of mRNA-degrading enzymes. It is involved in mRNA decay. Moderate expression in fetal brain and postnatal prefrontal cortex, amygdalaee and hypothalamus. High expression in immune cell types.	It has been reported that XRN1, which is a glial cell line-derived neurotrophic factor-inducible protein, forms together with FMRP and other proteins a multiprotein complex that is localized in the GW bodies in astrocytes and astrocytoma cells. GWBs are unique cytoplasmic structures that contain the mRNA binding protein GW182 and other proteins involved in mRNA processing pathways.	Mutations and/or CNVs affecting *XRN1* have never been reported in patients with ASD. Mutation in *FMR1*, which encodes the FMRP protein, is responsible for Fragile X syndrome, that is comorbidity with ASD.	Clifford *et al.*, 2007 Kielinen *et al.*, 2004 Moser *et al.*, 2007 Shimoyama *et al.*, 2003 Wang *et al.*, 2010
ZFX(-)* Chromatin remodeling	This gene encodes the Zinc finger protein, X-linked, which is a member of the krueppel C2H2-type zinc-finger protein family and probable plays a role as transcriptional activator. Good expression in postnatal temporal lobe, prefrontal cortex, thalamus and amygdalaee. High expression in immune cell types.	ZFX interacts with JARID1C (KDM5C), a lysine (K)-specific demethylase 5C that specifically demethylates Lys- 4 of histone H3, thereby playing a central role in histone code. It participates in transcriptional repression of neuronal genes by recruiting histone deacetylases and REST at neuron-restrictive silencer elements. ZFX is an essential transcriptional regulator of hematopoietic stem cell function. Furthermore, ZFX is required for pro-B to pre–B-cell transition and maintenance of mature recirculating B cells and B-1 cells.	Mutations and/or CNVs affecting *ZFX* have never been reported in patients with ASD. A *JARID1C* missense mutation affecting a highly conserved amino acid has been reported in a little boy diagnosed with idiopathic autism. A few *JARID1C*-regulated genes *SCN2A*, *CACNA1H*, *BDNF*, and *SLC18A1* have been associated with autism and cognitive dysfunction.	Adegbola *et al.*, 2008 Akbarian and Huang, 2009 Arenzana *et al.*, 2009
ZMYM5 or ZNF237(+) Transcriptional regulation	This gene encoder the zinc finger MYM-type 5 protein. Moderate expression in postnatal parietal lobe, cerebellum and cerebellum peduncles.	It has been reported that ZMYM5 may inhibit *Presenilin1* (*PS1*) transcription by forming an inhibitory complex with ERM, a member of the Ets transcription factors, which directly binds *PS1* promoter. Mutations in *PS1* are responsible of sporadic juvenile and familial cases of Alzheimer's disease. Besides the involvement of Presenilins in amyloid plaques formation, PSs regulate the cleavage of other signaling receptors and transducers such as Notch-1, ErbB4, DC44, and LDL-receptor-related proteins and cadherins. PSs also affect different other signaling molecules, such as Wnt signal transduction pathway, which regulates morphology, proliferation, and motility of the cell.	Mutations and/or CNVs affecting *ZNF237* have never been reported in patients with ASD. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Baulac *et al.*, 2003 De Ferrari and Moon, 2006 De Strooper, 2003 Kopan and Goate, 2000 Nizzari *et al.*, 2012 Okerlund and Cheyette, 2011 Pastoric and Das, 2007 Schroeter *et al.*, 1998 Wang *et al.*, 2010 Zhang *et al.*, 2012
ZNF138(-) Transcriptional regulation	This gene encodes the zinc finger protein 138 which is involved in transcriptional regulation. Moderate expression in fetal brain and in postnatal CNS, high expression in cerebellum.	ZNF138 is probably involved in development.	Mutations and/or CNVs affecting *ZNF138* have never been reported in patients with ASD.	Tommerup and Vissing, 1995
ZNF236(-)* Transcriptional regulation	This gene encodes the zinc finger protein 236 which is involved in transcriptional regulation. It is ubiquitous expressed with the highest levels in skeletal muscle and brain.		Mutations and/or CNVs affecting *ZNF236* have never been reported in patients with ASD.	

Tab. 4. Continued.

ZNF280A(-) Transcriptional regulation	This genes encoder the zinc finger protein 280A which acts as a transcription factor. Good expression in fetal brain and moderate expression in postnatal whole brain, in particular in amygdalaee.	Mutations and/or CNVs affecting *ZNF280A* have never been reported in patients with ASD.

[$] In this table only the genes included in rare CNVs which do not localize in recurrent genomic regions have been analyzed. (-), deleted or disrupted gene due to a rare deletion; (-)*, possible disrupted gene due to a rare duplication; (+), duplicated gene due to a rare duplication. The genes already implicated in ASD, due to mutations and/or CNVs or SNPs, are depicted in red and purple, respectively.

Genes implicated in CNS metabolism.
Genes implicated in synaptogenesis and synaptic plasticity.
Genes implicated in CNS-IS network.
Genes implicated in intracellular signaling and membrane trafficking.
Genes implicated in neurogenesis and neurodevelopment.
Genes implicated in transcriptional and translational regulation, and chromatin remodeling.

Genes whose function may be related to the IS development and function, within and outside the CNS.

ADHD, attention deficit hyperactivity disorder; AS, Angelman syndrome; BD, bipolar disorder; BWS, Beckwith-Wiedemann syndrome; CNS, central nervous system; CNV, copy number variation; CSF, cerebrospinal fluid; EP, epilepsy; ER, endoplasmic reticulon; HF-AU, high functioning autism; ID, intellectual disability; IS, immune system; MDD, major depressive disorder; MR, mental retardation; SCZ, schizophrenia; SNP, single nucleotide polymorphism; SRS, Siver-Russell syndrome; XLMR, X-linked mental retardation.

Tab. 4.1. Detailed list of the genes potentially perturbed by the identified rare CNVs and possible implicated in ASD pathogenesis (UCSC Genome Browser, hg19, February 2009)[§].

Gene name	Function and expression	Interactors and possible role in brain	Findings in ASDs or in other neuropsychiatric disorders	References
▪ **Patient 10, gain of 3.9 Mb at 2q14.2q14.3 (chr2:119130298-123004562)**				
★ ***CLASP1(+)*** Intracellular membrane trafficking: regulation of microtubule cytoskeleton dynamics	This gene encodes the cytoplasmic linker associated protein 1. CLASPs, such as CLASP1, are nonmotor microtubule-associated proteins. CLASP1 is involved in the regulation of microtubule dynamics at the kinetochore and throughout the spindle. High expression in fetal brain and in postnatal CNS. High expression in immune cell types.	It has been recently reported that CLASP protein (CLASP1 and CLASP2) function to both promote and restrict axon growth, thus suggesting that the opposing roles of CLASP are rooted in its unique microtubule (MT)-binding activities: CLASP supports axon extension when it binds to MT plus ends, whereas it restricts axon growth when bound along MT lattices. It directly interacts with CLIP2, the CAP-GLY domain containing linker protein 2, which seems to link microtubules to dendritic lamellar body (DLB), a membranous organelle predominantly present in bulbous dendritic appendages of neurons linked by dendrodendritic gap junctions. It may operate in the control of brain-specific organelle translocations.	Mutations and/or CNVs affecting *CLASP1* have never been reported in patients with ASD.	Akhmanova *et al.*, 2001 Al-Bassam *et al.*, 2010 Hur *et al.* 2011 Maiato *et al.*, 2003 Neukirchen and Bradke, 2011 Watanabe *et al.*, 2009 Wittmann and Waterman-Storer, 2005
C1QL2(+) CNS development	This gene encodes the complement component 1, q subcomponent-like 2. Highly expressed in the CNS.	Many member of the C1q family are secreted and play a crucial role in intercellular signaling. The gene expression of the C1ql subfamily in adult and developing mouse brain has been recently investigated. In adult brain the *C1ql1-C1ql3* mRNAs were mainly expressed in neurons and weak expression was found in glia-like structures. Moreover, although the *C1ql1-C1ql3* mRNAs were detectable as early as embryonic day 13, the C1ql2 mRNA was observed at later embryonic stages. Another member of the C1q family of proteins, Cbln, is highly expressed in the CNS and plays two fundamental roles in cerebellum synapses: the formation and stabilization of synaptic contact, and the control of functional synaptic plasticity by regulating the postsynaptic endocytotic pathway.	A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *C1QL2*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3.	Devillard *et al.*, 2010 Iijima *et al.*, 2010 Yuzaki, 2010

Tab. 4.1. Continued.

***DBI*(+)** Synaptic plasticity (GABA receptor modulation)	This gene encodes the diazepam binding inhibitor (GABA receptor modulator, acyl-CoA binding protein), which is a protein that binds medium- and long-chain acyl-CoA esters with very high affinity and may function as an intracellular carrier of acyl-CoA esters. It is also able to displace diazepam from the benzodiazepine (BZD) recognition site located on the GABA type A receptor. It is therefore possible that this protein also acts as a neuropeptide to modulate the action of the GABA receptor located in brain synapses. Moderate expression in fetal brain, good expression in postnatal cerebellum, thalamus, amygdalae, corpus callosum and spinal cord.	A potential imbalance between excitatory and inhibitory interneurons in the cortex may contribute to altered information processing in autism. Furthermore, reduced numbers of GABA and benzodiazepine receptors have been reported in the autistic brain, particularly in posterior cingulate cortex.	A CNV (loss) including *DBI* has recently been reported in an autistic patient. A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *DBI*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3. A SNP in *DBI* has been associated with anxiety disorders with panic attacks.	Devillard *et al.*, 2010 Griswold *et al.*, 2012 Oblak *et al.*, 2011 Thoeringer *et al.*, 2007
***EN1*(+)** Intracellular Wnt signaling	This gene encodes the engrailed homeobox 1 protein, which is a transcription factor that has been implicated in the control of pattern formation during CNS development. Low expression in fetal brain and moderate expression in postnatal occipital and temporal lobes, cerebellum, hypothalamus, and cervical ganglion.	En1 and En2 are important transcription factors whose role in CNS development is well known. They are involved in Wnt signaling pathway and, in particular, En1 togheter with β-catenin regulates transcription of β-catenin target genes in neuronal cells.	A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *EN1*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3. A single study reported no SNPs affecting *EN1* in 247 patients with SCZ, 98 patients with autism, and 56 patients with idiopathic MR. Conversely, a SNP in *EN2* has been associated with autism. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Alves dos Santos and Smidt, 2011 Benayed *et al.*, 2009 Chung *et al.*, 2011 De Ferrari and Moon, 2006 Devillard *et al.*, 2010 Laroche *et al.*, 2008 Okerlund and Cheyette, 2011 Sen *et al.*, 2010 Wang *et al.*, 2010 Yang *et al.*, 2010 Zhang *et al.*, 2012
***EPB41L5*(+)** Neurodevelopment	This gene encodes the erythrocyte membrane protein band 4.1 like 5. Moderate expression in fetal brain and in postnatal CNS.	EPB41L5 belongs to the family of the FERM proteins, which contain a FERM domain and are ubiquitous components of the cytocortex of animal cells where they are engaged in structural, transport, and signaling functions, thus contributing to animal morphogenesis. It has been demonstrated that the murine ortholog protein of the human Epb4.1L5, Lulu, helps anchor the actin-myosin contractile machinery to the cell membrane to allow the dynamic rearrangements of epithelia that mediate embryonic morphogenesis. In particular, this process is necessary for the organization of the neural plate (neuroepithelium polarity).	A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *EPB41L5*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3.	Devillard *et al.*, 2010 Lee *et al.*, 2007 Tepass, 2009

Tab. 4.1. Continued.

GLI2(+) Transcriptional regulation	This gene encodes the GLI family zinc finger 2 protein, which belongs to the C2H2-type zinc finger protein subclass of the Gli family. Members of this subclass are characterized as transcription factors which bind DNA through zinc finger motifs. It is thought to play a role during embryogenesis. High expression in fetal brain and low expression in postnatal CNS, except for cerebellum where the expression is good.	In mouse embryo Gli2 shows an essential role in the establishment of dorsoventral polarity in the vertebrate CNS. In addition, in mouse brain the Gli transcription factor are essential for thalamic development acting downstream the Sonichedgehog (Shh) signaling. In particular, Gli2 is the major activator, while Gli3 acts primarily as a repressor. Moreover, the expression of *sox2* gene, which is essential for the maintenance of neuronal stem cells (NSCs), is regulated by Gli2, by its binding to an enhancer that is vital for *sox2* expression in telencephalic neuroepithelial cells, which consist of NSCs and neural precursor cells.	Mutations and/or CNVs affecting *GLI2* have never been reported in patients with ASD. Defects in *GLI2* are the cause of holoprosencephaly type 9 (HPE9) also called pituitary anomalies with holoprosencephaly-like features. The primary features of this disease include defective anterior pituitary formation and pan-hypopituitarism, with or without overt forebrain cleavage abnormalities, and holoprosencephaly-like midfacial hypoplasia. A SNP in *GLI2* has been associated with tardive diskinesia in patients with chronic SCZ.	Greenbaum *et al.*, 2010 Haddad-Tovolli *et al.*, 2012 Matise *et al.*, 1998 Roessler *et al.*, 2003 Takanaga *et al.*, 2009
MARCO(+) Microglial cells maturation	This gene encodes the macrophage receptor with collagenous structure, which is a member of class A scavenger receptor family and it is part of the innate antimicrobial immune system. The protein MARCO acts as a homotrimer and binds both Gram.negative and Gram-positive bacteria. Low-moderate expression in fetal brain and in postnatal CNS. High expression in lymph nodes and monocytes.	Microglial cells originate from bone marrow and migrate in the brain when they finish their differentiation under the influence of growth factors and cytokines release by resident cells, such as granulocyte-macrophage colony stimulated factor (GM-CSF). GM-CSF treated microglial cells show enhanced ability to process antigens and enhanced antigen-presentation capacity. It has been demonstrated a strong up-regulation of *MARCO* mRNA in maturing microglial cells. Furthermore, during microglia maturation, MARCO induces a profound actin cytoskeleton rearrangement and a down-regulation of antigen-uptake function.	A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *MARCO*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3.	Devillard *et al.*, 2010 Gehrmann, 1996 Granucci *et al.*, 2003 Ling and Wong, 1993 Matyszak *et al.*, 1999 Ulvestad *et al.*, 1994
***PCDP1*(+)** Ciliogenesis and ciliary motility	This gene encodes the primary ciliary dyskinesia protein 1, which is required for ciliary motility. It is specifically expressed in brain ependymal cells.	PCDP1 plays an important role in ciliary and flagellar biogenesis and motility. Homozygous mice for mutations affecting *PCDP1* show the primary ciliary dyskinesia, which results from ciliary dysfunction and is commonly characterized by sinusitis, male infertility, hydrocephalus, and situs inversus.	A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *PCDP1*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3.	Devillard *et al.*, 2010 Lee *et al.*, 2008
★ ***PTPN4(+)*** Intracellular signaling	This gene encodes the protein tyrosine phosphatase, non-receptor type 4, which is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. This PTP has been shown to interact with glutamate receptor delta 2 and epsilon subunits, and is thought to play a role in signaling downstream of the glutamate receptors through tyrosine dephosphorylation. High expression in fetal brain and in postnatal whole brain, in particolar in thalamus and amygdalae. High expression in immune cell types.	PTPN4 associates directly with GRID2 and plays a role in signaling downstream of the GRID2 and/or in regulation of their activities through tyrosine dephosphorylation. GRID2 is selectively expressed in cerebellar Purkinje cells. It directly interacts with DLG2 and SHANK2 which act as adapter proteins in the postsynaptic density of excitatory synapses, interconnecting receptors of the postsynaptic membrane and the actin-based cytoskeleton.	A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *PTPN4*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3. A *de novo* CNV at 4q22.2 including *GRID2* has recently been reported in an autistic patient. Mutations and CNVs (*de novo* and inherited) affecting *SHANK2* have been reported in ASD patients.	Berkel *et al.*, 2010, 2012 Devillard *et al.*, 2010 He *et al.*, 2012 Hironaka *et al.*, 2000 Leblond *et al.*, 2012 Pinto *et al.*, 2010 Uemura *et al.*, 2004

Tab. 4.1. Continued.

★ ***RALB*(+)** Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encodes the v-ral simian leukemia viral oncogene homolog B protein, which is a GTP-binding protein that belongs to the small GTPase superfamily and Ras family of proteins. It is a multifuntional GTPase involved in a variety of cellular processes including gene expression, cell migration, cell proliferation, oncogenic transformation and membrane trafficking. Moderate expression in fetal brain and good expression in postnatal whole brain, in particular in cortex, amygdalae, and thalamus. High expression in immune cell types.	The Ras-like small GTPases, RalA and RalB, regulate a large variety of cellular processes including transcription, translation, cytoskeletal organization, membrane trafficking, cytokinesis, cell migration, cell proliferation, and cell survival. Recently, it has been demonstrated an involvement of RalA/B in projection neuron migration from the ventricular zone to the neocortical plate during mouse brain development. This process implies that the neurons become multipolar and move non-radially in the intermediate zone. Both Reelin, the Rap1 GTPase, and N-cadherin are important for multipolar neurons to polarize their migration towards the cortical plate. Indeed, Reelin regulates migration through Rap1 and Akt, and Rap1-regulated GTPases, RalA/B, Rac1 and Cdc42, are also involved.	A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *RALB*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3. The Rho GTPase Cdc42 is involved in neuronal morphogenesis, axonal guidance and synaptic plasticity by modulating the organization of actin cytoskeleton. The same pathway is involved in T-cell activation, migration, cell-cell adhesion. Point mutations and CNVs affecting *TSC1* and *TSC2* have been reported in patients with ASD and Tuberous Sclerosis 1 or 2. TSC2 activates CdC42, thus regulating cell adhesion and migration.	Devillard *et al.*, 2010 Fombonne *et al.*, 1997 Jossin and Cooper, 2011 Lewis *et al.*, 2004 Muzykewicz *et al.*, 2007 Shirakawa *et al.*, 2009 Wiznitzer, 2004 Wong, 2006
***RNU4ATAC*(+)** Neurodevelopment	This gene encodes the RNA, U4atac, small nuclear RNA, which is part of the U12-dependent minor spliceosome complex. No expression data are available at UCSC Genome Browser, release February 2009.	Homozygous defects in *RNU4ATAC* are a cause of microcephalic osteodysplastic primordial dwarfism type 1 (MOPD).	Mutations and/or CNVs affecting *RNU4ATAC* have never been reported in patients with ASD.	Abdel-Salam *et al.*, 2011
***SCTR*(+)** Synaptic plasticity: secretin receptor	This gene encodes the secretin receptor, which is a G protein-coupled receptor and belongs to the glucagon-VIP-secretin receptor family. It binds secretin which is the most potent regulator of pancreatic bicarbonate, electrolyte and volume secretion. Moderate expression in postnatal parietal lobe, prefrontal cortex, amygdalae, and hypothalamus.	Secretin is a peptide hormone released from the duodenum to stimulate the secretion of digestive juice by the pancreas. It also functions as a neuropeptide hormone in the brain. Secretin receptor-deficient mice show an impaired synaptic plasticity in the hippocampus, and abnormal social and cognitive behaviours, thus suggesting that the secretin receptor system has an important role in the CNS relating to social behaviour such as autism.	A *de novo* duplication of 4.2 Mb at 2q14.1q14.2, involving *SCTR*, has been detected in an autistic boy carrying the paracentric inversion inv(2)(q14.2q37.3), that was inherited from the healthy mother, and a terminal deletion at 2q37.3. Improved behavioral and language skills in autistic children who received porcine secretin have been firstly described in 1998. Subsequent controlled studies have either failed to confirm these results or have shown some behavioral improvements among a subset of children with autism. Secretin has also been described as being potentially therapeutic for other behavioral disorders such as SCZ.	Alamy et al., 2004 Devillard *et al.*, 2010 Esch and Carr, 2004 Horvath *et al.*, 1998 Kern *et al.*, 2002 Nishijima *et al.*, 2006 Sheitman *et al.*, 2004 Tay *et al.*, 2004 Yung *et al.*, 2001

Tab. 4.1. Continued.

▪ **Patient 14, gain of 442.5 kb at 9q34.3 (chr9:140527202-140969676)**				
CACNA1B*(-)* *de novo Synaptogenesis and synaptic plasticity	This gene encodes the calcium channel, voltage-dependent, N type, alpha 1B subunit. The protein encoded by this gene is the pore-forming subunit of an N-type voltage-dependent calcium channel, which controls neurotransmitter release from neurons. Good expression in fetal brain and moderate expression in postnatal CNS, in particular in cerebellum and thalamus.	Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization, and they are involved in a variety of calcium-dependent processes, including muscle contraction, hormone or neurotransmitter release, gene expression, cell motility, cell division and cell death. Clear function in presynaptic neurotransmitter release.	*CACNA1B* maps in the chromosomal region which is implicated in the 9q subtelomeric deletion syndrome (Kleefstra syndrome), which is comorbidi with ASD. In addition, GWAS have previously associated *CACNA1B* with SCZ and BD and a few CNVs (both gains and losses) affecting *CACNA1B* have been found in SCZ patients. Mutations affecting different CACNA genes, such as *CACNA1C*, *CACNA1F*, and *CACNA1H* have been reported in idiopathic or syndromic patients with ASD.	Anderlid *et al.*, 2002 Bhat *et al.*, 2012 Dawson *et al.*, 2002 Glessner *et al.*, 2010 Hemara-Wahanui *et al.*, 2005 Iwakoshi *et al.*, 2004 Kleefstra *et al.*, 2005, 2009 McMullan *et al.*, 2009 Moskvina *et al.*, 2009 Sahoo *et al.*, 2006 Splawski *et al.*, 2004, 2006
EHMT1*(-)* *de novo Chromatin remodeling	This gene encodes the euchromatic histone-lysine N-methyltransferase 1. The protein is a histone methyltransferase that is part of the E2F6 complex, which represses transcription, methylating the Lys-9 position of histone H3, which tags it for transcriptional repression. It could play a role in the G0/G1 cell cycle transition. Low expression in fetal brain and in postnatal CNS.	Alterations in RNA levels are frequently reported in brain of subjects diagnosed with autism, SCZ, depression and other psychiatric diseases. Recently it has been demonstrated that different epigenetic regulation, through histone lysine methylation, is present in post-morten human brain of patients affected by neuropsychiatric disorders vs. controls.	*EHMT1* point mutations and deletions of different size includine *EHMT1* cause the 9q subtelomeric deletion syndrome (Kleefstra syndrome), which is comorbidi with ASD. Balanced chromosomal abnormalities affecting *EHMT1* have been found in autistic patients.	Akbarian and Huang, 2009 Anderlid *et al.*, 2002 Dawson *et al.*, 2002 Iwakoshi *et al.*, 2004 Kleefstra *et al.*, 2005, 2009 McMullan *et al.*, 2009 Sahoo *et al.*, 2006 Talkowski *et al.*, 2012
▪ **Patient 23, loss of 748 kb, at 17q21.31 (chr17:43193251-43941693)**				
ACBD4*(-) *de novo Lipid metabolism	This gene encodes the acyl-CoA binding domain containing 4 protein, which is a member of the acyl-coenzyme A binding domain containing protein family. They are thought to play roles in acyl-CoA dependent lipid metabolism. Moderate expression in fetal brain and in postnatal CNS, in particular in prefrontal cortex, thalamus and hypothalamus.	Recently, brain transcriptome variation among behaviorally distinct strains of zebrafish has been reported including *acbd3* and *acbd4* genes. No data about the role in human brain are so far available.	Mutations and/or CNVs affecting *ACBD4* have never been reported in patients with ASD. *ACBD4* maps in the proximity of the genomic region involved in the 17q21.31 microdeletion/microduplication syndrome, which shows comorbidity with ASD. One ASD deleted patient and several ASD duplicated patients have so far been reported.	Betancur *et al.*, 2008 Drew *et al.*, 2012 Grisart *et al.*, 2009 Koolen *et al.*, 2008
★ ***CRHR1*(-) *de novo*** Synaptic plasticity: stress response modulation	This gene encodes the corticotropin releasing hormone receptor 1, a G-protein coupled receptor that binds neuropeptides of the corticotropin releasing hormone family that are major regulators of the hypothalamic-pituitary-adrenal pathway. The encoded protein is essential for the activation of signal transduction pathways that regulate diverse physiological processes including stress, reproduction, immune response and obesity. Good expression in fetal brain and postnatal CNS, in particular in cerebellum, amygdalae, and thalamus.	It has been demonstrated that CRHR1 critically controls behavioral adaptation to stress and is causally linked to emotional disorders. In particular, the lack of CRHR1 in murine forebrain glutamatergic circuits reduces anxiety and impairs neurotransmission in the amygdalae and hippocampus whereas selective deletion of CRHR1 in midbrain dopaminergic neurons increases anxiety-like behavior and reduces dopamine release in the prefrontal cortex. Moreover, CRHR1 is involved in CRH-induced hippocampal neuron apoptosis.	*CRHR1* maps within the genomic region involved in the 17q21.31 microdeletion/microduplication syndrome, which shows comorbidity with ASD. One ASD deleted patient and several ASD duplicated patients have so far been reported.	Amath *et al.*, 2012 Betancur *et al.*, 2008 Grisart *et al.*, 2009 Hsu *et al.*, 2012 Koolen *et al.*, 2008 Refojo *et al.*, 2011 Zhang *et al.*, 2012

Tab. 4.1. Continued.

★ ***FMNL1(-) de novo*** Neurodevelopment and macrophages motility and survival: actin cytoskeleton dynamics	This gene encodes the formin-like 1 protein, which is a formin-related protein. Formin-related proteins have been implicated in morphogenesis, cytokinesis, and cell polarity. FMNL1 may play a role in the control of cell motility and survival of macrophages. Low expression in fetal brain and moderate-good expression in postnatal whole brain, in particular in parietal and occipital lobe, amygdalae and hypothalamus. Very high expression in bone marrow and immune cell types.	FMNL1 is responsible for modifying actin at the macrophage podosome and may be involved in actin cytoskeleton dynamics during adhesion and migration within tissues. By analogies with other members of the same family, it may also have a role in neurogenesis. Indeed, recently it has been reported that Formin1 mediates the induction of dendritogenesis and synaptogenesis by neurogenin3 in mouse hippocampal neurons through a direct role in cytoskeleton dynamics.	Mutations and/or CNVs affecting *FMNL1* have never been reported in patients with ASD. *FMNL1* maps in the proximityof the genomic region involved in the 17q21.31 microdeletion/microduplication syndrome, which shows comorbidity with ASD. One ASD deleted patient and several ASD duplicated patients have so far been reported.	Betancur *et al.*, 2008 Grisart *et al.*, 2009 Koolen et al., 2008 Mersich *et al.*, 2010 Simon-Arceres *et al.*, 2011 Yu *et al.*, 2011

- **Patient 25, gain of 377 kb at 15q13.3 (chr15:32085731-32462701)**

CHRNA7(+) Synaptogenesis and synaptic plasticity	This gene encodes the cholinergic nicotinic receptor alpha 7. The nicotinic acetylcholine receptors (nAChRs) are members of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses. The nAChRs are thought to be hetero-pentamers composed of homologous subunits. The protein encoded by this gene forms a homo-oligomeric channel, displays marked permeability to calcium ions and is a major component of brain nicotinic receptors that are blocked by, and highly sensitive to, alpha-bungarotoxin. Once this receptor binds acetylcholine, it undergoes an extensive change in conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane. High expression in fetal brain and in postnatal CNS, in particular in cortex, amygdalae and thalamus.	In human fetal brain high levels of *CHRNA7* gene expression have been found in nuclei that receive sensory information, such as those of the neocortex and hippocampus, the thalamic nuclei, the reticular thalamic nucleus, the pontine nuclei and the superior olive complex, thus supporting a possible regulatory function for alpha 7-containing receptors in CNS development and in sensory processing, which may be involved in the pathological physiology of SCZ and autism.	*CHRNA7* maps within the genomic region involved in the 15q13.3 microdeletion/microduplication syndrome, which shows comorbidity with ASD. SNPs in the *CHRNA7* promoter have been associated with SCZ. CNVs sncompassing *CHRNA7* have been found in patients with ADHD, DD, and ID.	Ben-Shachar *et al.*, 2009 Guilmatre *et al.*, 2009 Hoppman-Chaney *et al.*, 2012 Leonard *et al.*, 2002 Masurel-Paulet *et al.*, 2010 Mikhail *et al.*, 2011 Miller *et al.*, 2009 Pagnamenta *et al.*, 2009 Pinto *et al.*, 2010 Sharp *et al.*, 2008 Szafranski *et al.*, 2010 van Bon *et al.*, 2009 Williams *et al.*, 2012

Tab. 4.1. Continued.

- **Patient 25, gain of 538 kb at 16p11.2 (chr16:29652999-30190568)**
- **Patient 38, gain 659 kb at 16p11.2 (chr16:29673954-30332581)**
- **Patient 39, gain 659 kb at 16p11.2 (chr16:29673954-30332581)**

***ALDOA*(+)** Brain metabolism: glycolysis	This gene encodes the aldolase A, fructose-bisphosphate protein, which is a glycolytic enzyme that catalyzes the reversible conversion of fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Three aldolase isozymes (A, B, and C), encoded by three different genes, are differentially expressed during development. Aldolase A is found in the developing embryo and is produced in even greater amounts in adult muscle, whereas is repressed in adult liver, kidney and intestine. Aldolase A shows levels similar to aldolase C in brain and other nervous tissue. Low expression in fetal brain and good expression in postnatal CNS.	Defects in *ALDOA* are the cause of glycogen storage disease type 12, also known as red cell aldolase deficiency, a metabolic disorder associated with increased hepatic glycogen and hemolytic anemia. It may lead to myopathy with exercise intolerance and rhabdomyolysis. Using the zebrafish as a tool, a set of 16p11.2 homologs has been recently identified. This set of genes is highly active during the first 5 days of development, and most genes in this region are required for nervous system development (including *aldoa*) – impacting brain morphology, eye development, axonal density or organization, and motor response. Screening for 16p11.2 genes whose function is sensitive to hemizygosity, the *aldoaa* and *kinesin family member 22* genes were identified as giving clear phenotypes when RNA levels were reduced by ~50%, suggesting that these genes are deletion dosage sensors.	*ALDOA* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***ASPHD1*(+)** Neurodevelopment	This gene encodes the aspartate beta-hydroxylase domain containing 1 protein. Good expression in fetal brain and in postnatal CNS, in particular in cerebellum.	The zebrafish loss of function model for the *ASPHD1* gene shows a weak phenotype with defective in brain morphology.	*ASPHD1* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***CDIPT*(+)** Intracellular signaling: phosphatidylinositol signaling system	This gene encodes the CDP-diacylglycerol-inositol 3-phosphatidyltransferase protein. Phosphatidylinositol breakdown products are ubiquitous second messengers that function downstream of many G protein-coupled receptors and tyrosine kinases regulating cell growth, calcium metabolism, and protein kinase C activity. Moderate expression in fetal brain and in postnatal CNS.	In mice, *CDIPT* mRNA had already expressed on the prenatal day 15 throughout the neuroaxis including the spinal cord. During the postnatal stages, *CDIPT* gene expression has been detected widely in the gray matters throughout the entire brain. The highest levels have bee reported in the olfactory mitral cells, the cerebral cortex, the hippocampal and dentate neuronal layer and the cerebellar Purkinje and granule cells. The zebrafish loss of function model for the *CDIPT* gene shows defective in brain morphology.	*CDIPT* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Saito *et al.*, 1998 Shinawi *et al.*, 2010 Weiss *et al.*, 2008

Tab. 4.1. Continued.

***CORO1A*(+)** CNS immunosurveillance, neurodevelopment: actin cytoskeleton dynamics	This gene encodes the coronin actin binding protein 1A, which is a member of the WD repeat protein family. Members of this family are involved in a variety of cellular processes, including cell cycle progression, signal transduction, apoptosis, and gene regulation. It is a crucial component of the cytoskeleton of highly motile cells, functioning both in the invagination of large pieces of plasma membrane, as well as in forming protrusions of the plasma membrane involved in cell locomotion. Good expression in fetal brain and very high expression in postnatal whole brain, in particular in cerebellum and amygdalae. Very high expression in thymus, bone marrow and immune cell types.	Coronins are a highly conserved family of actin regulatory genes. They regulate the actin cytoskeleton through antagonizing actin polymerization and promoting actin severing. Mice with mutations in *Coronin-1A*, expressed predominantly in hematopoeitic cells, are T-lymphocytopenic in part due to inability of mature T cells to be released from the thymus into the peripheral circulation, suggesting the role of actin cytoskeleton regulation in T cell homeostasis. In the CNS coronin 1A is predominantly expressed in microglial cells. The zebrafish loss of function model for the *CORO1A* gene shows defective in the neural tube formation. Recently, a patient carrying a heterozygous microdeletion at 16p11.2, affected by ADHD and a severe combined immunodeficiency, has been reported. The deletion encompassed the *CORO1A* gene and, in addition, a deletion of 2bp on the *CORO1A* allele, inherited from the healthy father, was found.	*CORO1A* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al*., 2012 Bijlsma *et al*., 2009 Christian *et al*., 2008 Fernandez *et al*., 2010 Foger *et al*., 2006 Hanson *et al*., 2010 Kumar *et al*., 2008 Marshall *et al*., 2008 Mochida *et al*., 1998 Pinto *et al*., 2010 Rosenfeld *et al*., 2010 Shinawi *et al*., 2010 Shiow *et al*., 2008, 2009 Uetrecht and Bear, 2006 Weiss *et al*., 2008
***C16orf53*(+)** Chromatin remodeling	This gene encodes the chromosome 16 open reading frame 53, which is a component of a Set1-like multiprotein histone methyltransferase complex. Good expression in fetal brain and in postnatal CNS, in particular in amygdalae and cerebellum.	The zebrafish loss of function model for *C16orf53* shows defective in brain morphology.	*C16orf53* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al*., 2012 Bijlsma *et al*., 2009 Cho *et al*., 2007 Christian *et al*., 2008 Fernandez *et al*., 2010 Hanson *et al*., 2010 Kumar *et al*., 2008 Marshall *et al*., 2008 Mochida *et al*., 1998 Pinto *et al*., 2010 Rosenfeld *et al*., 2010 Shinawi *et al*., 2010 Weiss *et al*., 2008
***DOC2A*(+)** Synaptic function and plasticity	This gene encodes the double C2-like domains, alpha protein. DOC2A is mainly expressed in brain and is suggested to be involved in Ca^{2+}-dependent neurotransmitter release through the interaction with UNC13B. It most probably regulates fusion of vesicles with membranes. Very high expression in fetal brain and in postnatal CNS, in particular in cortex, amygdalae, and cerebellum.	It has been reported that DOC2A-UNC13B interaction *in vitro* plays a role in a step before the final fusion of synaptic vesicles with the presynaptic plasma membrane in the evoked neurotransmitter release process. Furthermore, DOC2A binds to STXBP1, the syntaxin (STX) binding protein 1, thus regulating the interaction STXBP1-STX which is essential for the activity of the synaptic vesicle fusion machinery. *Doc2a* knockout mice exhibit defects in excitatory synaptic transmission and long-term potentiation, whereas knockdown of *doc2a* in zebrafish resulted in defective brain morphology, but apparently normal motor responses and axon tracts. Because the mice were studied at later stages, similar phenotypes could develop in older fish.	*DOC2A* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee et al., 2012 Bijlsma et al., 2009 Christian et al., 2008 Fernandez et al., 2010 Hanson *et al*., 2010 Kumar *et al*., 2008 Marshall *et al*., 2008 Mochida *et al*., 1998 Pinto *et al*., 2010 Rosenfeld *et al*., 2010 Sakaguchi *et al*., 1999 Shinawi *et al*., 2010 Weiss *et al*., 2008

Tab. 4.1. Continued.

***FAM57B*(+)** Neurodevelopment	This gene encodes the family with sequence similarity 57 protein. Good expression in fetal brain and in postnatal CNS.	The zebrafish loss of function model for *FAM57B* shows defective in brain morphology.	*FAM57BA* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***GDPD3*(+)** Neurodevelopment	This gene encodes the glycerophosphodiester phosphodiesterase domain containing 3 protein. Moderate expression in postnatal CNS.	The zebrafish loss of function model for *GDPD3* shows defective in brain morphology.	*GDPD3* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***HIRIP3*(+)** Neurodevelopment: histone metabolism	This gene encodes the HIRA interacting protein 3, which shares sequence similarity with Hir1p and Hir2p, the two corepressors of histone gene transcription characterized in the yeast, Saccharomyces cerevisiae. The structural features of the HIRA protein suggest that it may function as part of a multiprotein complex, probably involved in some aspects of chromatin and histone metabolism. Low expression in fetal brain and in postnatal CNS, except for amygdalae, cerebellum and thalamus where good levels of expression have been detected.	The zebrafish loss of function model for *HIRIP3* shows defective in brain morphology.	*HIRIP3*maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***INO80E*(+)** Chromatin remodeling, transcriptional regulation	This gene encodes the INO80 complex subunit E protein, which is a putative regulatory component of the chromatin remodeling INO80 complex that is involved in transcriptional regulation, DNA replication and probably DNA repair. Moderate expression in fetal brain and in postnatal whole brain.	The zebrafish loss of function model for *INO80E* shows very strong phenotype, suggesting early embryonic defects. In particular, it shows abnormal body length and defective neural tubes, resulting in anomalies in brain morphology.	*INO80E* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008

Tab. 4.1. Continued.

★ ***KCTD13*(+)** Neurodevelopment: actin cytoskeleton dynamics	This gene encodes the potassium channel tetramerisation domain containing 13 protein, which is the substrate-specific adapter of a BCR E3 ubiquitin-protein ligase complex involved in regulation of cytoskeleton structure. The BCRE3 ubiquitin ligase complex mediates the ubiquitination of RHOA, leading to its degradation by the proteasome, thereby regulating the actin cytoskeleton and cell migration. High expression in fetal brain and in postnatal CNS. High expression in immune cell types.	Overexpression of 16p11.2 human transcript in zebrafish embryos identified *KCTD13* as the sole message capable of inducing the microcephaly phenotype associated with the 16p11.2 duplication, whereas suppression of the same locus yielded the macrocephalic phenotype associated with the 16p11.2 deletion. Analyses of zebrafish and mouse embryos suggest that microcephaly is caused by decreased proliferation of neuronal progenitors with concomitant increase in apoptosis in the developing brain, whereas macrocephaly arises by increased proliferation and no changes in apoptosis.	*KCTD13* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD. A role for KCTD13 dosage changes is consistent with autism in both a recently reported family with a reduced 16p11.2 deletion, encompassing five genes such as *MVP*, *CDIPT*, *SEZ6L2*, *ASPHD*1 and *KCTD1*3, and a subject with a complex 16p11.2 rearrangement involving *de novo* structural alteration of *KCTD13*.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Crepel *et al.*, 2011 Fernandez *et al.*, 2010 Golzio *et al.*, 2012 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***KIF22*(+)** Intracellular membrane trafficking: regulation of microtubule cytoskeleton dynamics	This gene encodes the kinesin family member 22 protein, which is a member of the kinesin-like protein family. They are microtubule-dependent molecular motors that transport organelles within cells and move chromosomes during cell division. Studies with the Xenopus homolog suggest its essential role in metaphase chromosome alignment and maintenance. Moderate expression in fetal brain and in postnatal CNS.	In zebrafish loss of function model *KIF22* is required for nervous system development, impacting brain morphology, eye development, axonal density or organization, and motor response. *KIF22* was identified as giving a clear phenotype when RNA level was reduced by ~50%, suggesting that this gene is a deletion dosage sensor.	*KIF22* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***MAPK3-ERK1*(+)** Intracellular MAP kinase signaling	This gene encodes the mitogen-activated protein kinase 3 (ERK1), which is a member of the MAP kinase family. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act in a signaling cascade that regulates various cellular processes such as proliferation, differentiation, and cell cycle progression in response to a variety of extracellular signals. Good expression in fetal brain and in postnatal CNS.	MAPK3 interacts with PTPN11, the protein tyrosine phosphatase, non-receptor type 11, which has a role in signal transduction. The zebrafish loss of function model for *MAPK3* shows very strong phenotype, suggesting early embryonic defects. In particular, it shows abnormal body length and defective neural tubes, resulting in anomalies in brain morphology.	*MAPK3* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD. Rare single missense mutations affecting *MAPK3* have been reported in a few HF-AU patients. Up-regulation of the Ras/Raf/ERK1/2 signaling pathway has been found in the brain of autistic subjects and mouse animal model. Mutations affecting *PTPN11* cause Noonan syndrome which is comorbid with ASD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Ghaziuddin *et al.*, 1994 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Paul *et al.*, 1983 Pierpont *et al.*, 2009 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Schaaf *et al.*, 2011 Shinawi *et al.*, 2010 Swillen *et al.*, 1996 Weiss *et al.*, 2008 Yang *et al.*, 2011, 2012 Zou *et al.*, 2011

Tab. 4.1. Continued.

***MAZ*(+)** Neurodevelopment	This gene encodes the MYC-associated zinc finger protein, a purine-binding transcription factor. Moderate expression in fetal brain and in postnatal CNS.	It has been demonstrated that MAZ mediates enhancement of NMDA receptor subunit type 1 (NR1) promoter activity during neuronal differentiation. Furthermore, MAZ binds to DDC (deleted in colorectal carcinoma), which is a receptor for netrin required for axon guidance. DDC mediates axon attraction of neuronal growth cones in the developing nervous system upon ligand binding. In zebrafish loss of function model *MAZ* is required for brain development.	*MAZ* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD. Significantly differential *MAZ* gene expression in relation to age between a group of patients with SCZ and controls has been reported in peripheral blood lymphocytes.	Bataller *et al.*, 2003 Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Bowden *et al.*, 2006 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Okamoto *et al.*, 2002 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***MVP*(+)** Intracellular signaling: regulation of MAP kinase signaling	This gene encodes the major vault protein, which is the major component of the vault complex. Vaults are multi-subunit ribonucleoprotein structures that may be involved in nucleo-cytoplasmic transport. The encoded protein may play a role in multiple cellular processes by regulating the MAP kinase, JAK/STAT and phosphoinositide 3-kinase/Akt signaling pathways. Low expression in fetal brain and in postnatal CNS.	Recently, the cellular and subcellular expression of MVP in primate and rodent cerebral cortex, and in cortical neurons *in vitro* has been described. In prefrontal, somatosensory and hippocampal cortices, MVP was predominantly expressed in pyramidal neurons. Axons and particularly principal dendrites expressed MVP along individual microtubules, and in pre- and postsynaptic structures. Colocalization with microtubule-associated protein-2, tubulin, tau, and phalloidin has been observed in neurites and growth cones in culture. Immunoprecipitation coupled with reverse transcription PCR showed that MVP associates with mRNAs that are known to be translated in response to synaptic activity. In zebrafish loss of function model *MVP* is required for brain development.	*MVP* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Paspalas *et al.*, 2009 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***QPRT*(+)** Tryptophan metabolism	This gene encodes the quinolinate phosphoribosyltransferase, which is a key enzyme in catabolism of quinolinate, an intermediate in the tryptophan-nicotinamide adenine dinucleotide pathway. Quinolinate acts as a most potent endogenous exitotoxin to neurons. Low expression in postnatal CNS, in particular in amygdalae and thalamus. High expression in dendritic cells, NK-cells and monocytes.	QPRT converts quinolic acid (QUIN) to nicotinic acid ribonucleotide, a precursor in the synthesis of NAD^+, and carbon dioxide in the presence of Mg^{2+} and 5-phosphoribosyl-1-pyrophosphate. In the brain, QPRT is one of the rate-limiting enzymes of NAD^+ synthesis from Tryptophane, and therefore likely to influence QUIN levels in the CNS. Thus, QPRT activity is essential for the maintenance of cellular energy metabolism and DNA repair. A reduction in QPRT activity can be envisioned to lead to an accumulation of QUIN, and likely to induce a cytotoxic cascade within astrocytes and neurons. The zebrafish homolog of human *QPRT* is not known.	*QPRT* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD. One possible explanation for ASD pathogenesis is the modern theory of immunoexcitotoxicity (see the *ACMSD* gene).	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Braidy *et al.*, 2011 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Schwarcz *et al.*, 2012 Shinawi *et al.*, 2010 Weiss *et al.*, 2008

Tab. 4.1. Continued.

★ ***PPP4C(+)*** Neurodevelopment: microtubule cytoskeleton dynamics	This gene encodes the protein phosphatase 4, which is involved in many processes such as microtubule organization at centrosomes, maturation of spliceosomal snRNPs, apoptosis, DNA repair, tumor necrosis factor (TNF)-alpha signaling, activation of c-Jun N-terminal kinase MAPK8, regulation of histone acetylation, DNA damage checkpoint signaling, NF-kappa-B activation and cell migration. Moderate expression in fetal brain and in postnatal CNS. Very high expression in immune cell types.	It has been recently reported that PPP4R2, a regulatory subunit of the protein phosphatase 4 (PPP4C), displays a very dynamic intracellular localization in mouse and rat neuronal cell lines and in rat primary hippocampal neurons, strongly correlating with differentiation. Furthermore, in zebrafish loss of function model *PPP4C* is required for brain development.	*PPP4C* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD. NF-*κ*B is an important gene transcriptional factor that mediates cellular responses in inflammation, immunity, development, cell proliferation and apoptosis. Elevated levels of NF-κB have been reported in autistic patients vs. controls.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Bosio *et al.*, 2012 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Naik *et al.*, 2011 Philippe *et al.*, 2012 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
PRRT2(+) Neurodevelopment	This gene encodes the proline-rich transmembrane protein 2, which contains a proline-rich domain in its N-terminal half. Studies in mice suggest that it is predominantly expressed in brain and spinal cord in embryonic and postnatal stages. Good expression in fetal brain and in postnatal CNS. High expression in cerebellum.	Mutations in *PRRT2* are associated with paroxysmal kinesigenic dyskinesia (PKD), infantile convulsions with choreoathetosis (PKD with infantile seizures), and benign familial infantile seizures. There is a limited amount of research regarding the function of PRRT2; however, some indirect evidence suggested its role in the pathogenesis of PKD. First, PRRT2 was found to be mainly expressed in the basal ganglia, a brain area possibly involved in the PKD pathogenesis. Secondly, SNAP25, an interactive protein of PRRT2, is also expressed in the brain, especially the basal ganglia, participates in the regulation of neurotransmitter release and is involved in ADHD onset. No phenotype has been observed in the zebrafish loss of function model for *PRRT2* which is not expressed until the 48 hours of life.	*PRRT2* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Graham *et al.*, 2002 Hamano *et al.*,.1995 Hanson *et al.*, 2010 Hayashi *et al.*, 1997 Iwasaki *et al.*, 2004 Ko *et al.*, 2001 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Shirane *et al.*, 2001 Stelzl *et al.*, 2005 Volonte *et al.*, 2001 Wang *et al.*, 2011 Weiss *et al.*, 2008
SEZ6L2(+) Neurodevelopment	This gene encodes the seizure related 6 homolog (mouse)-like 2 protein, which is localized on the cell surface. Increased expression of this gene has been found in lung cancers, and the protein is therefore considered to be a novel prognostic marker for lung cancer. Very high expression in fetal brain and in postnatal CNS.	*In situ* analyses in whole mouse embryos have demonstrate that *Sez6l2* mRNA is expressed in the developing brain and spinal cord whereas in human fetal brain sections *SEZ6L2* is enriched in the cortical plate in the post mitotic neuron, in the ventricular zone, in the hippocampus, thalamus, ganglionic eminence, basal ganglia, and amygdalae and at lower levels in the pons and the putamen. The zebrafish loss of function model for the *SEZ6L2* gene shows a weak phenotype with defective in brain morphology.	*SEZ6L2* maps in the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity ASD, EP, SCZ, ID and ADHD. A coding variant in *SEZ6L2* has been significantly associated with ASD (datum not replicated).	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Konyukh *et al.*, 2011 Kumar *et al.*, 2008, 2009 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008

Tab. 4.1. Continued.

***SPN*(+)** T-cell activation	This gene encodes the sialophorin protein, which is a major sialoglycoprotein found on the surface of thymocytes, T lymphocytes, monocytes, granulocytes, and some B lymphocytes. Moderate expression in fetal brain and low expression in postnatale CNS, except for amygdalae and hypothalamus where expression is moderate. High expression in immune cell types.	SPN is part of a physiologic ligand-receptor complex involved in T-cell activation. During T-cell activation, this protein is actively removed from the T-cell-APC (antigen-presenting cell) contact site, suggesting a negative role in adaptive immune response.	*SPN* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
★ ***SULT1A3*(+)** CNS metabolism	This gene encodes the sulfotransferase family cytosolic 1A, phenol-preferring member 3 protein. Sulfotransferase enzymes catalyze the sulfate conjugation of many hormones, neurotransmitters, drugs, and xenobiotic compounds. Good expression in fetal brain and moderate expression in postnatal CNS. High expression in immune cell types.	SULT1A3 sulfates catecholamine neurotransmitters and its expression is highest in cytosol from superior temporal gyrus, hippocampus, and temporal lobe. SULT1A3 has been found in both neurons and glial cells.	*SULT1A3* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Salman *et al.*, 2009 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***TAOK2*(+)** Intracellular signaling: dendrite morphogenesis	This gene encodes the TAO kinase 2, which is a serine/threonine protein kinase that is involved in many different processes, including cell signaling, microtubule organization and stability, and apoptosis. Moderate expression in fetal brain and in postnatal CNS.	Very recently, it has been demonstrated that TAOK2 is essential for dendrite morphogenesis. TAOK2 downregulation impairs basal dendrite formation *in vivo* without affecting apical dendrites. Moreover, TAOK2 interacts with Neuropilin 1, a receptor protein that binds the secreted guidance cue Semaphorin 3A. Finally, Sema3A and TAOK2 modulate the formation of basal dendrites through the activation of the c-Jun N-terminal kinase (JNK). In zebrafish loss of function model *TAOK2* is required for brain development.	*TAOK2* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 de Anda *et al.*, 2012 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008
***YPEL3*(+)** Neurodevelopment	This gene encoder the yippee-like 3 protein. Good expression in fetal brain and in postnatal CNS.	YPEL3, a member of a recently discovered family of putative zinc finger motif coding genes consisting of YPEL1-5, is a p53-regulated gene. YPEL3 expression induced by DNA damage leads to p53 recruitment to a cis-acting DNA response element located near the human YPEL3 promoter. Physiologic induction of YPEL3 results in a substantial decrease in cell viability associated with an increase in cellular senescence. In zebrafish loss of function model *YPEL3* is required for brain development.	*YPEL3* maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.	Blaker-Lee *et al.*, 2012 Bijlsma *et al.*, 2009 Christian *et al.*, 2008 Fernandez *et al.*, 2010 Hanson *et al.*, 2010 Kelley *et al.*, 2010 Kumar *et al.*, 2008 Marshall *et al.*, 2008 Mochida *et al.*, 1998 Pinto *et al.*, 2010 Rosenfeld *et al.*, 2010 Shinawi *et al.*, 2010 Weiss *et al.*, 2008

Tab. 4.1. Continued.

- **Patient 27, gain of 2.6 Mb at 22q11.21 (chr22:18894835-21505417)**
- **Patient 32, loss of 120 kb at 22q11.21 (chr22:18890271-19010508)**

AIFM3*(+)** ***de novo Neurodevelopment	This nuclear gene encodes the apoptosis-inducing factor, mitochondrion-associated 3 protein. Moderate expression in fetal brain and in postnatal CNS.	Apoptosis-inducing factor (AIF) is implicated in caspase-independent apoptotic-like death. AIF released from mitochondria translocates to the nucleus, where it mediates some apoptotic events such as chromatin condensation and DNA degradation. During cerebellar development, a significant increase in the number of neurons with nuclear AIF localization has been reported in an age-dependent manner, suggesting the idea that AIF could be involved in apoptotic-like death of cerebellar granule neurons and that it could be an alternative mechanism of neuronal death during cerebellar development.	*AIFM3* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Blancas and Moran, 2011 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
ARVCF*(+)** ***de novo Neurodevelopment	This gene encodes the armadillo repeat gene deleted in velocardiofacial syndrome, which is a member of the catenin family. This family plays an important role in the formation of adherens junction complexes, which are thought to facilitate communication between the inside and outside environments of a cell. ARVCF belongs to the beta-catenin family. Good expression in fetal brain and in postnatal CNS.	It has been demonstrated by haplotype analyses performed on a 22q11.2 region including the *TXNRD2*, *COMT* and *ARVCF* genes, that a particular set of SNPs are over-transmitted in individuals with SCZ, a disorder characterized by specific cognitive impairments. Furthermore, the developmental impact of over-expression of an ~190 kb segment of human 22q11.2, which includes *TXNRD2*, *COMT* and *ARVCF*, on behaviors in bacterial artificial chromosome transgenic mice vs. wild-type mice has been determined. The collected data suggest that over-expression of this 22q11.2 segment enhances incentive learning and impairs the prolonged maintenance of working memory, but has no apparent effect on working memory per se, affect- and stress-related behaviors or motor capacity.	*ARVCF* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Chung *et al.*, 2011 De Ferrari and Moon, 2006 Fine *et al.*, 2005 Liu and Murray, 2012 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mas *et al.*, 2009 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Okerlund and Cheyette, 2011 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Sanders *et al.*, 2005 Sim *et al.*, 2012 Suzuki *et al.*, 2009 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006 Wang *et al.*, 2010 Zhang *et al.*, 2012
CDC45*(+)** ***de novo DNA replication	This gene encodes the cell division cycle 45 homolog (S. cerevisiae), which is an essential protein required to the initiation of DNA replication. Cdc45 is a member of the highly conserved multiprotein complex including Cdc6/Cdc18, the minichromosome maintenance proteins (MCMs) and DNA polymerase, which is important for early steps of DNA replication in eukaryotes. Expressed during neurogenesis.	It has been demonstrated that diminished dosage of the genes deleted in the 1.5 Mb 22q11 minimal critical deleted region, including *CDC45*, in a mouse model of 22q11 DiGeorge syndrome specifically compromises neurogenesis and subsequent differentiation in the cerebral cortex. In particular, CDC45 is expressed in the cortical ventricular and subventricular zones with highest level of expression during neurogenesis.	*CDC45* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Meechan *et al.*, 2009 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

CLDN5*(+)** ***de novo CNS immunosurveillance: brain blood barrier permeability	This gene encodes the claudin 5 protein. Claudins are integral membrane proteins and components of tight junction strands. Tight junction strands serve as a physical barrier to prevent solutes and water from passing freely through the paracellular space between epithelial or endothelial cell sheets. Moderate expression in fetal brain and good expression in postnatal CNS.	Claudin-5 is a key tight junction protein whose expression in the brain endothelial cells is critical to the function of brain blood barrier. In mouse model it has been recently demonstrated a regulation of CLDN5 expression by Tnf-α. Diminished levels of CLDN5 with tight junction structure alterations have been found in nueroinflammatory disease model. Mutations in this gene have been found in patients with velocardiofacial syndrome.	*CLDN5* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. A SNP in *CLDN5* has been associated with SCZ.	Antshel *et al.*, 2007 Aslam *et al.*, 2012 Bucan *et al.*, 2009 Fine *et al.*, 2005 Ishiguro *et al.*, 2008 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Sun *et al.*, 2004 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006 Wu *et al.*, 2010
CLTCL1*(+)** ***de novo Intracellular trafficking	This gene encodes the clathrin, heavy chain-like 1 protein, which is a member of the clathrin heavy chain family and encodes a major protein of the polyhedral coat of coated pits and vesicles. Moderate expression in fetal brain and in postnatal CNS. Good expression in amygdalae and thalamus.	*CLTCL1* encodes a member of the clathrin heavy chain family, which is involved in intracellular trafficking, that are important to glutamate receptor turnover. Chromosomal aberrations involving this gene are associated with meningioma, DiGeorge syndrome (DGS), and velo-cardio-facial syndrome (VCFS). In particular, a patient with features of DGS/VCFS, who carry a balanced (21;22)(p12;q11) translocation that interrupts only the *CLTCL1* gene, has been reported.	*CLTCL1* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. Recently, whole-exome sequencing of 16 autistic probands revealed validated homozygous, potentially pathogenic recessive mutations affecting candidate genes such as *UBE3B*, *CLTCL1*, *NCKAP5L*, and *ZNF18*, that segregated perfectly with the disease in 4/16 families. Moreover, it has been demonstrated that the mouse homologs of these four genes are upregulated in response to neuronal activity.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Chahrour *et al.*, 2012 Fine *et al.*, 2005 Holmes *et al.*, 1997 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
COMT*(+)** ***de novo Catecholamine metabolism	This gene encodes the catechol-O-methyltransferase protein, which catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, including the neurotransmitters dopamine, epinephrine, and norepinephrine. This O-methylation results in one of the major degradative pathways of the catecholamine transmitters. Low expression in fetal brain and moderate expression in postnatal whole brain, in particular in caudate nucleus, corpus callosum, thalamus, and hypothalamus.	The methylation of dopamine by COMT is an important mechanism for dopamine inactivation and dopaminergic tone in the CNS. Although COMT is expressed widely throughout the brain, it appears to play a particularly important role in dopamine flux in the prefrontal cortex. In the cortex, the dopamine transporter, which has a 1,000-fold higher affinity for dopamine than does COMT, is expressed at very low levels and does not appear to affect extracellular dopamine levels. Thus, inactivation of dopamine in the prefrontal cortex appears to rely preferentially on catabolic enzymes, including COMT. Different polymorphisms of *COMT* have been associated over the years with many neuropsychiatric disorders such as BD, anorexia nervosa, obsessive-compulsive disorders, and aggressive behaviour in SCZ.	*COMT* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. A SNP affecting *COMT* has been associated with ASD and probably correlates with a more aggressive phenotype.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Chen *et al.*, 2004 Fine *et al.*, 2005 Frisch *et al.*, 2001 Garris *et al.*, 1993 James *et al.*, 2006 Jones *et al.*, 2001 Li *et al.*, 1997 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Nieoullon, 2002 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Schindler *et al.*, 2000 Suzuki *et al.*, 2009 Szatmari *et al.*, 2007 Tunbridge *et al.*, 2006 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

CRKL*(+)** ***de novo Intracellular signaling	This gene encodes the v-crk sarcoma virus CT10 oncogene homolog (avian)-like, which is a protein kinase containing SH2 and SH3 domains that activates the RAS and JUN kinase signaling pathways and transforms fibroblasts in a RAS-dependent fashion. It is a substrate of the BCR-ABL tyrosine kinase, plays a role in fibroblast transformation by BCR-ABL, and may be oncogenic. Moderate expression in fetal brain and in postnatal cerebellum and thalamus.	The Crk and Crk-like (CrkL) adaptor proteins play important roles in numerous signaling pathways, bridging tyrosine kinase substrates to downstream signaling effectors by virtue of their phosphotyrosine-binding SH2 domains and their effector-binding SH3 domains. Crk and CrkL are known biochemically and genetically to be essential mediators of Reelin/Disabled-1 (Dab1) signaling, which governs proper mammalian brain development. Multimeric Reelin clusters its receptors as well as the receptor-bound intracellular scaffolding protein Dab1. Recently, 101 CrkL-SH3 binding proteins have been identified from embryonic murine brain. The identified proteins are enriched in the Crk/CrkL-SH3 binding motif and signaling activities regulating cell adhesion and motility. An atypical 0.8 Mb inherited duplication of 22q11.2, which encompasses 14 genes including *CRKL*, *ZNF74*, *PIK4CA*, *SNAP29* and *PCQAP* known to contribute to several aspects of the DGS/VCFS phenotype, has been recently described in a patient with psychomotor impairment, suggesting a role for these genes in neurodevelopment.	*CRKL* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Cheerathodi and Ballif, 2011 Fine *et al.*, 2005 Holmes *et al.*, 1997 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pebrel-Richard *et al.*, 2012 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
DGCR2*(+)** ***de novo Neurodevelopment	This gene encodes the DiGeorge syndrome critical region gene 2 protein, which is a novel putative adhesion receptor protein that could play a role in neural crest cell differentiation and migration. Moderate expression in fetal brain and in postnatal CNS.	DGCR2 could be involved in cell-cell or cell-matrix interactions required for normal cell differentiation and migration.	*DGCR2* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. The association between *DGCR2* and SCZ has been demonstrated through individual genotyping of 1,400 subjects. In a subsequnt gene expression analysis the risk allele of a coding SNP associated with SCZ was found to be associated with a reduced expression of *DGCR2*. This datum was not later replicated in a German sample. A *de novo* potentially disruptive mutation in *DGCR2* has been recently detected by exome sequencing analysis in a cohort of SCZ patient.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Georgi *et al.*, 2009 Ishiguro *et al.*, 2008 Liu *et al.*, 2007 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006 Xu *et al.*, 2011

Tab. 4.1. Continued.

***DGCR6*(-)*de novo** ***DGCR6*(-)** Synaptogenesis and synaptic plasticity	This gene encodes the DiGeorge syndrome critical region protein 6, which shares homology with the Drosophila melanogaster gonadal protein that participates in gonadal and germ cell development, and with the gamma-1 subunit of human laminin. This gene is a candidate for involvement in DiGeorge syndrome pathology and in SCZ. DGCR6 may play a role in neural crest cell migration into the third and fourth pharyngeal pouches. Low expression in fetal brain and high expression in postnatal CNS.	Recently, it has been demonstrated a role for DGCR6 in GABAB-receptor localization, which mediate slow inhibitory effects of the neurotransmitter gamma-aminobutyric acid (GABA) on synaptic transmission in the CNS.	*DGCR6* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. CNVs (loss) exclusively affecting *PRODH* and *DGCR6* and parentally inherited have been significantly associated with ASD. A significant relationship between the increased frequency of anxiety disorders and low *DGCR6* and *DGCR6L* expression has been recently reported in children with 22q11 DiGeorge syndrome.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Das Chakraborty *et al.*, 2012 Fine *et al.*, 2005 Guilmatre *et al.*, 2009 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Meechan *et al.*, 2009 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006 Zunner *et al.*, 2010
DGCR6L*(+)** ***de novo Synaptogenesis and synaptic plasticity?	This gene encodes the DiGeorge syndrome critical region gene 6-like protein. This gene, the result of a duplication at this locus, is one of two functional genes encoding nearly identical proteins that have similar expression patterns. The product of this gene is a protein that shares homology with the Drosophila gonadal protein, expressed in gonadal tissues and germ cells, and with the human laminin gamma-1 chain that functions in cell attachment and migration. DGCR6L may play a role in neural crest cell migration into the third and fourth pharyngeal pouches. Low expression in fetal brain and high expression in postnatal CNS, except for corpus callosum and spinal cord where the expression is low.		*DGCR6L* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. A significant relationship between the increased frequency of anxiety disorders and low *DGCR6* and *DGCR6L* expression has been recently reported in children with 22q11 DiGeorge syndrome.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Das Chakraborty *et al.*, 2012 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

DGCR8*(+)** ***de novo MicroRNA biogenesis	This gene encodes the DiGeorge syndrome critical region gene 8 protein, which is a subunit of the microprocessor complex which mediates the biogenesis of microRNAs from the primary microRNA transcript. This protein is required for binding the double-stranded RNA substrate and facilitates cleavage of the RNA by the ribonuclease III protein, Drosha. Good expression in fetal brain and moderate expression in postnatal CNS. Good expression in immune cell types.	It has been reported that *Dgcr8*+/- mice display reduced expression of a subset of microRNAs in the prefrontal cortex, a deficit that emerges over postnatal development. Layer V pyramidal neurons in the medial prefrontal cortex of *Dgcr8*+/- mice have altered electrical properties, decreased complexity of basal dendrites, and reduced excitatory synaptic transmission. These findings demonstrate that precise microRNA expression is critical for the postnatal development of prefrontal cortical circuitry. Similar defects in neuronal maturation resulting from microRNA deficiency could represent endophenotypes of certain neuropsychiatric diseases of developmental onset. Furthermore, mouse model of 22q11DS displays an age-dependent increase in hippocampal long-term potentiation (LTP), a form of synaptic plasticity underlying learning and memory. The sarco(endo)plasmic reticulum Ca(2+) ATPase (SERCA2), which is responsible for loading Ca(2+) into the endoplasmic reticulum (ER), is elevated in this mouse model. Screening of multiple mutant mouse lines revealed that haploinsufficiency of *Dgcr8* causes age-dependent, synaptic SERCA2 overexpression and increased LTP. Finally, SERCA2 is elevated in the brains of patients with SCZ, providing a link between mouse model findings and the human disease.	*DGCR8* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Earls *et al.*, 2012 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Schofield *et al.*, 2011 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
DGCR14*(+)** ***de novo Transcript maturation	This gene encodes the DiGeorge syndrome critical region gene 14 (DGCR14) protein, which may be a component of C complex spliceosomes. The orthologous protein in the mouse localizes to the nucleus. Moderate expression in fetal brain and in postnatal CNS, in particular in cortex, caudate nucleus and cerebellum.	DGCR14 is possibly involved pre-mRNA splicing. It has been demonstrated that the Df(16)A+/− mice which carry a microdeletion including the *Gscl* and *Dgcr14* genes, both located within the DiGeroge critical region, showed reduced synchrony of hippocampal theta with the neuronal activity of the prefrontal cortex. Moreover, it has been recently reported that loss of *Gscl* and *Dgcr14* affects the regulation of hippocampal theta and REM sleep, possibly contributing to the psychiatric symptoms frequently seen in patients who have 22q11 syndrome.	*DGCR14* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. Evidence of association between promoter polymorphisms in 22q11 gene *DGCR14* and SCZ have been detected by transmission disequilibrium test.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Funato *et al.*, 2010 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Sigurdsson *et al.*, 2010 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006 Wang *et al.*, 2006

Tab. 4.1. Continued.

GNB1L*(+)** ***de novo Intracellular signaling	This gene encodes the guanine nucleotide binding protein (G protein), beta polypeptide 1-like, which is a member of the WD repeat protein family. WD repeats are minimally conserved regions of approximately 40 amino acids typically bracketed by gly-his and trp-asp (GH-WD), which may facilitate formation of heterotrimeric or multiprotein complexes. Members of this family are involved in a variety of cellular processes, including cell cycle progression, signal transduction, apoptosis, and gene regulation. Detected at high levels in fetal brain and at low levels in postnatal CNS.	GNB1L shows homology to the human guanine nucleotide–binding protein b subunit (GNB1). GNB1 functions in G-protein–coupled receptor protein signaling pathways and intracellular signaling cascade. Recently, significant evidence for association between SCZ and *GNB1L* have been reported in a case–control association study. This observation, combined with the findings of reduced expression of *GNB1L* in postmortem brains of schizophrenics and the effect of heterozygous deletion of *Gnbl1* on prepulse inhibition, a SCZ endophenotype, in a mouse model, suggest that *GNB1L* is associated with SCZ phenotype observed in del22q11.2.	*GNB1L* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. A patient with ASD and SCZ, who carry a balanced translocation involving the 22q11.2 region and interrupting *GNB1L*, has been recently reported. Furthermore, private *GNB1L* missense variants in conserved residues, that affect residues in the WD40 repeat domains and are predicted to have deleterious effects on the protein, have been identified in three autistic families, thus supporting involvement of *GNB1L* in autism spectrum disorders as well.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Chen *et al.*, 2012 Fine *et al.*, 2005 Ishiguro *et al.*, 2010 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Meechan *et al.*, 2009 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Paylor *et al.*, 2006 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006 Williams *et al.*, 2008
★ ***GP1BB*(+)** ***de novo*** Neurodevelopment	This gene encodes the glycoprotein Ib (platelet), beta polypeptide, which is a heterodimeric transmembrane protein consisting of a disulfide-linked 140 kD alpha chain and 22 kD beta chain. It is part of the GPIb-V-IX system that constitutes the receptor for von Willebrand factor (VWF), and mediates platelet adhesion in the arterial circulation. GPIb alpha chain provides the VWF binding site, and GPIb beta contributes to surface expression of the receptor and participates in transmembrane signaling through phosphorylation of its intracellular domain. Very high expression in fetal brain and in postnatal CNS. Very high expression in whole blood, dendritic cells and monocytes.	Mutations in the *GPIBB* gene have been associated with Bernard-Soulier syndrome (BSS), velocardiofacial syndrome and giant platelet disorder. In particular, it has been recently reported on a four-year-old boy with a homozygous deletion comprising the *GP1BB* and *SEPT5* genes, located 5' to GP1BB. He presented with BSS, cortical dysplasia (polymicrogyria), developmental delay, and platelet secretion defect, thus supporting the possible effect of defect of these two genes in neurodevelopment.	*GP1BB* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. Both *GP1BB* and *SEPT5* have been previously suggested as autism candidate genes by molecular cytogenetic analysis and *in silico* studies.	Antshel *et al.*, 2007 Bartsch *et al.*, 2011 Bucan *et al.*, 2009 Fine *et al.*, 2005 Iurov *et al.*, 2010 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
GSC2(GSCL)*(+)** ***de novo Transcriptional regulation	This gene encodes the goosecoid homeobox 2 protein. Goosecoidlike (GSCL), a homeodomain-containing gene, resides in the critical region for VCFS/DGS on 22q11 and the encoded protein has a possible role in embryonic development as transcriptional regulator. Expressed in a limited number of adult tissues, including cerebral temporal and parietal lobes, as well as in early human development.	It has been demonstrated that the Df(16)A+/− mice which carry a microdeletion including the *Gscl* and *Dgcr14* genes, both located within the DiGeroge critical region, showed reduced synchrony of hippocampal theta with the neuronal activity of the prefrontal cortex. Moreover, it has been recently reported that loss of *Gscl* and *Dgcr14* affects the regulation of hippocampal theta and REM sleep, possibly contributing to the psychiatric symptoms frequently seen in patients who have 22q11 syndrome.	*GSCL* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Funato *et al.*, 2010 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

★ ***HIRA*(+)** ***de novo*** Embryonic development	This gene encodes the HIR histone cell cycle regulation defective homolog A (S. cerevisiae), which is a histone chaperone that preferentially places the variant histone H3.3 in nucleosomes. Orthologs of this gene in yeast, flies, and plants are necessary for the formation of transcriptionally silent heterochomatin. This gene plays an important role in the formation of the senescence-associated heterochromatin foci. These foci likely mediate the irreversible cell cycle changes that occur in senescent cells. It is considered the primary candidate gene in some haploinsufficiency syndromes such as DiGeorge syndrome, and insufficient production of the gene may disrupt normal embryonic development. Good expression in fetal brain and in postnatal caudate nucleus, amygdalae, and thalamus. High expression in immune cell types.	HIR/HIRA, one of the histone chaperones, was initially identified in yeast as negative regulators of histone gene expression. It has been confirmed that HIRA contains a conserved family of proteins found in various species including low eukaryotes, invertebrates and vertebrates. It is essential for proper development. Mutations of Hir/Hira genes result in very serious defects in normal development not only in yeast but also in advanced eukaryotes.	*HIRA* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006 Wang and Du, 2005
KLHL22*(+)** ***de novo Intracellular signaling: protein ubiquitination pathway	This gene encodes the kelch-like 22 (Drosophila) protein which is a substrate-specific adapter of a BCR (BTB-CUL3-RBX1) E3 ubiquitin ligase complex required for cell division. Good expression in fetal brain and in postnatal CNS, in particular in prefrontal cortex, cerebellum, amygdalae and hypothalamus.	The ubiquitin-proteasome system plays crucial roles in various aspects of neuronal development, such as axon formation, elongation and pruning, and synapse formation and elimination. The Cullin3 (Cul3)-based ubiquitin E3 ligases use BTB domain–containing proteins as substrate adaptors and, recently, KLHL20, a protein possessing a BTB domain and six kelch repeats, has been identified as such an adaptor. *KLHL20* mRNA is abundantly expressed in the brain of an embryonic day 14.5 (E14.5) mouse embryo, implying its role in neural development. In the adult mouse brain *KLHL20* mRNA is highly expressed in the hippocampus, especially in the dentate gyrus, where a lifelong neurogenesis occurs. By analogy, it is possible that also KLHL22 may be involved in neurodevelopment.	*KLHL22* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. The protein ubiquitination pathway has been previously implicated in ASD.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lee *et al.*, 2010 Lin *et al.*, 2011 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Segref and Hoppe, 2009 Szatmari *et al.*, 2007 Tai and Schuman, 2008 Vorstman *et al.*, 2006 Yi and Ehlers, 2007
LZTR1*(+)** ***de novo Neurodevelopment: Golgi complex stabilizator	This gene encodes the leucine-zipper-like transcription regulator 1 protein, which is a member of the BTB-kelch superfamily. Initially described as a putative transcriptional regulator based on weak homology to members of the basic leucine zipper-like family, the encoded protein subsequently has been shown to localize exclusively to the Golgi network where it may help stabilize the Golgi complex. Good expression in fetal brain and in postnatal CNS. In particular, high expression in the cortex.	It is a probable transcriptional regulator that may play a crucial role in embryogenesis.	*LZTR1* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

★ ***MED15(+) de novo*** Transcriptional regulation	This gene encodes the mediator complex subunit 15, which is a subunit of the multiprotein complexes PC2 and ARC/DRIP and may function as a transcriptional coactivator in RNA polymerase II transcription. Moderate expression in fetal brain and in postnatal CNS. Good expression in immune cell types.	Mediator is recruited to promoters by direct interactions with regulatory proteins and serves as a scaffold for the assembly of a functional preinitiation complex with RNA polymerase II and the general transcription factors. A possible involvement of the multiprotein complex MED15 in SCZ susceptibility was supported by the detection of an association in patients with SCZ vs. controls for an intragenic coding trinucleotide polymorphism. This datum was not later replicated. Recently, it has been reported that the mediator complex is used as a host RNA polymerase coactivator by herpes simplex, which diverts the polymerase activity toward viral RNA synthesis, thus suggesting a role of this protein in supporting cerebral infections which might be implicated in the onset of neurodevelopmental disorder.	*MED15* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. SNPs in *MED12* have been associated with ASD.	Antshel *et al.*, 2007 Beyer *et al.*, 2012 Bucan *et al.*, 2009 Carter, 2009 De Luca *et al.*, 2003 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Sandhu *et al.*, 2004 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
MRPL40(+) de novo Neurodevelopment and maintainance: mitochondrial protein synthesis	This nuclear gene encodes the mitochondrial ribosomal protein L40. Mammalian mitochondrial ribosomal proteins are encoded by nuclear genes and help in protein synthesis within the mitochondrion. Mitochondrial ribosomes (mitoribosomes) consist of a small 28S subunit and a large 39S subunit. Moderate expression in fetal brain and in postnatal CNS. MRPL40 appears more selectively, but not exclusively, associated with projecton neurons in the bulb, cortex and cerebellum. Expression was also detected in adult brain and enriched in brain synapses.	MRPL40 facilitates expression from the mitochondrial genes.	*MRPL40* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Accardi *et al.*, 2004 Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Maynard *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

★ ***PI4KA(+) de novo*** Phosphatidylinositol signaling system	This gene encodes the phosphatidylinositol 4-kinase, catalytic, alpha protein, which catalyzes the first committed step in the biosynthesis of phosphatidylinositol 4,5-bisphosphate. Good expression in fetal brain and in postnatal CNS. Good expression in immune cell types.	Recently, it has been demonstrated that reduced expression of individual phosphatidylinositol 4-kinase isozymes is associated with impaired survival of specific neuronal populations within the CNS. Furthermore, alterations to the concentrations of different phosphoinositide lipid species in the brain can have deleterious effects on clathrin-dependent membrane trafficking both in the Golgi-endosomal pathway and at the plasma membrane. Therefore, the four mammalian phosphatidylinositol 4-kinases modulate neuronal pools of phosphoinositide lipid and regulate intracellular membrane trafficking in the endocytic and secretory pathways. Dysfunctions in these enzymes have been associated with a broad spectrum of disorders including SCZ, BD, Lowe syndrome, age-related neurodegeneration, Alzheimer's disease and Down syndrome. An atypical 0.8 Mb inherited duplication of 22q11.2, which encompasses 14 genes including *CRKL*, *ZNF74*, *PIK4CA*, *SNAP29* and *PCQAP* known to contribute to several aspects of the DGS/VCFS phenotype, has been recently described in a patient with psychomotor impairment, suggesting a role for these genes in neurodevelopment.	*PI4KA* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. *PI4KA* has been previously suggested as autism candidate genes by molecular cytogenetic analysis and *in silico* studies.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Clayton *et al.*, 2012 Fine *et al.*, 2005 Iurov *et al.*, 2010 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pebrel-Richard *et al.*, 2012 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
PRODH(+)de novo ***PRODH(-)*** Proline metabolism	This nuclear gene encodes the proline dehydrogenase (oxidase) 1, which is a mitochondrial protein that catalyzes the first step in proline degradation. High expression in the fetal brain and in postnatal CNS.	Mutations in *PRODH* are associated with hyperprolinemia type 1 and susceptibility to schizophrenia 4 (SCZD4), which is a complex, multifactorial psychotic disorder or group of disorders characterized by disturbances in the form and content of thought, in mood (e.g. inappropriate affect), in sense of self and relationship to the external world (e.g. loss of ego boundaries, withdrawal), and in behavior (e.g bizarre or apparently purposeless behavior). There may be mild impairment of cognitive function *PRODH* is known to be dosage-sensitive in mouse neurodevelopment.	*PRODH* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. CNVs (loss) exclusively affecting *PRODH* and *DGCR6* and parentally inherited have been significantly associated with ASD. *De novo* CNVs (loss) affecting *PRODH* (demonstrated haploinsufficiency) have been reported in patients with ASD.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Guilmatre *et al.*, 2009 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Meechan *et al.*, 2009 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Nord *et al.*, 2011 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
RANBP1(+) de novo Intracellular RAN-GTP signaling	This gene encodes the RAN binding protein 1, which interacts specifically with GTP-charged RAN. RANBP1 does not activate GTPase activity of RAN but does markedly increase GTP hydrolysis by the RanGTPase-activating protein (RanGAP1). RANBP1 may act in an intracellular signaling pathway which may control the progression through the cell cycle by regulating the transport of protein and nucleic acids across the nuclear membrane. Moderate expression in fetal brain and in postnatal CNS.	Changes of hippocampal signaling protein levels, including the GTP-binding nuclear protein RAN, during postnatal brain development in the rat have been reported, thus supporting a developmental regulation of individual signaling proteins in the brain.	*RANBP1* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Weitzdörfer *et al.*, 2008 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

RTN4R*(+)** ***de novo Regulation of synaptogenesis during neurodevelopment	This gene encodes the reticulon 4 receptor precursor, an oligodendrocyte myelin glycoprotein and myelin-associated glycoprotein. This receptor mediates axonal growth inhibition and may play a role in regulating axonal regeneration and plasticity in the adult CNS. It is widespread expressed in the brain but highest levels are found in the gray matter.	It has been recently reported that RTN4R, also known as NgR1, inhibits the formation of new synapses in the postsynaptic neuron by signaling through the coreceptor TROY and RhoA, and functions in the dendrite as a barrier that limits excitatory synapse number during brain development, in particular in hippocampus.	*RTN4R* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Guilmatre *et al.*, 2009 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukaddes and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010; Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Wills *et al.*, 2012 Vorstman *et al.*, 2006
SEPT5*(+)** ***de novo Intracellular vescicle trafficking: cytoskeleton dynamics	This gene encodes the septin 5 protein, which is is a nucleotide binding protein, originally described in yeast as cell division cycle regulatory proteins. Septins are highly conserved in yeast, Drosophila, and mouse and appear to regulate cytoskeletal organization. Disruption of septin function disturbs cytokinesis and results in large multinucleate or polyploid cells. Very high expression in fetal brain and in postnatal CNS.	SEPT5 acts as a filament-forming cytoskeletal GTPase and may play a role in cytokinesis and platelet secretion. Indeed, septins polymerize into heterooligomeric protein complexes that form filaments, and can associate with cellular membranes, actin filaments and microtubules. SEPT5 is important for active membrane movement such as vesicle trafficking and exocytosis in non-dividing cells (i.e. platelets, neurons). In a heterologous system, SEPT5 overexpression has been shown to exert dopamine-dependent neurotoxicity. It has been recently reported on a four-year-old boy with a homozygous deletion comprising the *GP1BB* and *SEPT5* genes, located 5' to GP1BB. He presented with Bernard-Soulier syndrome, cortical dysplasia (polymicrogyria), developmental delay, and platelet secretion defect, thus supporting the possible effect of defect of these two genes in neurodevelopment.	*SEPT5* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. Both *GP1BB* and *SEPT5* have been previously suggested as autism candidate genes by molecular cytogenetic analysis and *in silico* studies.	Antshel *et al.*, 2007 Bartsch *et al.*, 2011 Bucan *et al.*, 2009 Fine *et al.*, 2005 Iurov *et al.*, 2010 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
SLC7A4*(+)** ***de novo Cellular trafficking: cationic amino acid transport	This gene encodes the solute carrier family 7 (orphan transporter), member 4, which is involved in the transport of the cationic amino acids (arginine, lysine and ornithine). Moderate expression in fetal brain and in postnatal CNS.	Cationic amino acid transporters (CAT) have important roles for normal brain functioning and various brain diseases. Recently, human cationic amino acid transporters 1, 2 and 3 (hCAT1, 2, and 3) have been mapped immunohistochemically throughout five adult human brains. All three hCAT1s were mainly localized in neurons, but were also found in numerous astrocytes, oligodendrocytes, plexus choroideus epithelial cells, and small blood vessels. The highest density of hCAT expressing neurons was observed in the hypothalamus, in some areas of the cerebral cortex, the thalamic reticular nucleus and the caudate nucleus, whereas weak to moderate expression was detected in the hippocampus, the prefrontal cortex (hCAT1 only), pons, brain stem and cerebellum.	*SLC7A4* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Jäger *et al.*, 2012 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

SLC25A1*(+)** ***de novo Cerebral mitochondrial metabolism	This gene encodes the solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1 (CTP), which is responsible for the movement of citrate across the mitochondrial inner membrane.	In mouse models, the citrate transporter Slc25a1 is expressed minimally, but apparently ubiquitously, throughout the brain. In addition, Slc25a1 is highly expressed in a subset of large cells in the globus pallidus. It is essential for mithochondrial metabolism.	*SLC25A1* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Kaplan *et al.*, 1995 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Maynard *et al.*, 2009 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
★ ***SNAP29*(+)** ***de novo*** Synaptic plasticity	This gene encodes the synaptosomal-associated protein, 29kDa, which is a member of the SNAP25 gene family and is involved in multiple membrane trafficking steps. Two other members of this gene family, SNAP23 and SNAP25, encode proteins that bind a syntaxin protein and mediate synaptic vesicle membrane docking and fusion to the plasma membrane. The protein encoded by this gene binds tightly to multiple syntaxins and is localized to intracellular membrane structures rather than to the plasma membrane. Moderate expression in fetal brain and postnatal CNS. High expression in immune cell types.	*In vitro* studies in cultured hyppocampal neurons have recently reported SNAP-29 as a candidate molecule regulating the disassembly of the SNARE complex. Indeed, SNAP-29 is present at synapses and regulates recycling of the SNARE complexes by competing with *α*-SNAP for binding to the SNAREs and consequently inhibiting disassembly of the SNARE complex. These findings suggest that SNAP-29 acts as a negative modulator for neurotransmitter release, probably by slowing recycling of the SNARE-based fusion machinery and synaptic vesicle turnover. In presynaptic neurons SNAP-29 binds to STX1A, which is also involved in docking of synaptic vesicles at presynaptic active zones. Moreover, SNAP-29 promoter is one of the targets of β-catenin. Defects in *SNAP29* are the cause of CEDNIK syndrome, which is a neurocutaneous syndrome characterized by cerebral dysgenesis, neuropathy, ichthyosis and palmoplantar keratoderma. An atypical 0.8 Mb inherited duplication of 22q11.2, which encompasses 14 genes including *CRKL*, *ZNF74*, *PIK4CA*, *SNAP29* and *PCQAP* known to contribute to several aspects of the DGS/VCFS phenotype, has been recently described in a patient with psychomotor impairment, suggesting a role for these genes in neurodevelopment.	*SNAP29* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. A polymorphism in the *SNAP29* gene promoter has been associated to SCZ. SNPs in *STX1A* have been associated with ASD and HF-AU. Moreover, in the postmortem anterior cingulate gyrus region of autistic patients, *STX1A* expression was found to be significantly lower than that of the control group.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 de Queiroz Soares *et al.*, 2012 Fine *et al.*, 2005 Guilmatre *et al.*, 2009 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Nakamura *et al.* 2008, 2011 Niklasson *et al.*, 2009 Pedrosa *et al.*, 2010 Pan *et al.*, 2005 Pebrel-Richard *et al.*, 2012 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Saito *et al.*, 2001 Sprecher et al., 2005 Su *et al.*, 2001 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

TBX1*(+)** ***de novo Transcriptional regulation	This gene encodes the T-box 1 (TBX1), transcript variant B protein, which belongs to a family of conserved transcription factors involved in the regulation of developmental processes. Moderate expression in fetal brain and in postnatal CNS.	Studies using mouse models of DiGeorge syndrome suggest a major role for *TBX1* in the molecular etiology of DGS/VCFS, which are characterized by neural-crest-related developmental defects. It has been reported that *Tbx1* heterozygous mice are impaired in social interaction, ultrasonic vocalization, memory-based behavioral alternation, working memory and thigmotaxis, compared with wild-type mice. Furthermore, Tbx1 mRNA and protein are ubiquitously expressed throughout the brain, but protein expression is enriched in regions that postnatally retain the capacity of neurogenesis. In postnatally derived hippocampal culture cells, Tbx1 levels are higher during proliferation than during differentiation, and expressed in neural progenitor cells, immature and matured neurons and glial cells.	*TBX1* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. An inactivating mutation of *TBX1* has been previously reported in a patient with Asperger syndrome.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Hiramoto *et al.*, 2011 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Paylor *et al.*, 2006 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
TRMT2A* (*HTF9C*)(+)** ***de novo Neurodevelopment?	This gene encodes the TRM2 tRNA methyltransferase 2 homolog A (S. cerevisiae) protein, which has an unknown function. However, it is orthologous to the mouse *Trmt2a* gene and contains an RNA methyltransferase domain. Expression of this gene varies during the cell cycle, with aberrant expression being a possible biomarker in certain breast cancers. Low expression in fetal brain and moderate expression in postnatal CNS.	TRMT2A probably functions as a regulator of cell cycle.	*TRMT2A* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. A SNP of the *HTF9C* gene has been previously associated with a deficit in sustained attention within SCZ in a Taiwanese cohort that might be an endophenotype of SCZ.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Liu *et al.*, 2007 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
TXNRD2*(+)** ***de novo Mitochondrial neuronal metabolism	This nuclear gene encodes the thioredoxin reductase 2, a mitochondrial dimeric NADPH-dependent FAD containing enzyme that catalyzes the reduction of the active site disulfide of thioredoxin and other substrates. TR is a member of a family of pyridine nucleotide-disulfide oxidoreductases and is a key enzyme in the regulation of the intracellular redox environment. Three thioredoxin reductase genes have been found that encode selenocysteine containing proteins. This gene partially overlaps the *COMT* gene on chromosome 22. Moderate expression in fetal brain and in postnatal CNS. Expression was also detected in adult brain and enriched in brain synapses.	TXNRD2 has been analyzed in mammalian cells and it has been demonstrated that its role in reducing thioredoxin appears to be essential for preventing cell death. The developmental impact of over-expression of an ~190 kb segment of human 22q11.2, which includes *TXNRD2*, *COMT* and *ARVCF*, on behaviors in bacterial artificial chromosome transgenic mice vs. wild-type mice has been determined. The collected data suggest that over-expression of this 22q11.2 segment enhances incentive learning and impairs the prolonged maintenance of working memory, but has no apparent effect on working memory per se, affect- and stress-related behaviors or motor capacity.	*TXNRD2* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. *TXNRD2* polymorphisms in the shared upstream promoter with *COMT* have been associated with SCZ. Moreover, TXNRD2 is apparently elevated in schizophrenic brain samples.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Maynard *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Prabakaran *et al.*, 2004 Ramelli *et al.*, 2008 Sanders *et al.*, 2005 Suzuki *et al.*, 2009 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

UFD1L*(+)** ***de novo Intracellular signaling: protein ubiquitination pathway	This gene encodes the ubiquitin fusion degradation 1 like (yeast) protein, which forms a complex with two other proteins, nuclear protein localization-4 and valosin-containing protein, and this complex is necessary for the degradation of ubiquitinated proteins. In addition, this complex controls the disassembly of the mitotic spindle and the formation of a closed nuclear envelope after mitosis. Moderate expression in fetal brain and in postnatal CNS.	Mutations in this gene have been associated with Catch 22 syndrome as well as cardiac and craniofacial defects. *UFD1L* encodes for the ubiquitin fusion degradation 1 protein, which is expressed in the medial telencephalon during mouse development. An association between SCZ and a single nucleotide functional polymorphism, located within the noncoding region upstream the first exon of the *UFD1L* gene, has been reported.	*UFD1L* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. The protein ubiquitination pathway has been previously implicated in ASD.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 De Luca *et al.*, 2001 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006
ZDHHC8*(+)** ***de novo Synaptic transmission: post-translational modification (palmytoilation)	This gene encodes the zinc finger, DHHC-type containing 8 protein, which is a member of the zinc finger DHHC domain-containing protein family. The encoded protein may function as a palmitoyltransferase and is localized in mitochondria. Moderate expression in fetal brain and in postnatal CNS. Expression was also detected in adult brain and enriched in brain synapses.	Post-translational modification of proteins by the lipid palmitate is critical for protein localization and function. Palmitoylation is regulated by the opposing enzymes palmitoyl acyltransferases (PATs) and acyl protein thioesterases, which add and remove palmitate from proteins, respectively. Palmitoylation is particularly important for a number of processes including neuronal development and synaptic activity in the central nervous system. Dysregulated palmitoylation contributes to neuropsychiatric disease. Indeed, in total six PATs (HIP14, HIP14L, ZDHHC8, ZDHHC9, ZDHHC12, and ZDHHC15) have been implicated in Huntington disease, Alzheimer disease, SCZ, MR, and infantile and adult onset forms of neuronal ceroid lipofuscinosis. Zdhhc8 is found primarily in apparent presynaptic processes, with an apparent preference for Zdhhc8 in glutmatergic versus GABAergic processes indicates an enhanced role for Zdhhc8 in excitatory synaptic transmission. *ZDHHC8* overexpression studies demonstrated an increased level of the corresponding protein and a subsequent increase in apoptotic cell death, perhaps leading to disrupted mitochondrial function.	*ZDHHC8* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. SNPs affecting *ZDHHC8* have been robustly associated to SCZ.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Liu *et al.*, 2002a and b Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Maynard *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Vorstman *et al.*, 2006 Young *et al.*, 2012
ZNF74*(+)** ***de novo Transcriptional regulation	This gene encoder the zinc finger protein 74. High expression in fetal brain and good expression in postnatal CNS.	A speciific *ZNF74* genotype has been associated with age-at-onset of SCZ. An atypical 0.8 Mb inherited duplication of 22q11.2, which encompasses 14 genes including *CRKL*, *ZNF74*, *PIK4CA*, *SNAP29* and *PCQAP* known to contribute to several aspects of the DGS/VCFS phenotype, has been recently described in a patient with psychomotor impairment, suggesting a role for these genes in neurodevelopment.	*ZNF74* maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pebrel-Richard *et al.*, 2012 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Szatmari *et al.*, 2007 Takase *et al.*, 2001 Vorstman *et al.*, 2006

Tab. 4.1. Continued.

▪ **Patient 29, gain of 8.0 Mb at 15q11.1q13.1 (chr15:20575646-28535051)** ▪ **Patient 50, loss of 211 kb at 15q11.2 (chr15:22873688-23085096)** ▪ **Patient 55, gain of 203 kb at 15q11.2 (chr15:22873688-23076420)**				
ATP10A*(+)** ***de novo Neurodevelopment?	This gene encodes the ATPase class V, type 10A protein, which belongs to the family of P-type cation transport ATPases, and to the subfamily of aminophospholipid-transporting ATPases. The aminophospholipid translocases transport phosphatidylserine and phosphatidylethanolamine from one side of a bilayer to another. This gene is maternally expressed. Good expression in fetal brain and in postnatal whole brain.	Defects in *ATP10A* contribute, in addition to mutations in *UBE3A*, to the onset of Angelman syndrome (AS). Allelic expression of *ATP10A* transcript in 16 human control brain samples has been recently examined: 10/16 exhibited biallelic expression while only 6/16 showed monoallelic expression. Contrary to the expectation for a maternally expressed imprinted gene, quantitative RT-PCR revealed significantly reduced *ATP10A* transcript in Prader-Willi syndrome brains with two maternal chromosomes due to uniparental disomy (PWS UPD). Investigation of factors that may influence allelic *ATP10A* expression status revealed that gender has a major affect, as females were significantly more likely to have monoallelic *ATP10A* expression than males. Moreover, a promoter polymorphism that disrupts binding of the transcription factor Sp1 also potentially contributes to allelic expression differences in females, thus supporting that monoallelic expression of human *ATP10A* is variable in the population and is influenced by both gender and common genetic variation. In mouse brain *ATP10A* is not imprinted.	*ATP10A* maps within the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. SNPs in *ATPA10* have been previously associated with ASD.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 DuBose *et al.*, 2010 Hogart *et al.*, 2008, 2010 Nurmi *et al.*, 2003 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005
C15orf2*(+)** ***de novo Neurodevelopment?	This gene encodes the chromosome 15 open reading frame 2 protein. This gene is biallelically expressed in adult testis and brain but is paternally imprinted in fetal brain. Defects in this gene may be associated with Prader-Willi syndrome. Moderate expression in fetal brain and in postnatal CNS.	*C15orf2* has no ortholog in rodents, but appears to be under strong positive selection in primates. *C15orf2* encodes a 1156 amino acid protein with six nuclear localisation sequences. Recently, it has been predicted a sequence similarity of C15orf2 to the nuclear pore complex (NPC) protein POM121 and it has been demonstrated that C15orf2 is located at the inner face of the nuclear envelope where it strongly associates with the NPC	*C15orf2* maps within the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Hogart *et al.*, 2010 Neumann *et al.*, 2012 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005

Tab. 4.1. Continued.

CYFIP1*(+)*de novo ***CYFIP1*(-)*de novo*** ***CYFIP1*(+)** Translational repression	This gene encodes the cytoplasmic FMR1 interacting protein 1, which is a component of the CYFIP1-EIF4E-FMR1 complex that binds to the mRNA cap and mediates translational repression. CYFIP1 promotes the translation repression activity of FMR1 in brain probably by mediating its association with EIF4E and mRNA. Moreover, it regulates formation of membrane ruffles and lamellipodia, plays a role in axon outgrowth throught its actin remodeling activity. Moderate expression in fetal brain and in postnatal CNS, particularly in amygdalae.	CYFIP1 is particularly enriched at synapses.	CNVs (*de novo* and inherited) between BP1 and BP2 encompassing *CYFIP1*, *NIPA1*, *NIPA2*, and *TUBGCP5*, are responsible for the 15q11.2 microdeletion/microduplication syndrome, which is comorbid with ASD and shows incomplete penetrance. A few rare deletions and common SNPs at 15q11.2, including *CYFIP1*, have been recently associated with SCZ in a Chinese Han population. Mutation affecting *FMR1* are responsible for the Fragile X syndrome which is comorbid with ASD.	Clifford *et al.*, 2007 Doornbos *et al.*, 2009 Kielinen *et al.*, 2004 Schenck *et al.*, 2003 Sempere Perez *et al.*, 2011 Wang *et al.*, 2010 van der Zwaag *et al.*, 2010 Zhao *et al.*, 2012
GABRA5*(+)** ***de novo Synaptic function and plasticity	This gene encodes the gamma-aminobutyric acid (GABA) A receptor, alpha 5. GABA is the major inhibitory neurotransmitter in the mammalian brain where it acts at GABA-A receptors, which are ligand-gated chloride channels. Chloride conductance of these channels can be modulated by agents such as benzodiazepines that bind to the GABA-A receptor. It is located at postsynaptic cell membrane. Very high expression in fetal brain and in postnatal CNS.	An altered expression of gamma-aminobutyric acid A (GABAA) and gammaaminobutyric acid B (GABAB) receptors has been reported in the brains of subjects with autism.	*GABRA5* maps within the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. *GABRA5* polymorphisms have been associated with BD, major depression and SCZ of a later-age onset.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Fatemi *et al.*, 2010 Hogart *et al.*, 2010 Oruc *et al.*, 1997 Papadimitriou *et al.*, 1998, 2001 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005
GABRB3*(+)** ***de novo Synaptic function and plasticity	This gene encoder the gamma-aminobutyric acid (GABA) A receptor, beta 3 (GABRB3), which is a member of the ligand-gated ionic channel family. The encoded protein is one of at least 13 distinct subunits of a multisubunit chloride channel that serves as the receptor for gamma-aminobutyric acid, the major inhibitory transmitter of the nervous system. Very high expression in fetal brain and in postnatal CNS, in particular in amygdalae.	An altered expression of gamma-aminobutyric acid A (GABAA) and gammaaminobutyric acid B (GABAB) receptors has been reported in the brains of subjects with autism.	*GABRB3* maps within the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. Polymorphisms in *GABRB3* have been reported in patients with ASD. Defects in *GABRB3* are associated with absence epilepsy type 5 (ECA5), which is a subtype of idiopathic generalized EP characterized by an onset at age 6-7 years, frequent absence seizures (several per day) and bilateral, synchronous, symmetric 3-Hz spike waves on EEG.	Bolton, 2004 Bonati *et al.*, 2007 Buxbaum *et al.*, 2002 Cook *et al.*, 1997, 1998 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Fatemi *et al.*, 2010 Hogart *et al.*, 2010 Kim *et al.*, 2006 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Tanaka *et al.*, 2008 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005

Tab. 4.1. Continued.

GABRG3*(+)** ***de novo Synaptic function and plasticity	This gene encodes the gamma-aminobutyric acid (GABA) A receptor, gamma 3 (GABRG3). GABA-A receptors are pentameric, consisting of proteins from several subunit classes: alpha, beta, gamma, delta and rho. The protein encoded by this gene is a gamma subunit, which contains the benzodiazepine binding site. GABA is the major inhibitory transmitter of the nervous system. Moderate expression in fetal brain and in postnatal CNS, in particolar in the cortex.	An altered expression of gamma-aminobutyric acid A (GABAA) and gammaaminobutyric acid B (GABAB) receptors has been reported in the brains of subjects with autism.	*GABRG3* maps within the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. Two SNPs in *GABRG3* have been associated with ASD.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Hogart *et al.*, 2010 Menold *et al.*, 2001 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005
MAGEL2*(+)** ***de novo Neurodevelopment	This gene encodes the MAGE-like protein 2. This gene is structurally similar to *NDN*, is also localized to the PWS chromosomal region, and is paternally imprinted, suggesting a possible role for it in PWS. MAGEL2 belongs to the MAGE/necdin family of proteins, which have roles in cell cycle, differentiation, and apoptosis. Good expression in fetal brain and in postnatal CNS, in particular in amygdalae, thalamus and hypothalamus.	*MAGEL2* is expressed in various brain regions, most notably the hypothalamus. Mice with a targeted deletion of *Magel2* display hypoactivity, blunted circadian rhythm, decreased fertility, and increased adiposity. It has been reported that in *Magel2*-null mice brain volume was reduced in specific regions, particularly in the parieto-temporal lobe of the cerebral cortex, the amygdalae, the hippocampus, and the nucleus accumbens, as measured by quantitative magnetic resonance imaging. Abnormal neurochemistry was detected in brain samples from adult mice, consisting of decreased serotonin and 5-hydroxyindoleacetic acid in the cortex and the hypothalamus, and decreased dopamine in the hypothalamus. *Magel2*-null mice displayed relatively normal motor and learning abilities, but exhibited abnormal behavior in novel environments.	*MAGEL2* maps within the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Hogart *et al.*, 2010 Mercer *et al.*, 2009 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005
MKRN3*(+)** ***de novo Probabably involved in protein ubiquitination pathway	This gene encodes the makorin ring finger protein 3, which contains a RING (C3HC4) zinc finger motif and several C3H zinc finger motifs. This gene is intronless and imprinted, with expression only from the paternal allele. Disruption of the imprinting at this locus may contribute to Prader-Willi syndrome. An antisense RNA of unknown function has been found overlapping this gene. Low expression in fetal brain and good expression in postnatal amygdalae, thalamus and hypothalamus.	MKRN3 encodes the zing finger protein 127 (ZNF127) which possibly function as a ribonucleoprotein. The intronless *ZNF127* gene is expressed ubiquitously and allele-specific analysis shows that *ZNF127* is expressed only from the paternal allele. Consistent with this expression pattern, in the brain the *ZNF127* 5' CpG island is completely unmethylated on the paternal allele but methylated on the maternal allele. ZNF127 probably belongs to the E3 ubiquitin ligase complex. Defects at this locus are not sufficient to cause PWS.	*MKRN3* maps within the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. The protein ubiquitination pathway has been previously implicated in ASD.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Hogart *et al.*, 2010 Jong *et al.*, 1999 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005

Tab. 4.1. Continued.

NDN*(+)** ***de novo Transcriptional regulation	This intronless gene encodes the necdin homolog (mouse) protein, and is located in the Prader-Willi syndrome deletion region. It is an imprinted gene and is expressed exclusively from the paternal allele. Studies in mouse suggest that the protein encoded by this gene may suppress growth in postmitotic neurons, facilitating the entry of the cell into the cycle arrest. High expression in fetal brain and in postnatal CNS.	NDN directly interacts with the transcription factor E2F1 via its transactivation domain and represses E2F1-dependent transcription. In addition, necdin interacts with NGFR, the nerve growth factor receptor, via its distinct intracellular domains.	*NDN* maps within the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Hogart *et al.*, 2010 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005
NIPA1*(+) *de novo ***NIPA1*(-) *de novo*** ***NIPA1*(-)*** Neurodevelopment and maintenance	This gene encodes the non imprinted in Prader-Willi/Angelman syndrome 1 protein, which is a magnesium transporter that associates with early endosomes and the cell surface in a variety of neuronal and epithelial cells. This protein may play a role in nervous system development and maintenance. Widely expressed with highest levels in neuronal tissues.	Two non imprinted genes in the 15q11.2 region, *CYFIP1* and *NIPA1*, are widely expressed during mouse brain development and are known to be implicated in axonal growth, neuronal connectivity, and neuronal morphology, thus being good candidates for ASD pathogenesis. Moreover, defects in *NIPA1* are the cause of spastic paraplegia autosomal dominant type 6 (SPG6). Spastic paraplegia is a degenerative spinal cord disorder characterized by a slow, gradual, progressive weakness and spasticity of the lower limbs.	CNVs (*de novo* and inherited) between BP1 and BP2 encompassing *CYFIP1*, *NIPA1*, *NIPA2*, and *TUBGCP5*, are responsible for the 15q11.2 microdeletion/microduplication syndrome, which is comorbid with ASD and shows incomplete penetrance. A few rare deletions and common SNPs at 15q11.2, including *NIPA1*, have been recently associated with SCZ in a Chinese Han population.	Doornbos *et al.*, 2009 Sempere Perez *et al.*, 2011 van der Zwaag *et al.*, 2010 Zhao *et al.*, 2012
NIPA2*(+) *de novo ***NIPA2*(-) *de novo*** ***NIPA2*(+)** Neurodevelopment	This gene encodes the non imprinted in Prader-Willi/Angelman syndrome 2 protein, which is a possible magnesium transporter. This gene is located adjacent to the imprinted domain in the Prader-Willi syndrome deletion region of chromosome 15. Low expression in fetal brain and high expression in postnatal amygdalae.	NIPA2 plays a role in magnesium metabolism and regulation of renal magnesium conservation. *NIPA2* heterozygous muutations cause the Childhood Absence Epilepsy in Chinese populations.	CNVs (*de novo* and inherited) between BP1 and BP2 encompassing *CYFIP1*, *NIPA1*, *NIPA2*, and *TUBGCP5*, are responsible for the 15q11.2 microdeletion/microduplication syndrome, which is comorbid with ASD and shows incomplete penetrance. A few rare deletions and common SNPs at 15q11.2, including *NIPA2*, have been recently associated with SCZ in a Chinese Han population.	Doornbos *et al.*, 2009 Goytan *et al.*, 2008 Jiang *et al.*, 2012 Sempere Perez *et al.*, 2011 van der Zwaag *et al.*, 2010 Zhao *et al.*, 2012
★ ***SNRPN*(+)** ***de novo*** Tissue-specific mRNA splicing regulation	This gene encodes the small nuclear ribonucleoprotein polypeptide N, which belongs to the snRNP SMB/SMN family. The protein plays a role in pre-mRNA processing, possibly tissue-specific alternative splicing events. Multiple transcription initiation sites have been identified and extensive alternative splicing occurs in the 5' untranslated region. Good expression in fetal brain and high expression in postnatal CNS. Good-high expression in immune cell types.	Defects in *SNURF-SNRPN* methylation cause Prader-Willy syndrome. Patients with the autoimmune disease systemic lupus erythematosus (SLE) have autoantibodies directed against some of the individual snRNP polypeptides. The most common autoantigen is called Sm. A wide range of non-specific symptoms can reveal neurolupus such as psychiatric disorders (mood disorders and schizoid personality)→clear role of anti-Sm autoantibodies in brain.	*SNRPN* maps in the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11-q13 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. Recently, a rare CNV disrupting the *SNRPN-SNURF* genes has been reported in an autistic boy without clinical signs of PWS/AS.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Hogart *et al.*, 2010 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Simonin *et al.*, 2004 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Talkowski *et al.*, 2012 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005

Tab. 4.1. Continued.

★ ***SNURF(+)de novo*** Neurodevelopment?	This gene encodes the SNRPN upstream reading frame protein, which is a highly basic protein localized to the nucleus. The evolutionarily constrained open reading frame is found on a bicistronic transcript which has a downstream ORF encoding the small nuclear ribonucleoprotein polypeptide N. The upstream coding region utilizes the first three exons of the transcript, a region that has been identified as an imprinting center. Multiple transcription initiation sites have been identified and extensive alternative splicing occurs in the 5' untranslated region but the full-length nature of these transcripts has not been determined. Moderate expression in fetal brain and very high expression in postnatal CNS. High expression in B- and T-cells.	Defects in *SNURF-SNRPN* methylation cause Prader-Willy syndrome.	*SNURF* maps in the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, this gene is involved in the 15q11-q13 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. Recently, a rare CNV disrupting the *SNRPN-SNURF* genes has been reported in an autistic boy without clinical signs of PWS/AS.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Hogart *et al.*, 2010 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Talkowski *et al.*, 2012 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005
★ ***TUBGCP5(+) de novo*** ***TUBGCP5(-) de novo*** ***TUBGCP5(+)**** Intracellular signaling: regulation of microtubule cytoskeleton dynamics	This gene encodes the tubulin gamma complex associated protein 5. Low expression in fetal brain and good expression in postnatal parietal lobe, prefrontal cortex, and hypothalamus. High expression in T-cells.	The gamma-tubulin complex is a large multiprotein complex, conserved among different species, that is required for microtubule nucleation at the centrosome. GCP5 and GCP6, like other components of the gamma-tubulin complex, localize to the centrosome and associate with microtubules, suggesting that the entire gamma-tubulin complex takes part in both of these interactions. Stoichiometry experiments revealed that there is a single copy of GCP5 and multiple copies of gamma-tubulin, GCP2, GCP3, and GCP4 within the gamma-tubulin complex. Thus, the gamma-tubulin complex is conserved in structure and function, suggesting that the mechanism of microtubule nucleation is conserved. Glycogen synthase kinase-3beta (GSK-3beta) is involved in the regulation of the dynamics of microtubule networks in cells. GSK-3beta interacts with GCP5 and both proteins partecipate in the proper formation of the mitotic spindles.	CNVs (*de novo* and inherited) between BP1 and BP2 encompassing *CYFIP1*, *NIPA1*, *NIPA2*, and *TUBGCP5*, are responsible for the 15q11.2 microdeletion/microduplication syndrome, which is comorbid with ASD and shows incomplete penetrance. A few rare deletions and common SNPs at 15q11.2, including *TUBGCP5*, have been recently associated with SCZ in a Chinese Han population.	Doornbos *et al.*, 2009 Izumi *et al.*, 2008 Murphy *et al.*, 2001 Sempere Perez et al., 2011 van der Zwaag *et al.*, 2010 Zhao *et al.*, 2012
cluster snoRNAs(+) de novo Neurodevelopment	These genes encode different classes of small nucleolar RNAs. High expression in the CNS.	A few small deletions of different class of *snoR*NA genes	The cluster of *snoRNA* genes map in the genomic region which is deleted in PWS and AS, that both are comorbid with ASD. Moreover, these genes are involved in the 15q11-q13 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Hogart *et al.*, 2010 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005

Tab. 4.1. Continued.

UBE3A*(+)** ***de novo Intracellular signaling: protein ubiquitination pathway	This genen encodes the ubiquitin protein ligase E3A, which is part of the ubiquitin protein degradation system. This imprinted gene is maternally expressed in brain and biallelically expressed in other tissues. Good-high expression in fetal brain and moderate expression in postnatal CNS.	Increased *UBE3A* gene dosage produce autism-related behavioral traits both in humans and mice that share the same conserved imprinting pattern. In particular, autism behavioral traits are weakly penetrant in individuals with mat dup15 (double dose of *UBE3A*), but highly penetrant in mat idic15 (triple dose of *UBE3A*). Indeed, *UBE3A* triple dosage reconstitute the three core autism traits in mice: defective social interaction, impaired communication, and increased repetitive stereotypic behavior. Moreover, in animals with increased *UBE3A* gene dosage, glutamatergic, but not GABAergic, synaptic transmission is suppressed as a result of reduced presynaptic release probability, synaptic glutamate concentration, and post-synaptic action potential coupling.	Maternally inherited deletion of this gene causes Angelman Syndrome (AS), which is an imprinting disorder characterized by severe motor and intellectual retardation, ataxia, hypotonia, EP, absence of speech, and characteristic facies. AS is caused by maternal deletion of chromosome 15, paternal uniparental disomy, imprinting defect, or *UBE3A* mutation. Over one-half of the patients with Angelman syndrome have ASD. Moreover, *UBE3A* is involved in the 15q11.2-q13.1 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. CNVs involving other E3 ligase genes have been previously reported in patients with ASD.	Bolton, 2004 Bonati *et al.*, 2007 Cook *et al.*, 1997 Depienne *et al.*, 2009 Descheemaeker *et al.*, 2006 Glessner *et al.*, 2009 Hogart *et al.*, 2010 Kishino *et al.*, 1997 Matsuura *et al.*, 1997 Pinto *et al.*, 2010 Sahoo *et al.*, 2006 Schroer *et al.*, 1998 Scheuerle and Wilson, 2011 Smith *et al.*, 2011 Steffenburg *et al.*, 1996 Szatmari *et al.*, 2007 Trillingsgaaard and Østergaard, 2004 Veltman *et al.*, 2005

- **Patinet 30, gain of 800 kb at 16p13.11 (chr16:15492317-16292235)**

***NDE1*(+)** Neurogenesis and neuronal migration: regulation of microtubule cytoskeleton dynamics	This gene encodes the nudE nuclear distribution gene E homolog 1 protein, which is a member of the nuclear distribution E family of proteins. This protein is localized at the centrosome and interacts with other centrosome components as part of a multiprotein complex that regulates dynein function. Moreover, it plays an essential role in microtubule organization, mitosis and neuronal migration. Moderate expression in postnatal prefrontal cortex, occipital lobe, thalamus, and hypothalamus. High expression in immune cell types.	NDE1 is known to interact with DISC1 (disrupted in schizophrenia, which has a role in neurogenesis and neuronal migration) and LIS1 (causing lissencephaly 1). Deficiency of the LIS1–NDE1 complex impairs cortical neurogenesis and neuronal migration frequently leading to EP, whereas DISC1-NDE1 deficiency appears to play a role in neuropsychiatric disorders, including SCZ and BD. Moreover, mutations in *NDE1* cause lissencephaly 4, a disorder characterized by lissencephaly, severe brain atrophy, microcephaly, and severe MR.	*NDE1* mapswithin the genomic region involved in the16p13.11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, ID, and SCZ. Inherited CNV (loss) involving *DISC1* has been reported in patients with ASD and SCZ. SNPs in *DISC1* in Chinese-Han populations have been associated with autism.	Barkicioglu *et al.*, 2011 de Kovel *et al.*, 2010 Hennah *et al.*, 2009 Hennah and Porteus, 2009 Pawlisz *et al.*, 2008 Pinto *et al.*, 2010 Ullmann *et al.*, 2007 Williams *et al.*, 2009 Zheng *et al.*, 2011

- **Patient 51, loss of 8.8 Mb at 2q14.3q21.3 (chr2:127083045-135910585)**

ACMSD(-) ***de novo*** Tryptophan metabolism	This gene encoder the aminocarboxymuconate semialdehyde decarboxylase. Quinolinate is derived from alpha-amino-beta-carboxy-muconate-epsilon-semialdehyde (ACMS). ACMSD can divert ACMS to a benign catabolite and thus prevent the accumulation of quinolinate from ACMS. Moderate expression in postnatal temporal lobe, occipital lobe, parietal lobe, prefrontal cortex, and cerebellum peduncles. Good expression in thalamus. Good expression in monocytes, NK-, T-, and B-cells.	The essential amino acid tryptophan is not only a precursor of serotonin but is also degraded to several other neuroactive compounds, including kynurenic acid, 3-hydroxykynurenine and quinolinic acid. In particulare, the quinolinate induce a neuronal excitotoxin due to its role as a NMDA receptor agonist. The synthesis of these metabolites is regulated by an enzymatic cascade, known as the kynurenine pathway, that is tightly controlled by the immune system. Dysregulation of this pathway, resulting in hyper-or hypofunction of active metabolites, is associated with neurodegenerative and other neurological disorders, such as Huntington's disease, Parkinson's disease and Alzheimer's disease, as well as with psychiatric diseases such as depression and SCZ.	Mutations and/or CNVs affecting *ACMSD* have never been reported in patients with ASD. One possible explanation for ASD pathogenesis is the modern theory of immunoexcitotoxicity. Indeed, chronic microglial activation is present in autistic brains from age 5 years to age 44 years, which result in an outpouring of neurotoxic levels of the excitotoxins, glutamate and quinolinic acid. Careful control of brain glutamate levels is essential to brain pathway development and excesses can result in arrest of neural migration, as well as dendritic and synaptic loss. In addition, certain cytokines, such as TNF-alpha, can, via its receptor, interact with glutamate receptors to enhance the neurotoxic reaction.	Blaylock, 2008 Schwarcz *et al.*, 2012

Tab. 4.1. Continued.

ARHGEF4*(-)** ***de novo Intracellular signaling: RhoA signaling implicated in regulation of actin cytoskeleton dynamics	This gene encodes the Rho guanine nucleotide exchange factor 4. Rho GTPases play a fundamental role in numerous cellular processes that are initiated by extracellular stimuli that work through G protein coupled receptors. ARHGEF4 acts as guanine nucleotide exchange factor for RHOA, RAC1 and CDC42 GTPases. The APC-ARHGEF4 complex seems to be involved in cell migration as well as in E-cadherin-mediated cell-cell adhesion. Very high expression in fetal brain and in postnatal CNS.	RhoGTPases play a pivotal role in regulating the actin cytoskeleton and influence cell polarity, microtubule dynamics, membrane-transport pathways, and transcription-factor activity. Numerous evidence has implicated RhoGTPases in neuronal morphogenesis, including cell migration, axonal growth and guidance, dendrite elaboration and plasticity, and synapse formation. RhoGEFs activate RhoGTPases by catalyzing the exchange of bound GDP for GTP, which induces a conformational change in the GTP-bound GTPase that allows its interaction with downstream effector proteins, thus playing a central role in defining the temporal and spatial activation of the corresponding GTPase within neuronal cells.	Recently, a rare small (~450 kb unique sequence) recurrent deletion in a previously linked attention-deficit hyperactivity disorder (ADHD) locus at 2q21.1 has been identified in five unrelated families with developmental delay/ID, ADHD, EP and other neurobehavioral abnormalities. Moreover, the reciprocal duplications have been identified in five unrelated families with autism, developmental delay, seizures and ADHD. The rearranged segment harbors five genes: *GPR148*, *FAM123C*, *ARHGEF4*, *FAM168B* and *PLEKHB2*. RhoGEFs have been previously implicated in human genetic disorders: - a mutation in the DH domain of *FGD1* *GEF* cosegregates with faciogenital dysplasia, a developmental disorder; - mutations in *ARHGEF6* are associated with X-linked nonsyndromic MR; - aberrant EphB/Ephexin5 signaling during the development of synapses may contribute to the abnormal cognitive function that occurs in Angelman syndrome and, possibly, ASD.	Boguski and McCormick, 1993 Bourne *et al*., 1990 Dharmadhikari *et al*., 2012 Etienne-Manneville and Hall, 2002 Hart *et al*., 1994 Kutsche *et al*., 2000 Margolis *et al*., 2010 Pasteris *et al*., 1994 Verhoeven *et al*., 2003
★ ***BIN1(-)*** ***de novo*** Synaptic function and plasticity	This gene encodes the bridging integrator 1 protein (amphiphysin 2), which is a nucleocytoplasmic adaptor protein. Isoforms that are expressed in the CNS may be involved in synaptic vesicle endocytosis and may interact with dynamin, synaptojanin, endophilin, and clathrin. Isoforms that are expressed in muscle and ubiquitously expressed isoforms localize to the cytoplasm and nucleus and activate a caspase-independent apoptotic process. Studies in mouse suggest that this gene plays an important role in cardiac muscle development. High expression in fetal brain and in postnatal CNS.	Amphiphysin is an intracellular protein involved in the synaptic vesicle cycle that promotes cleavage of clathrin-coated vesicles via binding of its Src homology 3 (SH3)—domain to dynamin. Acute blocking of the function of amphiphysin impairs synaptic vesicle endocytosis *in vitro*, leading to alteration of the presynaptic architecture with an increased number of clathrin coat intermediates and a decrease in the releasable vesicle pool. This results in a functionally relevant synaptic transmission failure, particularly at higher frequencies. In knockout mice with amphiphysin deficiency, stimulus-dependent vesicle recycling is reduced, resulting in learning deficits and an increased susceptibility to seizures, consistent with reduced CNS inhibition. In humans, paraneoplastic stiff person syndrome (SPS) is an autoimmune disease associated with autoantibodies to amphiphysin. One of the symptoms is anxiety and a reduced function of GABAergic synapses in amygdalae of stiff patients has been recently reported, thus supporting the link between the presence of auto-Ab against BIN1 and the perturbation of GABAergic signaling. Defects in *BIN1* are the cause of centronuclear myopathy autosomal recessive, also known as autosomal recessive myotubular myopathy.	Mutations and/or CNVs affecting *BIN1* have never been reported in patients with ASD.	Folli *et al*., 1993 Geis *et al*., 2010 Wigge and McMahon, 1998

Tab. 4.1. Continued.

CCDC115(-) de novo Neurogenesis	This gene encodes the coiled-coil domain containing 115 protein. Expressed throughout the brain.	The *CCDC115* (or *CCP1*) gene has been recently identified downstream of Fibroblast Growth Factor 2 (FGF2) by microarray analysis. The *CCP1* transcript is up-regulated upon FGF2 stimulation in primary cortical neuron culture derived from mouse embryonic telencephalon at embryonic day 14.5 (E14.5) and in neuroblastoma cell line, SK-N-SH. *In situ* hybridizations revealed that *CCP1* is expressed in the ventricular zone (VZ), a region of the developing cerebral cortex known to be composed of progenitor cells undergoing proliferation. It has been demonstrated that forced *CCP1* expression in mouse embryonic fibroblast and neuroblastoma SK-N-SH cell line increased cell proliferation, whereas down-regulation of *CCP1* expression by siRNA reduced it, thus suggesting that CCP1 regulates cell number by promoting proliferation and suppressing cell death.	Mutations and/or CNVs affecting *CCDC115* have never been reported in patients with ASD.	McConnell and Kaznowski, 1991 Pellicano *et al.*, 2006, 2010
ERCC3(-) de novo DNA repair and transcription	This gene encodes the excision repair cross-complementing rodent repair deficiency, complementation group 3 protein. ERCC3 is an ATP-dependent DNA helicase that functions in nucleotide excision repair and complements xeroderma pigmentosum group B mutations. It also is the 89 kDa subunit of basal transcription factor 2 (TFIIH) and thus functions in class II transcription. Moderate expression in fetal brain and in postnatal CNS, in particular in amygdalae, thalamus and hypothalamus.	Defects in *ERCC3* are the cause of xeroderma pigmentosum complementation group B, also known as xeroderma pigmentosum group B combined with Cockayne syndrome (XP/CS), an autosomal recessive pigmentary skin disorder characterized by solar hypersensitivity of the skin, high predisposition for developing cancers on areas exposed to sunlight and, in some cases, neurological abnormalities. Some XP-B patients present features of Cockayne syndrome, including dwarfism, sensorineural deafness, microcephaly, MR, pigmentary retinopathy, ataxia, decreased nerve conduction velocities. Furthermore, defects in *ERCC3* are a cause of trichothiodystrophy photosensitive, an autosomal recessive disease characterized by sulfur-deficient brittle hair and nails, ichthyosis, MR, impaired sexual development, abnormal facies and cutaneous photosensitivity correlated with a nucleotide excision repair (NER) defect. In order to explore the link between the defective gene and the neurological deficits in XP/CS, the expression of *ERCC3* mRNA in developing mice by *in situ* hybridisation was studied. *ERCC3* was found to be ubiquitously expressed in cells from all regions and all developmental stages, from 9 day post-coitum embryo, to 15 day post-natal brain. In post-natal brain, regional differences in expression correlated with cell density and there was no evidence of cell specific or developmental alterations in levels of expression. It is possible that the neurological defects apparent in XP-B are likely to arise pleiotypically from the participation of ERCC3 in interactions with other elements involved in particular aspects of neurodevelopmental control.	Mutations and/or CNVs affecting *ERCC3* have never been reported in patients with ASD.	Hubank and Mayne, 1994 Oh *et al.*, 2006

Tab. 4.1. Continued.

FAM123C*(-)** ***de novo Neurodevelopment	This gene encodes the family with sequence similarity 123C protein, which belongs to the FAM123 family. High expression in cerebellum.	Recently, the characterizeation of the *Wtx*/*Amer* genes (*FAM123*) has been reported during mouse embryonic development. The three members of the family, namely *Amer1* (*FAM123A*), *Amer2* (*FAM123B*) and *Amer3* (*FAM123C*) are expressed in a highly overlapping manner, in particular concerning neuroectoderm derivatives, yet they also have distinct temporal and tissue-specific signatures. Amer genes share to a certain extent expression in neurons of both central and peripheral nervous systems, and in many neural crest derivatives including sensory cranial ganglia, dorsal root ganglia, autonomic ganglia, and branchial arches. This spatial and temporal overlap of gene expression patterns may suggest that one of the original functions of an AMER ancestor protein may have been related to the development of the nervous system.	Recently, a rare small (~450 kb unique sequence) recurrent deletion in a previously linked attention-deficit hyperactivity disorder (ADHD) locus at 2q21.1 has been identified in five unrelated families with developmental delay/ID, ADHD, EP and other neurobehavioral abnormalities. Moreover, reciprocal duplications have been identified in five unrelated families with autism, developmental delay, seizures and ADHD. The rearranged segment harbors five genes: *GPR148*, *FAM123C*, *ARHGEF4*, *FAM168B* and *PLEKHB2*. Patients with germline mutations in *AMER1* display, in addition to the sclerosing skeletal dysplasia, CNS malformations, and learning disabilities, pointing toward an important role of AMER1 during neurogenesis.	Comai *et al.*, 2010 Dharmadhikari *et al.*, 2012 Jenkins *et al.*, 2009
FAM168B* (*MANI*)(-)** ***de novo Neurodevelopment	This gene encodes the family with sequence similarity 168, member B protein, also known as MANI, myelin-associated neurite-outgrowth inhibitor. Good expression in fetal brain and high expression in postnatal CNS, in particular in the cortex.	The protein MANI (myelin-associated neurite-outgrowth inhibitor) has been recently described: it localizes to neural membranes, promotes differentiation into catecholaminergic neurons, and one of its interacting protein is the cell division cycle protein 27 (Cdc27). Furthermore, MANI retards neuronal axonal growth as a positive effector of the protein Cdc27 expression and activity, thus suggesting that the novel MANI-Cdc27-APC pathway may be an important cascade that prevents neurons from extending axons, and providing implications for the potential treatment of neurodegenerative diseases.	Recently, a rare small (~450 kb unique sequence) recurrent deletion in a previously linked attention-deficit hyperactivity disorder (ADHD) locus at 2q21.1 has been identified in five unrelated families with developmental delay/ID, ADHD, EP and other neurobehavioral abnormalities. Moreover, reciprocal duplications have been identified in five unrelated families with autism, developmental delay, seizures and ADHD. The rearranged segment harbors five genes: *GPR148*, *FAM123C*, *ARHGEF4*, *FAM168B* and *PLEKHB2*.	Dharmadhikari *et al.*, 2012 Mishra *et al.*, 2011
GPR17*(-)** ***de novo Neurodevelopment	This gene encodes the G protein-coupled receptor 17. Moderate expression in fetal brain and high expression in postnatal CNS, in particular in amygdalae, thalamus and hypothalamus.	GPR17 is restricted to oligodendrocyte lineage cells in the CNS in a developmentally regulated manner. As development progress, GPR17 expression is downregulated and oligodendrocyte myelination begins. *In vitro*, GPR17 overexpression not only blocks differentiation of neural progenitor cells into oligodendrocytes but also inhibits terminal differentiation of primary oligodendrocytes precursor cells (OPCs). In transgenic mice, sustained GPR17 overexpression in oligodendrocytes results in myelination arrest and oligodendrocyte loss. Conversely, *GPR17* deletion accelerates OPC maturation *in vitro* and leads to an early-onset of myelination in the developing CNS. Taken together these data suggest that GPR17 acts to negatively regulate oligodendrocyte differentiation and myelination.	Mutations and/or CNVs affecting *GPR17* have never been reported in patients with ASD.	Chen *et al.*, 2009

Tab. 4.1. Continued.

GPR39(-) ***de novo*** Intracellular signaling	This gene encodes the G protein-coupled receptor 39. This receptor mediates its action by association with G proteins that activate a phosphatidylinositol-calcium second messenger system. It is involved in regulation of body weight, gastrointestinal mobility, hormone secretion and cell death. Zn^{2+} acts as an agonist. Good expression in fetal brain and in postnatal thalamus and hypothalamus.	The effects of Zn^{2+} on synaptic plasticity and neuronal excitability, and the effects of Zn^{2+} deficiency on learning and memory in mice and humans have been reported. Moreover, a Zn^{2+}-dependent metabotropic activity in hippocampal CA3 neurons has been detected, which is probably mediated by GPR39 that shows a Zn^{2+} receptor activity. This activity in brain areas rich in synaptic Zn^{2+} may represent the longsought link between dynamic changes in extracellular Zn^{2+} and neuronal metabotropic signaling mediated by this metal.	Mutations and/or CNVs affecting *GPR39* have never been reported in patients with ASD.	Besser *et al.*, 2009 Cole *et al.*, 2000 Kodirov *et al.*, 2006 Lopantsev *et al.*, 2003 Smart *et al.*, 2004
GPR148(-) ***de novo*** Neurodevelopment?	This gene encodes the G protein-coupled receptor 148, which is an orphan receptor. Expression is restricted to nervous system and testis.		Recently, a rare small (~450 kb unique sequence) recurrent deletion in a previously linked attention-deficit hyperactivity disorder (ADHD) locus at 2q21.1 has been identified in five unrelated families with developmental delay/ID, ADHD, EP and other neurobehavioral abnormalities. Moreover, reciprocal duplications have been identified in five unrelated families with autism, developmental delay, seizures and ADHD. The rearranged segment harbors five genes: *GPR148*, *FAM123C*, *ARHGEF4*, *FAM168B* and *PLEKHB2*.	Dharmadhikari *et al.*, 2012
HS6ST1(-) ***de novo*** Heparan sulfate biosynthesis	This gene encodes the heparan sulfate 6-O-sulfotransferase 1, which is a member of the heparan sulfate biosynthetic enzyme family. This enzyme is a type II integral membrane protein and is responsible for 6-O-sulfation of heparan sulfate. Specifically expressed in fetal brain.	Heparan sulfate (HS) interactions with secreted morphogens such as fibroblast growth factors, hedgehogs, and Wnts are essential for embryonic development. Formation of biologically relevant HS structures is a result of the coordinated action of various biosynthetic enzymes, of which HS 6-O-sulfotransferases (6OST) catalyze the transfer of sulfate groups to the 6-O position of glucosamine residues in HS. Three 6OST isoforms have been described in the mouse whose expression has been mapped during mouse organogenesis. 6OST transcripts are differentially expressed in several sites where heparin-binding growth factors are critical for development. In particular, 6OST1 is predominantly transcribed in epithelial and neural-derived tissues, 6OST2 is more mesenchymal, whereas 6OST3 appears at later stages and in a more restricted manner. It has been reported that mutant mice lacking the heparan sulfotransferases Hs2st or Hs6st1 display major axon guidance defects at the developing optic chiasm and corpus callosum.	Mutations and/or CNVs affecting *HS6ST1* have never been reported in patients with ASD. The association of autism and other symptoms of mental impairment with multiple exostoses in patients carrying mutations in HS/HSPG genes has been reported. More recently, genetic association has been found between autism and the HS3ST5 gene encoding one of the HS 3-O sulfotransferases in two large cohorts of European ancestry. Furthermore, a genome-wide scan for rare CNVs in 996 autism cases has identified four independent CNVs in the GPC5/GPC6 gene cluster, which encodes the glypican-5 and glypican-6 HSPGs in tandem array, on chromosome 13q22.	Bolton *et al.*,1995 Conway *et al.*, 2011 Ethell and Yamaguchi, 1999 Hsueh and Sheng 1999 Inatani *et al.*, 2003 Irie *et al.*, 2012 Ishikawa-Brush *et al.*, 1997 Kantor *et al.*, 2004 Li *et al.*, 2002 Matsumoto *et al.*, 2007 Pinto *et al.*, 2010 Pratt *et al.*, 2006 Sedita *et al.*, 2004

Tab. 4.1. Continued.

LYPD1(-) de novo Neurodevelopment	This gene encodes the LY6/PLAUR domain containing 1 protein. High expression in fetal brain and in postnatal CNS.	A large-scale systematic search for secreted proteins identified the gene *LYPD1* that is encoded at the antisense DNA strand corresponding to the genomic locus of *GPR39*. *LYPD1* was found highly expressed in all brain regions tested, with the highest levels observed in amygdalae and septum. However, the expression in peripheral tissues was large enough to be readily detected, with the heart showing the highest expression outside the CNS. The possible role of *LYPD1* in regulation *GPR39* expression must be still clarified.	Mutations and/or CNVs affecting *LYPD1* have never been reported in patients with ASD.	Clark *et al.*, 2003 Egerod *et al.*, 2007 McKee *et al.*, 1997
MAP3K2(-) de novo Intracellular signaling: MAP kinase and NF-kappa B signaling pathways	This gene encodes the mitogen-activated protein kinase kinase kinase 2, which is a member of serine/threonine protein kinase family. This kinase preferentially activates other kinases involved in the MAP kinase signaling pathway. This kinase has been shown to directly phosphorylate and activate Ikappa B kinases, and thus plays a role in NF-kappa B signaling pathway. This kinase has also been found to bind and activate protein kinase C-related kinase 2, which suggests its involvement in a regulated signaling process. Moderate expression in fetal brain and in postnatal CNS. Moderate expression in immune cell types.	ERK5, the extracellular signal-regulated kinase 5, is highly expressed in developing neurons of the CNS and plays a critical role in their survival. ERK5 is activated by neurotrophins including brain-derived neurotrophic factor (BDNF) and MEK5 is known to mediate BDNF stimulation of ERK5 in CNS neurons. It has been reported in rat cortical neurons that BDNF induces a sustained activation of ERK5 and activates Rap1, a small GTPase, as well as MAP3K2 (MEKK2), a MEK5 kinase. MAP3K2 directly binds to SH2D2A, which is involved in the control of T-cell activation.	Mutations and/or CNVs affecting *MAP3K2* have never been reported in patients with ASD. NF-κB is an important gene transcriptional factor that mediates cellular responses in inflammation, immunity, development, cell proliferation and apoptosis. Elevated levels of NF-κB have been reported in autistic patients vs. controls.	Malik *et al.*, 2011 Naik *et al.*, 2011 Philippe *et al.*, 2012 Sun *et al.*, 2001 Wang *et al.*, 2006
MGAT5(-) de novo Glycoprotein oligosaccharide biosynthesis	This gene encodes the mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase, which belongs to the glycosyltransferase family. It catalyzes the addition of beta-1,6-N-acetylglucosamine to the alpha-linked mannose of biantennary N-linked oligosaccharides present on the newly synthesized glycoproteins. It is one of the most important enzymes involved in the regulation of the biosynthesis of glycoprotein oligosaccharides. Alterations of the oligosaccharides on cell surface glycoproteins cause significant changes in the adhesive or migratory behavior of a cell. Increase in the activity of this enzyme has been correlated with the progression of invasive malignancies. Moderate expression in fetal brain and in postnatal temporal lobe, amygdalae and thalamus.	As known, the CNS is rich in glycoconjugates, located on cell surface and in extracellular matrix. The products of Golgi UDP-GlcNAc:N-acetylglucosaminyltransferases (encoded by *Mgat1*, *Mgat2*, *Mgat4* and *Mgat5*) act sequentially to generate the GlcNAc-branched complex-type N-glycans on glycoprotein receptors. It has been demonstrated in mice that functional alterations of these enzymes cause behavioural changes, suggesting in humans a possible role in the neurobiology of feelings and behaviours. Indeed, *Mgat5*(-/-) mice are not different from their wild-type littermates in physical and neurological assessments, anxiety level, startle reactivity and sensorimotor gating. However, they displayed a robust decrease in the immobility time in the forced swim test and the tail suspension test independent of locomotor activity, interpreted as a reduction in depression-like behavior.	Mutations and/or CNVs affecting *MGAT5* have never been reported in patients with ASD. An association between symptoms of depression and a SNP near to *MGAT5* and *NCKAP5* has been recently replicated in two independent samples.	Luciano *et al.*, 2012 Soleimani *et al.*, 2008

Tab. 4.1. Continued.

★ ***NCKAP5(-) de novo*** Intracellular membrane trafficking: regulation of actin cytoskeleton dynamics	This gene encodes the NCK-associated protein 5. Expressed in fetal and adult brain, leukocytes and fetal fibroblasts.	Regulation of actin dynamics through the Nck/N-WASp (neural Wiskott-Aldrich syndrome protein)/Arp2/3 pathway is essential for organogenesis (including brain development), cell invasiveness, T-cell activation, and pathogen infection.	Mutations and/or CNVs affecting *NCKAP5* have never been reported in patients with ASD. A SNP in *NCKAP5* has been strongly associated with BD in a sample of American individuals of European ancestry. Moreover, an association between symptoms of depression and a SNP near to *MGAT5* and *NCKAP5* has been recently replicated in two independent samples. Recessive mutations affecting *NCKAP5L* have been recently identified in an autistic patient.	Chahrour *et al.*, 2012 Ditlev *et al.*, 2012 Luciano *et al.*, 2012 Smith *et al.*, 2009
PLEKHB2(-) de novo Intracellular signaling and trafficking	This gene encodes the pleckstrin homology domain containing, family B (evectins) member 2 protein. High expression in fetal brain and in postnatal CNS.	PLEKHB2 (EVT-2) works as a coupling factors between extracellular signals and intracellular membrane biosynthesis and trafficking. Its pleckstrin homology domain typically binds signaling phospholipids that are generated consequent to receptor activation. In mouse studies it has been found that EVT-2 is expressed throught fetal and adult brain, except for the white matter where EVT-1 shows a specific expression.	Recently, a rare small (~450 kb unique sequence) recurrent deletion in a previously linked attention-deficit hyperactivity disorder (ADHD) locus at 2q21.1 has been identified in five unrelated families with developmental delay/ID, ADHD, EP and other neurobehavioral abnormalities. Moreover, reciprocal duplications have been identified in five unrelated families with autism, developmental delay, seizures and ADHD. The rearranged segment harbors five genes: *GPR148*, *FAM123C*, *ARHGEF4*, *FAM168B* and *PLEKHB2*.	Dharmadhikari *et al.*, 2012 Dowler *et al.*, 2000 Krappa *et al.*, 1999
★ ***RAB6C(-) de novo*** Intracellular membrane trafficking: regulation of actin cytoskeleton dynamics	This gene encodes the RAB6C protein, which is a small GTPase and belongs to the RAS oncogene family. High expression in fetal brain and in postnatal CNS. High expression in immune cell types.	Several members of the Rab family small GTPases that are key mediators of membrane trafficking, regulate axon-specific trafficking events. For example, Rab17 regulates dendritic morphogenesis and postsynaptic development in mouse hippocampal neurons. Moreover, Rab17 mediates dendrite growth and branching and does not regulate axon growth or branching. Rab4 and Rab5 GTPases are key players in the regulation of endocytosis as recently demonstrated in astrocytes, the most abundant glial cells in the brain.	Mutations and/or CNVs affecting *RAB6C* have never been reported in patients with ASD.	Mori *et al.*, 2012 Potokar *et al.*, 2012

Tab. 4.1. Continued.

RAB3GAP1(-) ***de novo*** Intracellular membrane trafficking	This gene encodes the RAB3 GTPase activating protein, catalytic subunit 1, which forms a heterodimer with a non-catalytic subunit to specifically regulate the activity of members of the Rab3 subfamily of small G proteins. This protein mediates the hydrolysis of GTP bound Rab3 to the GDP bound form. Moderate expression in fetal brain and good expression in postnatal cortex, caudate nucleus, amygdalae, thalamus, and corpus callosum.	Mutations in this gene are associated with Warburg micro syndrome, an autosomal recessive disorder characterized by severe ID, microcephaly, congenital cataract, microcornea, microphthalmia, agenesis, or hypoplasia of the corpus callosum and hypogenitalism. Synaptic vesicles contain several Rab proteins, including four Rab3 isoforms: Rab3A, Rab3B, Rab3C, and Rab3D. Rab3 is essential for the normal dynamics of neurotransmitter release and to maintain the long-term plasticity include long-term potentiation in hippocampal mossy-fiber synapses, cerebellar parallel-fiber synapses, corticostriatal and corticothalamic synapses, and cortico–lateral amygdalae synapses. In particular, recently it has been reported that Rab3B is required for long-term depression of hippocampal inhibitory synapses which may contribute to learning and memory, presumably by stabilizing circuits established in previous learning processes.	Mutations and/or CNVs affecting *RAB3GAP1* have never been reported in patients with ASD.	Castro-Alamancos and Calcagnotto, 1999 Fourcaudot *et al.* 2008 Linden and Ahn, 1999 Nicoll and Malenka 1995 Nicoll and Schmitz 2005 Salin *et al.*, 1996 Schlüter *et al.*, 2004 Schlüter *et al.*, 2006 Spencer and Murphy 2002 Südhof ,2004 Tsetsenis *et al.*, 2011
TUBA3D(-) ***de novo*** Neurodevelopment: organization of microtubule cytoskeleton	This gene encodes the tubulin, alpha 3d protein, which is a member of the alpha tubulin family. Tubulin is a major component of microtubules, which are composed of alpha- and beta-tubulin heterodimers and microtubule-associated proteins in the cytoskeleton. Microtubules maintain cellular structure, function in intracellular transport, and play a role in spindle formation during mitosis. High expression in fetal brain and in postnatal CNS.	It has been demonstrated that prenatal exposure to cocain affects CNS development altering, for example, cytoskeleton organization through a down-regulation of *TUBA3D* expression.	Mutations and/or CNVs affecting *TUBA3D* have never been reported in patients with ASD.	Lee *et al.*, 2009
TUBA3E(-) ***de novo*** Neurodevelopment: organization of microtubule cytoskeleton	This gene encodes the tubulin, alpha 3e protein, which is a member of the alpha tubulin family. Tubulin is a major component of microtubules, which are composed of alpha- and beta-tubulin heterodimers and microtubule-associated proteins in the cytoskeleton. Microtubules maintain cellular structure, function in intracellular transport, and play a role in spindle formation during mitosis. High expression in fetal brain and in postnatal CNS.	By analogy with other members of the same family, TUBA3E may be involved in neurodevelopment.	Mutations and/or CNVs affecting *TUBA3E* have never been reported in patients with ASD.	

Tab. 4.1. Continued.

- **Patient 52, gain of 1.4 Mb at 7q11.23 (chr7:72726578-74139390)**

★ ***BAZ1B(+)*** ***de novo*** Chromatin remodeling	This gene encodes the bromodomain adjacent to zinc finger domain, 1B protein, which is a member of the bromodomain protein family. The bromodomain is a structural motif characteristic of proteins involved in chromatin-dependent regulation of transcription. This gene is deleted in Williams-Beuren syndrome, a developmental disorder caused by deletion of multiple genes at 7q11.23. Good expression in fetal brain and in postnatal CNS, in particular in cortex, thalamus and spinal cord. High expression in immune cell types.	BAZ1B (WSTF) is a MAPK-dependent phosphoprotein (tyrosine-protein kinase) that plays a central role in chromatin remodeling and acts as a transcription regulator. It is involved in DNA damage response by phosphorylating 'Tyr-142' of histone H2AX (H2AXY142ph). H2AXY142ph plays a central role in DNA repair and acts as a mark that distinguishes between apoptotic and repair responses to genotoxic stress. It has been reported that during Xenopus laevis embryonic development WSTF is expressed differentially in neural tissue, especially during neurulae stages in the eye, in neural crest cells and the brain.	*BAZ1B* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD. Heterozygote (*baz1b*/-) and homozygote (-/-) mouse models show craniofacial abnormalities and cardiac malformations but no behavioural anomalies.	Ashe *et al.*, 2008 Berg *et al.*, 2007 Challman *et al.*, 2003 Cus *et al.*, 2006 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Gosch and Pankau, 1994 Herguner and Mukaddes, 2006 Kirchoff *et al.*, 2007 Klein-Tasman *et al.*, 2009 Lincoln *et al.*, 2007 Osborne, 2010 Oya *et al.*, 2009 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009 Yoshimura *et al.*, 2009
BCL7B(+) ***de novo*** IgE autoantigen	This gene encodes the B-cell CLL/lymphoma 7B protein, which is a member of the BCL7 family including BCL7A, BCL7B and BCL7C proteins. BCL7B contains a region that is highly similar to the N-terminal segment of BCL7A or BCL7C proteins. The BCL7A protein is encoded by the gene known to be directly involved in a three-way gene translocation in a Burkitt lymphoma cell line. Low expression in fetal brain and good expression in postnatal whole brain, in particular in cortex, cerebellum, caudate nucleus, and amygdalae. High expression in immune cell types.	Haploinsufficiency of *BCL7B* may be the cause of certain cardiovascular and musculo-skeletal abnormalities observed in WBS. Moreover, BCL7B causes an allergic reaction in human as it acts as an IgE autoantigen in atopic dermatitis patients with severe skin manifestations. The BCL7B atopy-related IgE autoantigens have been detected in serum bound to IgE antibodies, thus suggesting that intracellular IgE autoantigens can become released after tissue damage and may occur as IgE immune complexes. Via binding to antigen presenting cells as well as to effector cells, IgE autoantigen immune complexes may contribute to exacerbation and/or perpetuation of severe atopic diseases even in the absence of exogenous allergens.	*BCL7B* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Gosch and Pankau, 1994 Herguner and Mukaddes, 2006 Kirchhoff *et al.*, 2007 Klein-Tasman *et al.*, 2009 Lincoln *et al.*, 2007 Natter *et al.*, 1998 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009
CLDN3(+) ***de novo*** CNS immunosurveillance: blood-brain barrier maturation through Wnt/β-catenin pathway	This gene encodes the claudin 3 protein, which is a member of the claudin family. CLDN3 is an integral membrane protein and a component of tight junction strands. Tight junctions represent one mode of cell-to-cell adhesion in epithelial or endothelial cell sheets, forming continuous seals around cells and serving as a physical barrier to prevent solutes and water from passing freely through the paracellular space. Low expression in fetal brain and in postnatal CNS, except for postnatal cerebellum and amygdalae where the expression is good.	CLDN3 is predominantly present in brain endothelial cells (ECs), where it plays a specific role in the establishment and maintenance of blood-brain barrier (BBB) tight junction morphology. A major pathway regulating brain development is the canonical Wnt/wingless pathway acting via β-catenin (β-cat) stabilization. This favors translocation of β-cat to the nucleus, where it binds to transcription factors of the lymphoid enhancer factor (Lef)/T cell factor (TCF) family, and thus modulates gene transcription. Endothelial Wnt/β-cat signaling regulates induction and maintenance of BBB characteristics during embryonic and postnatal development. Endothelial specific stabilization of β-cat *in vivo* enhances barrier maturation, whereas inactivation of β-cat causes significant down-regulation of claudin3 (Cldn3), upregulation of plamalemma vesicle-associated protein, and BBB breakdown.	*CLDN3* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.	Becanovic *et al.*, 2006 Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Gosch and Pankau, 1994 Herguner and Mukaddes, 2006 Kirchhoff *et al.*, 2007 Klein-Tasman *et al.*, 2009 Liebner *et al.*, 2008 Lincoln *et al.*, 2007 Moon, 2005 Nitta *et al.*, 2003 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009 Wolburg *et al.*, 2003

Tab. 4.1. Continued.

CLDN4*(+)** ***de novo CNS immunosurveillance: blood-brain barrier maturation	This gene encodes the claudin 4, which is an integral membrane protein that belongs to the claudin family. The protein is a component of tight junction strands and may play a role in internal organ development and function during pre- and postnatal life. Very low expression in fetal brain and in postnatal CNS, except for postnatal thalamus where the expression is good.	CLDN4 is a tight junction protein involved in the blood-brain barrier integrity. It has been reported that polymorphisms in *CLDN4* may act as modulators of the phenotype in murine Experimental Autoimmune Encephalomyelitis model, which mimics the pathophysiology of multiple sclerosis (MS). Indeed, the syntenic region in human (7q11.23) has displayed suggestive linkage to MS.	*CLDN4* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Gosch and Pankau, 1994 Haines *et al.*, 1996 Herguner and Mukaddes, 2006 Kirchhoff *et al.*, 2007 Klein-Tasman *et al.*, 2009 Lincoln *et al.*, 2007 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009 Wolburg *et al.*, 2003
CLIP2*(+)** ***de novo Intracellular trafficking: axon growth throught microtubule cytoskeleton dynamics	This gene encodes the CAP-GLY domain containing linker protein 2, which belongs to the family of cytoplasmic linker proteins, which have been proposed to mediate the interaction between specific membranous organelles and microtubules. This protein was found to associate with both microtubules and an organelle called the dendritic lamellar body, a membranous organelle predominantly present in bulbous dendritic appendages of neurons linked by dendrodendritic gap junctions. CLIP2 may operate in the control of brain-specific organelle translocations. High expression in fetal brain and in postnatal CNS.	CLIP protein enable neuronal polarization by controlling the stabilization of microtubules and growth cone dynamics.In particular, CLIP2 regulates the cytoskeleton through the microtubule network, having a role in cytoskeleton remodeling. It has been previously reported that mouse models of *CLIP2* exhibited some degree of hippocampal dysfunction as evidenced by deficits in contextual fear conditioning and altered synaptic plasticity. However, the recently reported *Clip2* hetrozygotes and homozygotes show impaired motor coordination on some tasks, but no differences in anxiety or amygdalae function, suggesting that CLIP2 may contribute to coordination problems in WS but not to the characteristic behavioral profile.	*CLIP2* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Gosch and Pankau, 1994 Herguner and Mukaddes, 2006 Hoogenraad *et al.*, 2002, 2004 Kirchhoff *et al.*, 2007 Klein-Tasman *et al.*, 2009 Lincoln *et al.*, 2007 Neukirchen and Bradke, 2011 Osborne, 2010 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009 Vandeweyer *et al.*, 2012
★ ***EIF4H*(+)** ***de novo*** Translation regulation	This gene encodes the eukaryotic translation initiation factor 4H, which functions to stimulate the initiation of protein synthesis at the level of mRNA utilization. Moderate expression in fetal brain and in postnatal CNS. High expression in in immune cell types.	Protein synthesis is a tightly regulated, energy-consuming process. The control of mRNA translation into protein is fundamentally important for the fine-tuning of gene expression; additionally, precise translational control plays a critical role in many cellular processes, including development, cellular growth, proliferation, differentiation, synaptic plasticity, memory, and learning. Knockout mice deficient in *Eif4h* have been recently generated. These mice display growth retardation with a significant reduction of body weight that began from the first week of postnatal development. Neuroanatomical profiling results revealed a smaller brain volume in null mice compared with controls as well as altered brain morphology, where anterior and posterior brain regions were differentially affected. The inactivation of *Eif4h* also led to a reduction in both the number and complexity of neurons. Behavioral studies revealed severe impairments of fear-related associative learning and memory formation, thus suggesting that Eif4h might contribute to certain deficits associated with Williams-Beuren syndrome.	*EIF4H* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD. Rare single gene mutations in *EIF4E* have been previously reported in a few autistic patients and their unaffected fathers.	Berg *et al.*, 2007 Capossela *et al.*, 2012 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Gosch and Pankau, 1994 Herguner and Mukaddes, 2006 Kirchhoff *et al.*, 2007 Klein-Tasman *et al.*, 2009 Lincoln *et al.*, 2007 Neves-Pereira *et al.*, 2009 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009

Tab. 4.1. Continued.

★ ***FZD9(+)*** ***de novo*** Intracellular Wnt signaling pathway	This gene encodes the frizzled family receptor 9, which is a receptor for Wnt signaling proteins. Most of frizzled receptors are coupled to the beta-catenin canonical signaling pathway, which leads to the activation of disheveled proteins, inhibition of GSK- 3 kinase, nuclear accumulation of beta-catenin and activation of Wnt target genes. FZD9 may be involved in transduction and intercellular transmission of polarity information during tissue morphogenesis and/or in differentiated tissues. Good expression in fetal brain and in postnatal CNS.	Heterozygote and homozygotes mouse models for *FZD9* show diminished seizure threshold, abnormal hippocampal structure, splenomegaly, thymic atrophy, developing B-cell depletion, impaired spatial learning and memory. Moreover, it has been demonstrated a role for Fzd9 signaling in lymphoid development, particularly at points where B cells undergo self-renewal prior to further differentiation.	*FZD9* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Gosch and Pankau, 1994 Herguner and Mukaddes, 2006 Kirchhoff *et al.*, 2007 Klein-Tasman *et al.*, 2009 Lincoln *et al.*, 2007 Osborne, 2010 Qiao *et al.*, 2009 Ranheim *et al.*, 2005 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009 Zhao *et al.*, 2005
★ ***GTF2I(-)**** ***de novo*** Transcriptional regulation	This gene encodes the general transcription factor II, which is a multifunctional phosphoprotein with roles in transcription and signal transduction. High expression in fetal brain and in postnatal CNS. High expression in immune cell types.	The *GTF2I* and *GTF2IRD1* paralogs encode two principal members of the TFII-I family of transcription factors. In humans haploinsufficiency of these two genes are linked to the facial dysmorphism and cognitive defects of Williams syndrome. Homozygous deletion of *Gtf2i* in mice causes lethality during embryonic development with neural tube closure defects and exencephaly, whereas heterozygous animals show no gross changes in brain structure or development. Furthermore, heterozygous animals show no alterations in learning and memory, including spatial memory, but show alterations in the recognition of novel objects as well as increased social interaction with unfamiliar mice, reminiscent of the hypersociability observed in WBS patients.	*GTF21* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD. Two SNPs in *GTF2I* have recently been associated with ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Herguner and Mukaddes, 2006 Klein-Tasman *et al.*, 2009 Kirchoff *et al.*, 2007 Licoln *et al.*, 2007 Makeyev and Bayarsaihan, 2009 Malenfant *et al.*, 2012 Osborne, 2010 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Sakurai *et al.*, 2011 Van der Aa *et al.*, 2009
GTF2IRD1(+) ***de novo*** Transcriptional regulation	This gene encodes the GTF2I repeat domain containing 1 protein, which contains five GTF2I-like repeats and each repeat possesses a potential helix-loop-helix (HLH) motif. It functions as a transcription factor or as a positive transcriptional regulator under the control of Retinoblastoma protein. Moderate expression in fetal brain and in postnatal CNS.	The Gtf2ird1 mouse showed several phenotypes that overlap with Williams-Beuren syndrome (WBS). Both heterozygous and homozygous mice exhibited increased social interaction, reduced aggression and anxiety and impaired amygdalae-based learning and memory, which correlates with the high sociability, lack of social anxiety and disinhibition seen in individuals with WBS. Their hippocampal function appeared to be intact and they had no problems with spatial tasks. Serotonin metabolism was also altered in the frontal cortex of these mice, and subsequent studies have demonstrated selectively enhanced serotonin receptor 1A-mediated responses in layer V pyramidal neurons of the pre-frontal cortex, suggesting altered neurophysiology.	*GTF2IRD1* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Herguner and Mukaddes, 2006 Klein-Tasman *et al.*, 2009 Kirchoff *et al.*, 2007 Licoln *et al.*, 2007 Osborne, 2010 Proulx *et al.*, 2010 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Schneider *et al.*, 2012 Van der Aa *et al.*, 2009 Young *et al.*, 2008

Tab. 4.1. Continued.

LAT2*(+)** ***de novo T-cell activation	This gene encodes the linker for activation of T cell family, member 2 protein. Low expression in fetal brain and in postnatal CNS. Very high expression in spleen, peripheral blood lymphocytes, and germinal centers of lymph nodes. Present in B-cells, NK cells and monocytes.	T cells are essential for the adaptive immune response to pathogens. However, dysfunctional T cell activity has been implicated in numerous diseases, including the failure of organ transplants, allergic reactions, asthma, autoimmune disorders, and coronary artery disease. T cell responses to pathogens require the induction of the primary activating receptor, the T cell receptor (TCR), along with other costimulatory and adhesion receptors. Signal transduction pathways activated downstream of these receptors drive T cell responses required for the immune response and disease progression. Upon stimulation of the TCR and other receptors, the LAT proteins are phosphorylated at several tyrosines residues on their cytoplasmic tails. This leads to the binding of SH2 domain-containing proteins and their associated molecules and the formation of large multiprotein complexes. These dynamic and highly regulated signaling complexes facilitate the production of second messengers, activate downstream pathways, induce actin cytoskeleton polymerization, and stimulate the activity of multiple transcription factors. Thus, signaling pathways from several receptors feed into LATs, which then integrates this information and selectively induces pathways critical for T cell activation and the adaptive immune response.	*LAT2* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.	Bartelt and Houtman, 2012 Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Herguner and Mukaddes, 2006 Klein-Tasman *et al.*, 2009 Kirchoff *et al.*, 2007 Licoln *et al.*, 2007 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009
LIMK1*(+)** ***de novo Neurodevelopment: axon growth throught actin cytoskeleton dynamics	This gene encodes the LIM domain kinase 1 protein. LIM domains are highly conserved cysteine-rich structures containing 2 zinc fingers. LIMK1 is a serine/threonine kinase that regulates actin polymerization via phosphorylation and inactivation of the actin binding factor cofilin. This protein is ubiquitously expressed during development and plays a role in many cellular processes associated with cytoskeletal structure. This protein also stimulates axon growth and may play a role in brain development. High expression in fetal brain and in postnatal CNS.	It has been recently reported a novel interaction between LIMK1 and TrkB, which is required for the BDNF induced axonal elongation. BDNF induces TrkB dimerization, thus leading to LIMK1 dimerization and transphosphorylation independent of TrkB kinase activity, which could further enhance the activation and stabilization of LIMK1. Moreover, activated LIMK1 translocates to membrane fraction and phosphorylates its substrate cofilin, thus promoted actin polymerization and axonal elongation. Homozygote mice for *LIMK1* show abnormal dendrite spine morphology, altered hippocampal function, mild deficit in spatial learning and memory. *Limk1*-null mice show altered dendritic spine morphology in pyramidal neurons, a phenomenon that has previously been associated with other genetic disorders involving ID, such as Down, fragile X and Rubinstein–Taybi syndromes.	*LIMK1* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Herguner and Mukaddes, 2006 Kaufmann and Moser, 2000 Klein-Tasman *et al.*, 2009 Kirchoff *et al.*, 2007 Licoln *et al.*, 2007 Meng *et al.*, 2002 Osborne, 2010 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009

Tab. 4.1. Continued.

STX1A*(+)** ***de novo Synaptic plasticity	This gene encodes the syntaxin 1A, which is a member of the syntaxin superfamily. Syntaxins are nervous system-specific proteins implicated in the docking of synaptic vesicles with the presynaptic plasma membrane. Syntaxins bind synaptotagmin in a calcium-dependent fashion and interact with voltage dependent calcium and potassium channels via the C-terminal H3 domain. This gene product is a key molecule in ion channel regulation and synaptic exocytosis. Very high expression in fetal brain and postnatal CNS.	STX1A is part of the SNARE core complex containing SNAP25, VAMP2 and STX1A. Hemizygous deletion of *STX1A* in mice did not produce any obvious behavioral or cognitive phenotype, but mice homozygous for a truncated form of *STX1A* had altered synaptic plasticity, related to hippocampal dysfunction.	*STX1A* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD. SNPs in *STX1A* have been associated with ASD and HF-AU. Moreover, in the postmortem anterior cingulate gyrus region of autistic patients, *STX1A* expression was found to be significantly lower than that of the control group. SNPs in *SNAP25* have been associated to hyperactivity phenotype in ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Fujiwara *et al.*, 2006 Ghezzo *et al.*, 2009 Gillberg and Rasmussen, 1994 Herguner and Mukaddes, 2006 Klein-Tasman *et al.*, 2009 Kirchoff *et al.*, 2007 Licoln *et al.*, 2007 McRory *et al.*, 2008 Nakamura. *et al.* 2008, 2011 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009
TRIM50*(-) ***de novo*** Intracellular signaling: protein ubiquitination pathway	This gene encodes the tripartite motif protein 50A, which is an E3 ubiquitin-protein ligase. Good expression in fetal brain and in postnatal cingulate cortex, cerebellum, amygdalae and spinal cord.	Trim50 specifically interacts with E2 ubiquitin-conjugating enzymes and autoubiquitinates, showing that it can act as an E3 ubiquitin ligase.Thus hemizygosity of the TRIM50 E3 ubiquitin ligase possibly plays a role in the WBS phenotype as the result of accumulation of specific TRIM50 target substrates. It is unclear how a defective TRIM50 E3 ligase activity could influence some of the clinical manifestations of WBS. The specific expression of *TRIM50* in stomach, intestine, liver and brain suggests a possible involvement of *TRIM50* haploinsufficiency in the gastrointestinal pathologies and/or the cognitive profile of WBS patients.	*TRIM50* maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD. The protein ubiquitination pathway has been previously implicated in ASD.	Berg *et al.*, 2007 Challman *et al.*, 2003 Depienne *et al.*, 2007 Gillberg and Rasmussen, 1994 Herguner and Mukaddes, 2006 Klein-Tasman *et al.*, 2009 Kirchoff *et al.*, 2007 Licoln *et al.*, 2007 Micale *et al.*, 2008 Qiao *et al.*, 2009 Reiss *et al.*, 1985 Van der Aa *et al.*, 2009
▪ **Patient 53, loss of 11.1 Mb at 2q31.3q32.3 (chr2:181882353-193007633)**				
FRZB*(-)** ***de novo Intracellular Wnt signaling	This gene encodes the frizzled-related protein (SFRP3), which is a secreted protein that is involved in the regulation of bone development. Good expression in fetal brain and moderate expression in postnatal cerebellum, caudate nucleus, thalamus and spinal cord.	Defects in *FRZB* are a cause of female-specific osteoarthritis susceptibility. Although *FRZB* is primarily expressed in the cartilaginous cores of the long bone during embryonic and fetal development and in the appendicular skeleton, a role in regulating Wnt signaling during fetal and postnatal brain development has been proposed. Indeed, it has been demonstrated that in postnatal mouse cerebral cortex *Wnt* genes as well as SFRPs (secreted Frizzled-related proteins) are expressed in gene-specific regional and lamina patterns in each of the major subdivisions of the cerebral cortex: the olfactory bulb, the hippocampal formation, and the neocortex. In particular, FRZB (sFRP3) binds to Wnt3a and acts as Wnt-antagonist regulating cell proliferation induced by Wnt signaling. Recently, epigenetic silencing of *FRZB* has been detected in medulloblastoma sample as well as in other tumors, suggesting that *SFRP3* functions as a tumor suppressor. For example in medulloblastoma, FRZB acts as a melanoma migration and invasion suppressor by interfering with Wnt5a signaling.	Mutations and/or CNVs affecting *FRZB* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Ekström *et al.*, 2011 IMGSAC, 2001 Rabionet *et al.*, 2004 Shimogori *et al.*, 2004 Wawrzak *et al.*, 2007

Tab. 4.1. Continued.

GLS(-) ***de novo*** CNS metabolism: glutamine catabolism	This nuclear gene encodes the glutaminase 2 protein, which is a mitochondrial phosphate-activated glutaminase that catalyzes the hydrolysis of glutamine to stoichiometric amounts of glutamate and ammonia. It plays an important role in the regulation of glutamine catabolism and promotes mitochondrial respiration and increases ATP generation in cells by catalyzing the synthesis of glutamate and alpha-ketoglutarate. In addition, it increases cellular anti-oxidant function via NADH and glutathione production and may play a role in preventing tumor proliferation. Very high expression in fetal brain and in postnatal CNS.	Recent research in ASD has aroused interest in anterior cingulate cortex and in the neurometabolite glutamate. Using single-voxel proton magnetic resonance spectroscopy *in vivo* it has been demonstrated in anterior cingulate cortex of 8 children with ASD a significant elevation of glutamate + glutamine (Glx) peak compared to 10 typically developing controls, who were well matched for age. The hyperglutamatergic state may reflect an imbalance of excitation over inhibition in the brain as proposed in recent neurodevelopmental models of ASD.	Mutations and/or CNVs affecting *GLS* have never been reported in patients with ASD. Anomalies in glutamate metabolism may be one of the causes for ASD development. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Bejjani *et al.*, 2012 IMGSAC, 2001 Rabionet *et al.*, 2004 Shimmura *et al.*, 2011
GULP1(-) ***de novo*** Neurodevelopment	This gene encodes the GULP, engulfment adaptor PTB domain containing 1 protein, which is an adapter protein necessary for the engulfment of apoptotic cells by phagocytes. Several transcript variants, some protein coding and some thought not to be protein coding, have been found for this gene. Good expression in fetal brain and moderate expression in postnatal CNS, except for cerebellum where the expression is low.	It has been demonstrated that GULP1 is present in human hippocampal and neocortical neurons, where it interacts with the low-density lipoprotein receptor-related protein 1, LRP1, suggesting a potential relevance in Alzheimer's disease (AD). Moreover, GULP1 binds to the amyloid-β A4 precursor protein (APP). APP and its secreted form, sAPP, contribute to the development of neurons in hippocampus, a brain region critical for learning and memory. GULP1 colocalizes with APP in the Golgi and endoplasmic reticulum, and alters trafficking and processing of APP.	Mutations and/or CNVs affecting *GULP1* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Beyer *et al.*, 2012 Billnitzer *et al.*, 2012 IMGSAC, 2001 Rabionet *et al.*, 2004
HIBCH(-) ***de novo*** CNS metabolism: amino acid degradation	This nuclear gene encodes the 3-hydroxyisobutyryl-CoA hydrolase protein, which is a mitochondrial enzyme responsible for hydrolysis of both HIBYL-CoA and beta-hydroxypropionyl-CoA. It is involved in L-valine catabolism. Moderate expression in fetal brain and in postnatal caudate nucleus, amygdalae, thalamus, corpus callosum, and spinal cord.	Mutations in this gene have been associated with 3-hyroxyisobutyryl-CoA hydrolase deficiency. Defects in *HIBCH* are the cause of HIBCH deficiency, also known as deficiency of beta- hydroxyisobutyryl CoA deacylase or methacrylic aciduria. The enzyme defect results in accumulation of methacrylyl-CoA, a highly reactive compound, which readily undergoes addition reactions with free sulfhydryl groups. Affected individuals showed delayed development of motor skills, hypotonia, initial poor feeding, and a deterioration in neurological function during first stages of life. Recently, it has been reported the identification of six potential regulators of synaptic and axonal degeneration *in vivo*, including *HIBCH*, using mutant Drosophila lines, thus suggestimg that pathways not previously linked to synaptic formation, such as valine catabolism, may be involved in neurodevelopment.	Mutations and/or CNVs affecting *HIBCH* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	IMGSAC, 2001 Loupatty *et al.*, 2007 Rabionet *et al.*, 2004 Wishart *et al.*, 2012

Tab. 4.1. Continued.

INPP1*(-)** ***de novo Intracellular phosphatidylinositol signaling	This gene encodes the inositol polyphosphate-1-phosphatase protein, which is one of the enzymes involved in phosphatidylinositol signaling pathway. This enzyme removes the phosphate group at position 1 of the inositol ring from the polyphosphates inositol 1,4-bisphosphate and inositol 1,3,4-trisphophosphate. Good expression in fetal brain and in postnatal CNS.	Inositol and phosphatidylinositol phosphates are important for numerous cellular processes: neuronal survival, differentiation, neuroprotection, and transduction of signals from growth factors, neurotransmitters, and G protein coupled receptors.	Mutations and/or CNVs affecting *INPP1* have never been reported in patients with ASD. SNPs in genes implicated in phosphatidylinositol signaling pathway, suc as *INPP1*, *PIK3CG*, and *TSC2* have been previously associated with ASD, suggesting that phosphatidylinositol signaling may have a role in susceptibility to autism. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Berridge, 1993 Czech, 2000 Delmas *et al.*, 2002 IMGSAC, 2001 Rabionet *et al.*, 2004 Serajee *et al.*, 2003
ITGA4*(-)** ***de novo Neuroinflammation : focal adesion, leukocyte transendothelial migration	This gene encodes the integrin, alpha 4 protein, which belongs to the integrin alpha chain family of proteins. Integrins are heterodimeric integral membrane proteins composed of an alpha chain and a beta chain. This gene encodes an alpha 4 chain. Unlike other integrin alpha chains, alpha 4 neither contains an I-domain, nor undergoes disulfide-linked cleavage. Good expression in fetal brain and in postnatal CNS, in particular in thalamus and corpus callosum. Very high expression in immune cell types.	In experimental autoimmune encephalomyelitis, a model for multiple sclerosis, it has been recently reported that *in vitro* migration of CD8(+) T lymphocytes across blood-brain barrier-endothelial cells is dependent on α4 integrin, which is considered a mediator of neuroinflammation.	Mutations and/or CNVs affecting *ITGA4* have never been reported in patients with ASD. SNPs in *ITGA4* have been associated with ASD. Moreover, a positive association was found between one of these SNP markers and levels of a serum autoantibody directed to brain tissue, which was previously shown to be significantly more frequent in autistic patients than in age-matched controls, thus suggesting that *ITGA4* could be involved in a neuroimmune process thought to occur in autistic patients. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Conroy *et al.*, 2009 Correia *et al.*, 2009 Ifergan *et al.*, 2011 IMGSAC, 2001 Rabionet *et al.*, 2004
NAB1*(-)** ***de novo Transcriptional regulation	This gene encodes the NGFI-A binding protein 1, which acts as a transcriptional repressor for zinc finger transcription factors EGR1 and EGR2. Moderate expression in fetal brain and good expression in postnatal CNS, in particular in cortex, cerebellum, corpus callosum and spinal cord.	The early growth response protein 1, EGR1, is a transcriptional switch that regulates a number of diverse gene targets. It is strongly expressed in neurons in the adult brain, and its levels are altered by neurotransmitter release and neuronal activation. It can also exert long-lasting changes in gene expression and subsequent protein synthesis that mediate synaptic plasticity. EGR1 has been implicated in mediating a variety of behaviors that are dysregulated in MDD, including learning and memory, fear conditioning, drug addiction, and social interaction. The early growth response gene 2 (*EGR2*) is one of the susceptibility loci in BD. EGR2 is involved in cognitive function, myelination, and signal transduction related to neuregulin-ErbB receptor, Bcl-2 family proteins, and brain-derived neurotrophic factor. SNPs in *EGR2* have been associated to BD in a Korean sample.	Mutations and/or CNVs affecting *NAB1* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Cole *et al.*, 1989 IMGSAC, 2001 Kerman *et al.*, 2012 Kim *et al.*, 2012 Knapska and Kaczmarek, 2004 Malkani *et al.*, 2004 Rabionet *et al.*, 2004 Ressler *et al.*, 2002 Stack *et al.*, 2010 Valjent et al., 2006

Tab. 4.1. Continued.

NCKAP1(-) de novo Neuronal differentation: actin cytoskeleton organization	This gene encodes the NCK-associated protein 1. Very high expression in fetal brain and in postnatal CNS.	NCKAP1 (Nap1) is an adaptor protein that is thought to modulate actin nucleation by forming a pentameric complex with WAVE, PIR121, Abi1/2 and HSPC300. It is selectively expressed in the cortical plate region of the developing cortex, where neurons terminate their migration and begin their final laminar specific differentiation, characterized by the elaboration of distinct axonal and dendritic architecture. Functional analysis of Nap1 indicate that Nap1-mediated cytoskeletal rearrangements in the emerging cortical plate play an essential role in cortical neuronal differentiation underlying the formation of functional connectivity in cerebral cortex.	Mutations and/or CNVs affecting *NCKAP1* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Baumgartner *et al.*, 1995 Bladt *et al.*, 2003 Bogdan and Klambt, 2003 Hummel *et al.*, 2000 IMGSAC, 2001 Rabionet *et al.*, 2004 Soto *et al.*, 2002 Stradal *et al.*, 2004 Suzuki *et al.*, 2000 Yokota *et al.*, 2007
NEUROD1(-) de novo Transcriptional regulation	This gene encodes the neurogenic differentiation 1 protein, which is a member of the NeuroD family of basic helix-loop-helix (bHLH) transcription factors. The protein forms heterodimers with other bHLH proteins and activates transcription of genes that contain a specific DNA sequence known as the E-box. It regulates expression of the insulin gene, and mutations in this gene result in type II diabetes mellitus. Very high expression in fetal brain and in postnatal CNS.		Mutations and/or CNVs affecting *NEUROD1* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	IMGSAC, 2001 Rabionet *et al.*, 2004
ORMDL1(-) de novo Neurodevelopment?	This gene encodes the ORM1-like 1 (S. cerevisiae) protein, which is a negative regulator of sphingolipid synthesis. Expressed in brain.	By using an antibody specific for the C-terminus the expression of ORMDL1 (adoplin-1) has been determined in cultured neuronal cells and human brain tissues. Immunohistochemical analyses disclosed that adoplin proteins are primarily expressed in the neurons of cerebral cortices. Moreover, adoplin is localized mainly in the cell bodies and neurites of primary cortical neurons, which shows possible role(s) in nerve cells.	Mutations and/or CNVs affecting *ORMDL1* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Araki *et al.*, 2008 IMGSAC, 2001 Rabionet *et al.*, 2004
PDE1A(-) de novo Intracellular cAMP signaling	This gene encodes the phosphodiesterase 1A, calmodulin-dependent protein. Cyclic nucleotide phosphodiesterases (PDEs) play a role in signal transduction by regulating intracellular cyclic nucleotide concentrations through hydrolysis of cAMP and/or cGMP to their respective nucleoside 5-prime monophosphates. Good expression in fetal brain and in postnatal CNS, in particular in amygdalae, corpus callosum, and spinal cord.	It has been reported that intracranial self-stimulation to the murine lateral hypothalamus, a memory improving treatment, results in hippocampal changes in gene expression. For example, the *PDE1A* gene is overexpressed, suggesting a role of the intracellular calcium signaling in the development and improvement of cognitive function, by promoting neuroplasticity.	Mutations and/or CNVs affecting *PDE1A* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Huguet *et al.*, 2009 IMGSAC, 2001 Rabionet *et al.*, 2004

Tab. 4.1. Continued.

STAT1(-) de novo Modulation of neuroinflammation: intracellular STAT signaling	This gene encodes the signal transducer and activator of transcription 1.91kDa protein, which is a member of the STAT protein family. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein can be activated by various ligands including interferon-alpha, interferon-gamma, EGF, PDGF and IL6. This protein mediates the expression of a variety of genes, which is thought to be important for cell viability in response to different cell stimuli and pathogens. Moderate expression in fetal brain and in postnatal CNS. High expression in immune cell types.	The strong inflammatory response observed in neurodegenerative diseases can depend on the impairment of the endogenous control of microglial activation, triggering the release of potentially detrimental factors such as cytokines, nitric oxide (NO) and superoxide anion (O_2^-). By studying microglial and mixed glial cell cultures activation from neonatal rats after exposure to IFN-gamma and/or IL-1beta and TNF-alpha, it has been proposed that IL-1beta modulates IFN-gamma-induced production of oxidative molecules through cross talk between STAT1 and MAPK pathways, regulating the amplitude and duration of microglial activation. Modulation of ERK was observed at 30 min, whereas inhibition of pSTAT was observed later (at 4 h), indicating that it was an early and transient phenomenon. Mutations in *STAT1* causes the mendelian susceptibility to mycobacterial disease (autosomal dominant, recessive and X-linled), and the familial candidiasis type 7(autosomal dominant).	Mutations and/or CNVs affecting *STAT1* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	IMGSAC, 2001 Rabionet *et al.*, 2004 Tichauer *et al.*, 2007

Tab. 4.1. Continued.

STAT4(-) de novo Modulation of neuroinflammation: intracellular STAT signaling	This gene encodes the signal transducer and activator of transcription 4 protein, which is a member of the STAT family of transcription factors. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein is essential for mediating responses to IL12 in lymphocytes, and regulating the differentiation of T helper cells. Low expression in fetal brain and good expression in postnatal cortex, amygdalae and thalamus.	Genetic variations in *STAT4* are a cause of susceptibility to systemic lupus erythematosus and rheumatoid arthritis. Inflammatory cytokines are implemented in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. Moreover, the glia maturation factor (GMF), a brain protein, induces expression of proinflammatory cytokine/chemokine in the central nervous system, and GMF-deficient (knockout) mice are relatively resistant to EAE development after immunization with encephalitogenic MOG peptide. The expression evaluation of six murine *STAT* genes, which are known to regulate the cytokine-dependent signal transduction pathways in autoimmune inflammation, in the brains and spinal cords of wild type and GMF-knockout mice, revealed that the expressions of *STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5*, and *STAT6* genes were significantly upregulated in the wild type mice exhibiting EAE symptoms. Therefore, a significant suppression of STATs expression in GMF-knockout mice suggests GMF as an upstream effector of JAK/STAT signaling. In order to identify the molecular mechanisms underlying the pathological processes in multiple sclerosis, the gene expression profile in non-lesion containing tissue, the so-called normal-appearing white matter, has been studied. Genes known to be involved in anti-inflammatory and protective mechanisms such as *STAT6*, *JAK1*, *IL-4R*, *IL-10*, *Chromogranin C* and *Hif-1alpha* are consistently upregulated in the multiple sclerosis NAWM, and are mainly expressed in oligodendrocytes. On the other hand, genes involved in pro-inflammatory mechanisms, such as *STAT4*, *IL-1beta* and *MCSF*, were also upregulated but less regularly. *STAT4* expression was detected predominantly in microglia. Therefore, the upregulation of genes involved in anti-inflammatory mechanisms driven by oligodendrocytes may protect the CNS environment and thus limit lesion formation, whereas the activation of pro-inflammatory mechanisms in microglia may favour disease progression.	Mutations and/or CNVs affecting *STAT4* have never been reported in patients with ASD. A previous genomewide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	IMGSAC, 2001 Rabionet *et al.*, 2004 Zaheer *et al.*, 2007 Zeis *et al.*, 2008

Tab. 4.1. Continued.

TMEFF2(-) ***de novo*** Neurodevelopment	This gene encodes thetransmembrane protein with EGF-like and two pollistatin-like domains. High expression in fetal brain and in postnatal CNS, with highest level in amygdalae and corpus callosum.	TMEFF2 is a putative transmembrane protein whose survival effect has been measured using primary cultured neurons from several regions of fetal rat brain following treatment with a recombinant TMEFF2 protein fragment consisting of the putative extracellular domain. It has been reported that TMEFF2 increased survival of neurons from the hippocampus and midbrain, but not from the cerebral cortex, indicating that the survival effects of TMEFF2 are specific to certain cell types. Recombinant TMEFF2 also promoted survival of mesencephalic dopaminergic neurons. Furthermore, using *in situ* hybridization analysis it has been found that both *TMEFF* genes are widely expressed in rat brain, although they exhibit different patterns of expression, suggesting that they have specific roles in the CNS. In particular, TMEFF2 is highly expressed in the medial habenular, CA2, CA3 and dentate gyrus region of the hippocampus, corpus callosum, cerebellar cortex and cranial nerve nuclei (III, IV, VII, X, XII).	Mutations and/or CNVs affecting *TMEFF2* have never been reported in patients with ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Horie *et al.*, 2000 IMGSAC, 2001 Kanemoto *et al.*, 2001 Rabionet *et al.*, 2004
UBE2E3(-) ***de novo*** Intracellular signaling: protein ubiquitination pathway	This gene encodes the ubiquitin-conjugating enzyme E2E3. Ubiquitination involves at least three classes of enzymes: ubiquitin-activating enzymes, or E1s, ubiquitin-conjugating enzymes, or E2s, and ubiquitin-protein ligases, or E3s. This gene encodes a member of the E2 ubiquitin-conjugating enzyme family. Good expression in fetal brain and moderate expression in postnatal CNS.	Mutations and/or CNVs involving other E3 ligase genes have been reported in patients with ASD.	Mutations and/or CNVs affecting *UBE2E3* have never been reported in patients with ASD. The protein ubiquitination pathway has been previously implicated in ASD. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Bonati *et al.*, 2007 Glessner *et al.*, 2009 IMGSAC, 2001 Kishino *et al.*, 1997 Matsuura *et al.*, 1997 Rabionet *et al.*, 2004 Sahoo *et al.*, 2006 Scheuerle and Wilson, 2011 Smith *et al.*, 2011 Trillingsgaaard and Østergaard, 2004
ZNF804A(-) ***de novo*** Transcriptional regulation	This gene encodes the zinc finger protein 804A. High expression in fetal brain, especially in the developing hippocampus and the cortex, and good expression in postnatal CNS, in particular in adult cerebellum.		A CNV affecting *ZNF804A* has been recently reported in a patient with ASD. SNP in *ZNF804A* (rs1344706) has been associated to a higher risk to develop BD and SCZ, affecting both brain volume and neural connettivity. A previous genome-wide screen for autism reported strong evidence for linkage to chromosomes 2q (in particular 2q32), which was subsequently confirmed considering a region of 20-30 cM at 2q31q33.	Griswold *et al.*, 2012 IMGSAC, 2001 Rabionet *et al.*, 2004 Johnson *et al*, 2009 Lencz *et al.*, 2010 O'Donovan *et al.*, 2008 Owen *et al.*, 2009 Rabionet *et al.*, 2004 Talkowski *et al.*, 2012 Wassink *et al.*, 2012

Tab. 4.1. Continued.

▪ **Patient 57, gain of 3.8 Mb at 5q31.1q31.2 (chr5:133871536-137708167)**				
★ ***CAMLG(+)*** ***de novo*** Synaptogenesis and synaptic plasticity	This gene encodes the calcium modulating ligand protein. The immunosuppressant drug cyclosporin A blocks a calcium-dependent signal from the T-cell receptor (TCR) that normally leads to T-cell activation. When bound to cyclophilin B, cyclosporin A binds and inactivates the key signaling intermediate calcineurin. The protein encoded by this gene functions similarly to cyclosporin A, binding to cyclophilin B and acting downstream of the TCR and upstream of calcineurin by causing an influx of calcium. This integral membrane protein appears to be a new participant in the calcium signal transduction pathway, implicating cyclophilin B in calcium signaling, even in the absence of cyclosporine. High expression in fetal brain and in postnatal CNS, in particular in caudate nucleus and amygdalae. High expression in immune cell types.	CAMLG was first identified as a cyclophilin B binding protein and shown to implicate cyclophilin B in the calcium signal transduction pathway in T cell activation. CAMLG is reported to be involved in recycling and endocytic processing of GABAA receptors. In a cortical culture it has been demonstrated that the reduction of CAMLG translated into reduced GABAA receptors on the postsynaptic membrane with an effect specific to GABAA receptors since glutamate evoked current remained unaltered in these neurons. CAMLG binds to Tmub1, which is a protein containing a ubiquitin-like domain highly expressed in the nervous system. These proteins could work in concert to regulate cycling of receptors, such as GABA and glutamate receptors, to synaptic membranes.	Mutations and/or CNVs affecting *CAMLG* have never been reported in patients with ASD.	Bram and Crabtree, 1994 Yuan *et al.*, 2008 Zhang *et al.*, 2010
C5orf20 ***(DCNP1)(+)*** ***de novo*** T-cell activation, regulation of the corticotropin-releasing hormone in the brain	This gene encodes the dendritic cell nuclear protein 1. It is specifically expressed in dendritic cells (DCs), which are potent antigen-presenting cells involved in activating naive T cells to initiate antigen-specific immune response. Moderate expression in fetal brain and good expression in postnatal CNS, in particular in thalamus and hypothalamus. Good expression in dendritic cells.	*In situ* hybridization experiments have recently found a widespread distribution of *DCNP1* expression in the human brain at both the mRNA and protein levels as well as in the microglial cells. Furthermore, *DCNP1* is higher expressed in neurons of the paraventricular nucleus and supraoptic nucleus in patients with depression compared with controls. DCNP1 may play a role in the pathogenesis of the depressive disorder by upregulating the CRH (corticotropin-releasing hormone) promoter in the paraventricular nucleus. CRH neurons are the driving force for the hypothalamic-pituitary-adrenal axis, which is the final common pathway for the stress response and also a crucial system in the pathogenesis of depression.	Mutations and/or CNVs affecting *C5orf20* have never been reported in patients with ASD. A 1.2 Mb region of chromosome 5q31, including *DCNP1*, was previously identified in a genome-wide linkage analysis for autism. *DCNP1* has been proposed to be a novel candidate gene for major depression and this finding has been recently replicated.	Bosker *et al.*, 2011 Philippi *et al.*, 2005 Willis-Owen *et al.*, 2006 Zhou *et al.*, 2010
★ ***CDC23(+)*** ***de novo*** Intracellular signaling: protein ubiquitination pathway	This gene encodes the cell division cycle 23 homolog (S. cerevisiae) protein, which is essential for cell cycle progression through the G2/M transition. This protein is a component of anaphase-promoting complex (APC), which is composed of eight protein subunits and highly conserved in eukaryotic cells. Moderate expression in fetal brain and low expression in postnatal CNS. High expression in immune cell types.	CDC23 is a component of the anaphase promoting complex/cyclosome (APC/C), a cell cycle-regulated E3 ubiquitin ligase that controls progression through mitosis and the G1 phase of the cell cycle. The APC/C complex acts by mediating ubiquitination and subsequent degradation of target proteins. In particular, APC catalyzes the formation of cyclin B-ubiquitin conjugate that is responsible for the ubiquitin-mediated proteolysis of B-type cyclins. In Drosophila loss of zygotic expression of *cdc23* causes defects in the proliferation of brain neuroblasts and results in the absence of identified neuronal lineages in the central and peripheral nervous systems.	Mutations and/or CNVs affecting *CDC23* have never been reported in patients with ASD. The protein ubiquitination pathway has been previously implicated in ASD.	Glessner *et al.*, 2009 Kishino *et al.*, 1997 Matsuura *et al.*, 1997 Zhang *et al.*, 1991

Tab. 4.1. Continued.

★ ***CDC25C(+)*** ***de novo*** Intracellular signaling: regulation of cell cycle progression	This gene encodes the cell division cycle 25 homolog C (S. pombe) protein, is highly conserved during evolution and it plays a key role in the regulation of cell division. The encoded protein is a tyrosine phosphatase and belongs to the Cdc25 phosphatase family. It directs dephosphorylation of cyclin B-bound CDC2 and triggers entry into mitosis. It is also thought to suppress p53-induced growth arrest. Moderate expression in fetal brain and low expression in postnatal CNS, except for cortex where expression is high. High expression in immune cell types.	CDC25C functions as a dosage-dependent inducer in mitotic control, directly dephosphorylating CDK1 and activating its kinase activity. In early animal development, cell proliferation and differentiation are tightly linked and coordinated. In Xenopus laevis, four isoforms of cdc25 have been identified: cdc25A, cdc25B, cdc25C and cdc25D. These isoforms show a specific temporal and spatial expression: cdc25A and cdc25C are expressed both maternally and zygotically, whereas cdc25B and cdc25D are expressed zygotically. Both cdc25A and cdc25C are expressed mainly in prospective neural regions, whereas cdc25B is expressed preferentially in the CNS, such as the spinal cord and the brain. Interestingly, cdc25D is expressed in the epidermal ectoderm of the late-neurula embryo, and in the liver diverticulum endoderm of the mid-tailbud embryo.	Mutations and/or CNVs affecting *CDC25C* have never been reported in patients with ASD.	Nakajo *et al*., 2011
CXCL14(+) ***de novo*** Homeostasis of monocyte-derived macrophages, synaptic plasticity mediated by chemokine signaling	This gene encodes the chemokine (C-X-C motif) ligand 14 protein. It belongs to the cytokine gene family which encode secreted proteins involved in immunoregulatory and inflammatory processes. The protein encoded by this gene is structurally related to the CXC (Cys-X-Cys) subfamily of cytokines. It has been implicated that this cytokine is involved in the homeostasis of monocyte-derived macrophages rather than in inflammation. Moderate expression in fetal brain and high expression in postnatal CNS, except for cerebellum. Very low expression in immune cell types.	CXCL14 is a member of the CXC chemokine family. CXCL14 possesses chemoattractive activity for activated macrophages, immature dendritic cells and natural killer cells. *CXCL14*-deficient mice do not exhibit clear immune system abnormalities, suggesting that the function of CXCL14 can be compensated for by other chemokines. It has been recently reported that CXCL14 protein is present in a subset of hypothalamic neurons, thus suggesting its participation in hypothalamic functions such as control of autonomic nervous systems and/or in immune cell recruitment via the median eminence. During mouse development, *CXCL14* is not expressed in the nervous system prior to birth. Postnatally, *CXCL14* is highly expressed in many regions of the brain, including the cortex, basal ganglia, septum and hippocampus. In particular, in the hippocampal dentate gyrus (DG) CXCL14 is expressed by GABAergic interneurons, where it inhibits GABAergic transmission to nestin-EGFP-expressing neural stem/progenitor cells in the adult DG. In contrast CXCL12 enhanced the effects of GABA at these same synapses. Moreover, recent evidence revealed that CXCL14 participates in glucose metabolism, feeding behaviour-associated neuronal circuits, and anti-microbial defense	Mutations and/or CNVs affecting *CXCL14* have never been reported in patients with ASD. A 1.2 Mb region of chromosome 5q31, including CXCL14, was previously identified in a genome-wide linkage analysis for autism.	Banisadr *et al*., 2011 Hara and Tanegashima, 2012 Philippi *et al*., 2005 Yamamoto *et al*., 2011

Tab. 4.1. Continued.

DDX46(+) ★ ***de novo*** RNA metabolism	This gene encodes the DEAD (Asp-Glu-Ala-Asp) box polypeptide 46, which is a member of the DEAD box protein family. DEAD box proteins are putative RNA helicases implicated in a number of cellular processes involving alteration of RNA secondary structure, such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly. Based on their distribution patterns, some members of this family are believed to be involved in embryogenesis, spermatogenesis, and cellular growth and division. High expression in fetal brain and moderate expression in postnatal CNS. High expression in immune cell types.	The DExD/H-box RNA helicase family is a large protein group characterized by the presence of a helicase domain that is highly conserved from bacteria to humans. These proteins have been shown to play important roles in all aspects of RNA metabolism: pre-mRNA splicing, rRNA biogenesis, transcription, RNA stability and turnover, RNA export, and translation. It has been recently reported that a mutation in *Ddx46* is responsible for defects in the digestive organs and brain of the zebrafish mutant. Indeed, Ddx46 is specifically expressed in the digestive organs and brain of zebrafish and is required for premRNA splicing in these organs.	Mutations and/or CNVs affecting *DDX46* have never been reported in patients with ASD. A 1.2 Mb region of chromosome 5q31, including *DDX46*, was previously identified in a genome-wide linkage analysis for autism. A rare CNV affecting *DDX53* has been reported in a patient with ASD	Bleichert and Baserga, 2007 Hozumi *et al.*, 2012 Jankowsky, 2011 Philippi *et al.*, 2005 Pinto *et al.*, 2010 Rocak and Linder, 2004 Silverman *et al.*, 2003
FAM13B(+) ★ ***de novo*** Unknown function	This gene encodes the family with sequence similarity 13, member B protein, which shows an unknown function. High expression in fetal brain and in postnatal prefrontal cortex, amygdalae, and thalamus. High expression in immune cell types.		Mutations and/or CNVs affecting *FAM13B* have never been reported in patients with ASD.	
FAM53C(+) ★ ***de novo*** Unknown function	This gene encodes the family with sequence similarity 53, member C protein, which shows an unknown function. Good expression in fetal brain and in postnatal CNS. High expression in immune cell types.		Mutations and/or CNVs affecting *FAM53C* have never been reported in patients with ASD.	
FBXL21(+) ***de novo*** Intracellular signaling: protein ubiquitination pathway	This gene encodes the F-box and leucine-rich repeat protein 21, which is a member of the F-box protein family that is characterized by an approximately 40 amino acid motif, the F-box. The F-box proteins constitute one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination. Moderate expression in fetal brain and in postnatal parietal and occipital lobes, cerebellum, amygdalae, and spinal cord.	The *FBXL21* gene encodes an F-box containing protein, which is a component of the SCF (SKP1-cullin-F-box) ubiquitin protein ligase complex. The SCF complex is involved in the phosphorylation-dependent ubiquitination of targeted proteins, leading to the degradation of the targeted proteins. The function of the F-box protein in the SCF complex is to target specific proteins. Several neuron specific F-box proteins have been previously identified, including FBL2 and Parkin that are involved in NMDA receptor degradation and Parkinson's disease respectively. Furthermore, multiple linkage studies implicated the long arm of chromosome 5 as harboring susceptibility genes for SCZ.	Mutations and/or CNVs affecting *FBXL21* have never been reported in patients with ASD. A 1.2 Mb region of chromosome 5q31, including *FBXL21*, was previously identified in a genome-wide linkage analysis for autism. The protein ubiquitination pathway has been previously implicated in ASD. SNPs in *FBXL21* have been associated with SCZ in two Irish samples.	Cardozo and Pagano, 2004 Chen *et al.*, 2008 Glessner *et al.*, 2009 Gurling *et al.*, 2001 Kishino *et al.*, 1997 Kato *et al.*, 2005 Liao *et al.*, 2004 Matsuura *et al.*, 1997 Noda *et al.*, 2005 Paunio *et al.*, 2001 Philippi *et al.*, 2005 Sklar *et al.*, 2004

Tab. 4.1. Continued.

★ ***H2AFY(+)*** ***de novo*** Chromatin remodeling	This gene encodes the H2A histone family, member Y. This gene encodes a member of the histone H2A family. It replaces conventional H2A histones in a subset of nucleosomes where it represses transcription and participates in stable X chromosome inactivation. Moderate expression in fetal brain and in postnatal CNS. Very high expression in imuune cell types.	Histones play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. In particular, H2AFY inhibits the binding of transcription factors, interferes with the activity of remodeling SWI/SNF complexes, and inhibits histone acetylation by EP300 and recruits class I HDACs, which induces an hypoacetylated state of chromatin. It has been recently reported that the dynamic regulator of chromatin plasticity H2AFY is specifically overexpressed in the blood and frontal cortex of patients with Huntington disease compared with controls. This association precedes the onset of clinical symptoms, was confirmed in two mouse models, and was independently replicated in cross-sectional and longitudinal clinical studies.	Mutations and/or CNVs affecting *H2AFY* have never been reported in patients with ASD. A 1.2 Mb region of chromosome 5q31, including *H2AFY*, was previously identified in a genome-wide linkage analysis for autism. Nevrtheless, no association was found between a few SNPs in *H2AFY* and ASD.	Hu *et al.*, 2011 Philippi *et al.*, 2005, 2007
IL9(+) ***de novo*** Cytokine-mediated neurodevelopment and neuroinflammation	This gene encodes the interleukin 9, which is a cytokine that acts as a regulator of a variety of hematopoietic cells. This cytokine stimulates cell proliferation and prevents apoptosis. It functions through the interleukin 9 receptor (IL9R), which activates different signal transducer and activator (STAT) proteins and thus connects this cytokine to various biological processes. Low expression in fetal brain and moderate expression in postnatal temporal lobe, prefrontal cortex, cerebellum and thalamus. Good expression in NK-cells.	*IL9* has been identified as a candidate gene for asthma. Genetic studies on a mouse model of asthma demonstrated that this cytokine is a determining factor in the pathogenesis of bronchial hyperresponsiveness. In mammals, programmed cell death (PCD) is a central event during brain development. Trophic factors have been shown to prevent PCD in postmitotic neurons. Similarly, cytokines have neurotrophic effects involving regulation of neuronal survival. It has been reported that interleukin-9 and its receptor specifically control PCD of neurons in the murine newborn neocortex. IL-9 antiapoptotic action appeared to be time-restricted to early postnatal stages as both ligand and receptor transcripts were mostly expressed in neocortex between postnatal days 0 and 10. This period corresponds to the physiological peak of apoptosis for postmitotic neurons in mouse neocortex. IL-9 effects were mediated by the activation of the JAK/STAT pathway. Finally, IL-9 reduced the expression of the mitochondrial pro-apoptotic factor Bax whereas Bcl-2 level was not significantly affected. Moreover, n the experimental autoimmune encephalomyelitis mousel, which is animal model of multiple sclerosis, it has been reported that in the CNS IL-9 is produced by several Th cell subsets in the presence of IL-4 and that IL-9 receptor complex is constitutively expressed by astrocytes. IL-9 induces CCL-20 production by astrocytes to induce the migration of Th17 cells into the CNS, thus sustaining the neuroinflammatory process.	Mutations and/or CNVs affecting *IL9* have never been reported in patients with ASD. Elevated serum levels of a few interleukins have been previously reported in autistic patients.	Fontaine *et al.*, 2008 Zhou *et al.*, 2011

Tab. 4.1. Continued.

★ ***KIF20A(+)*** ***de novo*** Intracellular trafficking: retrograde transport from Golgi to ER	This gene encodes the kinesin family member 20A, which interacts with guanosine triphosphate (GTP)-bound forms of RAB6A and RAB6B and may act as a motor required for the retrograde RAB6 regulated transport of Golgi membranes and associated vesicles along microtubules. KIF20A has a microtubule plus end-directed motility. Good expression in fetal brain and low expression in postnatal CNS. Very high expression in eritroid and myeloid cell lineages and in dendritic cells.	KIF20A binds to Rab6B which is preferentially expressed in brain, especially in microglial cells, pericytes and Purkinje cells. In particular, Rab6B is abundant in SK-N-SH cells that can either differentiate into cells with a neuronal phenotype, or in cells exhibiting properties common to glial cells. Rab6B may also be abundant in human melanocytes. The interaction of Rab6B with Rabkinesin-6 (KIF20A) suggestes that Rab6B may regulate a transport route through a molecular machinery comparable to that of Rab6A, in particular a retrograde transport along microtubules from the Golgi apparatus to the endoplasmic reticulum.	Mutations and/or CNVs affecting *KIF20A* have never been reported in patients with ASD.	Opdam *et al.*, 2000 Sano *et al.*, 1990 Shinohara *et al.*, 1997
KLHL3(+) ***de novo*** Intracellular signaling: protein ubiquitination pathway	This gene encodes the kelch-like 3 (Drosophila) protein, which has an N-terminal BTB domain followed by a BACK domain and six kelch-like repeats in the C-terminus. These kelch-like repeats promote substrate ubiquitination of bound proteins via interaction of the BTB domain with the CUL3 (cullin 3) component of a cullin-RING E3 ubiquitin ligase (CRL) complex. Good expression in fetal brain and in postnatal CNS.	The ubiquitin-proteasome system plays crucial roles in various aspects of neuronal development, such as axon formation, elongation, and pruning, and synapse formation and elimination. The Cullin3 (Cul3)-based ubiquitin E3 ligases use BTB domain–containing proteins as substrate adaptors and, recently, KLHL20, a protein possessing a BTB domain and six kelch repeats, has been identified as such an adaptor. KLHL20 mRNA is abundantly expressed in the brain of an embryonic day 14.5 (E14.5) mouse embryo, implying its role in neural development. In the adult mouse brain KLHL20 mRNA is highly expressed in the hippocampus, especially in the dentate gyrus, where a lifelong neurogenesis occurs. By analogy, it is possible that also KLHL3 may be involved in neurodevelopment. Muatations in *KLHL3* cause pseudohypoaldosteronism type IID (PHA2D); a rare Mendelian syndrome featuring hypertension, hyperkalaemia and metabolic acidosis.	Mutations and/or CNVs affecting *KLHL3* have never been reported in patients with ASD. Another member of the same family of proteins, KLHL22, maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. The protein ubiquitination pathway has been previously implicated in ASD.	Antshel *et al.*, 2007 Bucan *et al.*, 2009 Fine *et al.*, 2005 Glessner *et al.*, 2009 Kishino *et al.*, 1997 Lee *et al.*, 2010 Lin *et al.*, 2011 Lo-Castro *et al.*, 2009 Marshall *et al.*, 2008 Matsuura *et al.*, 1997 Mukkades and Herguner, 2007 Niklasson *et al.*, 2009 Pinto *et al.*, 2010 Ramelli *et al.*, 2008 Segref and Hoppe, 2009 Szatmari *et al.*, 2007 Tai and Schuman, 2008 Vorstman *et al.*, 2006 Yi and Ehlers, 2007
★ ***LECT2(+)*** ***de novo*** Neurodevelopment	This gene encodes the leukocyte cell-derived chemotaxin 2, which is a secreted protein that acts as a chemotactic factor to neutrophils and stimulates the growth of chondrocytes and osteoblasts. A polymorphism in this gene may be associated with rheumatoid arthritis. Moderate expression in fetal brain and in postnatal CNS, except for cingulate cortex where the expression is good. High expression in fetal and adult liver. No expression has been detected in bone marrow.	Leukocyte cell-derived chemotaxin 2 (LECT2) was first isolated as a chemotactic factor from phytohemagglutinin-activated human T-cell leukemia SKW-3 cells. To elucidate LECT2 functions in brain, the influence of a deficiency of LECT2 on the morphology of cultured hippocampal neurons during neuronal development was investigated, and the expression of neurotrophins (NGF, BDNF, and NT-3) and their receptors (TrkA, TrkB, TrkC, and p75NTR) in these neurons was examined. It has been reported that the extension of axons and dendrites in neurons from LECT2-knockout mice was shorter than that in neurons from wild-type mice during culture and significantly less than that in wild-type mice after 4 days in culture. Moreover, neurons from LECT2-KO mice showed different expression of NGF, BDNF and NT-3 during culture compared to wild-type mice, suggesting that LECT2 regulates the extension of axons and dendrites and the expressions of NGF, BDNF and NT-3 during neuronal development.	Mutations and/or CNVs affecting *LECT2* have never been reported in patients with ASD.	Koshimizu and Ohtomi, 2010

Tab. 4.1. Continued.

NEUROG1(+) ***de novo*** Transcriptional regulation	This gene encodes the neurogenin 1 protein, which acts as a transcriptional regulator. It is involved in the initiation of neuronal differentiation, and associates with chromatin to enhancer regulatory elements in genes encoding key transcriptional regulators of neurogenesis. Good expression in postnatal thalamus, hypothalamus, amygdalae, and cerebellum.	In mouse beta-catenin/TCF complex appears to directly regulate the promoter of neurogenin 1, a gene implicated in cortical neuronal differentiation.	A 1.2 Mb region of chromosome 5q31, including *NEUROG1*, was previously identified in a genome-wide linkage analysis for autism. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders. SNPs in *NEUROG1* have been previously associated with SCZ.	Chung *et al.*, 2011 De Ferrari and Moon, 2006 Fanous *et al.*, 2007 Hirabayashi *et al.*, 2004 Ma *et al.*, 1999 Okerlund and Cheyette, 2011 Philippi *et al.*, 2005, 2007 Wang *et al.*, 2010 Zhang *et al.*, 2012
PITX1(+) ***de novo*** Transcriptional regulation	This gene encodes the paired-like homeodomain 1 protein, which is a member of the RIEG/PITX homeobox family, i.e., the bicoid class of homeodomain proteins. Members of this family are involved in organ development and left-right asymmetry. This protein acts as a transcriptional regulator involved in basal and hormone-regulated activity of prolactin. Moderate expression in fetal brain and in postnatal parietal lobe, prefrontal cortex, and hypothalamus.	PITX1 plays a role in the development of anterior structures, and in particular, the brain and facies and in specifying the identity or structure of hindlimb. Defects in *PITX1* are a cause of congenital clubfoot (CCF). Clubfoot is a congenital limb deformity defined as fixation of the foot in cavus, adductus, varus, and equinus (i.e. inclined inwards, axially rotated outwards, and pointing downwards) with concomitant soft tissue abnormalities. Clubfoot may occur in isolation or as part of a syndrome.	Mutations and/or CNVs affecting *PITX1* have never been reported in patients with ASD. A 1.2 Mb region of chromosome 5q31, including *PITX1*, was previously identified in a genome-wide linkage analysis for autism. Two SNPs in *PITX1* have been associated with ASD.	Philippi *et al.*, 2005, 2007
★ ***SAR1B(+)*** ***de novo*** Intracellular membrane trafficking	This gene encodes the SAR1 homolog B (S. cerevisiae) protein, which is a small GTPase that acts as a homodimer. The encoded protein is activated by the guanine nucleotide exchange factor PREB and is involved in protein transport from the endoplasmic reticulum to the Golgi. This protein is part of the COPII coat complex. Moderate expression in fetal brain and in postnatal prefrontal cortex, cerebellum, amygdalae, and thalamus. Good expression in immune cell types, in particular in dendritic cells, monocytes, and NK-cells.	SAR1B is involved in transport from the endoplasmic reticulum to the Golgi apparatus. Defects in *SAR1B* are the cause of chylomicron retention disease, also known as Anderson disease, which is an autosomal recessive disorder of severe fat malabsorption associated with failure to thrive in infancy. Furthermore, defects in *SAR1B* have also been associated with Marinesco-Sjögren syndrome, which is a progressive multisystem disease with autosomal recessive inheritance characterized by cataracts, MR, and cerebellar ataxia.	Mutations and/or CNVs affecting *SAR1B* have never been reported in patients with ASD.	Sakai *et al.*, 2008
★ ***SEC24A(+)*** ***de novo*** Intracellular membrane trafficking	This gene encodes the SEC24 family, member A protein, which belongs to a family of proteins that are homologous to yeast Sec24. This protein is a component of coat protein II (COPII)-coated vesicles that mediate protein transport from the endoplasmic reticulum. COPII acts in the cytoplasm to promote the transport of secretory, plasma membrane, and vacuolar proteins from the endoplasmic reticulum to the golgi complex. Moderate expression in fetal brain and in postnatal cortex, and cerebellum. Good expression in immune cell types, in particular in dendritic cells, NK-cells, and $CD4^+$ T-cells.	SEC24A interacts with SAR1B to form the COPII complex.	Mutations and/or CNVs affecting *SAR1B* have never been reported in patients with ASD.	Wendeler *et al.*, 2007

Tab. 4.1. Continued.

SLC25A48(+) de novo CNS metabolism: neuronal energy production	This nuclear gene encodes the solute carrier family 25, member 48, which is a mitochondrial protein. High expression in postnatal whole brain, in particular in prefrontal cortex, amygdalae and hypothalamus, and in spinal cord.	SLC25A48 is a member of the solute carrier family 25 proteins that function as transporters of a large variety of molecules including ATP/ADP and amino acids, and localize to the inner mitochondrial membrane. In particular, SLC25A48 is highly expressed in the CNS including the hypothalamus, pituitary and brainstem and has been shown to be important in healthy neurons for energy production and to have a role in neuronal signaling.	Mutations and/or CNVs affecting *SLC25A48* have never been reported in patients with ASD. Recently, a genome-wide association study has identified *SLC25A48* as candidate genes for Parkinson's disease in an Ashkenazi Jewish population.	Haitina *et al.*, 2006 Liu *et al.*, 2011 Palmieri, 2004
SPOCK1(+) de novo Neurogenesis: regulation of neuronal migration and axonal outgrowth	This gene encodes the sparc/osteonectin, cwcv and kazal-like domains proteoglycan 1 (testican 1), which is the protein core of a seminal plasma proteoglycan containing chondroitin- and heparan-sulfate chains. The protein's function is unknown, although similarity to thyropin-type cysteine protease-inhibitors suggests its function may be related to protease inhibition. Very high expression in fetal brain and in postnatal CNS.	Testican-1 is strongly expressed in the brain and has been reported to modulate neuronal attachment and matrix metalloproteinase activation. During mouse embryonic development SPOCK1 is actively expressed at the onset of neurogenesis during periods of neuron migration and axonal outgrowth. At a later developmental stage, its expression is particularly prevalent within developing synaptic fields. In particular, SPOCK1 is most prominently expressed in the thalamus, and is upregulated in activated astroglial cells of the cerebrum. The purified gene product has been shown to inhibit cell attachment and neurite extensions in culture. In the peripheral nervous system, SPOCK expression is also developmentally regulated particularly in dorsal root ganglion neurons. *SPOCK1* has been proposed as a candidate for modulating the expression and maintenance of tyrosine hydroxylase (TH) content in murine mesencephalic dopamine neurons *in vivo*. TH is the first and rate limiting enzyme in the biosynthesis of catecholamine neurotransmitters in the substantia nigra-ventral tegmental area and the mesotelencephalic dopamine system is implicated in normal and pathological behaviors related to motor function, motivation, and learning.	Mutations and/or CNVs affecting *SPOCK1* have never been reported in patients with ASD.	Charbonnier *et al.*, 2000 Edgell *et al.*, 2004 Röll *et al.*, 2006 Vadasz *et al.*, 2007
WNT8A(+) de novo Intracellular Wnt signaling pathway	This gene encodes the wingless-type MMTV integration site family, member 8A protein. The WNT gene family consists of structurally related genes which encode secreted signaling proteins. These proteins have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis. This gene is a member of the WNT gene family, and may be implicated in development of early embryos as well as germ cell tumors. No expression data are available in UCSC Genome Browser (hg19, release February 2009).	It has been reported in zebrafish that Wnt8 signaling emanating from lateral mesendodermal precursors is essential for neuroectodermal posteriorization starting from the organizing center, located at the midbrain-hindbrain boundary (MHB). Indeed, MHB patterns the midbrain and hindbrain primordia of the neural plate. Wnt8 is required for the initial subdivision of the neuroectoderm and graded Wnt8 activity mediates overall neuroectodermal posteriorization and thus determines the location of the MHB organizer.	Mutations and/or CNVs affecting *WNT8A* have never been reported in patients with ASD. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as of other neuropsychiatric disorders.	Chung *et al.*, 2011 De Ferrari and Moon, 2006 Okerlund and Cheyette, 2011 Rhinn *et al.*, 2005, 2009 Wang *et al.*, 2010 Zhang *et al.*, 2012

Tab. 4.1. Continued.

▪ **Patient 60, loss of 7.8 Mb at 5p15.33p15.31 (chr5:95243-7859564)**				
ADCY2(-) de novo Intracellular signaling	This gene encodes the adenylate cyclase 2 protein, which is a member of the family of adenylate cyclases. They are membrane-associated enzymes that catalyze the formation of the secondary messenger cyclic adenosine monophosphate (cAMP). This enzyme is insensitive to Ca(2+)/calmodulin, and is stimulated by the G protein beta and gamma subunit complex. High expression in fetal brain and in postnatal CNS, except for cerebellum, thalamus and hypothalamus.	Quite discrete patterns of expression of Ca2+/calmodulin-insensitive adenylyl cyclase were found in rat brain. Indeed, in some areas both species were co-expressed, but in others, little overlap was observed. The differential expression of the two mRNAs suggests that discrete roles may be fulfilled by the two adenylyl cyclases in neural tissues. Another member of the same family, ADCY1 (a Ca2+/calmodulin-sensitive adenylyl cyclase), is involved in calcium-signaling that is required for many brain functions, such as learning and memory.	Mutations and/or CNVs affecting *ADCY2* have never been reported in patients with ASD. The application of a genome-wide SNP microarray approach to a single multiplex consanguineous Pakistani family, affected by ID and distal myopathy, allow the localization of a single 2.5 Mb homozygosity-by-descent (HBD) locus in the region 5p15.32-p15.31, which includes *ADCY2*.	Khan *et al.*, 2012 Mons *et al.*, 1993 Stengel *et al.*, 1992 Wieczorek *et al.*, 2010
AHRR(-) de novo CNS metabolism: hydrocarbon detoxification	This gene encodes the aryl-hydrocarbon receptor repressor, which participates in the aryl hydrocarbon receptor (AhR) signaling cascade, that mediates dioxin toxicity, and is involved in regulation of cell growth and differentiation. It functions as a feedback modulator by repressing AhR-dependent gene expression. Moderate expression in fetal brain and good expression in postnatal whole brain, in particular in amygdalae, thalamus and hypothalamus.	The Aryl hydrocarbon receptor repressor shares structural similarities with Aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT). The AhRR is thought to be involved in transcriptional control of AhR-regulated genes by sequestering ARNT. *AhRR* mRNA expression pattern in untreated C57BL/6 mice varies across tissues with high levels in hearts and brains. In other tissues, *AhRR* mRNA expression was low. In contrast to wild-type animals, the tissue levels in *AhR*-/- mice were about two to three orders of magnitude lower. Treatment of wild-type animals with benzo(a)pyrene resulted in an induced AhRR expression in liver, spleen, lung and ovary. No significant induction of *AhRR* mRNA was found in brain and heart tissues, which have a constitutively high level of AhRR expression.	Mutations and/or CNVs affecting *AHRR* have never been reported in patients with ASD.	Bernshausen *et al.*, 2006
★ ***CEP72(-) de novo*** Neurogenesis and neuronal migration?	This gene encodes the centrosomal protein 72kDa, which is a member of the leucine-rich-repeat (LRR) superfamily of proteins. The protein is localized to the centrosome, a non-membraneous organelle that functions as the major microtubule-organizing center in animal cells. It is involved in the recruitment of key centrosomal proteins to the centrosome. Moderate expression in fetal brain and in postnatal CNS. High expression in immune cell types.	Several centrosomal proteins have been previously involved in neurodevelopment. For example, autosomal-recessive primary microcephaly (MCPH), a rare congenital disorder characterized by ID, reduced brain and head size, is due to mutations in seven known loci code for centrosomal proteins, such as CEP135 and STIL. Furthermore, *CDK5RAP2* and *CENPJ* have been reported expressed in neuroepithelia during prenatal neurogenesis and the proteins were found localized to the spindle poles of mitotic cells, suggesting that a centrosomal mechanism controls neuron number in the developing mammalian brain. By analogy, CEP72 may be involved in neurogenesis and neuronal migration.	Mutations and/or CNVs affecting *CEP72* have never been reported in patients with ASD.	Bond *et al.*, 2005 Hussain *et al.*, 2012

Tab. 4.1. Continued.

C5orf38(-) ***de novo*** Neurogenesis	This gene encodes the chromosome 5 open reading frame 38. Moderate expression in fetal brain and in postnatal CNS. Good expression in thalamus.	An open reading frame coding for an unknown protein, C5orf38, is identified in the promoter of *IRXA2* on chromosome 5p. This new gene is composed of four exons and it is orientated in a head-to-head manner to *IRXA2*. The expression profile of the gene analysed in 9 different human tissues reveals that it is expressed in a coordinated fashion with *IRXA2*. The gene is only found in the human and the chimpanzee genome, but not in the mouse or the rat genome, which suggests that it is unique for higher primates. As the identified bi-directional promoter not being a relic of an ancient compact genome, C5orf38 may play an important role in the evolution of higher primates in coordination with the *IRX* genes.	Mutations and/or CNVs affecting *C5orf38* have never been reported in patients with ASD.	Wu *et al.*, 2006
★ ***EXOC3(SEC6)(-)*** ***de novo*** Synaptogenesis: intracellulae membrane trafficking	This gene encodes the exocyst complex component 3, which is a component of the exocyst complex, a multiple protein complex essential for targeting exocytic vesicles to specific docking sites on the plasma membrane. Though best characterized in yeast, the component proteins and functions of exocyst complex have been demonstrated to be highly conserved in higher eukaryotes. At least eight components of the exocyst complex, including this protein, are found to interact with the actin cytoskeletal remodeling and vesicle transport machinery. The complex is also essential for the biogenesis of epithelial cell surface polarity. Moderate expression in fetal brain and good expression in postnatal CNS, in particular in cortex and cerebellum. High expression in immune cell types.	In a study of developing neurons, Sec6/8 complexes were found at the highest levels in regions of the brain undergoing synaptogenesis and in regions of cultured neurons where synapses will subsequently develop. In contrast, the level of Sec6/8 was downregulated in mature synapses. This led to the hypothesis that the main function of the Sec6/8 complex is in formation of synapses rather than in their function once formed. Moreover, it has been demonstrated that the interaction between Ral, a member of the Ras-GTPase family,which resides on the cytoplasmic side of vesicles, and the Sec6/8 exocyst complex, which resides on the inner surface of the plasma membrane, could promote the recruitment of synaptic vesicles from the reserve pool in the cytoplasm to the plasma membrane in response to stimuli like phorbol esters and calcium.	Mutations and/or CNVs affecting *EXOC3* have never been reported in patients with ASD.	Hazuka *et al.*, 1999 Hsu *et al.*, 1996, 1999 Polzin *et al.*, 2002
IRX1(-) ***de novo*** Neurogenesis	This gene encodes the iroquois homeobox 1 protein, which is a member of the Iroquois homeobox protein family. Homeobox genes in this family are involved in pattern formation in the embryo. No expression microarray data available.	Iroquois homeoproteins are prepatterning factors that positively regulate proneural genes and control neurogenesis. In zebrafish the Iroquois gene, *irx1*, homolog has been identified, which is also higly homologous to Xenopus *Xiro1*, Gallus *c-Irx1* and mouse *Irx1*. Expression of *irx1* was initially detected at the bud stage. By 16 h post-fertilization (hpf), *irx1* expression was exclusively limited to the prospective midbrain and hindbrain. Recently, loss of function studies in Xenopus clarified that Irx1 and Irx3 seem to have a predominant role during regionalization of the neural plate.	Mutations and/or CNVs affecting *IRX1* have never been reported in patients with ASD.	Cheng *et al.*, 2001 Rodríguez-Seguel *et al.*, 2009
IRX2(-) ***de novo*** Neurogenesis	This gene encodes the iroquois homeobox 2 protein, which is a member of the Iroquois homeobox gene family. Members of this family appear to play multiple roles during pattern formation of vertebrate embryos. No expression microarray data available.	The Iroquois (*Irx*) genes encode homeoproteins conserved during evolution. Vertebrate genomes contain six Irx genes organized in two clusters, IrxA (which harbors *Irx1*, *Irx2* and *Irx4*) and IrxB (which harbors *Irx3*, *Irx5* and *Irx6*). Loss-of-function study of all the early expressed Irx genes (*Irx1-5*) using specific morpholinos in Xenopus revealed that the five *Irx* genes display largely overlapping expression patterns and contribute to neural patterning. All *Irx* genes are required for proper formation of posterior forebrain, midbrain, hindbrain and, to a lesser an extent, spinal cord.	Mutations and/or CNVs affecting *IRX2* have never been reported in patients with ASD.	Rodríguez-Seguel *et al.*, 2009

Tab. 4.1. Continued.

IRX4(-) ***de novo*** Neurogenesis	This gene encodes the iroquois homeobox 4 protein. Moderate expression in postnatal parietal lobe, cerebellum, thalamus hypothalamus, subtalamic nucleus, and spinal cord. High expression in prostate and heart.	Loss-of-function study of all the early expressed Irx genes (*Irx1-5*) using specific morpholinos in Xenopus revealed that the five *Irx* genes display largely overlapping expression patterns and contribute to neural patterning. All *Irx* genes are required for proper formation of posterior forebrain, midbrain, hindbrain and, to a lesser an extent, spinal cord. Moreover, several findings support a role for IRX4 in heart development.	Mutations and/or CNVs affecting *IRX4* have never been reported in patients with ASD.	Garriock *et al.*, 2001 Rodríguez-Seguel *et al.*, 2009
★ ***LPCAT1(-)*** ***de novo*** CNS homeostasis: lipid metabolism	This gene encodes the lysophosphatidylcholine acyltransferase 1 protein, which catalyzes the conversion of LPC to phosphatidylcholine (PC) in the remodeling pathway of PC biosynthesis. High expression in fetal brain and in postnatal amygdalae. Very high expression in immune cell types.	Phosphatidylcholine (PC) is the major phospholipid of the brain and comprises almost half of vertebrate retinal phospholipids. Lyso-PC (LPC) is a bioactive proinflammatory lipid generated by the pathological metabolism of PC. Accumulation of LPC is associated with a host of diseases, including atherosclerosis, myocardial ischemia, neurodegeneration, inflammatory diseases, and diabetic complications. LPCAT1 is a lysophospholipid acyltransferase implicated in the anti-inflammatory response by its role in conversion of LPC to PC. In addition, the LPCAT1 enzyme also catalyzes the synthesis of platelet-activating factor (PAF), another potent inflammatory lipid, from lyso-PAF with use of acetyl-CoA as a substrate. In naive mice constant levels of PAF are produced by microglia and astrocytes, thus contributing to the maintenance of CNS homeostasis. In the CNS of experimental allergic encephalomyelitis (EAE) mice, which mimics multiple sclerosis, the blood-brain barrier is broken and inflammatory cells, such as T cells and macrophages, infiltrate the CNS. Thus, activated microglia and macrophages produce higher amounts of LPCAT1/2 and therefore a robust PAF production has been observed, contributing to the inflammatory process.	Mutations and/or CNVs affecting *LPCAT1* have never been reported in patients with ASD.	Cheng *et al.*, 2009 Kihara *et al.*, 2008 Matsumoto *et al.*, 2007 Nakanishi et al., 2006

Tab. 4.1. Continued.

★ ***NSUN2(-) de novo*** Translation regulation, epigenetic modifications	This gene encodes the NOP2/Sun domain family, member 2 protein, which is a methyltransferase that catalyzes the methylation of cytosine to 5-methylcytosine (m5C) at position 34 of intron-containing tRNA(Leu)(CAA) precursors. This modification is necessary to stabilize the anticodon-codon pairing and correctly translate the mRNA. Moderate expression in fetal brain and in postnatal cingulate cortex, cerebellum, and globus pallidus. High expression in B- and T-cells.	NSUN2 was the first SUN-domain-containing protein to be characterized in vertebrates, and it is strongly conserved from bacteria to humans.It functions in spindle assembly during mitosis as well as chromosome segregation. By studying cortical and cerebellar region dissections from whole brains of 3-month-old mice, NSUN2 staining was sporadically observed in some cortical and brain-stem neurons although the most striking localization was observed in Purkinje cells of the cerebellum. It has been hypothesized that NSUN2 deficiency at critical stages during brain development might play a role in translational regulation needed for proper synaptic plasticity and thus learning and memory. Another conceivable disease mechanism might involve impaired methylation of hemimethylated DNA, which is also one of the targets of NSUN2 activity. It is a well established fact that alterations in DNA methylation patterns can lead to changes in gene transcription patterns and can also promote mutational events. Such epigenetic modifications can also cause specific changes in brain functions, which is of particular interest with respect to the cognitive phenotype. For instance, both the metabotropic (GRM1–7) and ionotropic (e.g., NMDA, AMPA, and kainate) glutamate-receptor-encoding genes undergo dynamic, region-specific, and cell-specific changes in expression during the course of brain development.	Mutations and/or CNVs affecting *NSUN2* have never been reported in patients with ASD. The application of a genome-wide SNP microarray approach to a single multiplex consanguineous Pakistani family, affected by ID and distal myopathy, allow the localization of a single 2.5 Mb homozygosity-by-descent (HBD) locus in the region 5p15.32–p15.31, which includes *NSUN2*, thus identifying homozygous missense changes in *NSUN2* coding region which are responsible for an autosomal recessive form of ID. Interestingly, in addition to ID, *NSUN2*-mutation-positive individuals in the current study display features such as poor speech (dysarthria) and broad gait, which have previously been associated with cerebellar defects. Further *NSUN2* mutations have been recently identified in Kurdish and Iranian ID consanguineous families.	Abbasi-Moheb *et al.*, 2012 Akbarian and Huang, 2009 Hong *et al.*, 2003 Hussain *et al.*, 2009 Khan *et al.*, 2012 Kuss *et al.*, 2011 Robertson, 2005 Zhao *et al.*, 2003 Zschocke *et al.*, 2002
★ ***PDCD6(-) de novo*** Intracellular signaling for cell death	This gene encodes the programmed cell death 6, a calcium-binding protein belonging to the penta-EF-hand protein family, also known as ALG-2. Calcium binding is important for homodimerization and for conformational changes required for binding to other protein partners. This gene product participates in T cell receptor-, Fas-, and glucocorticoid-induced programmed cell death. In mice deficient for this gene product, however, apoptosis was not blocked suggesting this gene product is functionally redundant. Moderate expression in fetal brain and good expression in postnatal amygdalae, thalamus and hypothalamus. High expression in immune cell types.	By using post-mitotic cerebellar neuron cultures it has been found that the complex Alix/ALG-2 regulates cell death controlling both caspase-dependent and -independent pathways. As Alix is a regulator of the endo-lysosomal system, these findings suggest a molecular link between the endo-lysosomal system and the effectors of the cell death machinery.	*PDCD6* has been previously suggested as autism candidate gene by molecular cytogenetic analysis and in silico studies.	Iurov *et al.*, 2010 Trioulier *et al.*, 2004

Tab. 4.1. Continued.

★ ***SDHA(-) de novo*** CNS metabolism: mitochondrial oxidative phosphorylation	This nuclear gene encodes the mitochondrial succinate dehydrogenase complex, subunit A, flavoprotein, which is a major catalytic subunit of succinate-ubiquinone oxidoreductase, a complex of the mitochondrial respiratory chain. The complex is composed of four nuclear-encoded subunits and is localized in the mitochondrial inner membrane. Low expression in fetal brain and good expression in postnatal CNS. High expression in immune cell types.	Mutations in *SDHA* have been associated with mitochondrial complex II deficiency, which shows heterogeneous clinical manifestations. Clinical features include psychomotor regression in infants, poor growth with lack of speech development, severe spastic quadriplegia, dystonia, progressive leukoencephalopathy, muscle weakness, exercise intolerance, cardiomyopathy. Severe forms are known as Leigh Syndrome, a severe disorder characterized by bilaterally symmetrical necrotic lesions in subcortical brain regions.	Mutations and/or CNVs affecting *SDHA* have never been reported in patients with ASD.	Alston *et al.*, 2012 Horvath *et al.*, 2006
SLC6A3(-) de novo Trafficking: dopamine transport	This gene encodes the solute carrier family 6 protein, a dopamine transporter which is a member of the sodium- and chloride-dependent neurotransmitter transporter family. It terminates the action of dopamine by its high affinity sodium-dependent reuptake into presynaptic terminals. Low expression in fetal brain and moderate expression in postnatal parietal and occipital lobes, prefrontal cortex and thalamus. Good expression in amygdalae.	Dopaminergic circuits are central to many key brain functions, i.e., memory, locomotion, reward mechanisms, motivation and cognition. Thus, the disturbances in dopaminergic tone are implicated in a broad spectrum of neuropsychiatric disorders, including ADHD, Parkinson's disease (PD), SCZ, and diseases of addiction. The 3' UTR of *SLC6A3* contains a 40 bp tandem repeat, referred to as a variable number tandem repeat or VNTR, which can be present in 3 to 11 copies. Variation in the number of repeats is associated with several neuropsychiatric disorders. Moreover, defects in *SLC6A3* are the cause of dystonia-parkinsonism infantile, which is a neurodegenerative disorder characterized by infantile onset of parkinsonism and dystonia. Other neurologic features include global developmental delay, bradikinesia and pyramidal tract signs. It has been reportedt that PARK2, a multiprotein E3 ubiquitin ligase complex, increases dopamine uptake by enhancing the ubiquitination and degradation of misfolded SLC6A3, so as to prevent it from interfering with the oligomerization and cell surface expression of native SLC6A3. This function of parkin would enhance the precision of dopaminergic transmission, increase the efficiency of dopamine utilization, and reduce dopamine toxicity on neighboring cells.	VNTR polymorphisms in *SLC6A3* have been associated with idiopatic epiplepsy, ADHD, PD, addiction disorders. Moreover, specific *SLC6A3* alleles have been associated with more severe social anxiety and tic symptoms in ASD patients. CNVs involving *PARK2* have been reported in ASD patients.	Arias-Carrion *et al.*, 2010 Gadow *et al.*, 2008 Glessner *et al.*, 2009 Jiang *et al.*, 2004 Lafuente *et al.*, 2007 Scheuerle and Wilson, 2011 Shohamy and Adcock, 2010 Shumay *et al.*, 2011 Swanson *et al.*, 2007 Vernier *et al.*, 2004 Volkow *et al.*, 2007

Tab. 4.1. Continued.

SRD5A1(-) ***de novo*** CNS metabolism: neurosteroid byosynthesis	This gene encodes the steroid-5-alpha-reductase, alpha polypeptide 1, which catalyzes the conversion of testosterone into the more potent androgen, dihydrotestosterone (DHT). High expression in fetal brain and in postnatal CNS.	Allopregnanolone (ALLO) and tetrahydrodeoxycorticosterone (THDOC) are potent positive allosteric modulators of GABA action at GABAA receptors. ALLO and THDOC are synthesized in the brain from progesterone or deoxycorticosterone, respectively, by the sequential action of two enzymes: 5α-reductase (5α-R) type I and 3α-hydroxysteroid dehydrogenase (3α-HSD). The evaluation of 5α-R type I and 3α-HSD mRNA expression level in mouse brain by using *in situ* hybridization revealed that the two enzimes colocalize in cortical, hippocampal, and olfactory bulb glutamatergic principal neurons and in some output neurons of the amygdalae and thalamus. Moreover, they are significantly expressed in principal GABAergic output neurons, such as striatal medium spiny, reticular thalamic nucleus, and cerebellar Purkinje neurons, thus suggesting that ALLO and THDOC, which can be synthesized in principal output neurons, modulate GABA action at GABAA receptors, either with an autocrine or a paracrine mechanism or by reaching GABAA receptor intracellular sites through lateral membrane diffusion.	Mutations and/or CNVs affecting *SRD5A1* have never been reported in patients with ASD.	Agis-Balboa *et al.*, 2006
TPPP(-) ***de novo*** Neurodevelopment: microtubule cytoskeleton dynamics	This gene encodes the tubulin polymerization promoting protein, which has a role in maintaining the integrity of the microtubule network. Very high expression in fetal brain and in postnatal CNS.	Microtubules, which form a major part of the cytoskeleton, display many physiological functions in eukaryotic cells. The dynamic reorganizing ability and stability of microtubular systems show great variability in different tissues and at different stages of tissue development. TPPP is a recently discovered, brain-specific unstructured protein involved in brain function. It is found predominantly in oligodendrocytes in normal brain and its physiological function seems to be the dynamic stabilization of microtubular ultrastructures, as well as the projections of mature oligodendrocytes and ciliary structures.	*TPPP* has been previously suggested as autism candidate gene by molecular cytogenetic analysis and in silico studies.	Colello *et al.*, 2002 Hlavanda *et al.*, 2002 Iurov *et al.*, 2010 Orosz *et al.*, 2008 Seki *et al.*, 1999 Skjoerringe *et al.*, 2006 Tirian *et al.*, 2003 Zhou *et al.*, 2011
ZDHHC11(-) ***de novo*** Intracellular trafficking: protein palmitoylation	This gene encodes the zinc finger, DHHC-type containing 11 protein. The DHHC domain is required for palmitoyltransferase activity. High expression in fetal brain and in postnatal CNS.	Protein palmitoylation, a classical and common lipid modification, regulates diverse aspects of neuronal protein trafficking and function. Individual DHHC enzymes, which belong to a family of palmitoyltransferases, generate and maintain the specialized compartmentalization of substrates in polarized neurons. Moreover, protein palmitoylation is implicated in various aspects of pathophysiology, including neuronal development and synaptic plasticity.	Mutations and/or CNVs affecting *ZDHHC1* have never been reported in patients with ASD.	Fukata and Fukata, 2010

Tab. 4.1. Continued.

- **Patient 60, gain of 9.1 Mb at 18p11.32p11.22 (chr18:180229-9281969)**

ADCYAP1(+) de novo Synaptic transmission	This gene encodes the adenylate cyclase activating polypeptide 1 (PACAP) (pituitary), which stimulates adenylate cyclase and subsequently increases the cAMP level in target cells. Adenylate cyclase activating polypeptide 1 is not only a hypophysiotropic hormone, but also functions as a neurotransmitter and neuromodulator. Moderate expression in fetal brain and in postnatal CNS, in particular in thalamus.	The neuropeptide PACAP is an informational molecule released from stress-transducing neurons. It exerts post-synaptic effects required to complete hypothalamo-pituitary-adrenocortical (HPA) and hypothalamo-splanchnico-adrenomedullary (HSA) circuits activated by psychogenic and metabolic stressors. PACAP-responsive (in cell culture models) and PACAP-dependent (*in vivo*) transcriptomic responses in the adrenal gland, hypothalamus, and pituitary upon activation of these circuits have been identified. Gene products produced in response circuits during stress include additional neuropeptides and neurotransmitter biosynthetic enzymes and neuroprotective factors. PACAP is widely expressed throughout the brain and exerts its functions through the PACAP-specific receptor (PAC(1)). Recent studies reveal that genetic variants of the *PACAP* and *PAC(1)* genes are associated with mental disorders, and several behavioral abnormalities of *PACAP* knockout mice are reported, thus suggesting that PACAP has an important role in the regulation of locomotor activity, social behavior, anxiety-like behavior and, potentially, working memory.	Mutations and/or CNVs affecting *ADCYAP1* have never been reported in patients with ASD. In a genetic linkage study, fine-scale mapping of a locus for severe bipolar mood disorder on chromosome 18p11.3 suggests that the *PACAP* gene, which resides at 18p11.32, is located close to a BD locus. Recently, genetic association studies have also shown that genetic variants of the genes encoding *PACAP* or *PAC1* are associated with SCZ, MDDand post-traumatic stress disorder.	Hashimoto *et al.*,2007, 2010 Hattori *et al.*, 2012 Ishiguro *et al.*,2001 Koga *et al.*,2010 Lohoff *et al.*,2008 McInnes *et al.*,2001 Ressler *et al.*,2011 Stroth *et al.*, 2011
ARHGAP28(+) de novo Intracellular signaling: regulation of actin cytoskeleton dynamics	This gene encodes the Rho GTPase activating protein 28. Good expression in fetal brain and in postnatal thalamus. Good expression in immune cell types. Very high expression in testis.	Rho GTPases, RhoA and Cdc42, are involved in neuronal morphogenesis, axonal guidance and synaptic plasticity by modulating the organization of actin cytoskeleton. The same pathway is involved in T-cells activation, migration, cell-cell adhesion.	Mutations or CNVs affecting *ARHGAP28* have never been reported in patients with ASD. Point mutations and CNVs affecting TSC1 and TSC2 have been reported in patients with ASD and Tuberous Sclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates CdC42, thus regulating cell adhesion and migration.	Fombonne *et al.*, 1997 Lewis *et al.*, 2004 Muzykewicz *et al.*, 2007 Wiznitzer, 2004 Wong, 2006
DLGAP1(+) de novo Synaptogenesis and synaptic plasticity	This gene encodes the discs, large (Drosophila) homolog-associated protein 1. High expression in fetal brain an in postnatal CNS, in particolar in caudate nucleus, thalamus and corpus callosum. Very high expression in brain cortex.	DLGAP1 is a member of the neuronal postsynaptic density complex and directly interacts with other members of the postsynaptic density such as SHANK1, SHANK2, DLG1, and DLG4.	Mutations and/or CNVs affecting *DLGAP1* have never been reported in patients with ASD. Mutations and/or CNVs affecting several genes encoding preoteins of the postsynaptic density (e.g., *SHANK2*, *SHANK3*, *DLG1*, *DLG4*) have been reported in autistic patients. CNVs affecting *DLGAP2* have been reported in a few autistic patients.	Berkel *et al.*, 2010, 2012 Durand *et al.*, 2007 Feyder *et al.*, 2010 Leblond *et al.*, 2012 Marshall *et al.*, 2008 Moessner *et al.*, 2007 Peca *et al.*, 2011 Pinto *et al.*, 2010

Tab. 4.1. Continued.

EPB41L3(+) ***de novo*** Neurodevelopment: nerve conduction	This gene encodes the erythrocyte membrane protein band 4.1-like 3 (4.1B protein). Very high expression in fetal brain and in postnatal CNS and PNS.	Myelinated axons are organized into specialized domains critical to their function in saltatory conduction, i.e., nodes, paranodes, juxtaparanodes, and internodes. 4.1B is expressed by neurons, and at lower levels by Schwann cells, which also robustly express 4.1G. Immunofluorescence demonstrated that 4.1B is expressed subjacent to the axon membrane in all domains except the nodes. Mice deficient in 4.1B have preserved paranodes, in contrast to the juxtaparanodes, which are substantially affected in both the PNS and CNS.	Mutations and/or CNVs affecting *EPB41L3* have never been reported in patients with ASD.	Einheber *et al.*, 2012
LAMA1(+) ***de novo*** Neurogenesis	This gene encodes the laminin, alpha 1protein, which is thought to mediate the attachment, migration and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components. Moderate expression in fetal brain and in postnatal CNS.	To study the role of this crucial laminin chain during late developmental phases and organogenesis, a conditional Laminin α1 knockout-mouse model has been created. A strong defect in the organization of the adult cerebellum of *Lama1*(cko) mice has been reported. Indeed, the study of the postnatal cerebellum of *Lama1*(cko) animals revealed a disrupted basement membrane correlated to an unexpected excessive proliferation of granule cell precursors in the external granular layer, thus suggesting that Lama1 is essential for the proper cerebellum development.	Mutations and/or CNVs affecting *LAMA1* have never been reported in patients with ASD. A SNP in *LAMA1* has been recently reported in a patient with ASD.	Anney *et al.*, 2012 Heng *et al.*, 2011
★ ***NDUFV2(+)*** ***de novo*** CNS metabolism: mitochondrial oxidative phosphorylation	This nuclear gene encodes the mitochondrial NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa. The NADH-ubiquinone oxidoreductase complex (complex I) of the mitochondrial respiratory chain catalyzes the transfer of electrons from NADH to ubiquinone, and consists of at least 43 subunits. The complex is located in the inner mitochondrial membrane. This gene encodes the 24 kDa subunit of complex I, and is involved in electron transfer. Low expression in fetal brain and moderate expression in postnatal CNS. High expression in immune cell types.	Susceptibility loci for psychosis that includes BD, SCZ, psychosis not otherwise specified, and schizoaffective disorder, have been mapped at chromosome 18p11. In particular, SNPs in the *NDUFV2* gene promoter have been associated with BD and SCZ in a Japanese cohort. Moreover, *NDUFV2* expression levels have been found altered in post-mortem brain of schizophrenic subjects compared with controls.	Mutations and/or CNVs affecting *NDUFV2* have never been reported in patients with ASD.	Mukherjee *et al.*, 2006 Nakatani *et al.*, 2006 Washizuka *et al.*, 2004, 2006

Tab. 4.1. Continued.

PTPRM(+) ***de novo*** Intracellular signaling	This gene encodes the protein tyrosine phosphatase, receptor type, M, which is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. This PTP has been shown to mediate cell-cell aggregation through the interaction with another molecule of this PTP on an adjacent cell. This PTP can interact with scaffolding protein RACK1/GNB2L1, which may be necessary for the downstream signaling in response to cell-cell adhesion. Good expression in fetal brain and in postnatal CNS, in particular in thalamus, hypothalamus, and amygdalae.	Protein tyrosine phosphatases (PTPs) have emerged as a new class of signaling molecules that play important roles in the development and function of the CNS. They include both tyrosine-specific and dual-specific phosphatases. Based on their cellular localization they are also classified as receptor-like or intracellular PTP. However, the intracellular mechanisms by which these PTPs regulate cellular signaling pathways are not well understood. Recently, candidate blood biomarker genes for mood disorders have been identified: five genes are involved in myelination (*Mbp*, *Edg2*, *Mag*, *Pmp22* and *Ugt8*), and six genes are involved in growth factor signaling (*Fgfr1*, *Fzd3*, *Erbb3*, *Igfbp4*, *Igfbp6* and *Ptprm*). All of these genes have prior evidence of differential expression in human postmortem brains from mood disorder subjects.	Mutations and/or CNVs affecting *PTPRM* have never been reported in patients with ASD. A rare CNV affecting *PTPRT* has been reported in a patient with ASD.	Christian *et al.*, 2008 Le-Niculescu *et al.*, 2009 Paul and Lombroso, 2003
RAB12(+) ***de novo*** Intracellular membrane trafficking	This gene encodes the RAB12 protein, which is a member of the RAS oncogene family of small GTPases. Moderate expression in fetal brain and in postnatal CNS.	Plasma membrane receptor proteins play a key role in signal transduction and nutrient uptake. After endocytosis, receptor proteins are generally delivered to lysosomes for degradation or recycled back to the plasma membrane for recycling. Transferrin receptor (TfR) is a well-known representative of recycling receptor proteins, which are traveled between plasma membrane and recycling endosomes. It has been reported that the small GTPase Rab12 regulates membrane trafficking of TfR from recycling endosomes to lysosomes. As iron deficiency has been previously linked to cognitive impairments, recently the impact of iron deficiency on spatial learning and memory has been assessed in neonatal piglets as models of human infants. It has been established that neonatal iron deficiency leads to cognitive impairment, which may be due in part to a reduced iron concentration in the hippocampus.	Mutations and/or CNVs affecting *RAB12* have never been reported in patients with ASD.	
★ ***TGIF1(+)*** ***de novo*** Transcriptional regulation	This gene encodes the TGFB-induced factor homeobox 1, which is a member of the three-amino acid loop extension (TALE) superclass of atypical homeodomains. TALE homeobox proteins are highly conserved transcription regulators. This particular homeodomain binds to a previously characterized retinoid X receptor responsive element from the cellular retinol-binding protein II promoter. In addition, TGIF1 is an active transcriptional co-repressor of SMAD2 and may participate in the transmission of nuclear signals during development and in the adult. Low expression in fetal brain and in postnatal CNS. Good expression in immune cell types, in particular in monocytes, B- and T-cells.	TGIF is an atypical homeo-domain protein. *In vitro* studies have shown that TGIF can repress transcription mediated by either of two signaling pathways: TGF-beta and retinoic acid signaling. *In vivo* overexpression of *TGIF* in the developing chick neural tube demonstrated that TGIF plays an important role in regulating the expression of genes expressed in specific dorsal-ventral domains during neural development. Moreover, it has been reported that TGIF1 together with MEIS2, another TALE family member, regulate the transcription of the *D(1A)* gene, which encodes the predominant dopamine receptor in the striatum. The two proteins have opposite roles: while MEIS2 activates *D(1A)* transcription, TGIF1 represses the transcription of this gene. Recently, it has been suggested that TGIF1 may have a role in macrophage activation.	Mutations and/or CNVs affecting *TGIF1* have never been reported in patients with ASD. SNPs in *TGIF1* have been previously strongly associated with psychosis. Mutations in this gene are associated with holoprosencephaly type 4 (HPE-4), which is a structural anomaly of the brain associated with MR. HPE is the most common structural anomaly of the brain, in which the developing forebrain fails to correctly separate into right and left hemispheres. Holoprosencephaly is genetically heterogeneous and associated with several distinct facies and phenotypic variability.	Chavarría-Siles *et al.*, 2007 Chen *et al.*, 2003 Knepper *et al.*, 2006 Ramsey *et al.*, 2008 Yang *et al.*, 2000

Tab. 4.1. Continued.

THOC1(+) ***de novo*** Transcriptional elongation	This gene encodes the THO complex 1 protein, which is part of the TREX (transcription/export) complex, which includes TEX1, THO2, ALY, and UAP56. Expressed ubiquitously. High level in fetal brain and in postnatal CNS, in particular in cortex, caudate nucleus, corpus callosum, and spinal cord. Very high expression in immune cell types.		Mutations and/or CNVs affecting *THOC1* have never been reported in patients with ASD.	Matsui and Fukuda, 2011 Rytych *et al.*, 2012
USP14(-)* ***de novo*** Intracellular signaling: protein ubiquitination pathway	This gene encodes the ubiquitin specific peptidase 14 (tRNA-guanine transglycosylase), which is a member of the ubiquitin-specific processing family of proteases that is a deubiquitinating enzyme with His and Cys domains. This protein is located in the cytoplasm and cleaves the ubiquitin moiety from ubiquitin-fused precursors and ubiquitinylated proteins. High expression in fetal brain and in postnatal CNS. High expression in immune cell types.	Recently, it has been reported that homozygous ataxic mice (ax(J)) express reduced levels of the deubiquitinating enzyme Usp14. They develop severe tremors by 2-3 wk of age, followed by hindlimb paralysis, and death by 6-8 wk. While changes in the ubiquitin proteasome system often result in the accumulation of ubiquitin protein aggregates and neuronal loss, these pathological markers are not observed in the ax(J) mice. Instead, defects in neurotransmission were observed in both the central and peripheral nervous systems of ax(J) mice. These results imply that ax(J) nerve terminals are unable to recruit a sufficient number of vesicles to keep pace with physiological rates of transmitter release. Therefore, ubiquitination of synaptic proteins appears to play an important role in the normal operation of the neurotransmitter release machinery and in regulating the size of pools of synaptic vesicles.	Mutations and/or CNVs affecting *USP14* have never been reported in patients with ASD. The protein ubiquitination pathway has been previously implicated in ASD.	Bhattacharyya *et al.*, 2012

- **Patient 62, gain of 1.5 Mb at Xp22.31 (chrX:6551155-8032120)**
- **Patient 63, gain of 501 kb at Xp22.31 (chrX:7269569-7820659)**

PNPLA4(+) CNS metabolism: lipoprotein metabolism	This gene encodes the patatin-like phospholipase domain containing 4 protein, which is a member of the patatin-like family of phospholipases. The encoded enzyme has both triacylglycerol lipase and transacylase activities and may be involved in adipocyte triglyceride homeostasis. It is expressed in all tissues examined including the brain: low expression in postnatal CNS.	PNPLA4 has a role in the metabolism of structural phospholipids involved in the formation and repair of the neuronal membrane.	Mutations or CNVs affecting *PNPLA4* have never been reported in patients with ASD. In syndromic forms of X-linked ichthyosis (XLI), due to Xp22.31 microdeletion, it has been recently suggested a possible contribution of *PNPLA4* deficiency as one of the causes of neurological disorders among males, such as ADHD, autism and X-linked MR.	Carrascosa-Romero *et al.*, 2012

Tab. 4.1. Continued.

STS*(+)** ***STS*(-) CNS steroid metabolism	This gene encodes the steroid sulfatase isozyme S, which catalyzes the conversion of sulfated steroid precursors to estrogens during pregnancy. Mild expression in fetal brain and high expression in postnatal CNS.	STS mutations are known to cause X-linked ichthyosis, a keratinization disorder manifesting with mild erythroderma and generalized exfoliation of the skin within a few weeks after birth. Affected boys later develop large, polygonal, dark brown scales, especially on the neck, extremities, trunk, and buttocks. Recently, it has been demonstrated a function for STS in sulphate and no-sulfate steroids metabolism in CNS. Indeed, in the brain sulphated and non-sulfated steroids can influence the function of GABAA and NMDA receptors. Furthermore, based on work in mouse models, STS has been recently proposed as a novel ADHD candidate. Indeed, STS is expressed in regions of the developing brain relevant to ADHD pathology.	*STS* deletion alone is not sufficient to develop ASD, but if the XLI deletion involves also *NLGN4X* the risk to develop ASD is higher. CNVs (gains) including *STS* have been reported in ASD patients. Individuals with deletions encompassing *STS* or inactivating *STS* mutations are at elevated risk of developing ADHD.	Carrascosa-Romero *et al.*, 2012 Compagnone and Mellon, 2000 Kent *et al.*, 2008 Davies *et al.*, 2007 Li *et al.*, 2010 Stergiakouli *et al.*, 2011 Trent *et al.*, 2012

[§]In this table only the genes included in rare CNVs which localize in recurrent genomic regions or in regions previously implicated in ASD and/or in other neuropsychiatric disorders by linkage studies have been analyzed. (-), deleted or disrupted gene due to a rare deletion; (-)*, possible disrupted gene due to a rare duplication; (+), duplicated gene due to a rare duplication. The genes already implicated in ASD, due to mutations and/or CNVs, SNPs, or known syndromes which are comorbid with ASD are depicted in red, purple, and dark red, respectively.

Genes implicated in CNS metabolism.
Genes implicated in synaptogenesis and synaptic plasticity.
Genes implicated in CNS-IS network.
Genes implicated in intracellular signaling and membrane trafficking.
Genes implicated in neurogenesis and neurodevelopment.
Genes implicated in transcriptional and translational regulation, and chromatin remodeling.

★ Genes whose function may be related to the IS development and function, within and outside the CNS.

ADHD, attention deficit hyperactivity disorder; AS, Angelman syndrome; BD, bipolar disorder; BWS, Beckwith-Wiedemann syndrome; CNS, central nervous system; CNV, copy number variation; CSF, cerebrospinal fluid; DD, developmental delay; EP, epilepsy; ER, endoplasmic reticulon; GWAS, genome-wide association study; HF-AU, high functioning autism; ID, intellectual disability; IS, immune system; MDD, major depressive disorder; MR, mental retardation; SCZ, schizophrenia; SNP, single nucleotide polymorphism; SRS, Siver-Russell syndrome; XLMR, X-linked mental retardation.

DISCUSSION

6.1 Analysis of the identified rare CNVs: implication for genotype-phenotype correlation

The collected ASD cohort was, as expected, clinically heterogeneous, including different phenotypic categories ranging from full autism to high-functioning autism (HF-AU), Pervasive Developmental Disorder not otherwise specified (PDD-NOS), Asperger syndrome (AS), and syndromic autism (S-AU), which were classified according to the DSM-IV international criteria [Task Force on DSM-IV, 2000]. In particular, 15% of the patients were diagnosed as S-AU, in agreement with previous studies reporting a frequency of 10% of cases of autism in combination with facial dysmorphism and/or congenital malformations [Devlin and Scherer, 2012]. In addition, epilepsy was diagnosed in only 3.5% of the collected patients, in contrast to the reported frequency of 25% [Baird *et al*., 2006; Tuchman and Rapin, 2002].

Through the application of a genome-wide approach, copy number variations (CNVs) were determined, both rare (*de novo* and inherited) and those already reported in healthy controls according to the Database of Genomic Variants, which may be implicated in ASD. Specifically, 55% of the patients were found to be carriers of one or more rare CNV, and, in total, 120 rare CNVs were identified, 73 gains (60.8%) and 47 losses (39.2%), confirming that among rare CNVs the frequency of gains is higher than that of losses, as previously reported [Levy *et al*., 2011; Marshall *et al*., 2008; Pinto *et al*., 2010, Sanders *et al*., 2011; Sebat *et al*., 2007; Zhao *et al*., 2007]. Conversely, focusing on the subset of rare *de novo* CNVs, 60% of these variants are losses, and 40% are gains, in agreement with published data, which report a higher frequency of losses than gains among rare *de novo* CNVs [Levy *et al*., 2011; Marshall *et al*., 2008; Pinto *et al*., 2010, Sanders *et al*., 2011; Sebat *et al*., 2007; Zhao *et al*., 2007].

Furthermore, since recent studies on several ASD series analyzed by array CGH demonstrated a higher frequency of rare *de novo* CNVs in females than in males [Gilman *et al*., 2011; Levy *et al*., 2011], it has been hypothesized that rearrangements with a more severe effect and a higher penetrance are necessary for a female individual to develop ASD, compared to those anomalies necessary for the development of the same disease in males. This finding has not been reproduced in the cohort reported here, probably due to the small sample size. Indeed, rare *de novo* CNVs were found at the same percentage among female and male patients: 4/23 females (17.4%) and 16/92 males (17.4%).

In agreement with recently published data, the genotype-phenotype correlation in the present ASD series is rather complex [Schaaf *et al*., 2011]. First, rare CNVs, which may unveil, in part, the genetic causes underlying the disease, were found in only 55% of the patients, thus emphasizing the need to analyze large cohorts of autistic patients by means of different high-throughput

genome-wide approaches (e.g., array CGH, whole-exome sequencing) in order to increase the detection rate for these disorders.

In addition, most patients positive in the array CGH analysis (~76%) were found to be carriers of more than one CNV, which were present in different combinations (**Fig. 15**), thus supporting the existence of a genetic model characterized by oligogenic heterozygosity, i.e., the simultaneous presence in a single autistic patient of multiple heterozygous quantitative variants/rare mutations, both de novo and/or inherited, affecting multiple genes [Pinto *et al*., 2010; Schaaf *et al*., 2011]. Previous studies of parents and unaffected siblings for the presence of oligogenic events revealed that the vast majority of these combinations are unique to the probands [Schaaf *et al*., 2011], as confirmed in the present ASD series by siblings 38–39 (**Tab. 3**), although some exceptions have been reported [Schaaf *et al*., 2011], suggesting that some of these events are insufficient to cause autism alone. It can be speculated that the accumulation of several, if not many, of such inherited low-penetrating variants causes a genetic load, which ultimately crosses a given threshold and leads to clinical manifestation of the ASD in the respective individuals.

Therefore, when more rare CNVs are found in a single patient, it is difficult to attribute a "major" causative role to one of them, even in case of *de novo* CNVs, which are generally considered high-penetrance variants. Indeed, the more severe clinical pictures reported here (i.e., syndromic autism) do not always correlate with the finding of rare CNVs, neither with the CNV size nor with the presence of *de novo* CNVs in the subset of patients bearing rare CNVs (**Tab. 3**). Moreover, it cannot be excluded that "common" CNVs may have modulated the behavioural phenotype of the reported ASD patients, increasing the complexity of inter-individual genetic and phenotypic variability.

Interestingly, most of the identified rare *de novo* CNVs map either within genomic regions previously implicated in recurrent microrearrangements, which are responsible for some microdeletion/microduplication syndromes that are comorbid with ASD (**Tab. 3**, patients 14, 23, 27, 29, 50, 52, 62, and 63), or within genomic regions implicated by linkage studies in ASD or in other neurologic or neuropsychiatric disorders, such as bipolar and major depressive disorders, schizophrenia, and intellectual disability (ID) (**Tab. 3**, patients 51, 53, 57, and 60). Indeed, the 8.8 Mb genomic region found deleted in patient 51 at 2q14.3q21.3 includes a small region of 450 kb at 2q21.1, recurrent deletion of which has been associated with attention deficit hyperactivity disorder, ID, epilepsy, and other neurobehavioral abnormalities [Dharmadhikari *et al*., 2012]. More recently, reciprocal duplications have been identified in five unrelated families with autism, developmental delay, seizures, and attention deficit hyperactivity disorder [Dharmadhikari *et al*., 2012]. The rearranged segment harbors five genes, namely *GPR148*, *FAM123C*, *ARHGEF4*, *FAM168B*, and *PLEKHB2*, which are deleted in patient 51 (**Tabs. 3** and **4.1**).

Furthermore, in patients 53 and 57, rare large *de novo* CNVs affecting chromosomal regions previously suggested as ASD susceptibility loci by genome-wide linkage analyzes were identified, which map to 2q31.3q32.3 and 5q31.1q31.2, respectively (**Tab. 3**). Finally, both the 7.8 Mb loss at 5p15.33p15.21 and the 9.1 Mb gain at 18p11.32p11.22 identified in patient 60 have been proposed as candidate loci for ID and bipolar disorder, major depressive disorder, and schizophrenia, respectively [Hattori *et al*., 2012; Ishiguro *et al*., 2001; Kuss *et al*., 2011; Lohoff *et al*., 2008; McInnes *et al*., 2001], thus supporting the existence of shared neurological pathways among different neuropsychiatric diseases, as previously reported [Bateman and Gull; 2011; Elia *et al*., 2011a and b; Jarick *et al.*, 2012; Kirov *et al*., 2009; Lachman, 2008; Lionel *et al*., 2011; Shoukier *et al*., 2012; Stefansson *et al*., 2008; Utine *et al*., 2012; Vrijenhoek *et al*., 2008; Walsh *et al*., 2008; Williams *et al*., 2012].

Interestingly, in the ASD cohort reported here, the finding of rare *de novo* CNVs mapping in genomic regions responsible for recurrent genomic disorders does not correlate with the presence of a syndromic clinical picture in the carrying patients, with the exception of patient 14, who shows a mild syndromic phenotype (**Tabs. 3** and **4.1**). However, there are several possible explanations to clarify this discrepancy. Firstly, if the identified *de novo* CNV is smaller than that reported in association with the microdeletion/microduplication syndrome, it may not involve all the causative genes, although, conversely, it is possible that the rearrangement also perturbs the expression of genes localized outside its range by a position effect (**Tab. 3**). Furthermore, if the large *de novo* CNV is a duplication, it is difficult to predict the real effect of the rearrangement on the involved genes, which may lead to a haploinsufficiency, thus mimicking the effect of a deletion, as well as a mild or null perturbation of the gene expression or an increase of the corresponding protein. Indeed, Schaaf *et al*. recently reported the finding of point mutations with a mild effect in "syndromic" genes (i.e., missense mutations), such as *TSC1*, *TSC2*, *PTEN*, and *CACNA1C*, which in combination with mutations in other genes may have led to ASD development in a cohort of high-functioning autistic patients who do not show any syndromic clinical features [Schaaf *et al*., 2011], thus supporting our hypothesis. Therefore, a precise molecular characterization of the identified CNVs as well as a quantitative analysis of the gene transcripts is necessary to clarify their role in ASD.

For example, patient 14 carries a duplication of 442 kb at 9q34.3 (**Tab. 3**), which is included in the region responsible for the 9q subtelomeric deletion syndrome (Kleefstra syndrome), and shows a mild syndromic clinical picture not yet clearly defined as "Kleefstra". This syndrome, which is characterized by a typical gestalt, microcephaly, hypotonia, ID and, in some cases, ASD/autistic traits, is due to the deletion of the *EHMT1* gene, encoding a histone-methyltransferase, and, often, of the *CACNA1B* gene, which is located more distally and encodes a voltage-dependent calcium channel implicated in neurotransmitter release (**Tab. 4.1**) [Anderlid *et al*., 2002; Dawson *et al*.,

2002; Iwakoshi *et al*., 2004; Kleefstra *et al*., 2005, 2009; McMullan *et al*., 2009; Sahoo *et al*., 2006b]. Although the genomic instability of the chromosome 9q34 region has been well documented [Talkowski *et al*., 2012], often in association with neurodevelopmental disorders, only recently were a few autistic patients reported to bear complex deletion-duplication or duplication-triplication rearrangements involving *EHMT1*, thus suggesting that increased dosage of EHMT1 may be responsible for neurodevelopmental impairment and ASD [Yatsenko *et al*., 2012].

However, as both the duplication breakpoints fall within the gene coding sequences in patient 14 (**Fig. 18**), it is possible that both *EHMT1* and *CACNA1B* have been disrupted by the microrearrangement, thus resulting in gene haploinsufficiency as seen in microdeletion. To confirm this hypothesis, the duplication will be molecularly characterized, and any quantitative changes in *EHMT1* and *CACNA1B* expression will be evaluated. Furthermore, patient 14 bears another rare CNV, inherited from the mother, at Xq22.3, which likely disrupts, differently from what happens in the healthy mother, the only copy of the *IL1RAPL2* gene, encoding an interleukin receptor (**Tabs. 3** and **4.1**). By analogy with the function of another member of the same family, IL1RAPL1, IL1RAPL2 is likely involved in presynaptic calcium-dependent neurotransmitter release and differentiation of dendritic spines. Since mutations and CNVs affecting *IL1RAPL1* have been identified in patients with schizophrenia or autism with or without association to X-linked mental retardation [Piton *et al*., 2008], and SNPs in *IL1RAPL2* have recently been associated with ASD [Kantojarvi *et al*., 2011], this CNV may play a role in the onset of the disease.

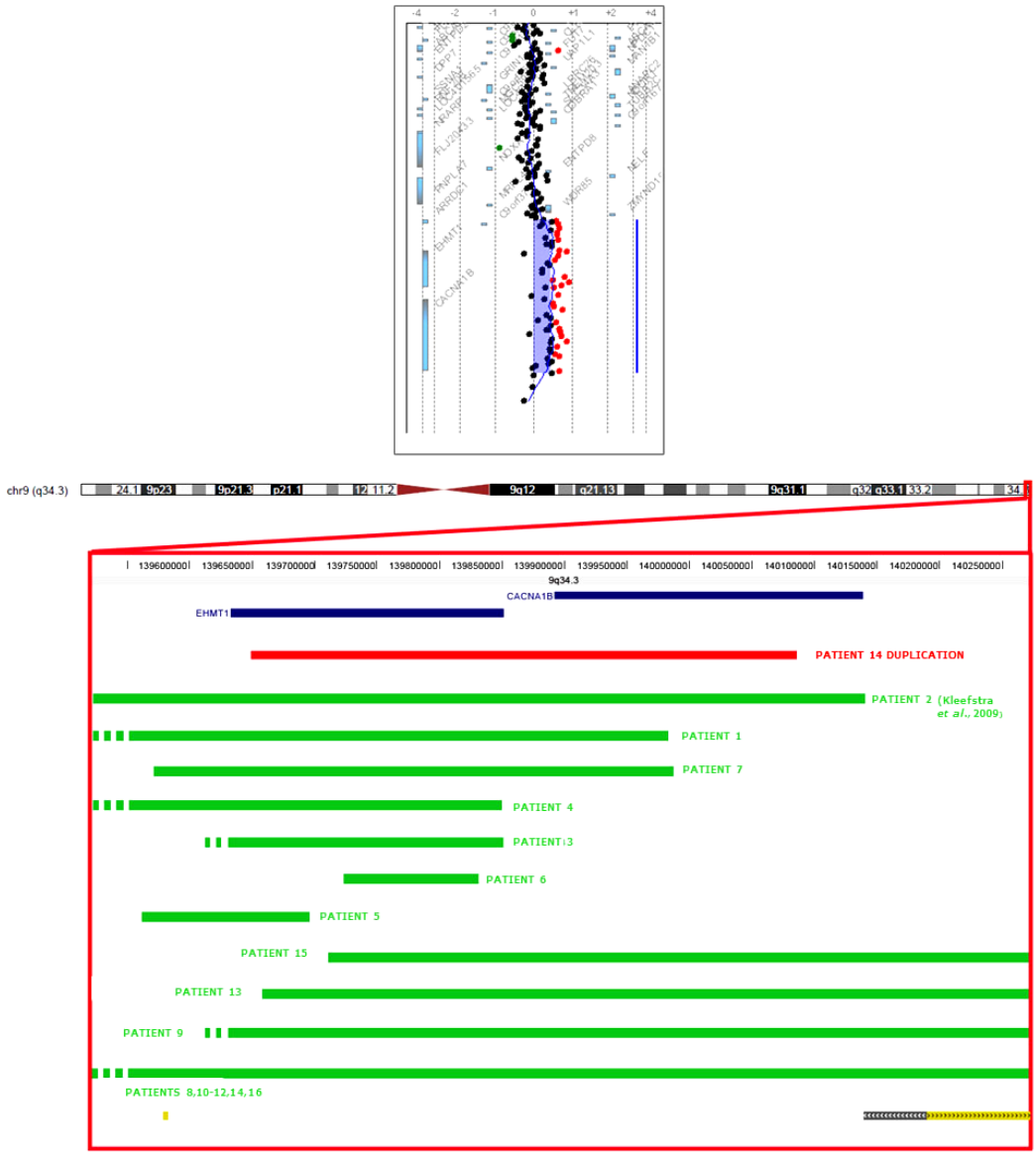

Fig 18. (**Top**) High-resolution array CGH (Agilent Technologies) identified a duplication of 442 kb at 9q34.3 in patient 14. (**Bottom**) Schematic view of the region involved in the 9q34.3 subtelomeric deletion syndrome. The most interesting genes mapped within this region are depicted in blue, previously reported deletions in green [Kleefstra *et al.*, 2009], the duplication identified in patient 14 in red, and the segmental duplications on the bottom line.

In patient 23, the *de novo* deletion of 749 kb at 17q21.31 includes part of the region involved in the 17q21.31 microdeletion/microduplication syndrome (**Tab. 3**, **Fig. 19**). The deletion syndrome is characterized by facial dysmorphism, hypotonia, ID, and ASD has been reported in only two patients [Betancur *et al.*, 2008; Cooper *et al.*, 2011], whereas this clinical characteristic has been demonstrated by several patients with the reciprocal duplication [Grisart *et al.*, 2009]. The minimal deleted region spans 424 kb [Koolen *et al.*, 2008; Sharp *et al.*, 2006] and encompasses six genes including *CRHR1*, encoding a G protein-coupled receptor that binds neuropeptides, and *MAPT*

encoding a protein that is involved in the assembly and stability of microtubules (**Tab. 4.1**). "Gain of function" mutations affecting *MAPT* cause autosomal dominant forms of fronto-temporal dementia [D'Souza *et al*., 1999; Hutton *et al*., 1998; Rademakers *et al*., 2004], and common variants have been associated with progressive paralysis [Pittman *et al*., 2005] and Alzheimer's disease [Myers *et al*., 2005]; therefore defects in this gene have also been suggested as candidates for ASD.

Interestingly, patient 23, who was diagnosed as PDD-NOS in the absence of a syndromic clinical picture, bears the deletion of only *CRHR1*, which is likely disrupted by the rearrangement (**Fig. 19**). This finding is in agreement with the recent report of three atypical deletions at 17q21.31 in three patients with the clinical signs of 17q21.31 microdeletion syndrome (one of the three patients was reported as autistic), which seems to exclude a pathogenetic role of *CRHR1* [Cooper *et al*., 2011]. Indeed, the critical region for this syndrome has been refined and focused to only three genes, *MAPT*, *STH*, and *KIAA1267*, thus supporting the involvement of *MAPT* in the onset of this syndrome, and, likely, in ASD [Cooper *et al*., 2011]. Although *MAPT* is not deleted in patient 23, it is not possible to exclude its dysregulation due to the identified microrearrangement, which may perturb gene expression by a position effect. Similar to the situation described in patient 14, in patient 23 the rare *de novo* CNV at 17q21.31 occurs in combination with a rare inherited CNV at 17q24, which likely disrupts the gene encoding PRKCA, a calcium-dependent kinase with roles in cell adhesion and differentiation, and may have contributed to ASD onset (**Tabs. 3** and **4**).

Finally, considering the rare inherited variants reported here, as expected, a few CNVs affect genomic regions, such as the 15q11.2 and 16p11.2 loci (**Tab. 3**, patients 25, 38, 39, 55), which are involved in recurrent genomic rearrangements that show incomplete penetrance and variable expressivity as they have been previously found in autistic patients as well as in healthy parents and in controls [Kumar *et al*., 2008; Marshall *et al*., 2008; Sempere-Perez *et al*., 2011; Weiss *et al*., 2008]. Interestingly, Nord *et al*. [Nord *et al*., 2011] recently described a decrease in the transcripts of several genes affected by rare CNVs in a group of autistic probands compared with their unaffected transmitting parents and a group of controls, suggesting that transcriptional anomaly may explain, at least in part, the phenotypic differences between patients and healthy parents or controls. Notably, however, a reduced mRNA level has also been reported in the transmitting parent compared with the non-transmitting parent [Nord *et al*., 2011].

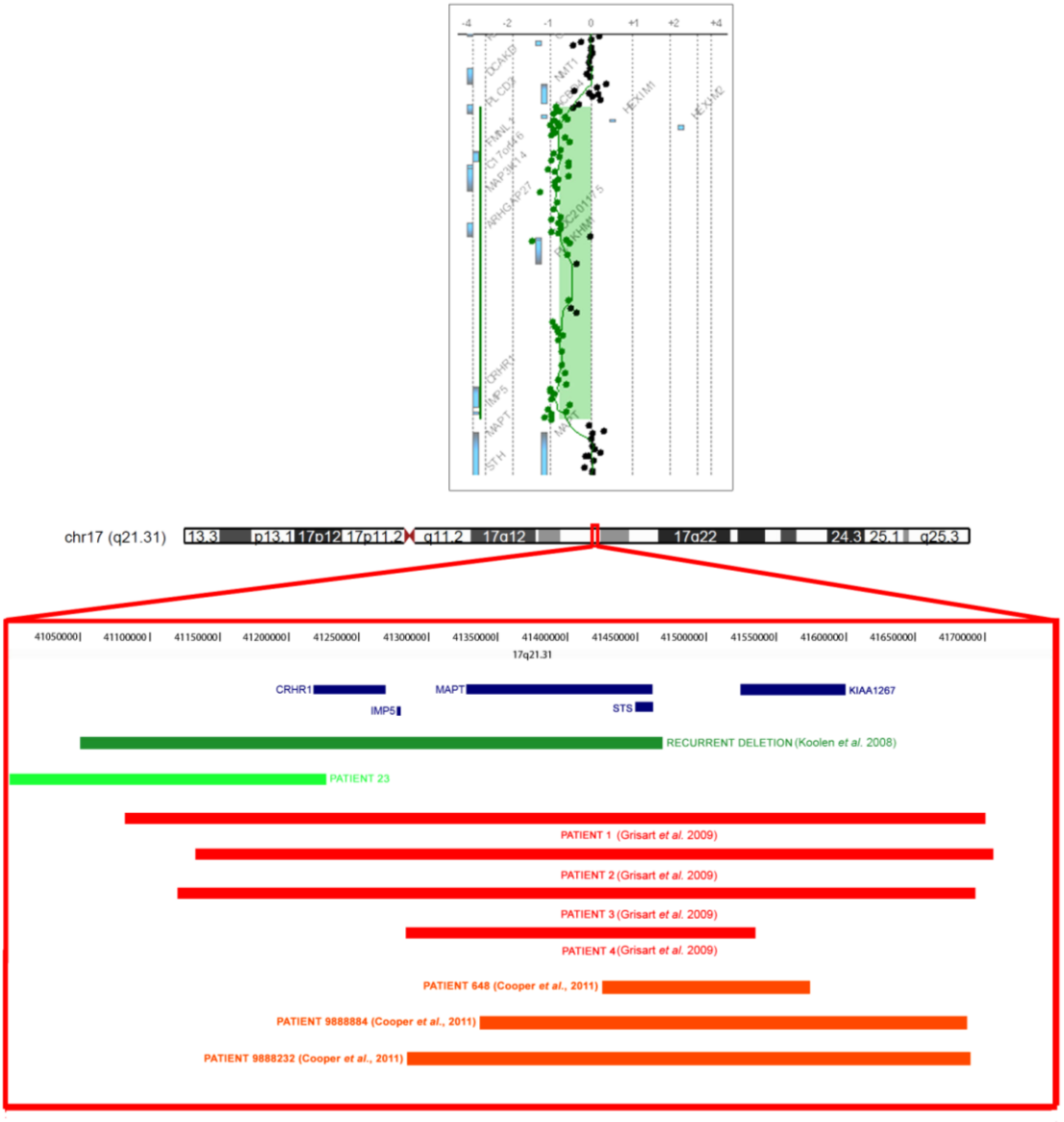

Fig. 19. (**Top**) High-resolution array CGH (Agilent Technologies) identified a deletion of 749 kb at 17q21.31 in patient 23. (**Bottom**) Schematic view of the region involved in the 17q21.31 microdeletion/microduplication syndrome. The most interesting genes mapped within this region are depicted in blue, the recurrent deletion in dark green [Koolen *et al*., 2008], the duplication identified in patient 23 in light green, the reciprocal duplications reported by Grisart *et al*. in red [Grisart *et al*., 2009], and the atypical deletions reported by Cooper *et al*. in orange [Cooper *et al*., 2011].

6.2 Identification of functional networks of genes potentially implicated in ASD

On the basis of the rare CNV gene content, it is possible to confirm in the present cohort the wide genetic heterogeneity associated with ASD. Indeed, the same affected loci were detected in only a few unrelated patients (*LCLAT1* in patients 7 and 33, and *MACROD2* in patients 3, 33, and the siblings 40–41), whereas most patients demonstrated specific subsets of rare CNVs in combinations characteristic for each patient, thus supporting the genetic complexity of ASD.

Moreover, most of the selected genes have never been reported in association with ASD, and thus they are suggested as new candidate loci (**Fig. 17**). They can be grouped into six functional networks that all contribute to CNS development and maintenance. Convergent pathways of action for the vast number of ASD genes proposed so far have been previously reported, most of which converge on synaptic function, thus confirming the neurological interpretation of ASD as a "synaptopathy" [Zoghbi and Bear, 2012]. Specifically, the selected genes are implicated in (**Tabs. 4** and **4.1**, **Fig. 17**):

- neurogenesis and neurodevelopment;
- transcriptional regulation and chromatin remodeling;
- CNS metabolism;
- synaptogenesis and synaptic plasticity;
- intracellular signaling and trafficking;
- local and systemic immune response.

Of note, although the schematic subdivision of genes above is proposed, it is clear that all the networks are deeply interconnected, and more than one network generally contribute to the same final neuronal function.

Neurogenesis and neurodevelopment (the genes affected by *de novo* CNVs are indicated in bold text, **Tabs 4** and **4.1**)

Sixty-six of the 276 selected genes contribute to neurogenesis or, more generally, to neurodevelopment acting directly on neuronal growth (axon outgrowth and patterning) (e.g., *ADAMTS9*, *CFDP1*, *CSH1*, ***EPB41L5***, ***FMNL1***, *GH2*, *CSPG5*, *DIP2A*, and *PLD5*), neuronal migration and organization into tissues (e.g., ***CEP72***, *FLNA*, ***LAMA1***, ***LIMK1***, ***NCKAP1***, *NDE1*, *PCNT*, *PPP4C*, ***SPOCK1***, ***TUBA3D/3E***, ***TPPP***, and *TMSB15B*), and cell cycle and apoptosis regulation (e.g., *MPHOSPH9* and ***AIFM3***) (**Fig. 20**). Most of the CNVs affecting these genes are parentally inherited, whereas the *de novo* variants map to recurrent genomic regions as well as regions previously associated with ASD or with other neurodevelopmental disorders by linkage studies (**Tabs. 3–4.1**). Both deletions and duplications were observed, confirming that a decrease in neuronal growth and migration, as well as an increase in the same processes, accompanied by dysregulation in the spatial organization of the neuronal layers, may result in the same final effect,

thus contributing to autism development.

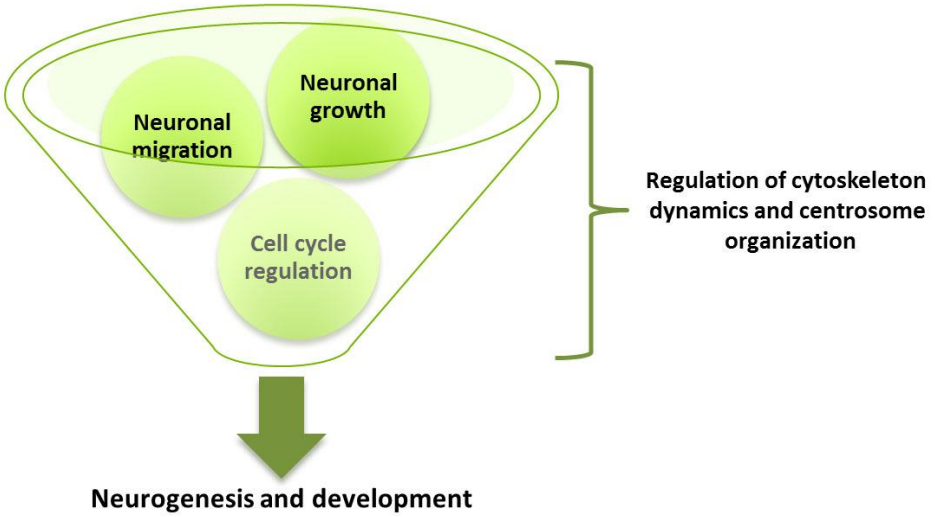

Fig. 20. Schematic view of the functional network of genes implicated in neurogenesis, subdivided on the basis of their function (green balls).

For example, the *CSPG5* gene (duplicated in patient 3, **Tab. 3**) encodes neuroglycan C, which is specifically expressed in the CNS and promotes neurite outgrowth that is, conversely, inhibited by both protein kinase C and phosphatidylinositol 3-kinase, both of which modulate CSPG5 function. *FMNL1* (deleted in patient 23) encodes a formin-related protein that mediates the induction of dendritogenesis and synaptogenesis by neurogenin 3 in mouse hippocampal neurons through a direct role in cytoskeleton dynamics. Moreover, both the *CSH1* and *GH2* genes encode hormones of the pituitary GH1/IGF-I axis that play an important role in CNS functions, including those associated with neuronal growth, development, and protection. It has been suggested that the GH1/IGF axis may play a role in influencing aspects of mood and cognition [Donahue *et al*., 2006; Lodygensky *et al*., 2008; Zearfoss *et al*., 2008], and, therefore, it is likely that deletion of both *CSH1* and *GH2* in patient 45 may have played a role in autism development. Of note, the deletion detected in patient 45 is maternally inherited, and the mother also shows autistic traits (**Tab. 3**).
Finally, ***LAMA1*** encodes the laminin alpha1 protein, which is thought to mediate the attachment, migration, and organization of cells into tissues during embryonic development. In the conditional laminin α1 knockout mouse, a strong defect in the organization of the adult cerebellum has been reported. Indeed, the postnatal cerebellum of these animals revealed a disrupted basement membrane correlated to an unexpected excessive proliferation of granule cell precursors in the external granular layer, thus suggesting that LAMA1 is essential for proper cerebellum development [Heng *et al*., 2011]. It can be speculated that overexpression of *LAMA1* in patient 60 (**Tab. 3**), due to a full gene duplication, may have contributed to autism onset by acting on

cerebellum organization during embryonic development, thus supporting the fundamental role of cerebellum anomalies in ASD as previously reported [Fatemi *et al*., 2012].

It is interesting to note that the shared pathway underlying most of the actions that ultimately give rise to neurogenesis and neurodevelopment is the regulation of actin and microtubule cytoskeleton dynamics as well as the organization of the centrosome, as confirmed by the identification of several genes that modulate these processes whose dysregulation may contribute to ASD development. For example, *FLNA* (potentially disrupted in patient 61, **Tab. 3**) encodes the filamin A protein, which is an actin binding protein that crosslinks actin filaments and links them to membrane glycoproteins. FLNA is involved in remodeling the cytoskeleton to affect changes in cell shape and migration, and a direct interaction between FLNA and SHANK3 has been recently reported in mouse brain extracts [Lian *et al*., 2012]. Moreover, ***LIMK1*** (duplicated in patient 52, **Tab. 3**) encodes the LIM domain kinase 1 protein, which is a serine/threonine kinase that regulates actin polymerization via phosphorylation and inactivation of the actin binding factor cofilin. LIMK1 stimulates axon growth and binds to TrkB, which is required for BDNF-induced axonal elongation. In *Limk1*-null mice, abnormal dendrite spine morphology as well as altered hippocampal function and mild deficits in spatial learning and memory have been reported [Osborne, 2010].

The deletion of ***NCKAP1*** seen in patient 53 may have resulted in an impairment of functional connectivity in the cerebral cortex (**Tab. 3**). NCKAP1 is an adaptor protein that is thought to modulate actin nucleation. It is selectively expressed in the cortical plate region of the developing cortex, where neurons terminate their migration and begin their final laminar-specific differentiation, characterized by the elaboration of distinct axonal and dendritic architecture [Yokota *et al*., 2007]. Similarly, overexpression of ***SPOCK1*** in patient 57 due to a full gene duplication (**Tab. 3**) may have abnormally modulated neuronal attachment and matrix metalloproteinase activation during neurodevelopment. During mouse embryonic development, SPOCK1 is actively expressed at the onset of neurogenesis during periods of neuronal migration and axonal outgrowth. At a later developmental stage, its expression is particularly prevalent within developing synaptic fields. In particular, SPOCK1 is most prominently expressed in the thalamus and is upregulated in activated astroglial cells of the cerebrum [Charbonnier *et al*., 2000; Edgell *et al*., 2004; Röll *et al*., 2006; Vadasz *et al*., 2007].

Both the ***TPPP*** and ***TUBA3D/3E*** genes encode proteins with a role in microtubule cytoskeleton organization. Indeed, the tubulin polymerization-promoting protein (TPPP) has a brain-specific role in the dynamic stabilization of microtubular ultrastructures as well as in the projections of mature oligodendrocytes and ciliary structures, whereas the tubulins alpha 3d and 3e are major components of microtubules. Thus, possible defects in microtubule assembly and organization due to ***TPPP*** and ***TUBA3D/3E*** deletions, identified in patients 60 and 51, respectively (**Tab. 3**), may be implicated

in ASD.

Finally, four of the collected genes encode proteins that are integral components of the centrosome structure, a non-membranous organelle that functions as the major microtubule-organizing centre in animal cells, with clear roles in centrosome organization. Specifically, PCNT (potentially disrupted in patient 28, **Tab. 3**), an integral component of the filamentous matrix of the centrosome, is involved in the initial establishment of organized microtubule arrays in both mitosis and meiosis; NDE1 (duplicated in patient 30) interacts with other centrosome components as part of a multiprotein complex that regulates dynein function; PPP4C (duplicated in patients 25, 38, and 39) acts in many processes, such as microtubule organization at centrosomes, maturation of spliceosomal snRNPs, apoptosis, DNA repair, tumour necrosis factor (TNF)-alpha signaling, regulation of histone acetylation, DNA damage checkpoint signaling, NF-kappa-B activation, and cell migration; and **CEP72** (deleted in patient 60) is involved in the recruitment of key centrosomal proteins to the centrosome. A few centrosomal proteins have been previously involved in neurodevelopment: mutations in the coding genes (e.g., *CEP135*, *STIL*, *CDK5RAP2*, and *CENPJ*) cause autosomal-recessive congenital disorders characterized by intellectual disability and reduced brain and head size, suggesting that a centrosomal mechanism controls neuron numbers in the developing mammalian brain [Bond *et al*., 2005; Hussain *et al*., 2012]. Therefore, it is likely that anomaly in centrosomal protein may also contribute to ASD onset.

Transcriptional and translational regulation and chromatin remodeling (the genes affected by *de novo* CNVs are indicated in bold, **Tabs. 4** and **4.1**)

Fifty-one of the 276 selected genes have reported functions correlated with neuronal nuclear activities, such as DNA replication, repair, transcriptional regulation, and chromatin remodeling, as well as with protein synthesis, which contribute to overall neurodevelopment (**Fig. 21**). Similar to the findings reported for the subset of genes involved in neurogenesis, both losses and gains were observed. Moreover, all the *de novo* CNVs, except for one detected in patient 24, map to recurrent genomic regions as well as to regions previously associated with ASD or with other neurodevelopmental disorders by linkage studies (**Tabs. 3–4.1**).

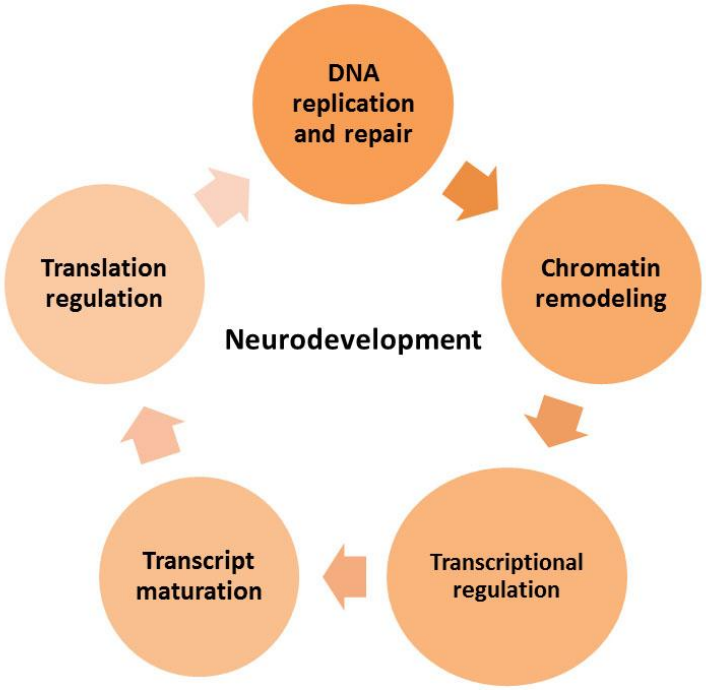

Fig. 21. Schematic view of the network of genes implicated in transcriptional and translational regulation, and in chromatin remodeling, subdivided on the basis of their function (light pink balls).

The largest functional category is that of transcriptional regulation, where the encoded proteins may act both as activators and repressors of transcription, playing a role, in most cases, in early embryonic development and thus regulating the differential expression of genes involved in neurodevelopment in a time-dependent manner. In particular, *RFX3* deletion is the only *de novo* variant that does not map to a recurrent genomic region (patient 24**, Tab. 3**). RFX3 is a transcriptional activator that acts as a transcription factor required for ciliogenesis. In mouse, it is strongly expressed in the ciliated ependymal cells of the subcommissural organ, choroid plexuses, and ventricular walls during embryonic and postnatal development [Benadiba *et al*., 2012; El-Zein *et al*., 2009], thus supporting a similar role in humans.

In addition to the well known genes implicated in transcriptional regulation and already associated with ASD, such as ***GTF1*** and ***GTF2IRD1*** at 7q11.23 (patient 52, **Tab. 3**), ***NDN*** at 15q11q13 (patient 29), and ***GSC2***, ***MED15*** and ***TBX1*** at 22q11.21 (patient 27), other genes may be suggested as ASD candidate genes after careful analysis. For example, ***GLI2*** encodes a member of the GLI family of zinc finger proteins that is thought to play a role during embryogenesis. In fact, in mouse embryo, GLI2 plays an essential role in the establishment of dorsoventral polarity and in thalamic development acting downstream of Sonic hedgehog (Shh) signaling [Haddad-Tovolli *et al*., 2012; Matise *et al*., 1998; Takanaga *et al*., 2009].

Furthermore, ***TGIF1***, which is duplicated in patient 60 along with ***THOC1*** (**Tab. 3**), encodes the TGFB-induced factor homeobox 1 protein, a member of the highly conserved TALE homeobox

protein family of transcription regulators. *In vivo* overexpression of TGIF in the developing chick neural tube demonstrated that TGIF plays an important role in regulating the expression of genes expressed in specific dorsal-ventral domains during neural development [Knepper *et al*., 2006]. Interestingly, ***NAB1***, which is deleted in patient 53 along with ***NEUROG1*** and ***ZNF804A*** (**Tab. 3**), encodes NGFI-A binding protein 1, which acts as a transcriptional repressor for zinc finger transcription factors EGR1 and EGR2. Both EGR1 and EGR2 have previously been implicated in mood disorders, such as major depressive disorder and bipolar disorder, respectively [Kerman *et al*., 2012; Kim *et al*., 2012]. Indeed, EGR1 is strongly expressed in neurons in the adult brain, where it can exert long-lasting changes in gene expression and subsequent protein synthesis that mediate synaptic plasticity, whereas EGR2 is involved in cognitive function, myelination, and signal transduction related to neuregulin-ErbB receptor, Bcl-2 family proteins, and brain-derived neurotrophic factor [Cole *et al*., 1989; Knapska and Kaczmarek, 2004; Malkani *et al*., 2004; Ressler *et al*., 2002; Valjent *et al*., 2006]. Dysregulation of EGR1/2 due to *NAB1* haploinsufficiency may be speculated to contribute to ASD development.

Finally, ***NEUROG1***, which is duplicated along with ***PITX1*** in patient 57 (**Tab. 3**), encodes neurogenin 1, which acts as a transcriptional regulator. It is involved in the initiation of cortical neuronal differentiation and associates with chromatin at enhancer regulatory elements in genes encoding key transcriptional regulators of neurogenesis. Interestingly, SNPs in *NEUROG1* have previously been associated with schizophrenia, whereas SNPs in *PITX1* have been associated with ASD [Fanous *et al*., 2007; Philippi *et al*., 2007]. *PITX1* encodes the paired-like homeodomain 1 protein, which plays a role in the development of anterior structures, in particular the brain and facies, and in specifying the identity or structure of hindlimbs [Philippi *et al*., 2007].

Interestingly, on the basis of the identified genes, anomalies in all the steps of neuronal nuclear activity may potentially be correlated with ASD pathology. Indeed, at least two genes are implicated in DNA replication, namely ***CDC45*** (duplicated in patient 27, **Tab. 3**), which encodes a protein necessary for the early steps of DNA replication in eukaryotes and is highly expressed during neurogenesis in cortical ventricular and subventricular zones, and *RNASEH2B* (deleted in patient 34), whose encoded protein specifically degrades the RNA of RNA:DNA hybrids. Of note, mutations affecting *RNASEH2B* cause Aicardi-Goutières syndrome type 2 (autosomal recessive and, rarely, autosomal dominant inheritance), which is characterized by brain malformations and cognitive dysfunction [Crow and Livingston, 2008]; thus, anomaly in this gene is also suggested in ASD development.

In addition, DNA repair must also be considered an essential process for proper neurogenesis. Indeed, the ERCC3 protein, whose corresponding gene demonstrated heterozygous deletion in patient 51 (**Tab. 3**), was found to be ubiquitously expressed in developing mouse brain from 9-day post-coitum embryo to 15-day postnatal brain, suggesting that defects may arise from ERCC3

interactions with other elements involved in particular aspects of neurodevelopmental control [Hubank and Mayne, 1994]. Moreover, mutations in *ERCC3* cause recessive forms of xeroderma pigmentosum group B combined with Cockayne syndrome (XP/CS), characterized by anomalies in skin pigmentation and, sometimes, neurological features such as microcephaly, intellectual disability, pigmentary retinopathy, ataxia, and decreased nerve conduction velocities [Oh *et al*., 2006].

Once mRNA has been transcribed, the translational process must begin, and specific proteins are required to form the initiation complex for translation. Rare mutations and balanced chromosomal abnormalities affecting *EIF4E*, encoding translation initiation factor 4E, have previously been reported in a small group of autistic boys and their unaffected fathers [Neves-Pereira *et al*., 2009]. Thus, the findings of rare CNVs involving ***EIF4H*** (fully duplicated in patient 52, **Tab. 3**) and *EIF2S3* (possibly disrupted in patient 1) seem to confirm a role of fine regulators of the translational process in ASD (**Tabs. 3–4.1)**. The control of mRNA translation into protein is, in fact, fundamentally important for the fine tuning of gene expression; additionally, precise translational control plays a critical role in many cellular processes, including development, cellular growth, proliferation, differentiation, synaptic plasticity, memory, and learning. Knockout mice deficient in *Eif4h* demonstrated a smaller brain volume compared with controls, altered brain morphology, and a reduction in both the number and complexity of neurons. Behavioural studies revealed severe impairments of fear-related associative learning and memory formation, thus suggesting that Eif4h, which in humans maps to 7q11.23, may contribute to certain deficits associated with Williams-Beuren syndrome [Capossela *et al*., 2012].

Similarly, when necessary translation must be finely repressed, one of the best-known genes encoding a protein that plays such a role is ***CYFIP1*** at 15q11.2, which is both deleted and duplicated in the reported cohort (patients 29, 50, and 55, **Tab. 3**). This gene encodes the cytoplasmic FMR1-interacting protein 1, which is a component of the CYFIP1-EIF4E-FMR1 complex that binds to the mRNA cap and mediates translational repression, thus promoting the translational repression activity of FMR1 in brain.

However, the complex process of transcription may also be regulated by the action of non-coding RNAs as well as by DNA epigenetic modifications also known as chromatin remodeling. As the evolution of the human brain has resulted in the emergence of higher-order cognitive abilities such as reasoning, planning, and social awareness, it is likely that in addition to larger brain size with greater complexity and capacity, a concomitant expansion of novel functional components and regulatory systems evolved [Barry and Mattick, 2012]. Recently, it has been suggested that RNA-directed epigenetic mechanisms have mediated human development and have contributed to brain plasticity, leading to the collateral emergence of psychiatric fragilities and conditions [Barry and Mattick, 2012]. In agreement with this hypothesis, ASD may be at least in part the result of

aberrant microRNA biogenesis, due to aberrations affecting, for example, ***DGCR8*** (duplicated in patient 27, **Tab. 3**), which encodes a subunit of the microprocessor complex that mediates the biogenesis of microRNAs from the primary microRNA transcript and plays a fundamental role in transcriptional regulation in the prefrontal cortex of developing mice [Schofield *et al*., 2011]. Similarly, defects in chromatin remodeling may contribute to ASD (e.g., ***BAZ1B***, ***EHMT1***, *JMJD2A*, *JMJD2C*, *SMARCC1*, and *SMYD3*), as confirmed by patients with Kleefstra/reciprocal duplication syndromes (patient 14, **Tab. 3**) [Kleefstra *et al*., 2005, 2009; Talkowski *et al*., 2012], both of which are comorbid with ASD and caused by aberrant *EHMT1* gene dosages leading to anomalies in histone methylation. Indeed, EHMT1 functions as a histone-lysine N-methyltransferase, whereas JMJD2A (deleted in patient 2, **Tab. 3**) and JMJD2C (possibly disrupted in siblings 38–39, **Tab. 3),** are histone-lysine demethylases. Of note, a SNP in *JMJD2C* has been previously associated with ASD in Finnish samples [Kantojärvi *et al*., 2010].

Moreover, during neural development, a switch from a stem/progenitor to a post-mitotic chromatin remodeling mechanism occurs as neurons exit the cell cycle and become committed to their adult state. Therefore, anomalies in chromatin remodeling may have contributed to ASD in the reported cohort. For example, SMARCC1, which belongs to the neural progenitors-specific chromatin remodeling complex (npBAF complex) and to the neuron-specific chromatin remodeling complex (nBAF complex), participates in this process, displaying helicase and ATPase activities (possibly disrupted in patient 3, **Tab. 3**). In mouse embryonic stem cells, Smarcc1 is necessary for heterochromatin formation and chromatin compaction during differentiation and plays important roles in facilitating mESC differentiation by coupling gene repression with global and local changes in chromatin structure [Marei *et al*., 2012; Schaniel *et al*., 2009]. Finally, ***BAZ1B*** maps to 7q11.23 (duplicated in patient 52, **Tab. 3**) and is deleted in the Williams-Beuren syndrome. It encodes a member of the bromodomain protein family, a MAPK-dependent phosphoprotein (tyrosine-protein kinase) that plays a central role in chromatin remodeling. Indeed, it is involved in DNA damage response by phosphorylating Tyr-142 of histone H2AX (H2AXY142ph). H2AXY142ph plays a central role in DNA repair and acts as a mark that distinguishes between apoptotic and repair responses to genotoxic stress [Cus *et al*., 2006; Oya *et al*., 2009]. Of note, heterozygote (Baz1b/-) and homozygote (-/-) mouse models show craniofacial abnormalities and cardiac malformations but no behavioural anomalies [Osborne, 2010].

Synaptogenesis and synaptic plasticity (the genes affected by *de novo* CNVs are indicated in bold, **Tabs. 4** and **4.1**)

Twenty-nine of the 276 selected genes are directly implicated in synaptogenesis and synaptic function and plasticity. In agreement with reported data that consider ASD as a synaptopathy [Zoghbi and Bear, 2012], most of the genes previously implicated in ASD (genes depicted in red in

Tabs. 4 and **4.1**) belong to this network and can be classified as either of the following:

1) genes localized pre-synaptically, which are involved in the docking of the synaptic vesicles onto the presynaptic membrane, vesicle recycling, membrane depolarization, and, consequently, neurotransmitter release;

2) genes localized post-synaptically, which encode either structural proteins that form the scaffolding complex known as postsynaptic density, adhesion molecules, or different types of neurotransmitter receptors such as glutamate, GABA, acetylcholine, and secretin receptors as well as synaptic signaling modulators (**Fig. 22**).

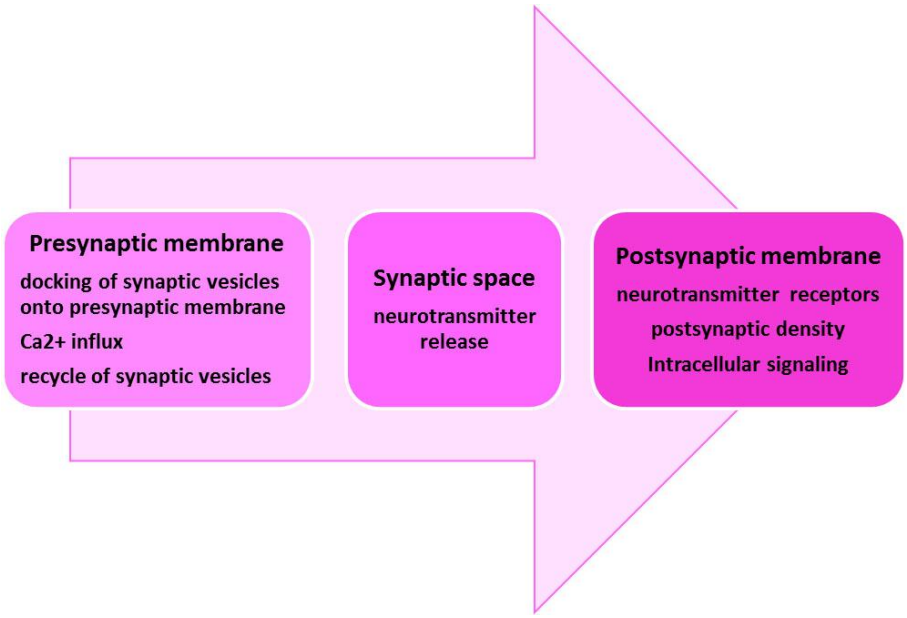

Fig. 22. Schematic view of the subsequent steps involved in synaptic function.

In observing a synapse from the presynaptic point of view, several known genes have been found to be affected by both *de novo* and inherited CNVs, such as ***CACNA1B*** (potentially disrupted in patient 14, **Tab. 3**) and *CACNA1C* (potentially disrupted in patients 38–39), which both encode voltage-dependent calcium channels responsible for intracellular Ca^{2+} influx that modulates neurotransmitter release. Additionally, *IL1RAPL2* (potentially disrupted in patient 14) may play a similar role. It encodes a member of the interleukin 1 receptor family that interacts with the neuronal calcium sensor 1 protein, thus playing a role in the downregulation of voltage-dependent calcium channel activity and in calcium-dependent exocytosis in excitatory synapses [Valnegri *et al*., 2011].

In addition, genes encoding proteins implicated in docking of synaptic vesicles to the membrane, such as *DOC2A* (duplicated in patients 25, 38, 39, **Tab. 3**), ***STX1A*** (duplicated in patient 52), and ***SNAP29*** (duplicated in patient 27), were detected. STX1A binds synaptotagmin in a calcium-dependent fashion and interacts with voltage-dependent calcium and potassium channels, thus

regulating calcium-dependent synaptic exocytosis. Furthermore, STX1A is part of the SNARE core complex, which also contains SNAP25 [Ghezzo *et al*., 2009]. SNAP29 forms a complex with SNAP23, SNAP25, and STX1A and acts as a negative modulator for neurotransmitter release, probably by slowing recycling of the SNARE-based fusion machinery and synaptic vesicle turnover [Pan *et al*., 2005; Su *et al.*, 2001]. Moreover, DOC2A binds to STXBP1, the syntaxin (STX) binding protein 1, thus regulating the STXBP1-STX interaction, which is essential for the activity of the synaptic vesicle fusion machinery [Mochida *et al*., 1998]. All the genes encoding these proteins map to recurrent genomic regions previously implicated in genomic disorders that are comorbid with ASD (**Tabs. 3-4.1**) [Betancur, 2011] as well as in other neuropsychiatric disorders, such as attention deficit hyperactivity disorder, intellectual disability, and schizophrenia, confirming the existence of functional networks of genes whose dysregulation is the shared pathological base for the development of this class of diseases.

Interestingly, the large *de novo* deletion detected in patient 60 at 5p15.33p15.31 (**Tab. 3**) involves, among others, the ***EXOC3*** gene, also known as ***SEC6***, which has not been previously suggested as an ASD candidate gene. This gene encodes the exocyst complex component 3, which is a component of the exocyst complex, a multiple-protein complex essential for targeting exocytic vesicles to specific docking sites on the plasma membrane. In a study of developing neurons, Sec6/8 complexes were found at the highest levels in regions of the brain undergoing synaptogenesis and in regions of cultured neurons where synapses will subsequently develop. By contrast, the level of Sec6/8 was downregulated in mature synapses [Hazuka *et al*., 1999; Hsu *et al*., 1996, 1999], leading to the hypothesis that the main function of the Sec6/8 complex is in the formation of synapses rather than in their function once formed.

Furthermore, two ASD candidate genes emerged from the present study, the *VAT1* and ***ADCYAP1*** genes (deleted in patient 25 and duplicated in patient 60, respectively, **Tab.3**). VAT1 is an abundant integral membrane protein of cholinergic synaptic vesicles and is thought to be involved in vesicular transport. In the CNS which uses acetylcholine as a neurotransmitter, is known to have a variety of neuromodulatory effects upon plasticity, arousal, and reward. Indeed, acetylcholine plays an important role in sustaining attention, learning, and short-term memory, and functional studies implicate the cholinergic system in the development of autism on the basis of neuronal nicotinic acetylcholine receptor losses in cerebral and cerebellar cortex and in the thalamus of autistic brains [Ray *et al*., 2005; Suzuki *et al*., 2011].

ADCYAP1 encodes adenylate cyclase activating polypeptide 1 (PACAP), which stimulates adenylate cyclase and subsequently increases the cAMP level in target cells. The neuropeptide PACAP is a molecule released from stress-transducing neurons. It exerts postsynaptic effects required to complete hypothalamo-pituitary-adrenocortical (HPA) and hypothalamo-splanchnico-adrenomedullary (HSA) circuits activated by psychogenic and metabolic stressors. PACAP is

widely expressed throughout the brain and exerts its functions through the PACAP-specific receptor PAC(1). Recent studies reveal that genetic variants of the *PACAP* and *PAC(1)* genes are associated with mental disorders, and several behavioural abnormalities of *PACAP* knockout mice have been reported, thus suggesting that PACAP has an important role in the regulation of locomotor activity, social behaviour, anxiety-like behaviour, and, potentially, working memory [Hattori *et al*., 2012; Stroth *et al*., 2011].

Among genes encoding structural proteins that function post-synaptically as transmembrane or scaffolding proteins, most have been reported previously, such as *NLGN4X* (potentially disrupted in patient 35, **Tab. 3**), *DLGAP2* (duplicated in patient 59), and *DLG2* (deleted in patient 26). Conversely, to the best of our knowledge, this study is the first to report a rare *de novo* CNV affecting *DLGAP1*, which was fully duplicated in patient 60 (**Tab. 3**). Additionally, DLGAP1 works at the postsynaptic density; therefore stoichiometric imbalances in the protein dosage may produce defects in scaffold formation, as previously suggested for SHANK3 [Toro *et al*., 2010].

Many transmembrane proteins encoded by this network of genes are actually neurotransmitter receptors, most of which have already been implicated in ASD. Specifically, CNVs affecting genes for glutamate receptors *GRID2* (deleted in patient 42, **Tab. 3**) and *GRM7* (potentially disrupted in patient 44) and receptors of GABA (***GABRA5/B3/G3***, duplicated in patient 29), acetylcholine (*CHRNA7*, duplicated in patient 25), and secretin (*SCTR*, duplicated in patient 10) have been identified.

Moreover, some interesting genes have been identified that modulate synaptic plasticity. First, ***CAMLG***, which is duplicated in patient 57 (**Tab. 3**), encodes a calcium modulating ligand protein that has been reported to be involved in recycling and endocytic processing of GABAA receptors. Indeed, in neuronal cortical cultures it has been demonstrated that a reduction of CAMLG translated to reduced GABAA receptors on the postsynaptic membrane with an effect specific to GABAA receptors, since glutamate-evoked current remained unaltered in these neurons [Yuan *et al*., 2008; Zhang *et al*., 2010]. Moreover, GABA signaling seems also to be modulated by the protein encoded by the *DBI* gene, which is fully duplicated in patient 10 (**Tab. 3**). This gene encodes the diazepam binding inhibitor, a GABA receptor modulator, which is able to displace diazepam from the benzodiazepine (BZD) recognition site located on the GABA type A receptor. It is possible that this protein acts as a neuropeptide to modulate the action of the GABA receptor located in brain synapses. Recently, a CNV involving loss of *DBI* was described in an autistic patient, and SNPs in *DBI* have been associated with anxiety and panic attacks [Griswold *et al*., 2012; Thoeringer *et al*., 2007]. Furthermore, functional studies reported reduced numbers of GABA and benzodiazepine receptors in autistic brain, particularly in posterior cingulate cortex [Oblak *et al*., 2011].

Finally, the ***CA4*** gene, which is fully duplicated in patient 16 as a result of a *de novo* duplication (**Tab. 3**), can be considered a good candidate for ASD development. It encodes carbonic anhydrase IV, which belongs to a large family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide. These enzymes are indirectly implicated in regulation of excitatory synaptic transmission, because the curtailment of extracellular alkaline shifts by extracellular carbonic anhydrases was shown to limit postsynaptic NMDA receptor activation during synchronous neural activity [Tong *et al*., 2000]. To date, defects in *CA4* have only been associated with retinitis pigmentosa type 17. However, rare single-gene mutations affecting *CA6*, another member of the same family, have been reported in a few autistic patients [Bucan *et al*., 2009].

CNS metabolism and homeostasis (the genes affected by *de novo* CNVs are indicated in bold, **Tabs. 4** and **4.1**)

Twenty-seven of the 276 selected genes play roles essential for proper neuronal metabolism, coding for genes involved in amino acid catabolism (e.g., ***ACMSD***, *BCHDHA*, *GLDC*, ***GLS***, *HIBCH*, ***PRODH***, and *QPRT*); neurotransmitter and neuropeptide maturation and degradation (e.g., ***COMT***, ***PREP***, and *SULT1A3*); glycolysis, Krebs cycle, and mitochondrial metabolism (e.g., *ALDOA*, *ME3*, *NDUFV2*, *SDHA*, *SLC25A48*, and ***TXNRD2***); and biosynthesis of heparan sulfate molecules, phospholipids and lipoproteins (***ABCD4***, ***HS6ST1***, *LCLAT1*, ***LPCAT1***, and ***NDST2***) (**Tabs. 4** and **4.1**, **Fig. 23**).

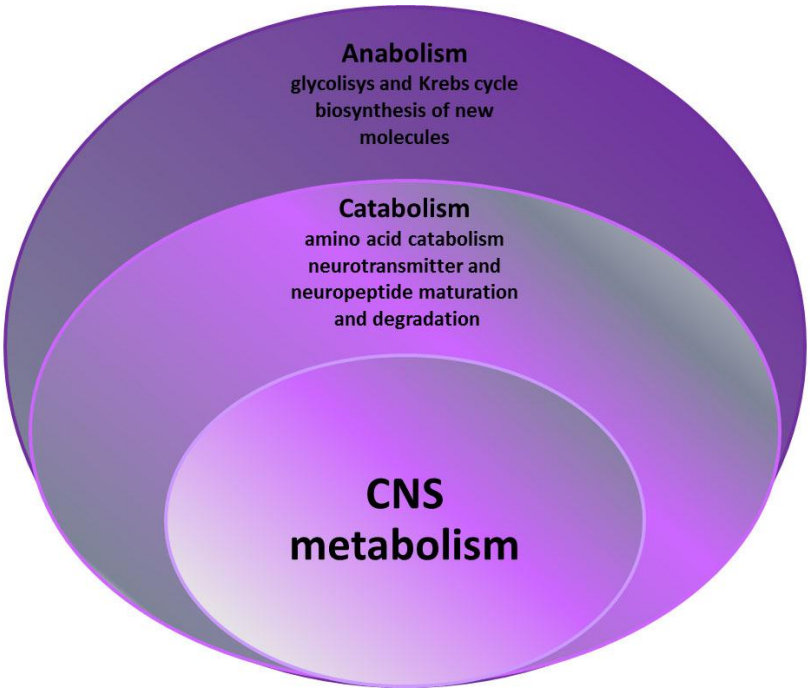

Fig. 23. Schematic view of the principal reactions implicated in CNS metabolism that are potentially dysregulated in the present ASD cohort.

Among the subset of genes that encode anabolic enzymes, those implicated in heparan sulfate (HS) biosynthesis appear as very interesting new ASD candidates on the basis of previous studies that report, for example, autism in patients with multiple exostoses due to mutations in HS genes [Bolton *et al.*,1995; Ishikawa-Brush *et al.*, 1997; Li *et al.*, 2002]. Both these genes, namely ***HS6ST1*** and ***NDST2***, are affected by *de novo* deletions (**Tab. 3,** patients 36 and 51, respectively) and encode sulfotransferase proteins, which are necessary for processing glucosamine and heparin polymers. Indeed, roles of HS in neural development have been well established using animal models that carry mutations in genes encoding enzymes involved in HS synthesis, thus revealing that HS is necessary for the specification of certain brain structures, such as the cerebellum and the olfactory bulbs, cortical neurogenesis, and a variety of axon path-finding processes [Conway *et al.*, 2011; Irie *et al.*, 2012]. Moreover, several pieces of evidence suggest a role for HS in synaptic function as well as in higher cognitive function. For example, in adult neurons, HS is enriched in synapses, especially in the postsynaptic membrane of dendritic spines [Ethell and Yamaguchi, 1999]. Thus, it may be speculated that the anomalies in HS genes identified in the two patients reported here may have played a role in ASD.

Another interesting gene appears to be ***LPCAT1***, which is included in a large *de novo* deletion identified in patient 60 (**Tab. 3**). It encodes the lysophosphatidylcholine acyltransferase 1 protein, which plays important roles in phospholipid metabolism and modulation of inflammation. Indeed, LPCAT1 catalyzes the conversion of lysophosphatidylcholine (LPC) to phosphatidylcholine (PC), which is the major phospholipid of the brain. Since LPC is a bioactive pro-inflammatory lipid whose accumulation is associated with atherosclerosis, myocardial ischemia, neurodegeneration, and inflammatory diseases, LPCAT1 is implicated in the anti-inflammatory response by its role in the conversion of LPC to PC. In addition, the LPCAT1 enzyme catalyzes the synthesis of platelet-activating factor (PAF), a potent inflammatory lipid, from lyso-PAF [Cheng *et al.*, 2009; Matsumoto *et al.*, 2007; Nakanishi *et al.*, 2006]. In naïve mice, constant levels of PAF are produced by microglia and astrocytes, thus contributing to the maintenance of CNS homeostasis. Conversely, in the CNS of experimental allergic encephalomyelitis mice, which mimic multiple sclerosis, the blood-brain barrier is broken. Inflammatory cells, such as T cells and macrophages, infiltrate the CNS, and higher amounts of LPCAT1/2 and therefore of PAF are produced by the activated microglia and macrophages, contributing to the inflammatory process [Kihara *et al.*, 2008].

Of note, other genes involved in CNS metabolism show implications in inflammation and strong relationships with immune cells, confirming that the role of the immune system is essential for correct CNS homeostasis. For example, ***PREP***, which was found deleted in patient 18 due to a *de novo* CNV, encodes a prolyl endopeptidase protein that has been reported to be involved in the maturation and degradation of peptide hormones and neuropeptides. Several neuropeptides

associated with learning, memory, and neurodegenerative disorders have been proposed as the substrates for PREP, suggesting a possible role for PREP in these processes. Indeed, *Prep* knockout mice demonstrate decreased synaptic spine density in the hippocampus, reduced hippocampal long-term potentiation, and impaired hippocampal-mediated learning and memory, thus revealing a possible role for PREP in mediating hippocampal plasticity and spatial memory formation [D'Agostino *et al*., 2012].

More interestingly, PREP directly binds TAC1, tachykinin precursor 1, thus participating in its maturation. Tachykinins (substance P, neurokinin A and B) are active neuropeptides that have been recognized as key mediators of neuro-immune interactions in neuroinflammation and some autoimmune diseases [Veres *et al*., 2009]. They are found throughout the CNS, with evidence for both neuronal and glial cells as being sources of them, and traditionally show well-defined functions as neurotransmitters modulating glutamatergic excitatory synaptic transmission. Furthermore, tachykinins may have a role in augmenting the immune functions of CNS glial cells resulting in the progression and duration of damaging inflammation within the CNS, which has already been observed in autistic brains. Indeed, elevated serum levels of neurokinin A have been recently reported in some autistic children compared to controls. Interestingly, levels of neurokinin A correlated to the severity of autism and to serum levels of anti-ribosomal P protein antibodies, thus supporting the pathogenic role of neurokinin A and its possible link to autoimmunity in autism [Mostafa and Al-Ayadhi, 2011]. Thus, it is a likely hypothesis that altered PREP levels within the CNS may interfere with tachykinin metabolism and the subsequent response to inflammatory stimuli, thus playing a role in ASD.

Finally, among the subset of genes that encode enzymes acting in amino acid catabolism, two are specifically involved in tryptophan catabolism, namely ***ACMSD***, deleted in patient 51, and *QPRT*, duplicated in patients 25, 38, and 39 (**Tab. 3**). ACMSD is the aminocarboxymuconate semialdehyde decarboxylase that converts alpha-amino-beta-carboxy-muconate-epsilon-semialdehyde to a benign catabolite, thus preventing the accumulation of quinolinate, whereas QPRT is the quinolinate phosphoribosyltransferase that converts quinolic acid to nicotinic acid ribonucleotide and carbon dioxide (**Fig. 24**) [Schwarcz *et al*., 2012]. Therefore, these enzymes are both implicated in limiting the cerebral levels of quinolate, which acts as a neuroactive compound, as well as other intermediates in tryptophan degradation, such as kynurenic acid and 3-hydroxykynurenine (**Fig. 24**). In particular, quinolinate is able to induce a neuronal excitotoxin due to its role as a NMDA receptor agonist.

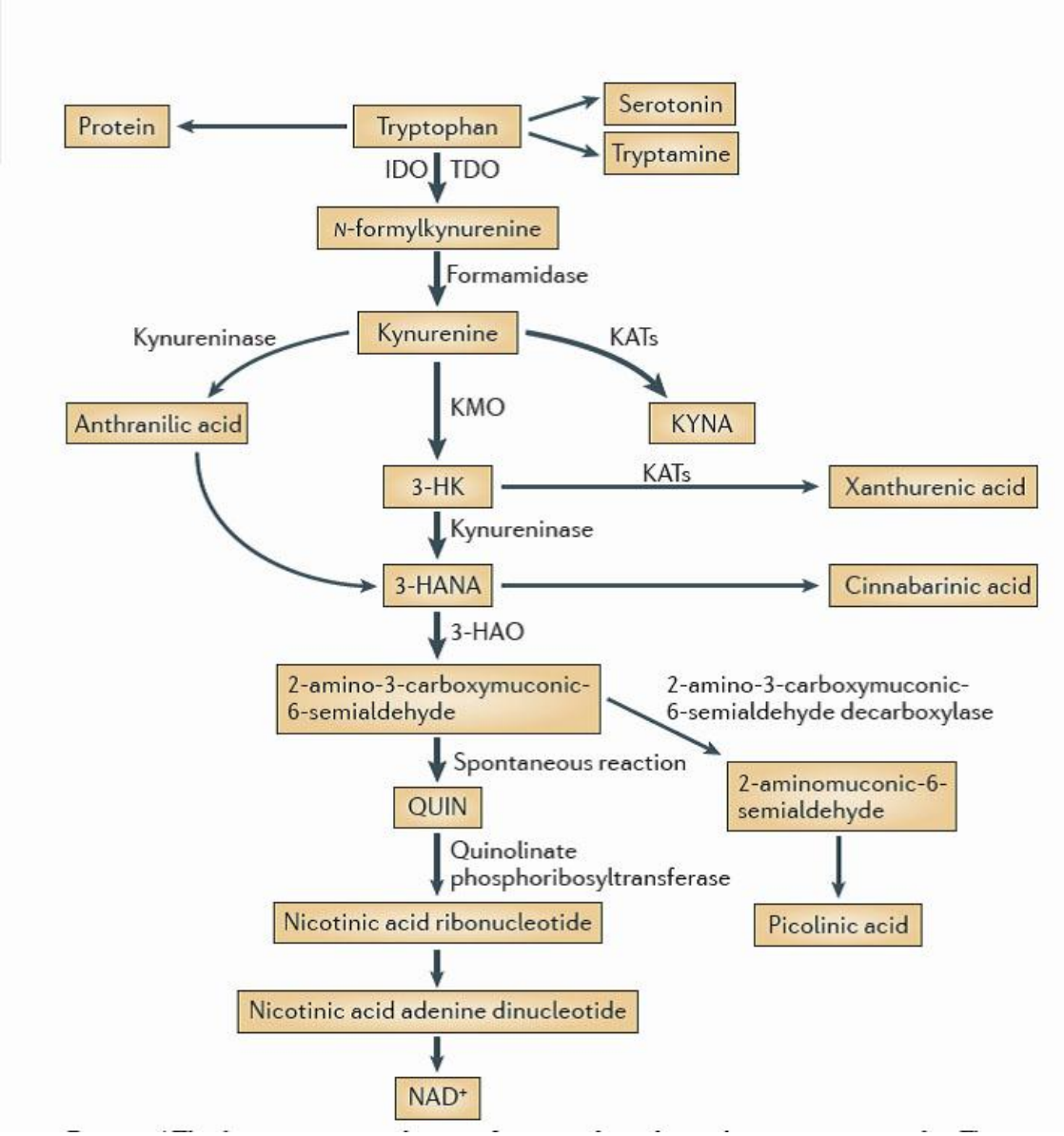

Fig. 24. The kynurenine pathway of tryptophan degradation in mammals [Schwarcz *et al.*, 2012].

The synthesis of these metabolites is regulated by an enzymatic cascade known as the kynurenine pathway, which is tightly controlled by the immune system. Indeed, as shown in **Fig. 25**, the two branches of the pathway are performed normally by non-neuronal cells, such as astrocytes and other glial cells, which metabolize tryptophan and intermediate metabolites from the blood vessels, thus producing quinolate (microglia) and kynurenic acid (astrocytes) [Schwarcz *et al.*, 2012]. Under normal conditions, in the periphery, the degradation of tryptophan and the subsequent formation of circulating kynurenines is normally regulated by steroid hormones, cytokines, and growth factors. Brain uptake of these kynurenines determines kynurenine pathway flux in the brain. Conversely, inflammatory conditions stimulate the kynurenine pathway both in the periphery and in the brain. Therefore, increased influx of brain-permeable metabolites leads to an excess of

kynurenines in the brain parenchyma. Furthermore, infiltrating cytokines stimulate the kynurenine pathway in activated microglial cells and blood-borne cells within the brain [Schwarcz *et al*., 2012].

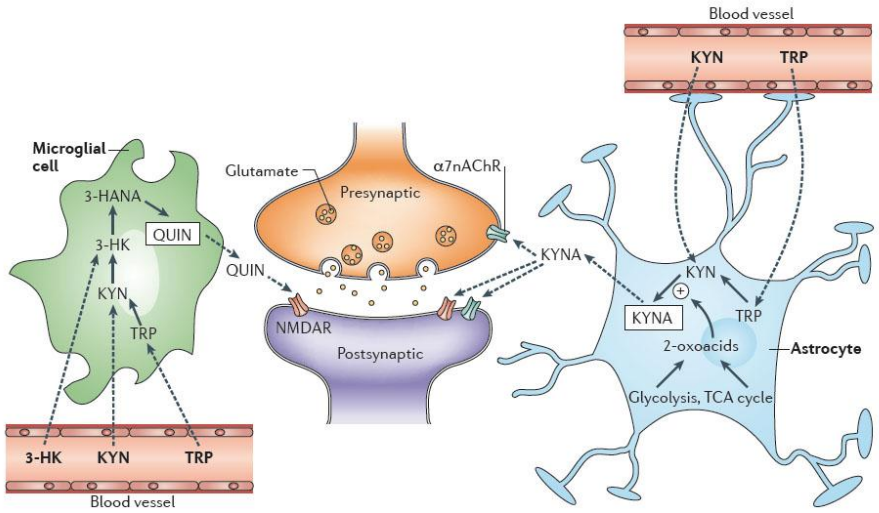

Fig. 25. Segregation of the two kynurenine pathway branches in the brain [Schwarcz et al., 2012].

It has been reported that dysregulation of this pathway, resulting in hyper- or hypofunction of active metabolites, is associated with neurodegenerative and other neurological disorders, such as Huntington's disease, Parkinson's disease, and Alzheimer's disease, as well as with psychiatric diseases, such as depression and schizophrenia. In this context, one possible explanation for ASD pathogenesis is the modern theory of immunoexcitotoxicity, that is, an excessive stimulation of glutamatergic synapses mediated by increased levels of neuroactive compounds produced by microglial cells, as hypothesized in a few patients reported here. Indeed, chronic microglial activation has been reported in autistic brains, which results in an outpouring of neurotoxic levels of the excitotoxins glutamate and quinolinic acid. Careful control of brain glutamate levels is essential to brain pathway development, and excesses can result in arrest of neural migration, as well as in dendritic and synaptic loss. In addition, certain cytokines, such as TNF-alpha, can, via their receptors, interact with glutamate receptors to enhance the neurotoxic reaction [Schwarcz et al., 2012].

Intracellular signaling and trafficking (the genes affected by *de novo* CNVs are indicated in bold, **Tabs. 4** and **4.1**)

Eighty-one of the 276 selected genes belong to a functional network implicated in intracellular signaling and trafficking, which acts in response to external signals that are first represented by synaptic neurotransmission. Of note, the present ASD cohort is enriched in rare CNVs affecting genes that act in this functional network, which, as expected, includes a few pathways already

suggested to be implicated in ASD pathogenesis: such as, the remodeling of actin cytoskeleton [Blanchoin *et al.*, 2000; Linseman and Loucks, 2008], which is deeply interconnected with the Wnt/β-catenin signaling pathway [Okerlund and Cheyette, 2011; Rosso *et al*., 2005; Salinas and Zou, 2008; Salinas *et al*., 199], the MAPK/ERK signaling pathway that ultimately regulates the mTOR pathway [Tavazoie *et al*., 2005; Wang *et al*., 2012], and the protein ubiquitination pathway [Ehlers, 2003; Glessner *et al*., 2009; Peça and Feng, 2012; Philpot *et al*., 2010] (**Fig. 26**).
However, most of the selected genes are new candidates, as they have not previously been associated with ASD (**Tabs. 4** and **4.1**).

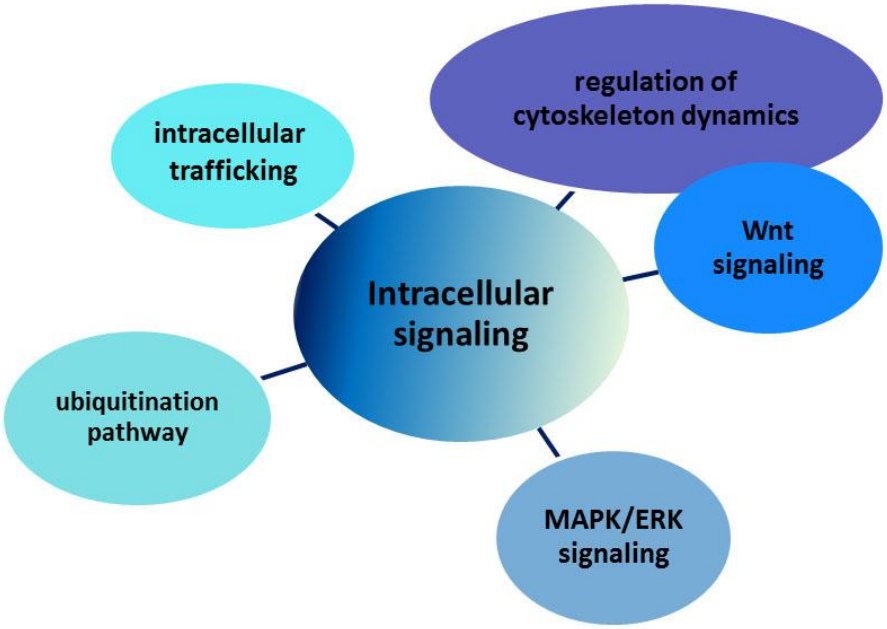

Fig. 26. Schematic view of the pathways (blue and light blue satellites) that act in concert to mediate the intracellular signaling processes.

One of the most important types of information that is transmitted through the synapse is related to the regulation of the morphology of the dendritic spines. It is mediated by the GTPase Rho family of proteins, such as RhoA/B, CdC42, and Rac1, whose role in ASD is well known. In addition, GTPase activity is regulated by different GEF (guanine nucleotide exchange factor), GDI (GDP dissociation inhibitor), and GAP (GTP-activating) proteins. Actin cytoskeleton remodeling is involved in neuronal morphogenesis, axonal guidance, and synaptic plasticity, and, pre-synaptically, it is necessary to mediate the docking of synaptic vesicles to the plasma membrane and subsequent neurotransmitter release. RhoGEFs have been previously implicated in human genetic disorders. For example, mutations in *ARHGEF6* have been associated with X-linked nonsyndromic mental retardation [Kutsche *et al*., 2000], and aberrant EphB/Ephexin5 signaling

during the development of synapses has been linked to the abnormal cognitive function that possibly occurs in ASD [Margolis *et al*., 2010].

Thus, it may be hypothesized that anomalies in *ARHGAP26* (potentially disrupted in patient 21, **Tab. 3**), ***ARHGAP28*** (duplicated in patient 60), ***ARHGEF4*** (deleted in patient 51), and *ARHGEF10* (duplicated in patient 59) may be implicated in the neurobehavioral disorders described in the affected patients. Recently, a rare small recurrent deletion in a region previously linked attention deficit hyperactivity disorder at 2q21.1, including *ARHGEF4,* has been identified in five unrelated families with developmental delay, intellectual disability, attention deficit hyperactivity disorder, and epilepsy and other neurobehavioral abnormalities, whereas the reciprocal duplications have been identified in five unrelated families with autism [Dharmadhikari *et al*., 2012].

Another GEF protein, DOCK8, was recently found to be deleted in two unrelated autistic patients who carry a 9p24 terminal deletion also including the *KANK1* gene [Lerer *et al*., 2005]. This finding is confirmed by the two rare duplications detected in patient 33, which potentially disrupt both the *DOCK8* and *KANK1* genes (**Tab. 3**). In particular, KANK1 functions in cytoskeleton formation in a RhoA-dependent manner by regulating actin polymerization, and recently it has been demonstrated that nucleo-cytoplasmic shuttling of human KANK protein accompanies intracellular translocation of beta-catenin and, therefore, beta-catenin-dependent transcription [Wang *et al*., 2006]. In addition, *CFL2* (duplicated in patient 17) encodes the cofilin 2 protein, which plays a role in the direct regulation of actin filament dynamics.

Interestingly, two genes encoding members of the Ras-like small GTPases that have not been previously associated with ASD were found to be affected by rare CNVs in the present cohort, namely *RALB* (deleted in patient 10, **Tab. 3**) and ***RAB6C*** (deleted in patient 51). RalA and RalB regulate a wide variety of cellular processes, including transcription, translation, cytoskeletal organization, membrane trafficking, cytokinesis, cell migration, cell proliferation, and cell survival. Recently, the involvement of RalA/B in projection neuron migration from the ventricular zone to the neocortical plate during mouse brain development has been demonstrated [Jossin and Cooper, 2011]. Moreover, several members of the Rab family small GTPases are key mediators of membrane trafficking and regulate axon-specific trafficking events. For example, Rab17 regulates dendritic morphogenesis and postsynaptic development in mouse hippocampal neurons [Mori *et al*., 2012], whereas Rab4 and Rab5 are key players in the regulation of endocytosis, as recently demonstrated in astrocytes, the most abundant glial cells in the brain [Potokar *et al*., 2012]. By analogy, it is likely that Rab6 may also be implicated in neurodevelopment.

Of note, all the genes involved in actin cytoskeleton remodeling show a high expression in immune cell types, as they are also involved in the maturation of dendritic cells, T cell activation, migration, and cell-cell adhesion, as well as formation of immunological synapses (**Tabs. 4** and **4.1**). Thus, it

is possible that a dysregulation of this pathway due to genetic defects may impact not only neurological but also immune system development.

Interestingly, a small percentage of genes encode proteins that play a role in the organization of the microtubules at the kinetochore (B9D2 and CLASP1) as well as motor proteins required for the transport of organelles along the microtubules (KIF2A and KIF22), whose encoding genes may be suggested as new ASD candidate loci. In particular, the kinesin proteins are microtubule-dependent molecular motors that transport organelles within cells, an essential process for axonal growth and elongation, and move chromosomes during cell division.

Wnt signaling is a key pathway that helps to organize development of the nervous system. It influences cell proliferation, cell fate, and cell migration in the developing nervous system, as well as axon guidance, dendrite development, and synapse formation. Given this wide range of roles, dysregulation of Wnt signaling could have any number of deleterious effects on neural development and thereby contribute in many different ways to the pathogenesis of neurodevelopmental disorders. Some major psychiatric disorders, including schizophrenia, bipolar disorder, and ASD, are, indeed, coming to be understood as involving subtle dysregulation of nervous system development, particularly of synapse formation and maintenance [Okerlund and Cheyette, 2011]. As shown in **Fig. 27**, a few genes, among those selected, participate in Wnt signaling and therefore may be new ASD candidates.

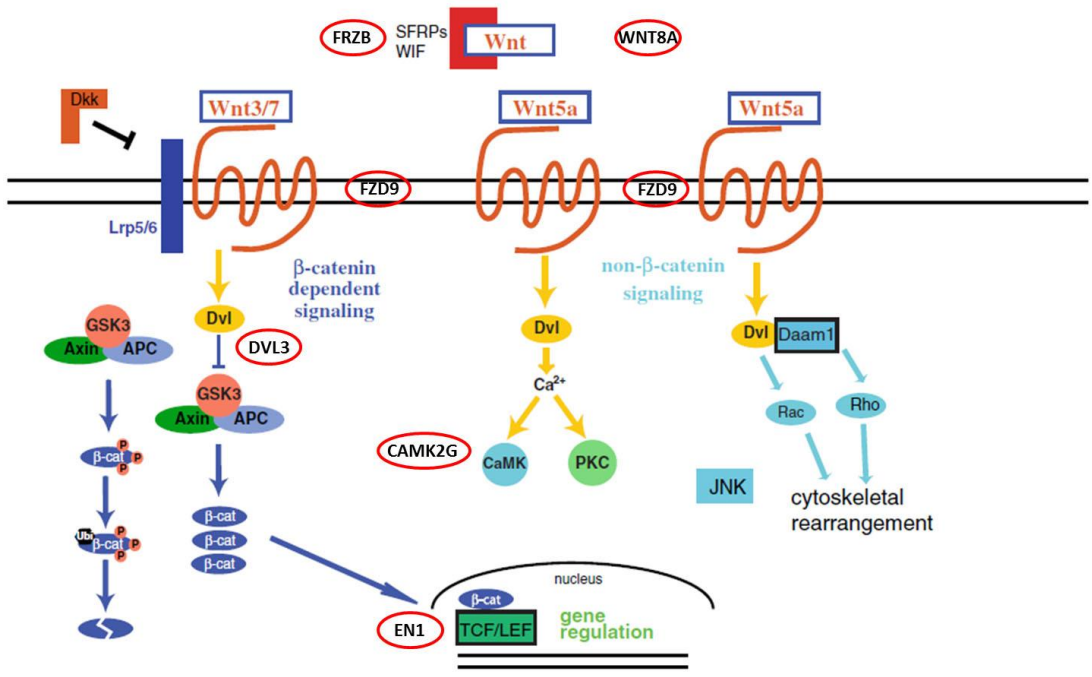

Fig. 27. Canonical (β-catenin-dependent signal, left) and non-canonical (β-catenin-independent signal, right) Wnt signaling pathways [Okerlund and Cheyette, 2011]. The new suggested ASD candidate genes are circled in red.

For example, *DVL3* (duplicated in patient 48, **Tab. 3**), the dishevelled (dsh) homolog 3 protein, is a member of a multi-gene family that shares strong similarity with the Drosophila dishevelled gene. DVL activation leads to its binding of AXIN, phosphorylation, and inhibition of glycogen synthase kinase-3β (GSK3β), and the regulation of several downstream targets, including β-catenin (**Fig. 27**) [Gao and Chen, 2010]. ***WNT8A*** (duplicated in patient 57) and ***FRZB*** (deleted in patient 53) encode secreted molecules that specifically bind receptor proteins, such as **FZD9** (duplicated in patient 52), thus activating the signaling cascade. Finally, *EN1* (duplicated in patient 10) encodes a transcription factor with a well known role in CNS development, and together with β-catenin regulates transcription of β-catenin target genes in neuronal cells [Alves dos Santos and Smidt, 2011], whereas ***CAMK2G*** (deleted in patient 36) is the only identified gene with a specific role in non-canonical Wnt signaling.

Furthermore, it is known that the Ras/Raf/ERK1/2 signaling pathway (**Fig. 28**) plays important roles in the genesis of neural progenitors and in learning and memory, as well as death-promoting apoptotic roles in neural cells. Upregulation of this pathway has been observed in the brains of autistic subjects and mouse models [Yang *et al*., 2011, 2012; Zou *et al*., 2011]. In addition, rare single missense mutations affecting *MAPK3* have been reported in a few HF-AU patients [Schaaf *et al*., 2011].

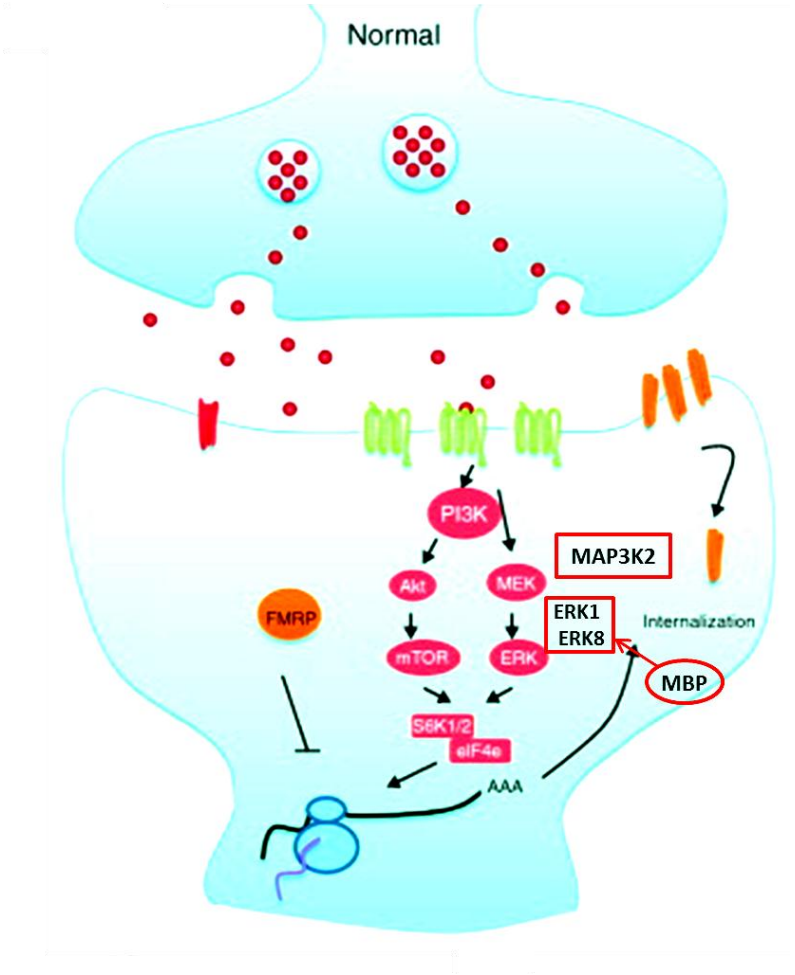

Fig. 28. Synaptic ERK1/2 signaling pathway [Wang *et al*., 2012]. The new suggested ASD candidate genes are circled in red. The AMPA (orange), NMDA (red), and mGlu (green) receptors are indicated at the postsynaptic membrane.

ERK1 (duplicated in patients 25, 38, and 39, **Tab. 3**), *ERK8* (deleted in patient 37), and ***MAP3K2*** (deleted in patient 51) have been found to be affected by rare CNVs in the present ASD series, and therefore they may be potentially implicated in ASD (**Fig. 28**). Interestingly, myelin basic protein, encoded by *MBP* (potentially disrupted in patient 42), which is a major constituent of the myelin sheath of oligodendrocytes and Schwann cells in the nervous system, is also related to the ERK signaling pathway. Indeed, MBP directly interacts with ERK1 and ERK2, and *in vitro* assays indicated a direct interaction between ERK8 and MBP (**Fig. 28**) [Abe *et al*., 2002].

Finally, due first of all to the well known implications of mutations affecting *UBE3A* in ASD pathogenesis, dysregulation of the ubiquitination pathway, which regulates the levels of synapse proteins and their turnover, has been previously implicated in ASD [Ehlers, 2003; Glessner *et al*., 2009; Peça and Feng, 2012; Philpot *et al*., 2010]. The finding of many genes encoding proteins that function in this pathway seems to confirm this observation. As shown in **Fig. 29**, the ubiquitin ligase **UBE3A** (duplicated in patient 29), which belongs to the E3 ubiquitin ligase family, works as

a single enzyme, directly binding the target protein as well as an ubiquitin conjugating enzyme (E2), whereas the SCF E3 ubiquitin ligase works as a complex.

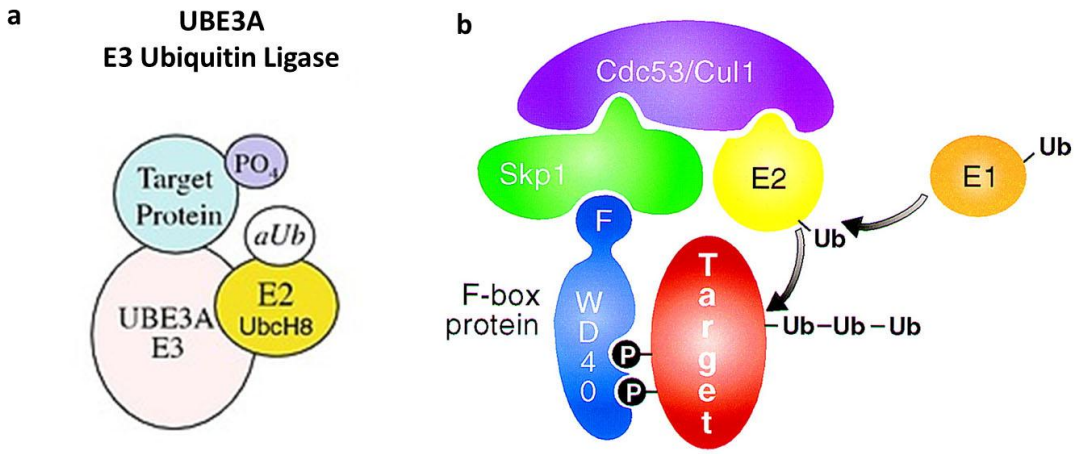

Fig. 29. E3 ubiquitin ligases. (a) UBE3A-type E3 ubiquitin ligase, which acts as a single protein; (B) SCF (Skp, Cullin, F-box containing complex) E3 ubiquitin ligase, which acts as a multiprotein complex.

Among the selected genes, ***TRIM50*** (potentially disrupted in patient 52, **Tab. 3**) encodes an E3 ubiquitin ligase; ***UBE2E3*** (deleted in patient 53) encodes a conjugating enzyme belonging to the family of E3 ubiquitin ligases; and ***KLHL22*** (duplicated in patient 27), ***KLHL3*** (duplicated in patient 57), and ***FBXL21*** (duplicated in patient 57) encode proteins of the SCF E3 ubiquitin ligase family. In particular, KLHL22 and KLHL3 are substrate adaptors for the ubiquitin ligase, whereas FBX22 is a specific F-box protein whose role is to target specific proteins. Furthermore, two of the selected genes, *USP9Y* (potentially disrupted in patient 19) and ***USP14*** (potentially disrupted in patient 60), code for peptidases that cleave the ubiquitin residues in order to recycle the proteins, thus contributing to protein turnover.

<u>CNS development and homeostasis mediate by the immune system, immunosurveillance and modulation of inflammation</u> (in bold are indicated the genes affected by *de novo* CNVs, **Tabs. 4** and **4.1**).

In agreement with published data, the CNV gene content analysis in the present ASD cohort has revealed deep interconnections between all the identified functional networks of genes and the immune system, thus supporting the hypothesis that ASD may be the result of defects in both the CNS and the immune system (**Tabs. 4** and **4.1**) [Onore *et al.*, 2012]. In addition, 19 of the 276 selected genes have been classified as genes encoding proteins which specifically play a role in mediating the cross-talk between the CNS and the immune system (**Fig. 30**).

Over the years several theories have been proposed to explain immune dysfunctions in ASD, howevere some questions are still unresolved:

- is there a strong genetic basis in patients with ASD, which may result in immune dysregulation?
- what role do viral or bacterial infections as well as maternal antibodies against foetus brain epitopes play in the CNS development during pregnancy or in the early months/years of life?
- why markers of chronic inflammation have been found in a subset of autistic patients? Is this phenomenon the consequence of genetic alterations, or is an abnormal response to non-genetic stimuli?

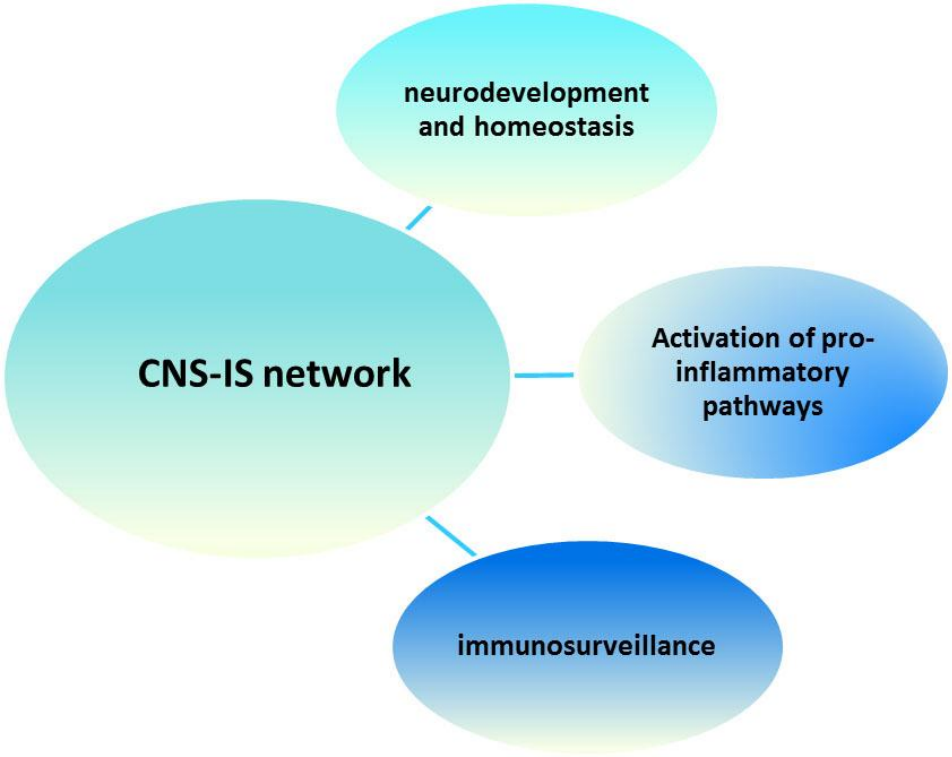

Fig. 30. Schematic view of the functional network of genes which are implicated in the cross-talk between the CNS and the IS. CNS, central nervous system; IS, immune system.

A few CNVs have been identified in the present ASD cohort whose gene content analysis might be of some help in answering to the above questions. First, a few genes are involved in neurodevelopment and CNS homeostasis, thus confirming that aberrations in immune genes may alter not only the immune response but also neurodevelopment, and that these events may have a genetic bases. For example, *MARCO* (duplicated in patient 10, **Tab.3**) is involved in microglial maturation, ***IL-9*** (duplicated in patient 57) is implicated in regulation of programmed cell death in developing brain, ***CXCL14*** (duplicated in patient 57) is involved in post-natal regulation of GABAergic transmission in specific areas of the brain, and ***TSPAN5*** (possibly disrupted in patient 6) encodes the tetraspanin 5, that mediates signal transduction events which play a role in the regulation of cell development, activation, growth and motility.

One of the first TSPAN5 interacting proteins is NOTCH2, that is involved in differentiation and synapse formation. Expression studies of autistic and matched control brains reported increased transcript levels of many immune system related genes such as *NOTCH2*. However, these expression patterns appear to be more associated with the late recovery phase of autoimmune brain disorders, than with the innate immune response characteristic of neurodegenerative diseases [Garbett *et al*., 2008]. Interestingly, rare single gene mutations affecting *TSPAN7*, another member of the tetraspanin family, have been reported in a few autistic patients [Piton *et al*., 2011].

Other genes of interest are implicated in CNS immunosurveillance, which is first mediated by the integrity of blood brain barrier, or in modulation of pro-inflammatory pathways. For example, in patient 55 the duplication of the *SELE* and *SELEL* genes has been identified (**Tab. 3**). These genes encode E-selectin and L-selectin, respectively, which are two members of the selectin family of proteins. E protein is generally found in cytokine-stimulated endothelial cells and is thought to be responsible for the accumulation of blood leukocytes at sites of inflammation by mediating the adhesion of cells to the vascular lining. P-selectin is expressed on the endothelium of the blood–CNS barrier and soluble L-selectin has been found in cerebrospinal fluid. Moreover, both P and L-selectin play important roles in the entry of circulating T-lymphocytes into the CNS. Therefore, it has been hypothesized that molecules not expressed in the brain may alter CNS function. Diminished expression of P-selectin has been associated with delayed neutrophil transmigration in neonatal rats [Engelhardt and Ransohoff , 2005; Onore *et al*., 2012]. Thus, decreased expression of P-selectin in individuals early in life may contribute to delayed leukocyte transmigration and increased susceptibility to infection, which may in turn damage neural tissues during CNS development. A decreased serum level of P- and L-selectin has been recently observed in a group of autistic subjects vs. controls, confirming a previous finding in a cohort of HF-AU patients, thus indicating an involvement of hypoactivity of T-lymphocytes in the pathophysiology of ASD [Iwata *et al*., 2008; Onore *et al*., 2012]. Similarly, it may be speculated that hyperactivity of T-lymphocytes, mediated by increased dosage of selectins, could contribute to ASD.
Conversely, the potential overexpression of the ***CLDN3***, ***CLDN4*** (duplicated in patient 52), and ***CLDN5*** (duplicated in patient 27) genes, which encode integral membrane proteins with a role in maintaining the integrity of the blood brain barrier, may play a role in CNS homeostasis as well as in protecting the CNS against infections [Nitta *et al*., 2003; Wolburg *et al*., 2003]. In addition, patient 52 was found to carry the duplication of the ***LAT2*** gene (**Tab. 3**), which encodes the linker for activation of T cell family, member 2. T cell responses to pathogens require the induction of the primary activating receptor, the T cell receptor (TCR), along with other costimulatory and adhesion receptors. Signal transduction pathways activated downstream of these receptors drive T cell responses required for the immune response and disease progression. One of this pathways is the

LAT signaling pathway which has the role to integrate the information and selectively induces pathways critical for T cell activation and the adaptive immune response [Bartelt and Houtman, 2012]. To date, it is not possible to predict if an up-regulation of the LAT signaling, possible due to the ***LAT2*** gene duplication, may have produced a T-cell hyper-responsiveness in patient 53; however, this hypothesis can not be excluded.

Similarly, other pathways, which are known to modulate inflammation, may be predicted to be dysregulated in a subset of the ASD patients reported here. The ***STAT1*** and ***STAT4*** genes are found deleted in patient 53 (**Tab. 3**), and both encode proteins with a pro-inflammatory role. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. In particular, STAT1 mediates the expression of a variety of genes, which is thought to be important for cell viability in response to different cell stimuli and pathogens. It has been proposed that the strong inflammatory response observed in neurodegenerative diseases may depend on the impairment of the endogenous control of microglial activation, and that the cross-talk between STAT1 and MAPK pathways may regulate the amplitude and duration of microglial activation [Zaheer *et al*., 2007].

Moreover, patient 53 bears the deletion of the ***ITGA4*** gene, which encodes an integral membrane protein that mediates the migration of T lymphocytes across blood-brain barrier-endothelial cells, and therefore it is considered a mediator of neuroinflammation [Ifergan *et al*., 2011]. Interestingly, SNPs in *ITGA4* have been previously associated with ASD [Correia *et al*., 2009]. Furthermore, a positive association was found between one of these SNP markers and levels of a serum autoantibody directed to brain tissue, which was previously shown to be significantly more frequent in autistic patients than in age-matched controls, thus suggesting that *ITGA4* could be involved in a neuroimmune process thought to occur in autistic patients [Correia *et al*., 2009].

Previous data reported elevated levels of NF-κB in autistic patients vs. controls [Malik *et al*., 2011; Naik *et al*., 2011]. NF-κB is an important gene transcriptional factor that mediates cellular responses in inflammation, immunity, development, cell proliferation and apoptosis. The *IKBKG* gene (duplicated in patient 61, **Tab.3**) encodes the inhibitor of kappa light polypeptide, which is the regulatory subunit of the inhibitor of kappaB kinase (IKK) complex, which, in turn, activates NF-kappaB resulting in activation of genes involved in inflammation, immunity, cell survival, and other pathways. Recently, it has been reported that brain abnormalities correlate with additional copies of the *IKBKG* gene [Ramocki *et al*., 2009, 2010]. Indeed, IKBKG overexpression causes impaired NF-κB signaling in skin fibroblasts derived from patients with white matter anomalies, thus further supporting the role of NF-κB signaling in astroglial cells for normal myelin formation of the CNS [Philippe *et al*., 2012]. Similarly, it is possible that an excessive induction of NF-κB, due to *IKBKG* gene duplication, may be involved in ASD pathogenesis.

Of note, most of the reported genes are affected by *de novo* large CNVs, thus their role in ASD pathogenesis must be interpreted in the context of a genomic disorder. However, the collected data support the hypothesis of the existence of a genetic basis, likely wide heterogeneous, that might explain, almost in part, the immune dysfunction observed in ASD patients. In addition, it is important to stress the idea that the genetic causes must be researched not only in anomalies affecting genes with a clear role in immunity but also in genes implicated in functional pathways which ultimately lead to CNS development, which also show high expression in immune cell types and, thus, may contribute to immune system development as well.

Nevertheless, further studies are necessary to replicate the hypothesized genetic aetiology of a part of immune system dysfunctions in ASD, although it will be more likely to replicate the implication of recurrent pathways rather than of recurrent genes, as already confirmed for the plethora of genes implicated in neurodevelopment. In addition, in order to validate genomic data the challenge will be to correlate in each patient the single genetic lesion with specific aberrations of the immune system.

REFERENCES

Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S *et al*. Mutations in *NSUN2* cause autosomal-recessive intellectual disability. *Am J Hum Genet* 2012;90:847-855.

Abdel-Salam GM, Miyake N, Eid MM, Abdel-Hamid MS, Hassan NA, Eid OM *et al*. A homozygous mutation in *RNU4ATAC* as a cause of microcephalic osteodysplastic primordial dwarfism type I (MOPD I) with associated pigmentary disorder. *Am J Med Genet A* 2011;155A:2885-2896.

Abdul-Rahman OA, Hudgins L. The diagnostic utility of a genetics evaluation in children with pervasive developmental disorders. *Genet Med* 2006;8:50-54.

Abe MK, Saelzler MP, Espinosa R 3rd, Kahle KT, Hershenson MB, Le Beau MM *et al.* ERK8, a new member of the mitogen-activated protein kinase family. *J Biol Chem* 2002;277:16733-16743.

Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. *Nat Rev Genet* 2008;9:341-355.

Accardi R, Oxelmark E, Jauniaux N, de Pinto V, Marchini A, Tommasino M. High levels of the mitochondrial large ribosomal subunit protein 40 prevent loss of mitochondrial DNA in null mmf1 Saccharomyces cerevisiae cells. *Yeast* 2004;21:539-548.

Adams DC, Karolak MJ, Larman BW, Liaw L, Nolin JD, Oxburgh L. Follistatin-like 1 regulates renal IL-1β expression in cisplatin nephrotoxicity. *Am J Physiol Renal Physiol* 2010;299:F1320-1327.

Adegbola A, Gao H, Sommer S, Browning M. A novel mutation in *JARID1C*/*SMCX* in a patient with autism spectrum disorder (ASD). *Am J Med Genet A* 2008;146A:505-511.

Agís-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E *et al*. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proc Natl Acad Sci USA* 2006;103:14602-14607.

Agrawal PB, Greenleaf RS, Tomczak KK, Lehtokari VL, Wallgren-Pettersson C, Wallefeld W *et al*. Nemaline myopathy with minicores caused by mutation of the *CFL2* gene encoding the skeletal muscle actin-binding protein, cofilin-2. *Am J Hum Genet* 2007;80:162-167.

Aguado F, Díaz-Ruiz C, Parlato R, Martínez A, Carmona MA, Bleckmann S *et al*. The CREB/CREM transcription factors negatively regulate early synaptogenesis and spontaneous network activity. *J Neurosci* 2009;29:328-333.

Akbarian S, Huang HS. Epigenetic regulation in human brain-focus on histone lysine methylation. *Biol Psychiatry* 2009;65:198-203.

Akhmanova A, Hoogenraad CC, Drabek K, Stepanova T, Dortland B, Verkerk T *et al*. Clasps are CLIP -115 and -170 associating proteins involved in the regional regulation of microtubule dynamics in motile fibroblasts. *Cell* 2001;104:923-935.

Alam S, Zinyk D, Ma L, Schuurmans C. Members of the Plag gene family are expressed in complementary and overlapping regions in the developing murine nervous system. *Dev Dyn* 2005;234:772-782.

Alamy SS, Jarskog LF, Sheitman BB, Lieberman JA. Secretin in a patient with treatment-resistant schizophrenia and prominent autistic features. *Schizophr Res* 2004;66:183-186.

Al-Bassam J, Kim H, Brouhard G, van Oijen A, Harrison SC, Chang F. CLASP promotes microtubule rescue by recruiting tubulin dimers to the microtubule. *Dev Cell* 2010;19:245-258.

Alexander AL, Lee JE, Lazar M, Boudos R, DuBray MB, Oakes TR *et al*. Diffusion tensor imaging of the corpus callosum in Autism. *Neuroimage* 2007;34:61-73.

Alford S, Schwartz E, Viana di Prisco G. The pharmacology of vertebrate spinal central pattern generators. *Neuroscientist* 2003;9:217-228.

Alston CL, Davison JE, Meloni F, van der Westhuizen FH, He L, Hornig-Do HT *et al*. Recessive germline *SDHA* and *SDHB* mutations causing leukodystrophy and isolated mitochondrial complex II deficiency. *J Med Genet* 2012;49:569-577.

Alves dos Santos MT, Smidt MP. En1 and Wnt signaling in midbrain dopaminergic neuronal development. *Neural Development* 2011,6:23

Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends Neurosci* 2008;31:137-145.

Amath A, Foster JA, Sidor MM. Developmental alterations in CNS stress-related gene expression following postnatal immune activation. *Neurosci* 2012;220:90-99.

Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999;23:185-188.

Anderlid BM, Schoumans J, Anneren G, Sahlen S, Kyllerman M, Vujic M *et al*. Subtelomeric rearrangements detected in patients with idiopathic mental retardation. *Am J Med Genet* 2002;107:275-284.

Anney R, Klei L, Pinto D, Almeida J, Bacchelli E, Baird G *et al*. Individual common variants exert weak effects on the risk for autism spectrum disorders. *Hum Mol Genet* 2012;21:4781-4792.

Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR *et al*. A genome-wide scan for common alleles affecting risk for autism. *Hum Mol Genet* 2010;19:4072-4082.

Antonacci F, Kidd JM, Marques-Bonet T, Ventura M, Siswara P, Jiang Z *et al*. Characterization of six human disease-associated inversion polymorphisms. *Hum Mol Genet* 2009;18:2555-2566.

Antshel KM, Aneja A, Strunge L, Peebles J, Fremont WP, Stallone K *et al*. Autistic spectrum disorders in velo-cardio facial syndrome (22q11.2 deletion). *J Autism Dev Disord* 2007;37:1776-1786.

Arai Y, Ohgane J, Yagi S, Ito R, Iwasaki Y, Saito K *et al*. Epigenetic assessment of environmental chemicals detected in maternal peripheral and cord blood samples. *J Reprod Dev* 2011;57:507-517.

Araki W, Takahashi-Sasaki N, Chui DH, Saito S, Takeda K, Shirotani K *et al*. A family of membrane proteins associated with presenilin expression and γ-secretase function. *FASEB J* 2008;22:819-827.

Arenzana TL, Smith-Raska MR, Reizis B. Transcription factor Zfx controls BCR-induced proliferation and survival of B lymphocytes. *Blood* 2009;113:5857-5867.

Arias-Carrión O, Stamelou M, Murillo-Rodríguez E, Menéndez-González M, Pöppel E. Dopaminergic reward system: a short integrative review. *Int Arch Med* 2010;3:24.

Ashe A, Morgan DK, Whitelaw NC, Bruxner TJ, Vickaryous NK, Cox LL *et al*. A genome-wide screen for modifiers of transgene variegation identifies genes with critical roles in development. *Genome Biol* 2008;9:R182.

Ashwood P, Corbett BA, Kantor A, Schulman H, Van de Water J, Amaral DG. In search of cellular immunophenotypes in the blood of children with autism. *Plos One* 2011a;6:e19299.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain Behav Immun* 2011b;25:40-45.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Altered T cell responses in children with autism. *Brain Behav Immun* 2011c;25:840-849.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. *J Neuroimmunol* 2011d;232:196-199.

Aslam M, Ahmad N, Srivastava R, Hemmer B. TNF-alpha induced NFκB signaling and p65 (RelA) overexpression repress Cldn5 promoter in mouse brain endothelial cells. *Cytokine* 2012;57:269-275.

Asperger H. Die autistischen Psychopathen im Kindesalter. *Archivfur Psychiatrie und Nervenkrankheiten* 1944;76-136.

Astrinidis A, Cash TP, Hunter DS, Walker CL, Chernoff J, Henske EP. Tuberin, the tuberous sclerosis complex 2 tumor suppressor gene product, regulates Rho activation, cell adhesion and migration. *Oncogene* 2002;21:8470-8476.

Atladottir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW *et al*. Association of family history of autoimmune diseases and autism spectrum disorders. *Pediatrics* 2009;124:687-694.

Atladottir HO, Thorsen P, Ostergaard L, Schendel DE, Lemcke S, Abdallah M *et al*. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *J Autism Dev Disord* 2010;40:1423-1430.

Auger AP, Jessen HM, Edelmann MN. Epigenetic organization of brain sex differences and juvenile social play behavior. *Horm Behav* 2011;59:358-363.

Autism and Developmental Disabilities Monitoring Network. Prevalence of autism spectrum disorders − Autism and Developmental Disabilities Monitoring Network, United States, 2006. *MMWR Surveill Summ* 2009,58:1-20.

Awadalla P, Gauthier J, Myers RA, Casals F, Hamdan FF, Griffing AR *et al*. Direct measure of the *de novo* mutation rate in autism and schizophrenia cohorts. *Am J Hum Genet* 2010;87:316-324.

Baas D, Meiniel A, Benadiba C, Bonnafe E, Meiniel O, Reith W *et al*. A deficiency in RFX3 causes hydrocephalus associated with abnormal differentiation of ependymal cells. *Eur J Neurosci* 2006;24:1020-1030.

Bachmann-Gagescu R, Mefford HC, Cowan C, Glew GM, Hing AV, Wallace S *et al*. Recurrent 200-kb deletions of 16p11.2 that include the SH2B1 gene are associated with developmental delay and obesity. *Genet Med* 2010;12:641-647.

Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M *et al*. A clinicopathological study of autism. *Brain* 1998;121:889-905.

Baird G, Robinson RO, Boyd S, Charman T. Sleep electroencephalograms in young children with autism with and without regression. *Dev Med Child Neurol* 2006;48:604-608.

Bakircioglu M, Carvalho OP, Khurshid M, Cox JJ, Tuysuz B, Barak T *et al*. The essential role of centrosomal NDE1in human cerebral cortex neurogenesis. *Am J Hum Genet* 2011;88:523-535.

Ballas N, Lioy DT, Grunseich C, Mandel G. Non–cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology: a cellular model for Rett syndrome. *Nat Neurosci* 2009;12:311-317.

Balmer D, Goldstine J, Rao YM, LaSalle JM. Elevated methyl-CpG-binding protein 2 expression is acquired during postnatal human brain development and is correlated with alternative polyadenylation. *J Mol Med* 2003;81:61-68.

Banisadr G, Bhattacharyya BJ, Belmadani A, Izen SC, Ren D, Tran PB *et al*. The chemokine BRAK/CXCL14 regulates synaptic transmission in the adult mouse dentate gyrus stem cell niche. *J Neurochem* 2011;119:1173-1182.

Baris HN, Tan WH, Kimonis VE, Irons MB. Diagnostic utility of array-based comparative genomic hybridization in a clinical setting. *Am J Med Genet A* 2007;143A:2523-2533.

Barnby G, Abbott A, Sykes N, Morris A, Weeks DE, Mott R *et al*. Candidate-gene screening and association analysis at the autism-susceptibility locus on chromosome 16p: evidence of association at *GRIN2A* and *ABAT*. *Am J Hum Genet* 2005;76:950-966.

Barnea-Goraly N, Kwon H, Menon V, Eliez S, Lotspeich L, Reiss AL. White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biol Psychiatry* 2004;55:323-326.

Baron MK, Boeckers TM, Vaida B, Faham S, Gingery M, Sawaya MR *et al*. An architectural framework that may lie at the core of the postsynaptic density. *Science* 2006;311:531-535.

Barrett S, Beck JC, Bernier R, Bisson E, Braun TA, Casavant TL *et al*. An autosomal genomic screen for autism. *Am J Med Genet* 1999;88:609-615.

Barry G, Mattick JS. The role of regulatory RNA in cognitive evolution. *Trends Cogn Sci* 2012;16:497-503.

Bartelt RR, Houtman JC. The adaptor protein LAT serves as an integration node for signaling pathways that drive T cell activation. *Wiley Interdiscip Rev Syst Biol Med* 2012 Nov 13. [Epub ahead of print]

Bartsch I, Sandrock K, Lanza F, Nurden P, Hainmann I, Pavlova A *et al*. Deletion of human *GP1BB* and *SEPT5* is associated with Bernard-Soulier syndrome, platelet secretion defect, polymicrogyria, and developmental delay. *Thromb Haemost* 2011;106:475-483.

Bataller L, Wade DF, Graus F, Rosenfeld MR, Dalmau J. The MAZ protein is an autoantigen of Hodgkin's disease and paraneoplastic cerebellar dysfunction. *Ann Neurol* 2003;53:123-127.

Bateman J, Gull D. Structural variations in attention-deficit hyperactivity disorder. *Lancet* 2011;377:378-379.

Baulac S, LaVoie M J, Kimberly WT, Strahle J, Wolfe MS, Selkoe DJ *et al*. Functional γ-secretase complex assembly in Golgi/trans-Golgi network: interactions among presenilin, nicastrin, Aph1, Pen-2, and γ-secretase substrates. *Neurobiol Dis* 2003;14:194-204.

Bauman M, Kemper TL. Histoanatomic observations of the brain in early infantile autism. *Neurology* 1985;35:866-874.

Baumgartner S, Martin D, Chiquet-Ehrismann R, Sutton J, Desai A, Huang I *et al*. The HEM proteins: a novel family of tissue-specific transmembrane proteins expressed from invertebrates through mammals with an essential function in oogenesis. *J Mol Biol* 1995;251:41-49.

Becanovic K, Jagodic M, Sheng JR, Dahlman I, Aboul-Enein F, Wallstrom E *et al*. Advanced intercross line mapping of Eae5 reveals *Ncf-1* and *CLDN4* as candidate genes for experimental autoimmune encephalomyelitis. *J Immunol* 2006;176:6055-6064.

Becker JA, Befort K, Blad C, Filliol D, Ghate A, Dembele D *et al*. Transcriptome analysis identifies genes with enriched expression in the mouse central extended amygdala. *Neuroscience* 2008;156:950-965.

Bejjani A, O'Neill J, Kim JA, Frew AJ, Yee1 VW, Ly R *et al*. Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism apectrum disorder demonstrated by ^{1}H MRS and ^{1}H MRSI. *Plos One* 2012;7:e38786.

Belichenko PV, Wright EE, Belichenko NP, Masliah E, Li HH, Mobley WC *et al*. Widespread changes in dendritic and axonal morphology in *Mecp2*-mutant mouse models of Rett syndrome: Evidence for disruption of neuronal networks. *J Comp Neurol* 2009;514:240-258.

Ben Shachar S, Lanpher B, German JR, Qasaymeh M, Potocki L, Nagamani SC *et al*. Microdeletion 15q13.3: a locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders. *J Med Genet* 2009;46:382-388.

Benadiba C, Magnani D, Niquille M, Morlé L, Valloton D, Nawabi H *et al*. The ciliogenic transcription factor RFX3 regulates early midline distribution of guidepost neurons required for corpus callosum development. *Plos Genet* 2012;8:e1002606.

Benayed R, Choi J, Matteson PG, Gharani N, Kamdar S, Brzustowicz LM *et al*. Autism-associated haplotype affects the regulation of the homeobox gene, ENGRAILED 2. *Biol Psychiatry* 2009;66:911-917.

Benayed R, Gharani N, Rossman I, Mancuso V, Lazar G, Kamdar S *et al*. Support for the homeobox transcription factor gene ENGRAILED 2 as an autism spectrum disorder susceptibility locus. *Am J Hum Genet* 2005;77:851-868.

Ben-Shachar S, Lanpher B, German JR, Qasaymeh M, Potocki L, Nagamani SC *et al*. Microdeletion 15q13.3: a locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders. *J Med Genet* 2009;46382-46388.

Ben-Yaakov K, Dagan SY, Segal-Ruder Y, Shalem O, Vuppalanchi D, Willis DE *et al*. Axonal transcription factors signal retrogradely in lesioned peripheral nerve. *EMBO J* 2012;31:1350-1363.

Berg JS, Brunetti-Pierri N, Peters SU, Kang SH, Fong CT, Salamone J *et al*. Speech delay and autism spectrum behaviors are frequently associated with duplication of the 7q11.23 Williams–Beuren syndrome region. *Genet Med* 2007;9:427-441.

Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U *et al*. Mutations in the *SHANK2* synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nat Genet* 2010;42:489-491.

Berkel S, Tang W, Treviño M, Vogt M, Obenhaus HA, Gass P *et al*. Inherited and *de novo SHANK2* variants associated with autism spectrum disorder impair neuronal morphogenesis and physiology. *Hum Mol Genet* 2012;21:344-357.

Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: a novel form of mercury poisoning. *Med Hypotheses* 2001;56:462-471.

Bernshausen T, Jux B, Esser C, Abel J, Fritsche E. Tissue distribution and function of the Aryl hydrocarbon receptor repressor (AhRR) in C57BL/6 and Aryl hydrocarbon receptor deficient mice. *Arch Toxicol* 2006;80:206-211.

Berridge MJ. Inositol trisphosphate and calcium signaling. *Nature* 1993;361:315-325.

Besser L, Chorin E, Sekler I, Silverman WF, Atkin S, Russell J *et al*. Synaptically-released zinc triggers metabotropic signaling via a zinc sensing receptor in the hippocampus. *J Neurosci* 2009;29:2890-2901.

Bessis A, Bechade C, Bernard D, Roumier A. Microglial control of neuronal death and synaptic properties. *Glia* 2007;55:233-238.

Betancur C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. *Brain Res* 2011;1380:42-77.

Betancur C, Moreno De Luca D, Gennetier A, Devillard F, Ginchat V, Assouline B *et al*. Chromosome 17q21.31 microdeletion in a patient with autism. Abstract (number 449) presented at the annual meeting of The American Society of Human Genetics, November 11-15 2008, Philadelphia, PA.

Beyer AS, von Einem B, Schwanzar D, Keller IE, Hellrung A, Thal DR *et al*. Engulfment adapter PTB domain containing 1 interacts with and affects processing of the amyloid-β precursor protein. *Neurobiol Aging* 2012;33:732-743.

Beyer KS, Klauck SM, Benner A, Poustka F, Poustka A. Association studies of the HOPA dodecamer duplication variant in different subtypes of autism. *Am J Med Genet* 2002;114:110-155.

Bhat S, Dao DT, Terrillion CE, Arad M, Smith RJ, Soldatov NM *et al*. CACNA1C (Ca(v)1.2) in the pathophysiology of psychiatric disease. *Prog Neurobiol* 2012;99:1-14

Bhat SS, Ladd S, Grass F, Spence JE, Brasington CK, Simensen RJ *et al*. Disruption of the *IL1RAPL1* gene associated with a pericentromeric inversion of the X chromosome in a patient with mental retardation and autism. *Clin Genet* 2008;73:94-96.

Bhattacharyya BJ, Wilson SM, Jung H, Miller RJ. Altered neurotransmitter release machinery in mice deficient for the deubiquitinating enzyme Usp14. *Am J Physiol Cell Physiol* 2012;302:C698-708.

Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH, van Haeringen A, Fransen van de Putte DE, Anderlid BM *et al*. Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. *Eur J Med Genet* 2009;52:77-87.

Billnitzer AJ, Barskaya I, Yin C, Perez RG. APP independent and dependent effects on neurite outgrowth are modulated by the receptor associated protein (RAP). *J Neurochem* 2012 Oct 13. [Epub ahead of print].

Bladt F, Aippersbach E, Gelkop S, Strasser GA, Nash P, Tafuri A *et al*. The murine Nck SH2/SH3 adaptors are important for the development of mesoderm-derived embryonic structures and for regulating the cellular actin network. *Mol Cell Biol* 2003;23:4586-4597.

Blaker-Lee A, Gupta S, McCammon JM, De Rienzo G, Sive H. Zebrafish homologs of genes within 16p11.2, a genomic region associated with brain disorders, are active during brain development, and include two deletion dosage sensor genes. *Dis Mod Mech* 2012;5:834-851.

Blancas S, Moran J. Role for apoptosis-inducing factor in the physiological death of cerebellar neurons. *Neurochem Int* 2011;58:934-942.

Blanchoin L, Pollard TD, Mullins RD. Interactions of ADF/cofilin, Arp2/3 complex, capping protein and profilin in remodeling of branched actin filament networks. *Curr Biol* 2000;10:1273-1282

Blaylock RL. A possible central mechanism in autism spectrum disorders, part 1. *Altern Ther Health Med* 2008;14:46-53.

Bleichert F, Baserga S. The long unwinding road of RNA helicases. *Mol Cell* 2007;27:339-352.

Boddaert N, Chabane N, Gervais H, Good CD, Bourgeois M, Plumet MH *et al*. Superior temporal sulcus anatomical abnormalities in childhood autism: a voxel-based morphometry MRI study. *NeuroImage* 2004;23:364-369.

Bogdan S, Klambt C. Kette regulates actin dynamics and genetically interacts with Wave and Wasp. *Development* 2003;130:4427-4437.

Boguski MS, McCormick F. Proteins regulating Ras and its relatives. *Nature* 1993;366:643-654.

Bolliger MF, Frei K, Winterhalter KH, Gloor SM. Identification of a novel neuroligin in humans which binds to PSD-95 and has a widespread expression. *Biochem J* 2001;356:581-588.

Bolton P, Powell J, Rutter M, Buckle V, Yates JR, Ishikawa-Brush Y *et al*. Autism, mental retardation, multiple exostoses and short stature in a female with 46,X,t(X;8)(p22.13;q22.1). *Psychiatr Genet* 1995;5:51-55.

Bolton PF, Veltman MW, Weisblatt E, Holmes JR, Thomas NS, Youings SA *et al*. Chromosome 15q11–13 abnormalities and other medical conditions in individuals with autism spectrum disorders. *Psychiatr Genet* 2004;14:131-137.

Bonati MT, Russo S, Finelli P, Valsecchi MR, Cogliati F, Cavalleri F *et al*. Evaluation of autism traits in Angelman syndrome: a resource to unfold autism genes. *Neurogenetics* 2007;8:169-178.

Bond J, Roberts E, Springell K, Lizarraga SB, Scott S, Higgins J *et al*. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. *Nat Genet* 2005;37:353-355.

Borck G, Mollà-Herman A, Boddaert N, Encha-Razavi F, Philippe A, Robel L *et al*. Clinical, cellular, and neuropathological consequences of *AP1S2* mutations: further delineation of a recognizable X-linked mental retardation syndrome. *Hum Mutat* 2008;29:966-974.

Borgonovo J, Capella P, Seltzer A, Sosa MA. Expression of coat proteins changes during postnatal development in selected areas of the rat brain. *Int J Dev Neurosci* 2012;30:333-341.

Bosio Y, Berto G, Camera P, Bianchi F, Ambrogio C, Claus P *et al*. PPP4R2 regulates neuronal cell differentiation and survival, functionally cooperating with SMN. *Eur J Cell Biol* 2012;91:662-674.

Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma D *et al*. Poor replication of candidate genes for major depressive disorder using genome-wide association data. *Mol Psychiatry* 2011;16:516-532.

Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: a conserved switch or diverse cell functions. *Nature* 1990;348:125-132.

Bowden NA, Weidenhofer J, Scott RJ, Schall U, Todd J, Michie PT *et al*. Preliminary investigation of gene expression profiles in peripheral blood lymphocytes in schizophrenia. *Schizophr Res* 2006;82:175-183.

Braidy N, Guillemin GJ, Grant R. Effects of kynurenine pathway inhibition on NAD metabolism and cell viability in human primary astrocytes and neurons. *Int J Tryptophan Res* 2011;4:29-37.

Bram RJ, Crabtree GR. Calcium signaling in T cells stimulated by a cyclophilin B-binding protein. *Nature* 1994;371:355-358.

Brambilla P, Hardan A, di Nemi SU, Perez J, Soares JC, Barale F. Brain anatomy and development in autism: review of structural MRI studies. *Brain Res Bull* 2003;61:557-569.

Brasch-Andersen C, Christiansen L, Tan Q, Haagerup A, Vestbo J, Kruse TA. Possible gene dosage effect of glutathione-S-transferases on atopic asthma: using real-time PCR for quantification of *GSTM1* and *GSTT1* gene copy numbers. *Hum Mutat* 2004;24:208-214.

Braun NN, Reutiman TJ, Lee S, Folsom TD, Fatemi SH. Expression of phosphodiesterase 4 is altered in the brains of subjects with autism. *Neuroreport* 2007;18:1841-1844.

Braunschweig D, Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Croen LA *et al*. Autism: maternally derived antibodies specific for fetal brain proteins. *Neurotoxicology* 2008;29:226-231.

Bremer A, Giacobini M, Eriksson M, Gustavsson P, Nordin V, Fernell E *et al*. Copy number variation characteristics in subpopulations of patients with autism spectrum disorders. *Am J Med Genet B Neuropsychiatr Genet* 2011;156:115-124.

Bret C, Hose D, Reme T, Kassambara A, Seckinger A, Meissner T *et al*. Gene expression profile of ADAMs and ADAMTSs metalloproteinases in normal and malignant plasma cells and in the bone marrow environment. *Exp Hematol* 2011;39:546-557.

Brown MA, Sims RJ 3rd, Gottlieb PD, Tucker PW. Identification and characterization of Smyd2: a split SET/MYND domain-containing histone H3 lysine 36-specific methyltransferase that interacts with the Sin3 histone deacetylase complex. *Mol Cancer* 2006;5:26.

Bucan M, Abrahams BS, Wang K, Glessner JT, Herman EI, Sonnenblick LI *et al*. Genome-wide analyses of exonic copy number variants in a family-based study point to novel autism susceptibility genes. *Plos Genet* 2009;5:e1000536.

Bultje RS, Castaneda-Castellanos DR, Jan LY, Jan YN, Kriegstein AR, Shi SH. Mammalian Par3 regulates progenitor cell asymmetric division via notch signaling in the developing neocortex. *Neuron* 2009;63:189-202.

Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, Takahashi TN *et al*. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline *PTEN* tumour suppressor gene mutations. *J Med Genet* 2005;42:318-321.

Buxbaum JD, Cai G, Chaste P, Nygren G, Goldsmith J, Reichert J *et al*. Mutation screening of the *PTEN* gene in patients with autism spectrum disorders and macrocephaly. *Am J Med Genet B Neuropsychiatr Genet* 2007;144B:484-491.

Buxbaum JD, Silverman JM, Smith CJ, Greenberg DA, Kilifarski M, Reichert J *et al*. Association between a *GABRB3* polymorphism and autism. *Mol Psychiatry* 2002;7:311-316.

Cacci E, Claasen JH, Kokaia Z. Microglia-derived tumor necrosis factor alpha exaggerates death of newborn hippocampal progenitor cells in vitro. *J Neurosci Res* 2005;80:789-797.

Capossela S, Muzio L, Bertolo A, Bianchi V, Dati G, Chaabane L *et al*. Growth defects and impaired cognitive-behavioral abilities in mice with knockout for Eif4h, a gene located in the mouse homolog of the Williams-Beuren syndrome critical region. *Am J Pathol* 2012;180:1121-1135.

Capponi S, Geroldi A, Fossa P, Grandis M, Ciotti P, Gulli R *et al*. HSPB1 and HSPB8 in inherited neuropathies: study of an Italian cohort of dHMN and CMT2 patients. *J Peripher Nerv Syst* 2011;16:287-294.

Cardozo T, Pagano M. The SCF ubiquitin ligase: insights into a molecular machine. *Nat Rev Mol Cell Biol* 2004;5:739-751.

Careaga M, Van de Water J, Ashwood P. Immune dysfunction in autism: a pathway to treatment. *Neurotherapeutics* 2010;7:283-292.

Carney RM, Wolpert CM, Ravan SA, Shahbazian M, Ashley-Koch A, Cuccaro ML *et al*. Identification of *MeCP2* mutations in a series of females with autistic disorder. *Pediatr Neurol* 2003;28:205-211.

Carrascosa-Romero MC, Suela J, Alfaro-Ponce B, Cepillo-Boluda AJ. X-chromosome-linked ichthyosis associated to epilepsy, hyperactivity, autism and mental retardation, due to the Xp22.31 microdeletion. *Rev Neurol* 2012;54:241-248.

Carter CJ. Schizophrenia susceptibility genes directly implicated in the life cycles of pathogens: Cytomegalovirus, Influenza, Herpes simplex, Rubella, and Toxoplasma gondii. *Schizophr Bull* 2009;35:1163-1182.

Carter NP. Methods and strategies for analyzing copy number variation using DNA microarrays. *Nature genetics* 2007;39:S16-21.

Castro-Alamancos MA, Calcagnotto ME. Presynaptic long-term potentiation in corticothalamic synapses. *J Neurosci* 1999;19:9090-9097.

Chahrour M, Yung SY, Shaw C, Zhou X, Wong STC, Qin J *et al*. MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* 2008;320:1224-1229.

Chahrour MH, Yu TW, Lim ET, Ataman B, Coulter ME, Hill RS *et al*. Whole-exome sequencing and homozygosity analysis implicate depolarization-regulated neuronal genes in autism. *Plos Genet* 2012;8:e1002635.

Challman TD, Barbaresi WJ, Katusic SK, Weaver A. The yield of the medical evaluation of children with pervasive developmental disorders. *J Autism Dev Disord* 2003;33:187-192.

Chang SH, Chiang SY, Chiu CC, Tsai CC, Tsai HH, Huang CY *et al*. Expression of anti-cardiolipin antibodies and inflammatory associated factors in patients with schizophrenia. *Psychiatric Res* 2011;187:341-346.

Chao HT, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J *et al*. Dysfunction in GABA signaling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* 2010;468:263-269.

Charbonnier F, Chanoine C, Cifuentes-Diaz C, Gallien CL, Rieger F, Alliel PM *et al*. Expression of the proteoglycan SPOCK during mouse embryo development. *Mech Dev* 2000;90:317-321.

Chavarría-Siles I, Walss-Bass C, Quezada P, Dassori A, Contreras S, Medina R *et al*. TGFB-induced factor (*TGIF*): a candidate gene for psychosis on chromosome 18p. *Mol Psychiatry* 2007;12:1033-1041.

Cheerathodi M, Ballif BA. Identification of CrkL-SH3 binding proteins from embryonic murine brain: implications for Reelin signaling during brain development. *J Proteome Res* 2011;10:4453-4462.

Chen F, Ogawa K, Nagarajan RP, Zhang M, Kuang C, Chen Y. Regulation of TG-interacting factor by transforming growth factor-beta. *Biochem J* 2003;371:257-263.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S *et al*. Functional analysis of genetic variation in catechol-O-methyltransferase (*COMT*): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* 2004;75:807-821.

Chen RZ, Akbarian S, Tudor M, Jaenisch R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nat Genet* 2001;27:327-331.

Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC *et al*. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science* 2003;302:885-889.

Chen X, Wang X, Sun C, Chen Q, O'Neill FA, Walsh D *et al*. *FBXL21* association with schizophrenia in Irish family and case–control samples. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:1231-1237.

Chen Y, Wu H, Wang S, Koito H, Li J, Ye F *et al*. The oligodendrocyte-specific G-protein coupled receptor GPR17 is a cell-intrinsic timer of myelination. *Nat Neurosci* 2009;12:1398-1406.

Chen YZ, Matsushita M, Girirajan S, Lisowski M, Sun E, Sul Y *et al*. Evidence for involvement of *GNB1L* in autism. *Am J Med Genet B Neuropsychiatr Genet* 2012;159B:61-71.

Cheng CW, Hui C, Strähle U, Cheng SH. Identification and expression of zebrafish Iroquois homeobox gene *irx1*. *Dev Genes Evol* 2001;211:442-444.

Cheng L, Han X, Shi Y. A regulatory role of LPCAT1 in the synthesis of inflammatory lipids, PAF and LPC, in the retina of diabetic mice. *Am J Physiol Endocrinol Metab* 2009;297:E1276-1282.

Ching MS, Shen Y, Tan WH, Jeste SS, Morrow EM, Chen X *et al*. Deletions of *NRXN1* (neurexin-1) predispose to a wide spectrum of developmental disorders. *Am J Med Genet B Neuropsychiatr Genet* 2010;153B:937-947.

Chiocchetti A, Pakalapati G, Duketis E, Wiemann S, Poustka A, Poustka F *et al*. Mutation and expression analyses of the ribosomal protein gene *RPL10* in an extended German sample of patients with autism spectrum disorder. *Am J Med Genet A* 2011;155A:1472-1475.

Cho YW, Hong T, Hong S, Guo H, Yu H, Kim D *et al*. PTIP associates with MLL3- and MLL4-containing histone H3 lysine 4 methyltransferase complex. *J Biol Chem* 2007;282:20395-20406.

Choi J, Ababon MR, Matteson PG, Millonig JH. Cut-like homeobox 1 and nuclear factor I/B mediate ENGRAILED2 autism spectrum disorder-associated haplotype function. *Hum Mol Genet* 2012;21:1566-1580.

Choi KH, Zepp ME, Higgs BW, Weickert CS, Webster MJ. Expression profiles of schizophrenia susceptibility genes during human prefrontal cortical development. *J Psychiatry Neurosci* 2009;34:450-458.

Christian SL, Brune CW, Sudi J, Kumar RA, Liu S, Karamohamed S *et al*. Novel submicroscopic chromosomal abnormalities detected in autism spectrum disorder. *Biol Psychiatry* 2008:63:1111-1117.

Christie DA, Lemke CD, Elias IM, Chau LA, Kirchhof MG, Li B *et al*. Stomatin-like protein 2 binds cardiolipin and regulates mitochondrial biogenesis and function. *Mol Cell Biol* 2011;31:3845-3856.

Chubykin AA, AtasoyD, Etherton MR, Brose N, Kavalali ET, Gibson JR *et al*. Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. *Neuron* 2007;54:919-931.

Chung RH, Ma D, Wang K, Hedges DJ, Jaworski JM, Gilbert JR *et al*. An X chromosome-wide association study in autism families identifies *TBL1X* as a novel autism spectrum disorder candidate gene in males. *Mol Autism* 2011;2:18.

Clark HF, Gurney AL, Abaya E, Baker K, Baldwin D, Brush J *et al*. The secreted protein discovery initiative (SPDI), a large-scale effort to identify novel human secreted and transmembrane proteins: a bioinformatics assessment. *Genome Res* 2003;13:2265-2270.

Clayton EL, Minogue S, Waugh MG. Phosphatidylinositol 4-Kinases and PI4P metabolism in the nervous system: roles in psychiatric and neurological diseases. *Mol Neurobiol* 2012 Oct 10. [Epub ahead of print].

Clifford S, Dissanayake C, Bui QM, Huggins R, Taylor AK, Loesch DZ. Autism spectrum phenotype in males and females with fragile X full mutation and premutation. *J Autism Dev Disord* 2007;37:738-747.

Cody H, Pelphrey K, Piven J. Structural and functional magnetic resonance imaging of autism. *Int J Dev Neurosci* 2002;20:421-438.

Cole AJ, Saffen DW, Baraban JM, Worley PF. Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature* 1989;340:474-476.

Cole JT, Sweatt AJ, Hutson SM. Expression of mitochondrial branched-chain amino transferase and α-keto-acid dehydrogenase in rat brain: implications for neurotransmitter metabolism. *Front Neuroanatomy* 2012;6:18.

Cole TB, Robbins CA, Wenzel HJ, Schwartzkroin PA, Palmiter RD. Seizures and neuronal damage in mice lacking vesicular zinc. *Epilepsy Res* 2000;39:153-169.

Colello RJ, Fuss B, Fox MA, Alberti J. A proteomic approach to rapidly elucidate oligodendrocyte-associated proteins expressed in the myelinating rat optic nerve. *Electrophoresis* 2002;23:144-151.

Coleman M, Blass JP. Autism and lactic acidosis. *J Autism Dev Disord* 1985;15:1-8.

Comai G, Boutet A, Neirijnck Y, Schedl A. Expression patterns of the *Wtx/Amer* gene family during mouse embryonic development. *Dev Dyn* 2010;239:1867-1878.

Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. *Front Neuroendocrinol* 2000;21:1-56.

Conroy J, Cochrane L, Anney RJ, Sutcliffe JS, Carthy P, Dunlop A *et al*. Fine mapping and association studies in a candidate region for autism on chromosome 2q31-q32. *Am J Med Genet B Neuropsychiatr Genet* 2009;150B:535-544.

Constantino JN, Zhang Y, Frazier T, Abbacchi AM, Law P. Sibling recurrence and the genetic epidemiology of autism. *Am J Psychiatry* 2010,167:1349-1356.

Conway CD, Howe KM, Nettleton NK, Price DJ, Mason JO, Pratt T. Heparan sulfate sugar modifications mediate the functions of slits and other factors needed for mouse forebrain commissure development. *J Neurosci* 2011;31:1955-1970.

Cook EH Jr, Courchesne RY, Cox NJ, Lord C, Gonen D, Guter SJ *et al*. Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. *Am J Hum Genet* 1998;62:1077-1083.

Cook EH Jr, Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C *et al*. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am J Hum Genet* 1997;60:928-934.

Cook EH Jr, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. *Nature* 2008,455:919-923.

Cook EH, Leventhal BL. The serotonin system in autism. *Curr Opin Pediatr* 1996;8:348-354.

Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C *et al*. A copy number variation morbidity map of developmental delay. *Nat Genet* 2011;43:838-846.

Correia C, Coutinho AM, Almeida J, Lontro R, Lobo C, Miguel TS *et al*. Association of the alpha4 integrin subunit gene (*ITGA4*) with autism. *Am J Med Genet B Neuropsychiatr Genet* 2009;150B:1147-1151.

Cottrell CE, Bir N, Varga E, Alvarez CE, Bouyain S, Zernzach R *et al*. Contactin 4 as an autism susceptibility locus. *Aut Res* 2011;4:189-199.

Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD *et al*. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology* 2001;57:245-254.

Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP *et al*. Mapping early brain development in autism. *Neuron* 2007;56:399-413.

Courchesne E, Yeung-Courchesne R, Press GA, Hesselink JR, Jernigan TL. Hypoplasia of cerebellar vermal lobules VI and VII in autism. *N Engl J Med* 1988;31821:1349-1354.

Cousins SL, Stephenson FA. Identification of N-methyl-D-aspartic acid (NMDA) receptor subtype-specific binding sites that mediate direct interactions with scaffold protein PSD-95. *J Biol Chem* 2012;287:13465-13476.

Crepel A, Steyaert J, De la Marche W, De Wolf V, Fryns JP, Noens I *et al*. Narrowing the critical deletion region for autism spectrum disorders on 16p11.2. *Am J Med Genet B Neuropsychiatr Genet* 2011;156:243-245.

Croen LA, Braunschweig D, Haapanen L, Yoshida CK, Fireman B, Grether JK *et al*. Maternal midpregnancy autoantibodies to fetal brain protein: the early markers for autism study. *Biol Psychiatry* 2008;64:583-588.

Croen LA, Grether JK, Yoshida CK, Odouli R, Van de Water J. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. *Arch Pediatr Adolesc Med* 2005;159:151-157.

Crow YJ, Livingston JH. Aicardi-Goutières syndrome: an important Mendelian mimic of congenital infection. *Dev Med Child Neurol* 2008;50:410-416.

Cubelos B, Sebastián-Serrano A, Beccari L, Calcagnotto ME, Cisneros E, Kim S *et al*. *Cux1* and *Cux2* regulate dendritic branching, spine morphology and synapses of the upper layer neurons of the cortex. *Neuron* 2010;66:523-535.

Curran S, Bolton P, Rozsnyai K, Chiocchetti A, Klauck SM, Duketis E *et al*. No association between a common single nucleotide polymorphism, rs4141463, in the *MACROD2* gene and autism spectrum disorder. *Am J Med Genet B Neuropsychiatr Genet* 2011;156B:633-639.

Cus R, Maurus D, Kühl M. Cloning and developmental expression of WSTF during Xenopus laevis embryogenesis. *Gene Expr Patterns* 2006;6:340-346.

Cuscó I, Medrano A, Gener B, Vilardell M, Gallastegui F, Villa O *et al*. Autism-specific copy number variants further implicate the phosphatidylinositol signaling pathway and the glutamatergic synapse in the etiology of the disorder. *Hum Mol Genet* 2009;18:1795-1804.

Czech MP. PIP2 and PIP3: complex roles at the cell surface. *Cell* 2000;100:603-606.

D'Agostino G, Kim JD, Liu ZW, Jeong JK, Suyama S, Calignano A *et al*. Prolyl Endopeptidase-deficient mice have reduced synaptic spine density in the ca1 region of the hippocampus, impaired LTP, and spatial learning and memory *Cereb Cortex* 2012 Jul 5.[Epub ahead of print]

Da Silva JS, Medina M, Zuliani C, Di Nardo A, Witke W, Dotti CG. RhoA/ROCK regulation of neuritogenesis via profilin IIa–mediated control of actin stability. *J Cell Biol* 2003;162:1267-1279.

Das Chakraborty R, Bernal AJ, Schoch K, Howard TD, Ip EH, Hooper SR *et al*. Dysregulation of DGCR6 and DGCR6L: psychopathological outcomes in chromosome 22q11.2 deletion syndrome. *Transl Psychiatry* 2012;2:e105.

Davidson G, Niehrs C. Emerging links between CDK cell cycle regulators and Wnt signaling. *Trends Cell Biol* 2010;20:453-460.

Davies W, Humby T, Isles AR, Burgoyne PS, Wilkinson LS. X-monosomy effects on visuospatial attention in mice: a candidate gene and implications for Turner syndrome and attention deficit hyperactivity disorder. *Biol Psychiatry* 2007;61:1351-1360.

Davis MM, Olausson P, Greengard P, Taylor JR, Nairn AC. Regulator of calmodulin signaling knockout mice display anxiety-like behavior and motivational deficits. *Eur J Neurosci* 2012;35:300-308.

Dawson AJ, Putnam S, Schultz J, Riordan D, Prasad C, Greenberg CR *et al*. Cryptic chromosome rearrangements detected by subtelomere assay in patients with mental retardation and dysmorphic features. *Clin Genet* 2002;62:488-494.

de Anda FC, Rosario AL, Durak O, Tran T, Gräff J, Meletis K *et al*. Autism spectrum disorder susceptibility gene *TAOK2* affects basal dendrite formation in the neocortex. *Nat Neurosci* 2012;15:1022-1031.

De Ferrari GV, Moon RT. The ups and downs of Wnt signaling in prevalent neurological disorders. *Oncogene* 2006;25:7545-7553.

de Kovel CGF, Trucks H, Helbig I, Mefford HC, Baker C, Leu C *et al*. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. *Brain* 2010;133:23-32.

De Luca A, Conti E, Grifone N, Amati F, Spalletta G, Caltagirone C *et al*. Association study between CAG trinucleotide repeats in the *PCQAP* gene (PC2 glutamine/Q-rich-associated protein) and schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 2003;116B:32-35.

De Luca A, Pasini A, Amati F, Botta A, Spalletta G, Alimenti S *et al*. Association study of a promoter polymorphism of *UFD1L* gene with schizophrenia. *Am J Med Genet* 2001;105:529-533.

de Queiroz Soares DC, Dutra RL, D'angioli Costa Quaio CR, Melaragno MI, Kulikowski LD, Torres LC *et al*. Role of *SNAP29*, *LZTR1* and *P2RXL1* genes on immune regulation in a patient with atypical 0.5Mb deletion in 22q11.2 region. *Clin Immunol* 2012;145:55-58.

De Strooper B. Aph-1, Pen-2, and nicastrin with presenilin generate an active γ-secretase complex. *Neuron* 2003;38:9-12.

Dean B, Keriakous D, Scarr E, Thomas EA. Gene expression profiling in Brodmann's area 46 from subjects with schizophrenia. *Aust N Z J Psychiatry* 2007;41:308-320.

Delatycki MB, Danks A, Churchyard A, Zhou XP, Eng C. *De novo* germline *PTEN* mutation in a man with Lhermitte-Duclos disease which arose on the paternal chromosome and was transmitted to his child with polydactyly and Wormian bones. *J Med Genet* 2003;40:e92.

Delmas P, Wanaverbecq N, Abogadie FC, Mistry M, Brown DA. Signaling microdomains define the specificity of receptor-mediated InsP(3) pathways in neurons. *Neuron* 2002;34:209-220.

Dennis M, Lockyer L, Lazenby AL, Donnelly RE, Wilkinson M, Schoonheyt W. Intelligence patterns among children with high-functioning autism, phenylketonuria, and childhood head injury. *J Autism Dev Disord* 1999;29:5-17.

Depienne C, Heron D, Betancur C, Benyahia B, Trouillard O, Bouteiller D *et al*. Autism, language delay and mental retardation in a patient with 7q11 duplication. *J Med Genet* 2007;44:452-458.

Depienne C, Moreno-De-Luca D, Heron D, Bouteiller D, Gennetier A, Delorme R *et al*. Screening for genomic rearrangements and methylation abnormalities of the 15q11–q13 region in autism spectrum disorders. *Biol Psychiatry* 2009;66:349-359.

Descheemaeker MJ, Govers V, Vermeulen P, Fryns JP. Pervasive developmental disorders in Prader–Willi syndrome: the Leuven experience in 59 subjects and controls. *Am J Med Genet A* 2006;140:1136-1142.

Deverman BE, Patterson PH. Cytokines and CNS development. *Neuron* 2009;64:61-78.

Devillard F, Guinchat V, Moreno-De-Luca D, Tabet AC, Gruchy N, Guillem P *et al*. Paracentric inversion of chromosome 2 associated with cryptic duplication of 2q14 and deletion of 2q37 in a patient with autism. *Am J Med Genet A* 2010;152A:2346-2354.

Devillard F, Guinchat V, Moreno-De-Luca D, Tabet AC, Gruchy N, Guillem P *et al*. Paracentric inversion of chromosome 2 associated with cryptic duplication of 2q14 and deletion of 2q37 in a patient with autism. *Am J Med Genet Part A* 2010;152A:2346-2354.

Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder. *Curr Opin Genet Dev* 2012;22:229-237.

Dhami GK, Ferguson SS. Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis. *Pharmacol Ther* 2006;111:260-271.

Dhar SU, del Gaudio D, German JR, Peters SU, Ou Z, Bader PI *et al*. 22q13.3 deletion syndrome: clinical and molecular analysis using array CGH. *Am J Med Genet A* 2010;152A:573-581.

Dharmadhikari AV, Kang SH, Szafranski P, Person RE, Sampath S, Prakash SK *et al*. Small rare recurrent deletions and reciprocal duplications in 2q21.1, including brain-specific *ARHGEF4* and *GPR148*. *Hum Mol Genet* 2012;21:3345-3355.

Dhillon S, Hellings JA, Butler MG. Genetics and mitochondrial abnormalities in autism spectrum disorders: a review. *Curr Genomics* 2011;12:322-332.

Díaz-Ruiz C, Parlato R, Aguado F, Ureña JM, Burgaya F, Martínez A *et al*. Regulation of neural migration by the CREB/CREM transcription factors and altered Dab1 levels in *CREB*/*CREM* mutants. *Mol Cell Neurosci* 2008;39:519-528.

Diehl F, Brown MA, van Amerongen MJ, Novoyatleva T, Wietelmann A, Harriss J *et al*. Cardiac deletion of Smyd2 is dispensable for mouse heart development. *Plos One* 2010;5:e9748.

Ditlev JA, Michalski PJ, Huber G, Rivera GM, Mohler WA, Loew LM *et al*. Stoichiometry of Nck-dependent actin polymerization in living cells. *J Cell Biol* 2012;197:643-658.

Djurovic S, Le Hellard S, Kähler AK, Jönsson EG, Agartz I, Steen VM *et al*. Association of MCTP2 gene variants with schizophrenia in three independent samples of Scandinavian origin (SCOPE). *Psychiatry Res* 2009;168:256-258.

Donahue CP, Kosik KS, Shors TJ. Growth hormone is produced within the hippocampus where it responds to age, sex, and stress. *Proc Natl Acad Sci USA* 2006;103:6031-6036.

Donati G, Brighenti E, Vici M, Mazzini G, Trere D, Montanaro L *et al*. Selective inhibition of rRNA transcription down regulates E2F-1: a new p53-independent mechanism linking cell growth to cell proliferation. *J Cell Sci* 2011;124:3017-3028.

Doornbos M, Sikkema-Raddatz B, Ruijvenkamp CA, Dijkhuizen T, Bijlsma EK, Gijsbers AC *et al*. Nine patients with a microdeletion 15q11.2 between breakpoints 1 and 2 of the Prader-Willi critical region, possibly associated with behavioural disturbances. *Eur J Med Genet* 2009;52:108-115.

Dowdle WE, Robinson JF, Kneist A, Sirerol-Piquer MS, Frints SG, Corbit KC *et al*. Disruption of a ciliary B9 protein complex causes Meckel syndrome. *Am J Hum Genet* 2011;89:94-110.

Dowler S, Currie RA, Campbell DG, Deak M, Kular G, Downes CP *et al*. Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. *Biochem J* 2000;351:19-31.

Drew RE, Settles ML, Churchill EJ, Williams SM, Balli S, Robison BD. Brain transcriptome variation among behaviorally distinct strains of zebrafish (Danio rerio). *BMC Genomics* 2012;13:323.

D'Souza I, Poorkaj P, Hong M, Nochlin D, Lee VM, Bird TD *et al*. Missense and silent tau gene mutations cause frontotemporal dementia with parkinsonism-chromosome 17 type, by affecting multiple alternative RNA splicing regulatory elements. *Proc Natl Acad Sci USA* 1999;96:5598-5603.

DuBose AJ, Johnstone KA, Smith EY, Hallett RA, Resnick JL. *Atp10a*, a gene adjacent to the PWS/AS gene cluster, is not imprinted in mouse and is insensitive to the PWS-IC. *Neurogenetics* 2010;11:145-151.

Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F *et al*. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet* 2007;39:25-27.

Earls LR, Fricke RG, Yu J, Berry RB, Baldwin LT, Zakharenko SS. Age-dependent microRNA control of synaptic plasticity in 22q11 deletion syndrome and schizophrenia. *J Neurosci* 2012;32:14132-14144.

Edgell CJ, BaSalamah MA, Marr HS. Testican-1: a differentially expressed proteoglycan with protease inhibiting activities. *Int Rev Cytol* 2004;236:101-122.

Egerod KL, Holst B, Petersen PS, Hansen JB, Mulder J, Hokfelt T *et al*. GPR39 splice variants versus antisense gene *LYPD1*: expression and regulation in gastrointestinal tract, endocrine pancreas, liver, and white adipose tissue. *Mol Endocrinol* 2007;21:1685-1698.

Ehlers MD. Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat Neurosci* 2003;6:231-242.

Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ *et al*. Reversal of learning deficits in a $Tsc2^{+/-}$ mouse model of tuberous sclerosis. *Nat Med* 2008;14:843-848.

Einheber S, Meng X, Rubin M, Lam I, Mohandas N, An X *et al*. The 4.1B cytoskeletal protein regulates the domain organization and sheath thickness of myelinated axons. *Glia* 2012 Oct 26. [Epub ahead of print]

Ekström EJ, Sherwood V, Andersson T. Methylation and loss of Secreted Frizzled-Related Protein 3 enhances melanoma cell migration and invasion. *Plos One* 2011;6:e18674.

El Zein L, Ait-Lounis A, Morlé L, Thomas J, Chhin B, Spassky N *et al*. RFX3 governs growth and beating efficiency of motile cilia in mouse and controls the expression of genes involved in human ciliopathies. *J Cell Sci* 2009;122:3180-3189.

Elia J, Gai X, Hakonarson H, White PS. Structural variations in attention-deficit hyperactivity disorder. *Lancet* 2011a;377:377-378.

Elia J, Glessner JT, Wang K, Takahashi N, Shtir CJ, Hadley D *et al*. Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder. *Nat Genet* 2011b;44:78-84.

Engelhardt B, Ransohoff RM. The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. *Trends Immunol* 2005;26:485-495.

Enstrom AM, Van de Water JA, Ashwood P. Autoimmunity in autism. *Curr Opin Invest Drugs* 2009;10:463-473.

Esch BE, Carr JE. Secretin as a treatment for autism: a review of the evidence. *J Autism Dev Disord* 2004;34:543-556.

Esumi S, Kakazu N, Taguchi Y, Hirayama T, Sasaki A, Hirabayashi T *et al*. Monoallelic yet combinatorial expression of variable exons of the protocadherin-alpha gene cluster in single neurons. *Nat Genet* 2005;37:171-176.

Ethell IM, Yamaguchi Y. Cell surface heparan sulfate proteoglycan syndecan-2 induces the maturation of dendritic spines in rat hippocampal neurons. *J Cell Biol* 1999;144:575-586.

Etienne-Manneville S, Hall A. Rho GTPases in cell biology. *Nature* 2002;420:629-635.

Evers M, Cunningham-Rundles C, Hollander E. Heat shock protein 90 antibodies in autism. *Mol Psychiatry* 2002;7(Suppl. 2):S26–S28.

Fagiolini M, Fritschy JM, Löw K, Möhler H, Rudolph U, Hensch TK. Specific GABAA circuits for visual cortical plasticity. *Science* 2004;303:1681-1683.

Fanciulli M, Norsworthy PJ, Petretto E, Dong R, Harper L, Kamesh L *et al*. *FCGR3B* copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. *Nat Genet* 2007;39:721-723.

Fanous AH, Chen X, Wang X, Amdur RL, O'Neill FA, Walsh D *et al*. Association between the 5q31.1 gene neurogenin1 and schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 2007;144B:207-214.

Fatemi SH, Aldinger KA, Ashwood P, Bauman ML, Blaha CD, Blatt GJ *et al*. Consensus paper: pathological role of the cerebellum in autism. *Cerebellum* 2012;11:777-807.

Fatemi SH, Halt AR, Stary JM, Realmuto GM, Jalali-Mousavi M. Reduction in anti-apoptotic protein Bcl-2 in autistic cerebellum. *NeuroReport* 2001a;12:929-933.

Fatemi SH, Reutiman TJ, Folsom TD, Rooney RJ, Patel DH, Thuras PD. mRNA and protein levels for GABAAα4, α5, β1 and GABABR1 receptors are altered in brains from subjects with autism. *J Autism Dev Disord* 2010;40:743-750.

Fatemi SH, Snow AV, Stary JM, Araghi-Niknam M, Reutiman TJ, Lee S *et al*. Reelin signaling is impaired in autism. *Biol Psychiatry* 2005;57:777-787.

Fatemi SH, Stary JM, Halt AR, Realmuto GR. Dysregulation of Reelin and Bcl-2 proteins in autistic cerebellum. *J Autism Dev Disord* 2001b;31:529-535.

Fedirko N, Avshalumov M, Rice ME, Chesler M. Regulation of postsynaptic Ca^{2+} influx in hippocampal CA1 pyramidal neurons via extracellular carbonic anhydrase. *J Neurosci* 2007;27:1167-1175.

Feng J, Schroer R, Yan J, Song W, Yang C, Bockholt A *et al*. High frequency of neurexin 1beta signal peptide structural variants in patients with autism. *Neurosci Lett* 2006;409:10-13.

Fernandez BA, Roberts W, Chung B, Weksberg R, Meyn S, Szatmari P *et al*. Phenotypic spectrum associated with *de novo* and inherited deletions and duplications at 16p11.2 in individuals ascertained for diagnosis of autism spectrum disorder. *J Med Genet* 2010;47:195-203.

Fernandez BA, Roberts W, Chung B, Weksberg R, Meyn S, Szatmari P *et al*. Phenotypic spectrum associated with *de novo* and inherited deletions and duplications at 16p11.2 in individuals ascertained for diagnosis of autism spectrum disorder *J Med Genet* 2010;47:195-203.

Fernandez T, Morgan T, Davis N, Klin A, Morris A, Farhi A *et al*. Disruption of Contactin 4 (CNTN4) results in developmental delay and other features of 3p deletion syndrome. *Am J Hum Genet* 2008;82:1385.

Feyder M, Karlsson RM, Mathur P, Lyman M, Bock R, Momenan R *et al*. Association of mouse *Dlg4* (PSD-95) gene deletion and human *DLG4* gene variation with phenotypes relevant to autism spectrum disorders and Williams' syndrome. *Am J Psychiatry* 2010;167:1508-1517.

Fine SE, Weissman A, Gerdes M, Pinto-Martin J, Zackai EH, McDonald-McGinn DM *et al*. Autism spectrum disorders and symptoms in children with molecularly confirmed 22q11.2 deletion syndrome. *J Autism Dev Disord* 2005;35:461-470.

Fisher DA, Smith JF, Pillar JS, St. Denis SH, Cheng JB. Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. *J Biol Chem* 1998;273:15559-15564.

Foger N, Rangell L, Danilenko DM, Chan AC. Requirement for coronin 1 in T lymphocyte trafficking and cellular homeostasis. *Science* 2006;313:839-842.

Folli F, Solimena M, Cofiell R, Austoni M, Tallini G, Fassetta G *et al*. Autoantibodies to a 128-kd synaptic protein in three women with the stiff-man syndrome and breast cancer. *N Engl J Med* 1993;328:546-551.

Folstein SE, Rosen-Sheidley B. Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat Rev Genet* 2001;2:943-955.

Fombonne E, Du Mazaubrun C, Cans C, Grandjean H. Autism and associated medical disorders in a French epidemiological survey. *J Am Acad Child Adolesc Psychiatry* 1997;36:1561-1569.

Fombonne E. Epidemiology of pervasive developmental disorders. *Pediatr Res* 2009;65:591-598.

Fontaine RH, Cases O, Lelièvre V, Mesplès B, Renauld JC, Loron G *et al*. IL-9/IL-9 receptor signaling selectively protects cortical neurons against developmental apoptosis. *Cell Death Differ* 2008;15:1542-1552.

Fourcaudot E, Gambino F, Humeau Y, Casassus G, Shaban H, Poulain B *et al*. (2008) cAMP/PKA signaling and RIM1alpha mediate presynaptic LTP in the lateral amygdala. *Proc Natl Acad Sci USA* 2008; 105:15130–15135.

Freitag CM. The genetics of autistic disorders and its clinical relevance: a review of the literature. *Mol Psychiatry* 2007;12:2-22.

Frisch A, Laufer N, Danziger Y, Michaelovsky E, Leor S, Carel C *et al*. Association of anorexia nervosa with the high activity allele of the COMT gene: a family-based study in Israeli patients. *Mol Psychiatry* 2001;6:243-245.

Fujiwara T, Mishima T, Kofuji T, Chiba T, Tanaka K, Yamamoto A *et al*. Analysis of knockout mice to determine the role of HPC-1/syntaxin 1A in expressing synaptic plasticity. *J Neurosci* 2006;26:5767-5776

Fukao T, Tanabe M, Terauchi Y, Ota T, Matsuda S, Asano T *et al*. PI3K-mediated negative feedback regulation of IL-12 production in DCs. *Nat Immunol* 2002;3:875-881.

Fukata Y, Fukata M. Protein palmitoylation in neuronal development and synaptic plasticity. *Nat Rev Neurosci* 2010;11:161-175.

Funato H, Sato M, Sinton CM, Gautron L, Williams SC, Skach A *et al*. Loss of Goosecoid-like and DiGeorge syndrome critical region 14 in interpeduncular nucleus results in altered regulation of rapid eye movement sleep. *Proc Natl Acad Sci USA* 2010;107:18155-18160.

Gadow KD, Roohi J, DeVincent CJ, Hatchwell E. Association of ADHD, tics, and anxiety with dopamine transporter (DAT1) genotype in autism spectrum disorder. *J Child Psychol Psychiatry* 2008;49:1331-1338.

Gant JC, Thibault O, Blalock EM, Yang J, Bachstetter A *et al*. Decreased number of interneurons and increased seizures in neuropilin 2 deficient mice: Implications for autism and epilepsy. *Epilepsia* 2009;50:629-645.

Gao C, Chen YG. Dishevelled: the hub of Wnt signaling. *Cell Signal* 2010;22:717-727.

Gao Y, Chen X, Shangguan S, Bao Y, Lu X, Zou J *et al*. Association study of *PARD3* gene polymorphisms with neural tube defects in a Chinese Han population. *Reprod Sci* 2012;19:764-771.

Garbett K, Ebert PJ, Mitchell A, Lintas C, Manzi B, Mirnics K *et al*. Immune transcriptome alterations in the temporal cortex of subjects with autism. *Neurobiol Dis* 2008;30:303-311.

Garden GA, Moller T. Microglia biology in health and disease. *J Neuroimmune Pharmacol* 2006;1:127-137.

Garriock RJ, Vokes SA, Small EM, Larson R, Krieg PA. Developmental expression of the Xenopus Iroquois-family homeobox genes, *Irx4* and *Irx5*. *Dev Genes Evol* 2001;211:257-260.

Garris PA, Collins LB, Jones SR, Wightman RM. Evoked extracellular dopamine in vivo in the medial prefrontal cortex. *J Neurochem* 1993;61:637-647.

Gasque P, Neal JW, Singhrao SK, McGreal EP, Dean YD, Van BJ *et al*. Roles of the complement system in human neurodegenerative disorders: pro-inflammatory and tissue remodeling activities. *Mol Neurobiol* 2002;25:1-17.

Gauthier J, Spiegelman D, Piton A, Lafrenière RG, Laurent S, St-Onge J *et al*. Novel *de novo SHANK3* mutation in autistic patients. *Am J Med Genet B Neuropsychiatr Genet* 2009;150B:421-424.

Gehrmann J. Microglia: a sensor to threats in the nervous system? *Res Virol* 1996;147:79-88.

Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Nataf R *et al*. Biomarkers of environmental toxicity and susceptibility in autism. *J Neurol Sci* 2009;280:101-108.

Geis C, Weishaupt A, Hallermann S, Grunewald B, Wessig C, Wultsch T *et al*. Stiff person syndrome-associated autoantibodies to amphiphysin mediate reduced GABAergic inhibition. *Brain* 2010:133;3166-3180.

Gemelli T, Berton O, Nelson ED, Perrotti LI, Jaenisch R, Monteggia LM. Postnatal loss of methyl-CpG binding protein 2 in the forebrain is sufficient to mediate behavioral aspects of Rett syndrome in mice. *Biol Psychiatry* 2006;59:468-476.

Georgi A, Schumacher J, Leon CA, Wolf AV, Klein K, Böhenz KV *et al*. No association between genetic variants at the *DGCR2* gene and schizophrenia in a German sample. *Psychiatr Genet* 2009;19:104.

Geschwind DH. Advances in autism. *Annu Rev Med* 2009;60:367-380.

Ghandour H, Cullere X, Alvarez A, Luscinskas FW, Mayadas TN. Essential role for Rap1 GTPase and its guanine exchange factorCalDAG-GEFI in LFA-1 but not VLA-4 integrin-mediated human T-cell adhesion. *Blood* 2007;110:3682-3690.

Gharani N, Benayed R, Mancuso V, Brzustowicz LM, Millonig JH. Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. *Mol Psychiatry* 2004;9:474-484.

Ghaziuddin M, Bolyard B, Alessi N, Autistic disorder in Noonan syndrome. *J Intellect Disabil Res*. 1994;38:67-72.

Ghezzo A, Guerini FR, Bolognesi E, Matteoli M, Manca S, Sotgiu S *et al*. Neuropsycological gender differences in healthy individuals and in pediatric neurodevelopmental disorders. A role for SNAP-25. *Med Hypotheses* 2009;73:978-980

Giger RJ, Cloutier JF, Sahay A, Prinjha RK, Levengood DV, Moore SE *et al*. Neuropilin-2 is required in vivo for selective axon guidance responses to secreted semaphorins. *Neuron* 2000;25:29-41.

Gillberg C, Cederlund M, Lamberg K, Zeijlon L. Brief report: ''the autism epidemic''. The registered prevalence of autism in a Swedish urban area. *J Autism Dev Disord* 2006,36:429-435.

Gillberg C, Rasmussen P. Brief report: four case histories and a literature review of Williams syndrome and autistic behavior. *J Autism Dev Disord* 1994;24:381-393.

Gillberg C. Chromosomal disorders and autism. *J Autism Dev Disord* 1998;28:415-425.

Gillberg IC, Gillberg C, Ahlsen G. Autistic behaviour and attention deficits in tuberous sclerosis: a population-based study. *Dev Med Child Neurol* 1994;36:50-56.

Gilman SR, Iossifov I, Levy D, Ronemus M, Wigler M, Vitkup D. Rare *de novo* variants associated with autism implicate a large functional network of genes involved in formation and function of synapses. *Neuron* 2011;70:898-907.

Gingell K, Parmar R, Sungum-Paliwal S. Autism and multiple pituitary deficiency. *Dev Med Child Neurol* 1996;38:545-549.

Glessner JT, Reilly MP, Kim CE, Takahashi N, Albano A, Hou C *et al*. Strong synaptic transmission impact by copy number variations in schizophrenia. *Proc Natl Acad Sci USA* 2010;107:10584-10589.

Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S *et al*. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. *Nature* 2009;459:569-573.

Goebel P, Montalbano A, Ayers N, Kompfner E, Dickinson L, Webb CF *et al*. High frequency of matrix attachment regions and Cut-like protein x/CCAAT-displacement protein and B cell regulator of IgH transcription binding sites flanking Ig V region genes. *J Immunol* 2002;169:2477-2487.

Goines P, Van de Water J. The immune system's role in the biology of autism. *Curr Opin Neurol* 2010;23:111-117.

Golzio C, Willer J, Talkowski ME, Oh EC, Taniguchi Y, Jacquemont S *et al*. KCTD13 is a major driver of mirrored neuroanatomical phenotypes of the 16p11.2 copy number variant. *Nature* 2012;485:363-367.

Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, Catano G *et al*. The influence of *CCL3L1* gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* 2005;307:1434-1440.

Goorden SM, van Woerden GM, van der Weerd L, Cheadle JP, Elgersma Y. Cognitive deficits in $Tsc1^{+/-}$ mice in the absence of cerebral lesions and seizures. *Ann Neurol* 2007;62:648-655.

Gosch A, Pankau R. "Autistic" behavior in two children with Williams–Beuren syndrome. *Am J Med Genet* 1994;53:83-84.

Goytain A, Hines RM, Quamme GA. Functional characterization of NIPA2, a selective Mg2+ transporter. *Am J Physiol Cell Physiol* 2008;295:C944-953.

Graham ME, Washbourne P, Wilson MC, Burgoyne RD. Molecular analysis of SNAP-25 function in exocytosis. *Ann N Y Acad Sci* 2002;971:210–221.

Granucci F, Petralia F, Urbano M, Citterio S, Di Tota F, Santambrogio L *et al*. The scavenger receptor MARCO mediates cytoskeleton rearrangements in dendritic cells and microglia. *Blood* 2003;102:2940-2947.

Greenbaum L, Alkelai A, Rigbi A, Kohn Y, Lerer B. Evidence for association of the GLI2 gene with tardive dyskinesia in patients with chronic schizophrenia. *Mov Disord* 2010;25:2809-2817.

Griffith E, Walker S, Martin CA, Vagnarelli P, Stiff T, Vernay B *et al*. Mutations in Pericentrin cause Seckel syndrome with defective ATR-dependent DNA damage signaling. *Nat Genet* 2008;40:232-236.

Griggs BL, Ladda S, Saula RA, DuPonta BR, Srivastava AK. Dedicator of cytokinesis 8 is disrupted in two patients with mental retardation and developmental disabilities. *Genomics* 2008,91:195-202.

Grigorenko EL, Han SS, Yrigollen CM, Leng L, Mizue Y, Anderson GM *et al*. Macrophage migration inhibitory factor and autism spectrum disorders. *Pediatrics* 2008;122:e438-e445.

Grisart B, Willatt L, Destree A, Fryns JP, Rack K, de Ravel T *et al*. 17q21.31 microduplication patients are characterised by behavioural problems and poor social interaction. *J Med Genet* 2009;46:524-530.

Griswold AJ, Ma D, Cukier HN, Nations LD, Schmidt MA, Chung RH *et al*. Evaluation of copy number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. *Hum Mol Genet* 2012;21:3513-3523.

Groehler AL, Lannigan DA. A chromatin-bound kinase, ERK8, protects genomic integrity by inhibiting HDM2-mediated degradation of the DNA clamp PCNA. *J Cell Biol* 2010;190:575-586.

Guilmatre A, Dubourg C, Mosca AL, Legallic S, Goldenberg A, Drouin-Garraud V *et al*. Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism and mental retardation. *Arch Gen Psychiatry* 2009;66:947-956.

Guilmatre A, Dubourg C, Mosca AL, Legallic S, Goldenberg A, Drouin-Garraud V *et al*. Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism, and mental retardation. *Arch Gen Psychiatry* 2009;66:947-956.

Gulledge AT, Kampa BM, Stuart GJ. Synaptic integration in dendritic trees. *J Neurobiol* 2005;64:75-90.

Gupta S, Aggarwal S, Rashanravan B, Lee T. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. *J Neuroimmunol* 1998;85:106-109.

Gurling HM, Kalsi G, Brynjolfson J, Sigmundsson T, Sherrington R, Mankoo BS *et al*. Genome-wide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24and 20q12.1–11.2. *Am J Hum Genet* 2001;68:661-673.

Guy J, Hendrich B, Holmes M, Martin JE, Bird A. A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat Genet* 2001;27:322-326.

Haddad-Tóvolli R, Heide M, Zhou X, Blaess S, Alvarez-Bolado G. Mouse thalamic differentiation: Gli-dependent pattern and Gli-independent prepattern. *Front Neurosci* 2012;6:27.

Hadjikhani N, Joseph RM, Snyder J, Tager-Flusberg H. Anatomical differences in the mirror neuron system and social cognition network in autism. *Cereb Cortex* 2006;16:1276-1282.

Hagerman RJ, Rivera SM, Hagerman PJ. The Fragile X family of disorders: a model for autism and targeted treatments. *Curr Pediatr Rev* 2008;4:40-52.

Haines JL, Ter-Minassian M, Bazyk A, Gusella JF, Kim DJ, Terwedow H *et al*. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatability complex. *Nat Genet* 1996;13:469-471.

Haitina T, Lindblom J, Renström T, Fredriksson R. Fourteen novel human members of mitochondrial solute carrier family 25 (SLC25) widely expressed in the central nervous system. *Genomics* 2006;88:779-790.

Hamamoto R, Furukawa Y, Morita M, Iimura Y, Silva FP, Li M *et al*. SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. *Nat Cell Biol* 2004;6:731-740.

Hamano S, Tanaka Y, Nara T, Nakanishi Y, Shimizu M. Paroxysmal kinesigenic choreoathetosis associated with prenatal brain damage. *Acta Paediatr Jpn* 1995;37:401-404.

Hamel B, Monaghan-Benson E, Rojas RJ, Temple BRS, Marston DJ, Burridge K *et al*. SmgGDS is a guanine nucleotide exchange factor that specifically activates RhoA and RhoC. *J Biol Chem* 2011;286:12141-12148.

Hamosh A, Scharer G, Van Hove J. Glycine Encephalopathy. In: Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP, eds. *GeneReviews*. Seattle (WA): University of Washington, Seattle; 1993-2002 [updated November 2009].

Han JM, Sahin M. TSC1/TSC2 signaling in the CNS. *FEBS Lett* 2011;585:973-980.

Handa V, Goldwater D, Stiles D, Cam M, Poy G, Kumari D *et al*. Long CGG-repeat tracts are toxic to human cells: implications for carriers of Fragile X premutation alleles. *FEBS Lett* 2005;579:2702-2708.

Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* 2007;10:1387-1394.

Hanson E, Nasir RH, Fong A, Lian A, Hundley R, Shen Y *et al*. Cognitive and behavioral characterization of 16p11.2 deletion syndrome. *J Dev Behav Pediatr* 2010;31:649-657.

Hara T, Tanegashima K. Pleiotropic functions of the CXC-type chemokine CXCL14 in mammals. *J Biochem* 2012;151:469-476.

Harada Y, Tanaka Y, Terasawa M, Pieczyk M, Habiro K, Katakai T *et al*. DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses. *Blood* 2012;119:4451-4461.

Hardan AY, Girgis RR, Lacerda AL, Yorbik O, Kilpatrick M, Keshavan MS *et al*. Magnetic resonance imaging study of the orbitofrontal cortex in autism. *J Child Neurol* 2006a;21:866-871.

Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ. An MRI study of increased cortical thickness in autism. *Am J Psychiatry* 2006b;163:1290-1292.

Hart MJ, Eva A, Zangrilli D, Aaronson SA, Evans T, Cerione RA *et al*. Cellular transformation and guanine nucleotide exchange activity are catalyzed by a common domain on the dbl oncogene product. *J Biol Chem* 1994;269:62-65.

Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T *et al*. Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. *Mol Psychiatry* 2007;12:1026-1032.

Hashimoto R, Hashimoto H, Shintani N, Ohi K, Hori H, Saitoh O *et al*. Possible association between the pituitary adenylate cyclase-activating polypeptide (PACAP) gene and major depressive disorder. *Neurosci Lett* 2010;468:300-302.

Hassel B, Schreff M, Stube EM, Blaich U, Schumacher S. CALEB/NGC interacts with the Golgi-associated protein PIST. *J Biol Chem* 2003;278:40136-40143.

Hata Y, Butz S, Sudhof TC. CASK: A novel dlg/PSD95 homolog with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neurexins. *J Neurosci* 1996;16:2488-2494.

Hattori S, Takao K, Tanda K, Toyama K, Shintani N, Baba A *et al*. Comprehensive behavioral analysis of pituitary adenylate cyclase-activating polypeptide (PACAP) knockout mice. *Front Behav Neurosci* 2012;6:58.

Hayashi MK, Tang C, Verpelli C, Narayanan R, Stearns MH, Xu RM *et al*. The postsynaptic density proteins Homer and Shank form a polymeric network structure. *Cell* 2009;137:159-171.

Hayashi R, Hanyu N, Yahikozawa H, Yanagisawa N. Ictal muscle discharge pattern and SPECT in paroxysmal kinesigenic choreoathetosis. *Electromyogr Clin Neurophysiol* 1997;37:89-94.

Hazlett HC, Poe M, Gerig G, Smith RG, Provenzale J, Ross A *et al*. Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. *Arch Gen Psychiatry* 2005;62:1366-1376.

Hazlett HC, Poe MD, Gerig G, Smith RG, Piven J. Cortical gray and white brain tissue volume in adolescents and adults with autism. *Biol Psychiatry* 2006;59:1-6.

Haznedar MM, Buchsbaum MS, Metzger M, Solimando A, Spiegel-Cohen J, Hollander E. Anterior cingulate gyrus volume and glucose metabolism in autistic disorder. *Am J Psychiatry* 1997;154:1047-1050.

Hazuka CD, Foletti DL, Hsu SC, Kee Y, Hopf FW, Scheller RH. The sec6/8 complex is located at neurite outgrowth and axonal synapse-assembly domains. *J Neurosci* 1999;19:1324-1334.

He WZ, Liu WQ, Zhong XQ, Chen XL, Li SY, Zhang HM *et al*. Analysis of *de novo* copy number variations in a family affected with autism spectrum disorders using high-resolution array-based comparative genomic hybridization. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2012;29:266-269.

Heller T. Ueber Dementia infantilis. *Zeitschrift fuer die Erforschung und Behandlung des Jugendlichen Schwachsinns* 1908;2:17-28.

Hemara-Wahanui A, Berjukow S, Hope CI, Dearden PK, Wu SB, Wilson-Wheeler J *et al*. A *CACNA1F* mutation identified in an X-linked retinal disorder shifts the voltage dependence of Cav1.4 channel activation. *Proc Natl Acad Sci USA* 2005;102:7553-7558.

Hendrich B, Bird A. Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol Cell Biol* 1998;18:6538-6547.

Heng C, Lefebvre O, Klein A, Edwards MM, Simon-Assmann P, Orend G *et al*. Functional role of laminin α1 chain during cerebellum development. *Cell Adh Migr* 2011;5:480-489

Hennah W, Porteous D. The DISC1 pathway modulates expression of neurodevelopmental, synaptogenic and sensory perception genes. *Plos One* 2009;4:e4906.

Hennah W, Thomson P, McQuillin A, Bass N, Loukola A, Anjorin A *et al*. DISC1 association, heterogeneity and interplay in schizophrenia and bipolar disorder. *Mol Psychiatry* 2009;14:865-873.

Henningsson S, Jonsson L, Ljunggren E, Westberg L, Gillberg C, Rastam M *et al*. Possible association between the androgen receptor gene and autism spectrum disorder. *Psychoendrocrinology* 2009;34:752-761.

Herbert MR, Harris GJ, Adrien KT, Ziegler DA, Makris N, Kennedy DN *et al*. Abnormal asymmetry in language association cortex in autism. *Ann Neurol* 2002;52:588-596.

Herbert MR, Russo JP, Yang S, Roohi J, Blaxill M, Kahler SG *et al*. Autism and environmental genomics. *NeuroToxicology* 2006;27:671-684.

Herbert MR, Ziegler DA, Deutsch CK, O'Brien LM, Kennedy DN, Filipek PA *et al*. Brain asymmetries in autism and developmental language disorder: a nested whole-brain analysis. *Brain* 2005;128:213-226.

Herbert MR, Ziegler DA, Makris N, Filipek PA, Kemper TL, Normandin JJ *et al*. Localization of white matter volume increase in autism and developmental language disorder. *Ann Neurol* 2004;55:530-540.

Herguner S, Mukaddes NM. Autism and Williams syndrome: a case report. *World J Biol Psychiatry* 2006;7:186-188.

Hernández-Deviez DJ, Mackay-Sim A, Wilson JM. A Role for ARF6 and ARNO in the regulation of endosomal dynamics in neurons. *Traffic* 2007;8:1750-1764.

Hernández-Deviez DJ, Roth MG, Casanova JE, Wilson JM. ARNO and ARF6 regulate axonal elongation and branching through downstream activation of Phosphatidylinositol 4-Phosphate 5-Kinase. *Mol Biol Cell* 2004;15:111-120.

Hernández-Deviez DJ, Wilson JM. Functional assay of ARNO and ARF6 in neurite elongation and branching. *Methods Enzymol* 2005;404:242-252.

Heuer L, Ashwood P, Schauer J, Goines P, Krakowiak P, Hertz-Picciotto I *et al*. Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. *Autism Res* 2008;1:275-283.

Himmelheber AM, Sarter M, Bruno JP. Increases in cortical acetylcholine release during sustained attention performance in rats. *Brain Res Cogn Brain Res* 2000;9:313-325.

Hirabayashi Y, Itoh Y, Tabata H, Nakajima K, Akiyama T, Masuyama N *et al*. The Wnt/b-catenin pathway directs neuronal differentiation of cortical neural precursor cells. *Development* 2004;131:2791-2801.

Hiramoto T, Kang G, Suzuki G, Satoh Y, Kucherlapati R, Watanabe Y *et al*. *Tbx1*: identification of a 22q11.2 gene as a risk factor for autism spectrum disorder in a mouse model. *Hum Mol Genet* 2011;20:4775-4785.

Hirokawa N, Takemura R. Kinesin superfamily proteins and their various functions and dynamics. *Exp Cell Res* 2004;301:50-59.

Hironaka K, Umemori H, Tezuka T, Mishina M, Yamamoto T. The protein-tyrosine phosphatase PTPMEG interacts with glutamate receptor d2 and e subunits. *J Biol Chem* 2000;275:16167-16173.

Hlavanda E, Kovacs J, Olah J, Orosz F, Medzihradszky KF *et al*. Brain specific p25 protein binds to tubulin and microtubules and induces aberrant microtubule assemblies at substoichiometric concentrations. *Biochemistry* 2002;41:8657-8664.

Hogart A, Patzel KA, LaSalle JM. Gender influences monoallelic expression of ATP10A in human brain. *Hum Genet* 2008;124:235-242.

Hogart A, Wu D, LaSalle JM, Schanen NC. The comorbidity of autism with the genomic disorders of chromosome 15q11.2-q13. *Neurobiol Dis* 2010;38:181-191.

Holmes AS, Blaxill MF, Haley BE. Reduced levels of mercury in first baby haircuts of autistic children. *Int J Toxicol* 2003;22:277-285.

Holmes SE, Riazi MA, Gong W, McDermid HE, Sellinger BT, Hua A *et al*. Disruption of the clathrin heavy chain-like gene (*CLTCL*) associated with features of DGS/VCFS: a balanced (21;22)(p12;q11) translocation. *Hum Mol Genet* 1997;6:357-367.

Holt R, Sykes NH, Conceição IC, Cazier JB, Anney RJ, Oliveira G *et al*. CNVs leading to fusion transcripts in individuals with autism spectrum disorder. *Eur J Hum Genet* 2012;20:1141-1147.

Hong C, Bollen AW, Costello JF. The contribution of genetic and epigenetic mechanisms to gene silencing in oligodendrogliomas. *Cancer Res* 2003;63:7600-7605.

Hoogenraad CC, Akhmanova A, Galjart N, De Zeeuw CI. LIMK1 and CLIP-115: linking cytoskeletal defects to Williams syndrome. *Bioessays* 2004;26:141-150.

Hoogenraad CC, Koekkoek B, Akhmanova A, Krugers H, Dortland B, Miedema M *et al*. Targeted mutation of *Cyln2* in the Williams syndrome critical region links *CLIP-115* haploinsufficiency to neurodevelopmental abnormalities in mice. *Nat Genet* 2002;32:116-127.

Hoppman-Chaney N, Wain K, Seger P, Superneau D, Hodge J. Identification of single gene deletions at 15q13.3: further evidence that *CHRNA7* causes the 15q13.3 microdeletion syndrome phenotype. *Clin Genet* 2012 Jul [Epub ahead of print].

Horie M, Mitsumoto Y, Kyushiki H, Kanemoto N, Watanabe A, Taniguchi Y *et al*. Identification and characterization of TMEFF2, a novel survival factor for hippocampal and mesencephalic neurons. *Genomics* 2000;67:146-152.

Horvath K, Stefanatos G, Sokolski KN, Wachtel R, Nabors L, Tildon JT. Improved social and language skills after secretin administration in patients with autistic spectrum disorders. *J Assoc Acad Minor Phys* 1998;9:9-15.

Horváth R, Abicht A, Holinski-Feder E, Laner A, Gempel K, Prokisch H *et al*. Leigh syndrome caused by mutations in the flavoprotein (Fp) subunit of succinate dehydrogenase (SDHA). *J Neurol Neurosurg Psychiatry* 2006;77:74-76.

Hozumi S, Hirabayashi R, Yoshizawa A, Ogata M, Ishitani T, Tsutsumi M *et al*. DEAD-Box protein Ddx46 is required for the development of the digestive organs and brain in zebrafish. *Plos One* 2012;7:e33675.

Hsu DT, Mickey BJ, Langenecker SA, Heitzeg MM, Love TM, Wang H *et al*. Variation in the corticotropin-releasing hormone receptor 1 (*CRHR1*) gene influences fMRI signal responses during emotional stimulus processing. *Neurosci* 2012;32:3253-3260.

Hsu SC, Hazuka CD, Foletti DL, Scheller RH. Targeting vesicles to specific sites on the plasma membrane: the role of the sec6/8 complex. *Trends Cell Biol* 1999;9:150-153.

Hsu SC, Ting AE, Hazuka CD, Davanger S, Kenny JW, Kee Y *et al*. The mammalian brain rsec6/8 complex. *Neuron* 1996;17:1209-1219.

Hsueh YP, Sheng M. Regulated expression and subcellular localization of syndecan heparan sulfate proteoglycans and the syndecan-binding protein CASK/LIN-2 during rat brain development. *J Neurosci* 1999;19:7415-7425.

Hu Y, Chopra V, Chopra R, Locascio JJ, Liao Z, Ding H *et al*. Transcriptional modulator H2A histone family, member Y (H2AFY) marks Huntington disease activity in man and mouse. *Proc Natl Acad Sci USA* 2011;108:17141-17146.

Hubank M, Mayne L. Expression of the excision repair gene, *ERCC3* (excision repair cross-complementing), during mouse development. *Brain Res Dev Brain Res* 1994;81:66-76.

Huguet G, Aldavert-Vera L, Kádár E, Peña de Ortiz S, Morgado-Bernal I, Segura-Torres P. Intracranial self-stimulation to the lateral hypothalamus, a memory improving treatment, results in hippocampal changes in gene expression. *Neuroscience* 2009;162:359-374.

Hulea L, Nepveu A. CUX1 transcription factors: from biochemical activities and cell-based assays to mouse models and human diseases. *Gene* 2012;497:18-26.

Hummel T, Leifker K, Klambt C. The Drosophila HEM-2/NAP1 homolog KETTE controls axonal pathfinding and cytoskeletal organization. *Genes Dev* 2000;14:863-873.

Hur E, Saijilafu, Lee BD, Kim S, Xu W, Zhou F. GSK3 controls axon growth via CLASP-mediated regulation of growth cone microtubules. *Genes Dev* 2011;25:1968-1981.

Hussain MS, Baig SM, Neumann S, Nürnberg G, Farooq M, Ahmad I *et al*. A truncating mutation of *CEP135* causes primary microcephaly and disturbed centrosomal function. *Am J Hum Genet* 2012;90(5):871-878.

Hussain S, Benavente SB, Nascimento E, Dragoni I, Kurowski A, Gillich A *et al*. The nucleolar RNA methyltransferase Misu (NSun2) is required for mitotic spindle stability. *J Cell Biol* 2009;186:27-40.

Hutton M, Lewis J, Dickson D, Yen SH, McGowan E. Analysis of tauopathies with transgenic mice. *Trends Mol Med* 2001;7:467-470.

Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y *et al*. Detection of large-scale variation in the human genome. *Nat Genet* 2004;36:949-951.

Ifergan I, Kebir H, Alvarez JI, Marceau G, Bernard M, Bourbonnière L *et al*. Central nervous system recruitment of effector memory CD8+ T lymphocytes during neuroinflammation is dependent on α4 integrin. *Brain* 2011;134:3560-3577.

Iijima T, Miura E, Watanabe M, Yuzaki M. Distinct expression of C1q-like family mRNAs in mouse brain and biochemical characterization of their encoded proteins. *Eur J Neurosci* 2010;31:1606-1615.

Inatani M, Irie F, Plump AS, Tessier-Lavigne M, Yamaguchi Y. Mammalian brain morphogenesis and midline axon guidance require heparan sulfate. *Science* 2003;302:1044-1046.

International Molecular Genetic Study of Autism Consortium (IMGSAC). A full genome screen for autism with evidence for linkage to a region on chromosome 7q. *Hum Mol Genet* 1998;7:571-578.

International Molecular Genetic Study of Autism Consortium (IMGSAC). A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. *Am J Hum Genet* 2001;69:570-581.

International Multiple Sclerosis Genetics Consortium (IMSGC). Esposito F, Patsopoulos NA, Cepok S, Kockum I, Leppä V, Booth DR *et al*. *IL12A, MPHOSPH9/CDK2AP1* and *RGS1* are novel multiple sclerosis susceptibility loci. *Genes Immun* 2010;11:397-405.

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J *et al*. *De novo* gene disruptions in children on the autistic spectrum. *Neuron* 2012;74:285-299.

Irie F, Badie-Mahdavi H, Yamaguchi Y. Autism-like socio-communicative deficits and stereotypies in mice lacking heparan sulfate. *Proc Natl Acad Sci USA* 2012;109:5052-5056.

Irie M, Hata Y, Takeuchi M, Ichtchenko K, Toyoda A, Hirao K *et al*. Binding of neuroligins to PSD-95. *Science* 1997;277:1511-1515.

Ishiguro H, Imai K, Koga M, Horiuchi Y, Inada T, Iwata N *et al*. Replication study for associations between polymorphisms in the *CLDN5* and *DGCR2* genes in the 22q11 deletion syndrome region and schizophrenia. *Psychiatr Genet* 2008;18:255-256.

Ishiguro H, Koga M, Horiuchi Y, Noguchi E, Morikawa M, Suzuki Y *et al*. Supportive evidence for reduced expression of *GNB1L* in schizophrenia. *Schizophr Bull* 2010;36:756-765.

Ishiguro H, Ohtsuki T, Okubo Y, Kurumaji A, Arinami T. Association analysis of the pituitary adenylcyclase activating peptide gene (*PACAP*) on chromosome 18p11 with schizophrenia and bipolar disorders. *J Neural Transm* 2001;108:849-854.

Ishikawa-Brush Y, Powell JF, Bolton P, Miller AP, Francis F, Willard HF *et al*. Autism and multiple exostoses associated with an X;8 translocation occurring within the GRPR gene and 3′ to the SDC2 gene. *Hum Mol Genet* 1997;6:1241-1250.

Iurov IIu, Vorsanova SG, Saprina EA, Iurov IuB. Identification of candidate genes of autism on the basis of molecular cytogenetic and in silico studies of the genome organization of chromosomal regions involved in unbalanced rearrangements. *Genetika* 2010;46:1348-1351.

Ivaschenko TE., Sideleva OG, Baranov VS. Glutathione- S-transferase micro and theta gene polymorphisms as new risk factors of atopic bronchial asthma. *J Mol Med* 2002;80:39-43.

Iwakoshi M, Okamoto N, Harada N, Nakamura T, Yamamori S, Fujita H *et al*. 9q34.3 deletion syndrome in three unrelated children. *Am J Med Genet A* 2004;126A:278-283.

Iwasaki Y, Nakamura T, Hamada K. Late-onset of idiopathic paroxysmal kinesigenic choreoathetosis: a case report. *Rinsho Shinkeigaku* 2004;44:365-368.

Iwashita S, Nobukuni T, Tanaka S, Kobayashi M, Iwanaga T, Tamate HB *et al*. Partial nuclear localization of a bovine phosphoprotein, BCNT, that includes a region derived from a LINE repetitive sequence in Ruminantia. *Biochim Biophys Acta* 1999;1427:408-416.

Iwata Y, Suzuki K, Nakamura K, Matsuzaki H, Sekine Y, Tsuchiya KJ *et al*. Increased levels of serum soluble L-selectin in unmedicated patients with schizophrenia. *Schizophr Res* 2007;89:154-160.

Iwata Y, Tsuchiya KJ, Mikawa S, Nakamura K, Takai Y, Suda S *et al*. Serum levels of P-selectin in men with high-functioning autism. *Br J Psychiatry* 2008;193:338-339.

Izumi N, Fumoto K, Izumi S, Kikuchi A. GSK-3beta regulates proper mitotic spindle formation in cooperation with a component of the gamma-tubulin ring complex, GCP5. *J Biol Chem* 2008;283:12981-12991.

Jabara HH, McDonald DR, Janssen E, Massaad MJ, Ramesh N, Borzutzky A *et al*. DOCK8 functions as an adaptor that links TLR-MyD88 signaling to B cell activation. *Nat Immunol* 2012;:13:612-620.

Jacquemont ML, Sanlaville D, Redon R, Raoul O, Cormier-Daire V, Lyonnet S *et al*. Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. *J Med Genet* 2006;43:843-849.

Jäger K, Wolf S, Dobrowolny H, Steiner J, Nave H, Maronde E *et al*. Differential topochemistry of three cationic amino acid transporter proteins, hCAT1, hCAT2 and hCAT3, in the adult human brain. *Amino Acids* 2012 Jul 3. [Epub ahead of print].

Jaillard S, Andrieux J, Plessis G, Krepischi AC, Lucas J, David V *et al*. 5q12.1 deletion: delineation of a phenotype including mental retardation and ocular defects. *Am J Med Genet A* 2011;155A:725-731.

Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B *et al*. Linkage and association of the glutamate receptor 6 gene with autism. *Mol Psychiatry* 2002;7:302-310.

Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC *et al*. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet* 2003;34:27-29.

Jamain S, Radyushkin K, Hammerschmidt K, Granon S, Boretius S, Varoqueaux F *et al*. Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. *Proc Natl Acad Sci* 2008;105:1710-1715.

James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH *et al*. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am J Med Genet B Neuropsychiatr Genet* 2006;141B:93547-93556.

Jankowsky E. RNA helicases at work: binding and rearranging. *Trends Biochem Sci* 2011;36:19-29.

Jansen EJ, Martens GJ. Novel insights into V-ATPase functioning: distinct roles for its accessory subunits ATP6AP1/Ac45 and ATP6AP2/(pro) renin receptor. *Curr Protein Pept Sci* 2012;13:124-133.

Jarick I, Volckmar AL, Pütter C, Pechlivanis S, Nguyen TT, Dauvermann MR *et al*. Genome-wide analysis of rare copy number variations reveals PARK2 as a candidate gene for attention-deficit/hyperactivity disorder. *Mol Psychiatry* 2012;Nov 20. [Epub ahead of print]

Jaworski J. ARF6 in the nervous system. *Eur J Cell Biol* 2007;86:513-524.

Jenkins ZA, van Kogelenberg M, Morgan T, Jeffs A, Fukuzawa R, Pearl E *et al*. Germline mutations in WTX cause a sclerosing skeletal dysplasia but do not predispose to tumorigenesis. *Nat Genet* 2009;41:95-100.

Jha P, Sheth D, Ghaziuddin M. Autism spectrum disorder and Klinefelter syndrome. *Eur Child Adolesc Psychiatry* 2007;16:305-308.

Jiang H, Jiang Q, Feng J. Parkin increases dopamine uptake by enhancing the cell surface expression of dopamine transporter. *J Biol Chem* 2004;279:54380-54386.

Jiang M, Gao Y, Yang T, Zhu X, Chen J. Cyclin Y, a novel membrane-associated cyclin, interacts with PFTK1. *FEBS Lett* 2009;583:2171-2178.

Jiang Y, Zhang Y, Zhang P, Sang T, Zhang F, Ji T *et al*. *NIPA2* located in 15q11.2 is mutated in patients with childhood absence epilepsy. *Hum Genet* 2012;131:1217-1224.

Johnson MB, Kawasawa YI, Mason CE, Krsnik Z, Coppola G, Danoviæ D *et al*. Functional and evolutionary insights into human brain development through global transcriptome analysis. *Neuron* 2009;62:494-509.

Jones G, Zammit S, Norton N, Hamshere ML, Jones SJ, Milham C *et al*. Aggressive behaviour in patients with schizophrenia is associated with catechol-O-methyltransferase genotype. *Br J Psychiatry* 2001;179:351-355.

Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N *et al*. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet* 1998;19187-19191.

Jong MT, Gray TA, Ji Y, Glenn CC, Saitoh S, Driscoll DJ *et al*. A novel imprinted gene, encoding a RING zinc-finger protein, and overlapping antisense transcript in the Prader-Willi syndrome critical region. *Hum Mol Genet* 1999;8:783-793.

Jordan C, Li HH, Kwan HC, Francke U. Cerebellar gene expression profiles of mouse models for Rett syndrome reveal novel MeCP2 targets. *BMC Med Genet* 2007;8:36.

Jossin Y, Cooper JA. Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. *Nat Neurosci* 2011;14: 697-703.

Jossin Y, Goffinet AM. Reelin signals through phosphatidylinositol 3-kinase and Akt to control cortical development and through mTor to regulate dendritic growth. *Mol Cell Biol* 2007;27:7113-7124.

Ju BG, Solum D, Song EJ, Lee KJ, Rose DW, Glass CK *et al*. Activating the PARP-1 sensor component of the groucho/ TLE1 corepressor complex mediates a CaMKinase IIdelta-dependent neurogenic gene activation pathway. *Cell* 2004;119:815-829.

Juenger H, Holst MI, Duffe K, Jankowski J, Baader SL. Tetraspanin-5 (Tm4sf9) mRNA expression parallels neuronal maturation in the cerebellum of normal and L7En-2 transgenic mice. *J Comp Neurol* 2005;483:318-328.

Jungers KA, Le Goff C, Somerville RP, Apte SS. Adamts9 is widely expressed during mouse embryo development. *Gene Expr Patterns* 2005;5:609-617.

Just MA, Cherkassky VL, Keller TA, Minshew NJ. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain* 2004;127:1811-1821.

Juurlink BH, Paterson PG. Review of oxidative stress in brain and spinal cord injury: suggestions for pharmacological and nutritional management strategies. *J Spinal Cord Med* 1998;21:309-334.

Kajizuka M, Miyachi T, Matsuzaki H, Iwata K, Shinmura C, Suzuki K *et al*. Serum levels of platelet-derived growth factor BB homodimers are increased in male children with autism. *Prog Neuropsychopharmacol Biol Psychiatry* 2010;34:154-158.

Kakinuma N, Roy BC, Zhu Y, Wang Y, Kiyama R. Kank regulates RhoA-dependent formation of actin stress fibers and cell migration via 14-3-3 in PI3K – Akt signaling. *J Cell Biol* 2008;181:537-549.

Kalkman HO. Potential opposite roles of the extracellular signal-regulated kinase (ERK) pathway in autism spectrum and bipolar disorders. *Neurosci Biobehav Rev* 2012;36:2206-2213.

Kamnasaran D, Chen CP, Devriendt K, Mehta L, Cox DW. Defining a holoprosencephaly locus on human chromosome 14q13 and characterization of potential candidate genes. *Genomics* 2005;85:608-621.

Kanaho Y, Funakoshi Y, Hasegawa H. Phospholipase D signaling and its involvement in neurite outgrowth. *Biochim Biophys Acta* 2009;1791:898-904.

Kanemoto N, Horie M, Omori K, Nishino N, Kondo M, Noguchi K *et al*. Expression of TMEFF1 mRNA in the mouse central nervous system: precise examination and comparative studies of TMEFF1 and TMEFF2. *Brain Res Mol Brain Res* 2001;86:48-55.

Kannan K, Jain SK. Oxidative stress and apoptosis. *Pathophysiology* 2000;7:153-163.

Kanner L. Autistic disturbances of affective contact. *The Nervous Child* 1943;2:217-250.

Kantojärvi K, Kotala I, Rehnström K, Ylisaukko-Oja T, Vanhala R, von Wendt TN *et al*. Fine mapping of Xq11.1-q21.33 and mutation screening of *RPS6KA6*, *ZNF711*, *ACSL4*, *DLG3*, and *IL1RAPL2* for autism spectrum disorders (ASD). *Autism Res* 2011;4:228-233.

Kantojärvi K, Onkamo P, Vanhala R, Alen R, Hedman M, Sajantila A *et al*. Analysis of 9p24 and 11p12-13 regions in autism spectrum disorders: rs1340513 in the *JMJD2C* gene is associated with ASDs in Finnish sample. *Psychiatr Genet* 2010;20:102-108.

Kantor DB, Chivatakarn O, Peer KL, Oster SF, Inatani M, Hansen MJ *et al*. Semaphorin 5A is a bifunctional axon guidance cue regulated by heparan and chondroitin sulfate proteoglycans. *Neuron* 2004;44:961-975.

Kaplan RS, Mayor JA, Gremse DA, Wood DO. High level expression and characterization of the mitochondrial citrate transport protein from the yeast Saccharomyces cerevisiae. *J Biol Chem* 1995;270:4108-4114.

Kato A, Rouach N, Nicoll RA, Bredt DS. Activity-dependent NMDA receptor degradation mediated by retrotranslocation and ubiquitination. *Proc Natl Acad Sci USA* 2005;102:5600-5605.

Kaufmann WE, Moser HW. Dendritic anomalies in disorders associated with mental retardation. *Cereb Cortex* 2000;10:981-991.

Kelley KD, Miller KR, Todd A, Kelley AR, Tuttle R, Berberich SJ. YPEL3, a p53-regulated gene that induces cellular senescence. *Cancer Res* 2010;70:3566-3575.

Kemper TL, Bauman M. Neuropathology of infantile autism. *J Neuropathol Exp Neurol* 1998;57:645-652.

Kemper TL, Bauman ML. The contribution of neuropathologic studies to the understanding of autism. *Neurol Clin* 1993;11:175-187.

Kent L, Bowdin S, Kirby GA, Cooper WN, Maher ER. Beckwith Weidemann syndrome: a behavioral phenotype-genotype study. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:1295-1297.

Kent L, Emerton J, Bhadravathi V, Weisblatt E, Pasco G, Willatt LR *et al*. X-linked ichthyosis (steroid sulfatase deficiency) is associated with increased risk of attention deficit hyperactivity disorder, autism and social communication deficits. *J Med Genet* 2008;45:519-524.

Kent L, Emerton J, Bhadravathi V, Weisblatt E, Pasco G, Willatt LR *et al*. X-linked ichthyosis (steroid sulfatase deficiency) is associated with increased risk of attention deficit hyperactivity disorder, autism and social communication deficits. *J Med Genet* 2008;45:519-524.

Kent L, Evans J, Paul M, Sharp M. Comorbidity of autistic spectrum disorders in children with Down syndrome. *Dev Med Child Neurol* 1999;41:153-158.

Kerman IA, Bernard R, Bunney WE, Jones EG, Schatzberg AF, Myers RM *et al*. Evidence for transcriptional factor dysregulation in the dorsal raphe nucleus of patients with major depressive disorder. *Front Neurosci* 2012;6:135.

Kern JK, Van Miller S, Evans PA, Trivedi MH. Efficacy of porcine secretin in children with autism and pervasive developmental disorder. *J Autism Dev Disord* 2002;32:153-160.

Khan MA, Rafiq MA, Noor A, Hussain S, Flores JV, Rupp V *et al*. Mutation in *NSUN2*, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. *Am J Hum Genet* 2012;90:856-863.

Kidd JM, Cooper GM, Donahue WF, Hayden HS, Sampas N, Graves T *et al*. Mapping and sequencing of structural variation from eight human genomes. *Nature* 2008;453:56-64

Kielinen M, Rantala H, Timonen E, Linna SL, Moilanen I. Associated medical disorders and disabilities in children with autistic disorder: a population-based study. *Autism* 2004;8:49-60.

Kifayathullah LA, Arunachalam JP, Bodda C, Agbemenyah HY, Laccone FA, Mannan AU. MeCP2 mutant protein is expressed in astrocytes as well as in neurons and localizes in the nucleus. *Cytogenet Genome Res* 2010;129:290-297.

Kihara Y, Yanagida K, Masago K, Kita Y, Hishikawa D, Shindou H *et al*. Platelet-activating factor production in the spinal cord of experimental allergic encephalomyelitis mice via the group IVA cytosolic phospholipase A2-lyso-PAFAT axis. *J Immunol* 2008;181:5008-5014.

Kim HG, Kishikawa S, Higgins AW, Seong IS, Donovan DJ, Shen Y *et al*. Disruption of neurexin 1 associated with autism spectrum disorder. *Am J Hum Genet* 2008;82:199-207.

Kim SA, Kim JH, Park M, Cho IH, Yoo HJ. Association of *GABRB3* polymorphisms with autism spectrum disorders in Korean trios. *Neuropsychobiology* 2006;54:160-165.

Kim SH, Song JY, Joo EJ, Lee KY, Shin SY, Lee YH *et al*. Genetic association of the *EGR2* gene with bipolar disorder in Korea. *Exp Mol Med* 2012;44:121-129.

Kim SK, Lee JY, Park HJ, Kim JW, Chung JH. Association study between polymorphisms of the *PARD3* gene and schizophrenia. *Exp Ther Med* 2012;3:881-885.

Kim SY, Chung HS, Sun W, Kim H. Spatiotemporal expression pattern of non-clustered protocadherin family members in the developing rat brain. *Neuroscience* 2007;147:996-1021.

Kim SY, Yasuda S, Tanaka H, Yamagata K, Kim H. Non-clustered protocadherin. *Cell Adh Migr* 2011;5:97-105.

Kim YW, Kim Y, Kim EH, Koh D, Sun W, Kim H. Expression and subcellular localization of thymosin beta15 following kainic acid treatment in rat brain. *Biochem Biophys Res Commun* 2008;371:664-669.

Kirbach BB, Golenhofen N. Differential expression and induction of small heat shock proteins in rat brain and cultured hippocampal neurons. *J Neurosci Res* 2011;89:162-175.

Kirchhoff M, Bisgaard AM, Bryndorf T, Gerdes T. MLPA analysis for a panel of syndromes with mental retardation reveals imbalances in 5.8% of patients with mental retardation and dysmorphic features, including duplications of the Sotos syndrome and Williams–Beuren syndrome regions. *Eur J Med Genet* 2007;50:33-42.

Kirov G, Grozeva D, Norton N, Ivanov D, Mantripragada KK, Holmans P *et al*. Support for the involvement of large copy number variants in the pathogenesis of schizophrenia. *Hum Mol Genet* 2009;18:1497-1503

Kishi N, Macklis JD. MECP2 is progressively expressed in post-migratory neurons and is involved in neuronal maturation rather than cell fate decisions. *Mol Cell Neurosci* 2004;27:306-321.

Kishino T, Lalande M, Wagstaff J. *UBE3A*/*E6-AP* mutations cause Angelman syndrome. *Nat Genet* 1997;15:70-73.

Klauck SM, Felder B, Kolb-Kokocinski A, Schuster C, Chiocchetti A, Schupp I *et al*. Mutations in the ribosomal protein gene RPL10 suggest a novel modulating disease mechanism for autism. *Mol Psychiatry* 2006;11:1073-1084.

Kleefstra T, Smidt M, Banning MJ, Oudakker AR, Van Esch H, de Brouwer AP *et al*. Disruption of the gene Euchromatin Histone Methyl Transferase1 (*Eu-HMTase1*) is associated with the 9q34 subtelomeric deletion syndrome. *J Med Genet* 2005;42:299-306.

Kleefstra T, van Zelst-Stams WA, Nillesen WM, Cormier-Daire V, Houge G, Foulds N *et al*. Further clinical and molecular delineation of the 9q subtelomeric deletion syndrome supports a major contribution of EHMT1 haploinsufficiency to the core phenotype. *J Med Genet* 2009;46:598-606.

Kleiman RJ, Chapin DS, Christoffersen C, Freeman J, Fonseca KR, Geoghegan KF *et al*. Phosphodiesterase 9A regulates central cGMP and modulates responses to cholinergic and monoaminergic perturbation in vivo. *J Pharmacol Exp Ther* 2012;341:396-409.

Klein-Tasman BP, Phillips KD, Lord C, Mervis CB, Gallo FJ. Overlap with the autism spectrum in young children with Williams syndrome. *J Dev Behav Pediatr* 2009;30:289-299.

Klevernic IV, Martin NM, Cohen P. Regulation of the activity and expression of ERK8 by DNA damage. *FEBS Lett* 2009;583:680-684.

Klevernic IV, Stafford MJ, Morrice N, Peggie M, Morton S, Cohen P. Characterization of the reversible phosphorylation and activation of ERK8. *Biochem J* 2006;394:365-373.

Knapska E, Kaczmarek L. A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? *Prog Neurobiol* 2004;74:183-211.

Knepper JL, James AC, Ming JE. TGIF, a gene associated with human brain defects, regulates neuronal development. *Dev Dyn* 2006;235:1482-1490.

Ko CH, Kong CK, Ngai WT, Ma KM. Ictal (99m)Tc ECD SPECT in paroxysmal kinesigenic choreoathetosis. *Pediatr Neurol* 2001;24 225-227.

Kodirov SA, Takizawa S, Joseph J, Kandel ER, Shumyatsky GP, Bolshakov VY. Synaptically released zinc gates long-term potentiation in fear conditioning pathways. *Proc Natl Acad Sci USA* 2006;103:15218-15223.

Koga M, Ishiguro H, Horiuchi Y, Inada T, Ujike H, Itokawa M *et al*. Replication study of association between *ADCYAP1* gene polymorphisms and schizophrenia. *Psychiatr Genet* 2010;20:123-125.

Konishi H, Matsuzaki H, Tanaka M, Takemura Y, Kuroda S, Ono Y *et al*. Activation of protein kinase B (Akt/RAC-protein kinase) by cellular stress and its association with heat shock protein Hsp27. *FEBS Lett* 1997;410:493-498.

Konyukh M, Delorme R, Chaste P, Leblond C, Lemiere N, Nygren G *et al*. Variations of the candidate *SEZ6L2* gene on chromosome 16p11.2 in patients with autism spectrum disorders and in human populations. *Plos One* 2011;6:e17289.

Koolen DA, Sharp AJ, Hurst JA, Firth HV, Knight SJ, Goldenberg A *et al*. Clinical and molecular delineation of the 17q21.31 microdeletion syndrome. *J Med Genet* 2008;45:710-720.

Kopan R, Goate A. A common enzyme connects Notch signaling and Alzheimer's disease. *Genes Dev* 2000;14:2799-2806.

Korade Z, Mirnics K. Gene expression: the autism disconnect. *Nature* 2011;474:294-295.

Korbel JO, Urban AE, Affourtit JP, Godwin B, Grubert F, Simons JF *et al*. Paired-end mapping reveals extensive structural variation in the human genome. *Science* 2007;318:420-426.

Koshimizu Y, Ohtomi M. Regulation of neurite extension by expression of LECT2 and neurotrophins based on findings in LECT2-knockout mice. *Brain Res* 2010;1311:1-11.

Kousaka A, Mori Y, Koyama Y, Taneda T, Miyata S, Tohyama M. The distribution and characterization of endogenous protein arginine N-methyltransferase 8 in mouse CNS. *Neuroscience* 2009;163:1146-1157.

Krappa R, Nguyen A, Burrola P, Deretic D, Lemke G. Evectins: vesicular proteins that carry a pleckstrin homology domain and localize to post-Golgi membranes. *Proc Natl Acad Sci USA* 1999; 96:4633-4638.

Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA *et al*. Recurrent 16p11.2 microdeletions in Autism. *Hum Mol Genet* 2008;17:628-638.

Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA *et al*. Recurrent 16p11.2 microdeletions in autism. *Hum Mol Genet* 2008;17:628-638.

Kumar RA, Marshall CR, Badner JA, Babatz TD, Mukamel Z, Aldinger KA *et al*. Association and mutation analyses of 16p11.2 autism candidate genes. *Plos One* 2009;4:e4582.

Kumar V, Zhang MX, Swank MW, Kunz J, Wu GY. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. *J Neurosci* 2005;25:11288-11299.

Kuss AW, Garshasbi M, Kahrizi K, Tzschach A, Behjati F, Darvish H *et al*. Autosomal recessive mental retardation: homozygosity mapping identifies 27 single linkage intervals, at least 14 novel loci and several mutation hotspots. *Hum Genet* 2011;129:141-148.

Kutsche K, Yntema H, Brandt A, Jantke I, Nothwang HG, Orth U *et al*. Mutations in *ARHGEF6*, encoding a guanine nucleotide exchange factor for Rho GTPases, in patients with X-linked mental retardation. *Nat Genet* 2000;26:247-50.

Kwon C, Qian L, Cheng P, Nigam V, Arnold J, Srivastava D. A regulatory pathway involving Notch1/beta-catenin/Isl1 determines cardiac progenitor cell fate. *Nat Cell Biol* 2009;11:951-957.

Lachman HM. Copy variations in schizophrenia and bipolar disorder. *Cytogenetic and genome research* 2008;123:27-35.

Lafuente A, Bernardo M, Mas S, Crescenti A, Aparici M, Gassó P *et al*. Dopamine transporter (DAT) genotype (VNTR) and phenotype in extrapyramidal symptoms induced by antipsychotics. *Schizophr Res* 2007;90:115-122.

Lainhart JE, Piven J, Wzorek M, Landa R, Santangelo SL, Coon H *et al*. Macrocephaly in children and adults with autism. *J Am Acad Child Adolesc Psych* 1997;36:282-290.

Lamprianou S, Chatzopoulou E, Thomas JL, Bouyain S, Harroch S. A complex between contactin-1 and the protein tyrosine phosphatase PTPRZ controls the development of oligodendrocyte precursor cells. *Proc Natl Acad Sci USA* 2011;108:17498-17503.

Laroche F, Ramoz N, Leroy S, Fortin C, Rousselot-Paillet B, Philippe A *et al*. Polymorphisms of coding trinucleotide repeats of homeogenes in neurodevelopmental psychiatric disorders. *Psychiatr Genet* 2008;18:295-301.

László A, Horváth E, Eck E, Fekete M. Serum serotonin, lactate and pyruvate levels in infantile autistic children. *Clin Chim Acta* 1994;229:205-207.

Laumonnier F, Bonnet-Brilhault F, Gomot M, Blanc R, David A, Moizard MP *et al*. X-linked mental retardation and autism are associated with a mutation in the *NLGN4* gene, a member of the neuroligins family. *Am J Hum Genet* 2004;74:552-557.

Lawson-Yuen A, Saldivar JS, Sommer S, Picker J. Familial deletion within *NLGN4* associated with autism and Tourette syndrome. *Eur J Hum Genet* 2008;16:614-618.

Leblond CS, Heinrich J, Delorme R, Proepper C, Betancur C, Huguet G *et al*. Genetic and functional analyses of *SHANK2* mutations suggest a multiple hit model of autism spectrum disorders. *Plos Genet* 2012;8:e1002521.

Lee C, Scherer SW. The clinical context of copy number variation in the human genome. *Expert Rev Mol Med* 2010;12:e8.

Lee CT, Lehrmann E, Hayashi T, Amable R, Tsain SY, Chen J *et al*. Gene expression profiling reveals distinct cocaine-responsive genes in human fetal CNS cell types. *Addict Med* 2009;3:218-226.

Lee J, Sayegh J, Daniel J, Clarke S, Bedford MT. PRMT8, a new membrane-bound tissue-specific member of the protein arginine methyltransferase family. *J Biol Chem* 2005;280:32890-32896.

Lee JD, Silva-Gagliardi NF, Tepass U, McGlade CJ, Anderson KV. The FERM protein Epb4.1l5 is required for organization of the neural plate and for the epithelial-mesenchymal transition at the primitive streak of the mouse embryo. *Development* 2007;134:2007-2016.

Lee L, Campagna DR, Pinkus JL, Mulhern H, Wyatt TA, Sisson JH *et al*. Primary ciliary dyskinesia in mice lacking the novel ciliary protein Pcdp1. *Mol Cell Biol* 2008;28:949-957.

Lee LC, Zachary AA, Leffell MS, Newschaffer CJ, Matteson KJ, Tyler JD *et al*. HLA-DR4 in families with autism. *Pediatr Neurol* 2006;35:303-307.

Lee YR, Yuan WC, Ho HC, Chen CH, Shih HM, Chen RH. The Cullin 3 substrate adaptor KLHL20 mediates DAPK ubiquitination to control interferon responses. *EMBO J* 2010;29:1748-1761.

Lehtimäki KA, Peltola J, Liimatainen S, Haapala AM, Arvio M. Cardiolipin and β(2)-Glycoprotein I antibodies associate with cognitive impairment and seizure frequency in developmental disorders. *Seizure* 2011;20:438-441.

Lencz T, Szeszko PR, DeRosse P, Burdick KE, Bromet EJ, Bilder RM *et al*. Schizophrenia risk gene, *ZNF804A*, influences neuroanatomical and neurocognitive phenotypes. *Neuropsychopharmacology* 2010;35:2284-2291.

Le-Niculescu H, Kurian SM, Yehyawi N, Dike C, Patel SD, Edenberg HJ *et al*. Identifying blood biomarkers for mood disorders using convergent functional genomics. *Mol Psychiatry* 2009;14:156-174.

Leonard S, Gault J, Hopkins J, Logel J, Vianzon R, Short M *et al*. Association of promoter variants in the alpha7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. *Arch Gen Psychiatry* 2002;59:1085-1096.

Lerer I, Sagi M, Meiner V, Cohen T, Zlotogora J, Abeliovich D. Deletion of the *ANKRD15* gene at 9p24.3 causes parent-of-origin-dependent inheritance of familial cerebral palsy. *Hum Mol Genet* 2005;14:3911-3920.

Levenga J, deVrij FM, Oostra BA, Willemsen R. Potential therapeutic interventions for fragile X syndrome. *Trends Mol Med* 2010;16:516-527.

Levy D, Ronemus M, Yamrom B, Lee YH, Leotta A, Kendall J *et al*. Rare *de novo* and transmitted copy-number variation in autistic spectrum disorders. *Neuron* 2011;70:886-897.

Lewis JC, Thomas HV, Murphy KC, Sampson JR. Genotype and psychological phenotype in tuberous sclerosis. *J Med Genet* 2004;41:203-207.

Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F *et al*. Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell* 1992;69:905-914.

Li F, Shen Y, Köhler U, Sharkey FH, Menon D, Coulleaux L *et al*. Interstitial microduplication of Xp22.31: causative of intellectual disability or benign copy number variant? *Eur J Med Genet* 2010;53:93-99.

Li H, Yamagata T, Mori M, Momoi MY. Association of autism in two patients with hereditary multiple exostoses caused by novel deletion mutations of *EXT1*. *J Hum Genet* 2002;47:262-265.

Li J, Liu X, Wang H, Zhang W, Chan DC, Shi Y. Lysocardiolipin acyltransferase 1 (ALCAT1) controls mitochondrial DNA fidelity and biogenesis through modulation of MFN2 expression. *Proc Natl Acad Sci USA* 2012;109:6975-6980.

Li N, Zhao CT, Wang Y, Yuan XB. The transcription factor Cux1 regulates dendritic morphology of cortical pyramidal neurons. *Plos One* 2010;5:e10596.

Li T, Vallada H, Curtis D, Arranz M, Xu K, Cai G *et al*. Catechol-O-methyltransferase Val158Met polymorphism: frequency analysis in Han Chinese subjects and allelic association of the low activity allele with bipolar affective disorder. *Pharmacogenetics* 1997;7:349-353.

Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM *et al*. Elevated immune response in the brain of autistic patients. *J Neuroimmunol* 2009;207:111-116.

Lian G, Lu J, Hu J, Zhang J, Cross SH, Ferland RJ *et al*. Filamin a regulates neural progenitor proliferation and cortical size through Wee1-dependent Cdk1 phosphorylation. *J Neurosci* 2012;32:7672-7684.

Liao EH, Hung W, Abrams B, Zhen M. An SCF-like ubiquitin ligase complex that controls presynaptic differentiation. *Nature* 2004;430:345-350.

Libbey JE, Coon HH, Kirkman NJ, Sweeten TL, Miller JN, Stevenson EK *et al*. Are there enhanced MBP autoantibodies in autism? *J Autism Dev Disord* 2008;38:324-332.

Liebner S, Corada M, Bangsow T, Babbage J, Taddei A, Czupalla CJ *et al*. Wnt/beta-catenin signaling controls development of the blood-brain barrier. *J Cell Biol* 2008;183:409-417.

Lin MY, Lin YM, Kao T, Chuang HH, Chen RH. PDZ-RhoGEF ubiquitination by Cullin3–KLHL20 controls neurotrophin-induced neurite outgrowth. *J Cell Biol* 2011;193:985-994.

Lincoln AJ, Searcy YM, Jones W, Lord C. Social interaction behaviors discriminate young children with autism and Williams syndrome. *J Am Acad Child Adolesc Psychiatry* 2007;46:323-331.

Linden DJ, Ahn S. Activation of presynaptic cAMP-dependent protein kinase is required for induction of cerebellar long-term potentiation. *J Neurosci* 1999;19:10221-10227.

Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia* 1993;7:9-18.

Linseman DA, Loucks FA. Diverse roles of Rho family GTPases in neuronal development, survival, and death. *Front Biosci* 2008;13:657-676 .

Lintas C, Persico AM. Autistic phenotypes and genetic testing: state-of-the-art for the clinical geneticist. *J Med Genet* 2009;46:1-8.

Lionel AC, Crosbie J, Barbosa N, Goodale T, Thiruvahindrapuram B, Rickaby J *et al*. Rare copy number variation discovery and cross-disorder comparisons identify risk genes for ADHD. *Sci Transl Med* 2011;3:95ra75.

Lisik W, Gong Y, Tejpal N, Skelton TS, Bremer EG, Kloc M *et al*. Intragraft gene expression profile associated with the induction of tolerance by allochimeric MHC I in the rat heart transplantation model. *Genesis* 2010;48:8-19.

Liu H, Abecasis GR, Heath SC, Knowles A, Demars S, Chen YJ *et al*. Genetic variation in the 22q11 locus and susceptibility to schizophrenia. *Proc Natl Acad Sci USA* 2002a;99:16859-16864.

Liu H, Heath SC, Sobin C, Roos JL, Galke BL, Blundell ML *et al*. Genetic variation at the 22q11 *PRODH2*/*DGCR6* locus presents an unusual pattern and increases susceptibility to schizophrenia. *Proc Natl Acad Sci USA* 2002b;99:3717-3722.

Liu X, Cheng R, Verbitsky M, Kisselev S, Browne A, Mejia-Sanatana H *et al*. Genome-wide association study identifies candidate genes for Parkinson's disease in an Ashkenazi Jewish population. *BMC Med Genet* 2011;12:104.

Liu XB, Murray KD. Neuronal excitability and calcium/calmodulin-dependent protein kinase type II: location, location, location. *Epilepsia* 2012;53:45-52.

Liu YL, Fann CS, Liu CM, Chang CC, Yang WC, Wu JY *et al*. *HTF9C* gene of 22q11.21 region associates with schizophrenia having deficit-sustained attention. *Psychiatr Genet* 2007;17:333-338.

Lo-Castro A, Galasso C, Cerminara C, El-Malhany N, Benedetti S, Nardone AM *et al*. Association of syndromic mental retardation and autism with 22q11.2 duplication. *Neuropediatrics* 2009;40:137-140.

Lodygensky GA, Seghier ML, Warfield SK, Tolsa CB, Sizonenko S, Lazeyras F *et al*. Intrauterine growth restriction affects the preterm infant's hippocampus. *Pediatr Res* 2008;63:438-443.

Lohoff FW, Bloch PJ, Weller AE, Ferraro TN, Berrettini WH. Association analysis of the pituitary adenylate cyclase-activating polypeptide (*PACAP*/*ADCYAP1*) gene in bipolar disorder. *Psychiatr Genet* 2008;18:53-58.

Lombard J. Autism: a mitochondrial disorder? *Med Hypotheses* 1998;50:497-500.

Lopantsev V, Wenzel HJ, Cole TB, Palmiter RD, Schwartzkroin PA. Lack of vesicular zinc in mossy fibers does not affect synaptic excitability of CA3 pyramidal cells in zinc transporter 3 knockout mice. *Neurosci* 2003;116:237-248.

Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L *et al*. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. *J Autism Dev Disord* 1989;19:185-212.

Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* 1994;24:659-685.

Loupatty FJ, Clayton PT, Ruiter JPN, Ofman R, Ijlst L, Brown GK *et al*. Mutations in the gene encoding 3-hydroxyisobutyryl-coa hydrolase results in progressive infantile neurodegeneration. *Am J Hum Genet* 2007;80:195-199.

Luan X, Diekwisch TG. CP27 affects viability, proliferation, attachment and gene expression in embryonic fibroblasts. *Cell Prolif* 2002;35:207-219.

Luciano M, Huffman JE, Arias-Vásquez A, Vinkhuyzen AA, Middeldorp CM, Giegling I *et al*. Genome-wide association uncovers shared genetic effects among personality traits and mood states. *Am J Med Genet B Neuropsychiatr Genet* 2012;159B:684-695.

Ma Q, Fode C, Guillemot F, Anderson DJ. Neurogenin1 and neurogenin2 control two distinct waves of neurogenesis in developing dorsal root ganglia. *Genes Dev* 1999,13:1717-1728.

Maas NM, Van de Putte T, Melotte C, Francis A, Schrander-Stumpel CT, Sanlaville D *et al***.** The C20orf133 gene is disrupted in a patient with Kabuki syndrome. *J Med Genet* 2007;44:562-569.

MacLaren EJ, Charlesworth P, Coba MP, Grant SGN *et al*. Knockdown of mental disorder susceptibility genes disrupts neuronal network physiology in vitro. *Mol Cell Neurosci* 2011;47:93-99.

Maezawa I, Jin LW. Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. *J Neurosci* 2010;30:5346-5356.

Maezawa I, Swanberg S, Harvey D, LaSalle JM, Jin LW. Rett syndrome astrocytes are abnormal and spread MeCP2 deficiency through gap junctions. *J Neurosci* 2009;29:5051-5061.

Maiato H, Rieder CL, Earnshaw WC, Sunkel CE. How do kinetochores CLASP dynamic microtubules? *Cell Cycle* 2003;2:511-514.

Makeyev AV, Bayarsaihan D. Alternative splicing and promoter use in TFII-I genes. *Gene* 2009;433:16-25.

Malenfant P, Liu X, Hudson ML, Qiao Y, Hrynchak M, Riendeau N *et al*. Association of *GTF2i* in the Williams-Beuren syndrome critical region with autism spectrum disorders. *J Autism Dev Disord* 2012;42:1459-1469.

Malik M, Tauqeer Z, Sheikh AM, Wen G, Nagori A, Yang K *et al*. NF-κB signaling in the brain of autistic subjects. *Mediators Inflamm* 2011;2011:785265.

Malkani S, Wallace KJ, Donley MP, Rosen, JB. Anegr-1 (zif268) antisense oligodeoxynucleotide infused into the amygdala disrupts fear conditioning. *Learn Mem* 2004;11:617-624.

Manning MA, Cassidy SB, Clericuzio C, Cherry AM, Schwartz S, Hudgins L *et al*. Terminal 22q deletion syndrome: a newly recognized cause of speech and language disability in the autism spectrum. *Pediatrics* 2004;114:451-457.

Manzi B, Loizzo AL, Giana G, Curatolo P. Autism and metabolic diseases. *J Child Neurol* 2008; 233:307-314.

Marei HE, Ahmed AE, Michetti F, Pescatori M, Pallini R, Casalbore P *et al*. Gene expression profile of adult human olfactory bulb and embryonic neural stem cell suggests distinct signaling pathways and epigenetic control. *Plos One* 2012;7:e33542.

Margolis SS, Salogiannis J, Lipton DM, Mandel-Brehm C, Wills ZP, Mardinly AR *et al*. EphB-mediated degradation of the RhoA GEF Ephexin5 relieves a developmental brake on excitatory synapse formation. *Cell* 2010;143:442-455.

Marshall CR, Lionel AC, Scherer SW. Copy Number Variations in Autism Spectrum Disorders. In: The Neuroscience of Autism Spectrum Disorders. Eds. Buxbaum & Hof, Academic Press, 14 December 2012.

Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J *et al*. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008;82:477-488.

Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y *et al*. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science* 2003;302:890-893.

Martins-de-Souza D, Maccarrone G, Wobrock T, Zerr I, Gormanns P, Reckow S *et al*. Proteome analysis of the thalamus and cerebrospinal fluid reveals glycolysis dysfunction and potential biomarkers candidates for schizophrenia. *J Psychiatr Res* 2010;44:1176-1189.

Mas S, Bernardo M, Parellada E, Garcia-Rizo C, Gasso P, Alvarez S *et al*. *ARVCF* single marker and haplotypic association with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2009;33:1064-1069.

Masurel-Paulet A, Andrieux J, Callier P, Cuisset JM, Le Caignec C, Holder M *et al*. Delineation of 15q13.3 microdeletions. *Clin Genet* 2010;78:149-161.

Matise MP, Epstein DJ, Park HL, Platt KA, Joyner AL. Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. *Development* 1998;125:2759-2770.

Matsui T, Fukuda M. Small GTPase Rab12 regulates transferrin receptor degradation: Implications for a novel membrane trafficking pathway from recycling endosomes to lysosomes. *Cell Logist* 2011;1:155-158.

Matsumoto T, Kobayashi T, Kamata K. Role of lysophosphatidylcholine (LPC) in atherosclerosis. *Curr Med Chem* 2007;14:3209-3220.

Matsumoto Y, Irie F, Inatani M, Tessier-Lavigne M, Yamaguchi Y. Netrin-1/DCC signaling in commissural axon guidance requires cell-autonomous expression of heparan sulfate. *J Neurosci* 2007;27:4342-4350.

Matsumoto-Taniura N, Pirollet F, Monroe R, Gerace L, Westendorf JM. Identification of novel M phase phosphoproteins by expression cloning. *Mol Biol Cell* 1996;7:1455-1469.

Matsuura T, Sutcliffe JS, Fang P, Galjaard RJ, Jiang YH, Benton CS *et al*. *De novo* truncating mutations in E6-AP ubiquitin–protein ligase gene (*UBE3A*) in Angelman syndrome. *Nat Genet* 1997;15:74-77.

Matyszak MK, Denis-Donini S, Citterio S, Longhi R, Granucci F, Ricciardi-Castagnoli P. Microglia induce myelin basic protein-specific T cell anergy or T cell activation, according to their state of activation. *Eur J Immunol* 1999;29:3063-3076.

Maynard TM, Meechan DW, Dudevoir ML, Gopalakrishna D, Peters AZ, Heindel CC *et al*. Mitochondral localization and function of a subset of 22q11 deletion syndrome candidate genes. *Mol Cell Neurosci* 2008;39:439-451.

McAlonan GM, Cheung V, Cheung C, Suckling J, Lam GY, Tai KS *et al*. Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. *Brain* 2005;128:268-276.

McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S *et al*. Microduplications of 16p11.2 are associated with schizophrenia. *Nat Genet* 2009;41:1223-1227.

McConnell SK, Kaznowski CE. Cell cycle dependence of laminar determination in developing neocortex. *Science* 1991,254:282-285.

McInnes LA, Service SK, Reus VI, Barnes G, Charlat O, Jawahar S *et al*. Fine-scale mapping of a locus for severe bipolar mood disorder on chromosome 18p11.3 in the Costa Rican population. *Proc Natl Acad Sci USA* 2001;98:11485-11490.

McKee KK, Tan CP, Palyha OC, Liu J, Feighner SD, Hreniuk DL *et al*. Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors. *Genomics* 1997;46:426-434.

McKinney C, Merriman ME, Chapman PT, Gow PJ, Harrison AA, Highton J *et al*. Evidence for an influence of chemokine ligand 3-like 1 (*CCL3L1*) gene copy number on susceptibility to rheumatoid arthritis. *Ann Rheum Dis* 2008;67:409-413.

McMillan DR, Kayes-Wandover KM, Richardson JA, White PC. Very large G Protein-coupled Receptor-1, the largest known cell surface protein, is highly expressed in the developing central nervous system. *J Biol Chem* 2002;277:785-792.

McMullan DJ, Bonin M, Hehir-Kwa JY, de Vries BB, Dufke A, Rattenberry E *et al*. Molecular karyotyping of patients with unexplained mental retardation by SNP arrays: a multicenter study. *Hum Mutat* 2009;30:1082-1092.

McRory JE, Rehak R, Simms B, Doering CJ, Chen L, Hermosilla T *et al*. Syntaxin 1A is required for normal in utero development. *Biochem Biophys Res Commun* 2008;375:372-377.

Meechan DW, Tucker ES, Maynard TM, LaMantia AS. Diminished dosage of 22q11 genes disrupts neurogenesis and cortical development in a mouse model of 22q11 deletion/DiGeorge syndrome. *Proc Natl Acad Sci USA* 2009;106:16434-16445.

Mekinian A, Lachassinne E, Nicaise-Roland P, Carbillon L, Motta M, Vicaut E *et al*. European registry of babies born to mothers with antiphospholipid syndrome. *Ann Rheum Dis* 2012 May 15. [Epub ahead of print]

Meng Y, Zhang Y, Tregoubov V, Janus C, Cruz L, Jackson M *et al*. Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Neuron* 2002;35:121-133.

Menold MM, Shao Y, Wolpert CM, Donnelly SL, Raiford KL, Martin ER *et al*. Association analysis of chromosome 15 gabaa receptor subunit genes in autistic disorder. *J Neurogenet* 2001;15:245-259.

Mercer RE, Kwolek EM, Bischof JM, van Eede M, Henkelman RM, Wevrick R. Regionally reduced brain volume, altered serotonin neurochemistry, and abnormal behavior in mice null for the circadian rhythm output gene *Magel2*. *Am J Med Genet B Neuropsychiatr Genet* 2009;150B:1085-1099.

Merikangas AK., Corvin AP, Gallagher L. Copy-number variants in neurodevelopmental disorders: promises and challenges. *Trends in genetics* 2009;25:536-544.

Mersich AT, Miller M, Chkourko H, Blystone SD. The Formin FRL1 (FMNL1) is an essential component of macrophage podosomes. *Cytoskeleton* 2010;67:573-585.

Mersich AT, Miller M, Chkourko H, Blystone SD. The Formin FRL1 (FMNL1) is an essential component of macrophage podosomes. *Cytoskeleton* 2010;67:573-585.

Micale L, Fusco C, Augello B, Napolitano LM, Dermitzakis ET, Meroni G *et al*. Williams-Beuren syndrome *TRIM50* encodes an E3 ubiquitin ligase. *Eur J Hum Genet* 2008;16:1038-1049.

Mikhail FM, Lose EJ, Robin NH, Descartes MD, Rutledge KD, Rutledge SL *et al*. Clinically relevant single gene or intragenic deletions encompassing critical neurodevelopmental genes in patients with developmental delay, mental retardation, and/or autism spectrum disorders. *Am J Med Genet A* 2011;155A:2386-2396.

Miles JH, Takahashi TN, Bagby S, Sahota PK, Vaslow DF, Wang CH *et al*. Essential versus complex autism: definition of fundamental prognostic subtypes. *Am J Med Genet A* 2005;135:171-180.

Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP *et al*. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet* 2010;86:749-764.

Miller DT, Shen Y, Weiss LA, Korn J, Anselm I, Bridgemohan C *et al*. Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatric disorders. *J Med Genet* 2009;46:242-248.

Minshew NJ, Williams DL. The new neurobiology of autism: cortex, connectivity, and neuronal organization. *Arch Neurol* 2007;64:945-950.

Mishra M, Akatsu H, Heese K. The novel protein MANI modulates neurogenesis and neurite-cone growth. *J Cell Mol Med* 2011;15:1713-1725.

Missler M, Zhang W, Rohlmann A, Kattenstroth G, Hammer RE, Gottmann K *et al*. a-Neurexins couple Ca^{2+} channels to synaptic vesicle exocytosis. *Nature* 2003;423:939-948.

Mochida S, Orita S, Sakaguchi G, Sasaki T, Takai Y. Role of the Doc2a–Munc13–1 interaction in the neurotransmitter release process. *Proc Natl Acad Sci USA* 1998;95:11418-11422.

Moessner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J *et al*. Contribution of *SHANK3* mutations to autism spectrum disorder. *Am J Hum Genet* 2007;81:1289-1297.

Mogha A, Guariglia SR, Debata PR, Wen GY, Banerjee P. Serotonin 1A receptor-mediated signaling through ERK and PKCα is essential for normal synaptogenesis in neonatal mouse hippocampus. *Transl Psychiatry* 2012;2:e66.

Monaco AP, Bailey AJ. Autism. The search for susceptibility genes. *Lancet* 2001;358:S3.

Mons N, Yoshimura M, Cooper DM. Discrete expression of Ca2+/calmodulin-sensitive and Ca(2+)-insensitive adenylyl cyclases in the rat brain. *Synapse* 1993;14:51-59.

Monteleone I, Monteleone G, Fina D, Caruso R, Petruzziello C, Calabrese E *et al*. A functional role of flip in conferring resistance of Crohn's disease lamina propria lymphocytes to FAS-mediated apoptosis. *Gastroenterology* 2006;130:389-397.

Moon RT. Wnt/beta-catenin pathway. *Sci STKE* 2005;2005:cm1.

Moretti P, Zoghbi HY. MeCP2 dysfunction in Rett syndrome and related disorders. *Curr Opin Genet Dev* 2006;16:276-281.

Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J *et al*. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biol Psychiatry* 2010;68:368-376.

Mori Y, Matsui T, Furutani Y, Yoshihara Y, Fukuda M. Small GTPase Rab17 regulates dendritic morphogenesis and postsynaptic development of hippocampal neurons. *J Biol Chem* 2012;287:8963-8973.

Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS *et al*. Identifying autism loci and genes by tracing recent shared ancestry. *Science* 2008;321:218-223.

Moser JJ, Eystathioy T, Chan EK, Fritzler MJ. Markers of mRNA stabilization and degradation, and RNAi within astrocytoma GW bodies. *J Neurosci Res* 2007;85:3619-3631.

Moskvina V, Craddock N, Holmans P, Nikolov I, Pahwa JS, Green E *et al*. Gene-wide analyses of genome-wide association data sets: evidence for multiple common risk alleles for schizophrenia and bipolar disorder and for overlap in genetic risk. *Mol Psichiatry* 2009;14:252-260.

Mostafa GA, Al-Ayadhi LY. A lack of association between hyperserotonemia and the increased frequency of serum anti-myelin basic protein auto-antibodies in autistic children. *J Neuroinflammation* 2011;8:71.

Mostafa GA, Al-Ayadhi LY. The possible link between the elevated serum levels of neurokinin A and anti-ribosomal P protein antibodies in children with autism. *J Neuroinflammation* 2011;8:180.

Mount RH, Charman T, Hastings RP, Reilly S, Cass H. Features of autism in Rett syndrome and severe mental retardation. *J Autism Dev Disord* 2003;33:435-442.

Muhle R, Trentacoste SV, Rapin I. The genetics of autism. *Pediatrics* 2004;113:e472-486.

Mukaddes NM, Herguner S. Autistic disorder and 22q11.2 duplication. *World J Biol Psychiatry* 2007;8:127-130.

Mukherjee O, Meera P, Ghosh S, Kubendran S, Kiran K, Manjunath KR *et al*. Evidence of linkage and association on 18p11.2 for psychosis. *Am J Med Genet B Neuropsychiatr Genet* 2006;141B:868-873.

Muller RA, Chugani DC, Behen ME, Rothermel RD, Muzik O, Chakraborty PK *et al*. Impairment of dentato-thalamocortical pathway in autistic men: language activation data from positron emission tomography. *Neurosci Lett* 1998;245:1-4.

Muñoz-Yunta JA, Palau-Baduell M, Salvadó-Salvadó B, Valls-Santasusana A, Rosendo-Moreno N, Clofent-Torrentó M *et al*. Autism, epilepsy and genetics. *Rev Neurol* 2008;46 Suppl 1:S71-77.

Murakami JW, Courchesne E, Press GA, Yeung-Courchesne R, Hesselink JR. Reduced cerebellar hemisphere size and its relationship to vermal hypoplasia in autism. *Arch Neurol* 1989;46:689-694.

Murphy SM, Preble AM, Patel UK, O'Connell KL, Dias DP, Moritz M *et al*. GCP5 and GCP6: two new members of the human gamma-tubulin complex. *Mol Biol Cell* 2001;12:3340-3352.

Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? *Neuro Endocrinol Lett* 2005;26:439-446.

Muzykewicz DA, Newberry P, Danforth N, Halpern EF, Thiele EA. Psychiatric comorbid conditions in a clinic population of 241 patients with tuberous sclerosis complex. *Epilepsy Behav* 2007;11:506-513.

Naik US, Gangadharan C, Abbagani K, Nagalla B, Dasari N, Manna SK. A study of Nuclear Transcription Factor-Kappa B in childhood autism. *Plos One* 2011;6:e19488.

Nakajo N, Deno YK, Ueno H, Kenmochi C, Shimuta K, Sagata N. Temporal and spatial expression patterns of Cdc25 phosphatase isoforms during early Xenopus development. *Int J Dev Biol* 2011;55:627-632.

Nakamura K, Anitha A, Yamada K, Tsujii M, Iwayama Y, Hattori E *et al*. Genetic and expression analyses reveal elevated expression of syntaxin 1A (STX1A) in high functioning autism. *Int J Neuropsychopharmacol* 2008;11:1073-1084.

Nakamura K, Iwata Y, Anitha A, Miyachi T, Toyota T, Yamada S *et al*. Replication study of Japanese cohorts supports the role of STX1A in autism susceptibility. *Prog Neuropsychopharmacol Biol Psychiatry* 2011;35:454-458.

Nakanishi H, Shindou H, Hishikawa D, Harayama T, Ogasawara R, Suwabe A *et al*. Cloning and characterization of mouse lung-type acyl-CoA:lysophosphatidylcholine acyltransferase 1 (LPCAT1). Expression in alveolar type II cells and possible involvement in surfactant production. *J Biol Chem* 2006;281:20140-20147.

Nakanishi K, Aono S, Hirano K, Kuroda Y, Ida M, Tokita Y *et al*. Identification of neurite outgrowth-promoting domains of neuroglycan C, a brain-specific chondroitin sulphate proteoglycan, and involvement of phosphatidylinositol 3-kinase and protein kinase C signaling pathways in neuritogenesis. *J Biochem* 2006;281:24970-24978.

Nakatani N, Hattori E, Ohnishi T, Dean B, Iwayama Y, Matsumoto I *et al*. Genome-wide expression analysis detects eight genes with robust alterations specific to bipolar I disorder: relevance to neuronal network perturbation. *Hum Mol Genet* 2006;15:1949-1962.

Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN *et al*. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* 1998;393:386-389.

Natter S, Seiberler S, Hufnagl P, Binder BR, Hirschl AM, Ring J *et al*. Isolation of cDNA clones coding for IgE autoantigens with serum IgE from atopic dermatitis patients. *FASEB J* 1998;12:1559-1569.

Neale BM, Kou Y, Liu L, Maayan A, Samocha KE, Sabo A *et al*. Patterns and rates of exonic *de novo* mutations in autism spectrum disorders. *Nature* 2012;485: 242-245.

Neukirchen D, Bradke F. Cytoplasmic linker proteins regulate neuronal polarization through microtubule and growth cone dynamics. *J Neurosci* 2011;31:1528-1538.

Neumann LC, Markaki Y, Mladenov E, Hoffmann D, Buiting K, Horsthemke B. The imprinted *NPAP1/C15orf2* gene in the Prader-Willi syndrome region encodes a nuclear pore complex associated protein. *Hum Mol Genet* 2012;21:4038-4048.

Neves-Pereira M, Müller B, Massie D, Williams JH, O'Brien PC, Hughes A *et al*. Deregulation of EIF4E: a novel mechanism for autism. *J Med Genet* 2009;46:759-765.

Newschaffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, Levy SE *et al*. The epidemiology of autism spectrum disorders. *Annu Rev Public Health* 2007;28:235-258.

Nicoll RA, Malenka RC. Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* 1995;377:115-118.

Nicoll RA, Schmitz D. Synaptic plasticity at hippocampal mossy fibre synapses. *Nat Rev Neurosci* 2005;6:863-876.

Nie D, Di Nardo A, Han JM, Baharanyi H, Kramvis I, Huynh T *et al*. Tsc2–Rheb signaling regulates EphA-mediated axon guidance. *Nat Neurosci* 2010;13:163-172.

Nieoullon A. Dopamine and the regulation of cognition and attention. *Prog Neurobiol* 2002;67:53-83.

Niklasson L, Rasmussen P, Oskarsdottir S, Gillberg C. Autism, ADHD, mental retardation and behavior problems in 100 individuals with 22q11 deletion syndrome. *Res Dev Disabil* 2009;30:763-773.

Nishijima I, Yamagata T, Spencer CM, Weeber EJ, Alekseyenko O, Sweatt JD *et al*. Secretin receptor-deficient mice exhibit impaired synaptic plasticity and social behavior. *Hum Mol Genet* 2006;15:3241-3250.

Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N *et al*. Size-selective loosening of the blood-brain barrier in claudin-5 – deficient mice. *J Cell Biol* 2003;161:653-660 .

Niu S, Yabut O, D'Arcangelo G. The Reelin signaling pathway promotes dendritic spine development in hippocampal neurons. *J Neurosci* 2008;28:10339-10348

Nizzari M, Thellung S, Corsaro A, Villa V, Pagano A, Porcile C *et al*. Neurodegeneration in Alzheimer disease: role of amyloid precursor protein and presenilin1 intracellular signaling. *J Toxicol* 2012;2012:187297.

Noda K, Kitami T, Gai WP, Chegini F, Jensen PH, Fujimura T *et al*. Phosphorylated IkappaBalpha is a component of Lewy body of Parkinson's disease. *Biochem Biophys Res Commun* 2005;331:309-317.

Noor A, Whibley A, Marshall CR, Gianakopoulos PJ, Piton A, Carson AR *et al*. Disruption at the *PTCHD1* locus on Xp22.11 in autism spectrum disorder and intellectual disability. *Sci Transl Med* 2010;2:49ra68.

Nord AS, Roeb W, Dickel DE, Walsh T, Kusenda M, Lewis O'Connor K *et al*. Reduced transcript expression of genes affected by inherited and *de novo* CNVs in autism. *Eur J of Hum Genet* 2011;19:727-731.

Novarino G, El-Fishawy P, Kayserili H, Meguid NA, Scott EM, Schroth J *et al*. Mutations in BCKD-kinase lead to a potentially treatable form of autism with epilepsy. *Science* 2012; 338:394-397

Nuber UA, Kriaucionis S, Roloff TC, Guy J, Selfridge J, Steinhoff C *et al*. Up-regulation of glucocorticoid-regulated genes in a mouse model of Rett syndrome. *Hum Mol Genet* 2005;14:2247-2256.

Numata S, Iga J, Nakataki M, Tayoshi S, Tanahashi T, Itakura M *et al*. Positive association of the pericentrin (*PCNT)* gene with major depressive disorder in the Japanese population. *J Psychiatry Neurosci* 2009;34:195-198.

Nurmi EL, Amin T, Olson LM, Jacobs MM, McCauley JL, Lam AY *et al*. Dense linkage disequilibrium mapping in the 15q11-q13 maternal expression domain yields evidence for association in autism. *Mol Psychiatry* 2003;8:624-634.

O'Donnell WT, Warren ST. A decade of molecular studies of fragile X syndrome. *Annu Rev Neurosci* 2002;25:315-338.

O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V *et al*. Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* 2008;40:1053-1055.

O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S *et al*. Exome sequencing in sporadic autism spectrum disorders identifies severe *de novo* mutations. *Nat Genet* 2011;43:585-589.

O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP *et al*. Sporadic autism exomes reveal a highly interconnected protein network of *de novo* mutations. *Nature* 2012;485:246-250.

Oblak AL, Rosene DL, Kemper TL, Bauman ML, Blatt GJ. Altered posterior cingulate cortical cyctoarchitecture, but normal density of neurons and interneurons in the posterior cingulate cortex and fusiform gyrus in autism. *Autism Res* 2011;4:200-211.

Odell D, Maciulis A, Cutler A, Warren L, McMahon WM, Coon H *et al*. Confirmation of the association of the C4B null allelle in autism. *Hum Immunol* 2005;66:140-145.

Oh KS, Khan SG, Jaspers NG, Raams A, Ueda T, Lehmann A *et al*. Phenotypic heterogeneity in the XPB DNA helicase gene (ERCC3): xeroderma pigmentosum without and with Cockayne syndrome. *Hum Mutat* 2006;27:1092-1103.

Okamoto S, Sherman K, Bai G, Lipton SA. Effect of the ubiquitous transcription factors, SP1 and MAZ, on NMDA receptor subunit type 1 (NR1) expression during neuronal differentiation. *Brain Res Mol Brain Res* 2002;107:89-96.

Okerlund ND, Cheyette BN. Synaptic Wnt signaling-a contributor to major psychiatric disorders? *J Neurodev Disord* 2011;3:162-174.

Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain Behav Immun* 2012;26:383-392.

Onore CE, Nordahl CW, Young GS, Van de Water JA, Rogers SJ, Ashwood P. Levels of soluble platelet endothelial cell adhesion molecule-1 and P-Selectin are decreased in children with Autism Spectrum Disorder. *Biol Psychiatry* 2012 Jun 19. [Epub ahead of print]

Opdam FJ, Echard A, Croes HJ, van den Hurk JA, van de Vorstenbosch RA, Ginsel LA *et al*. The small GTPase Rab6B, a novel Rab6 subfamily member, is cell-type specifically expressed and localised to the Golgi apparatus. *J Cell Sci* 2000;113:2725-2735.

Orlova KA, Crino PB. The tuberous sclerosis complex. *Ann NY Acad Sci* 2010;1184:87-105.

Orosz F, Ovadi J. TPPP orthologs are ciliary proteins. *FEBS Lett* 2008;582:3757-3764.

Oruc L, Verheyen GR, Furac I, Ivezić S, Jakovljević M, Raeymaekers P *et al*. Positive association between the *GABRA5* gene and unipolar recurrent major depression. *Neuropsychobiology* 1997;36:62-64.

Osborne LR. Animal models of Williams syndrome. *Am J Med Genet C Semin Med Genet* 2010;154C:209-219.

Osterweil EK, Krueger DD, Reinhold K, Bear MF. Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. *J Neurosci* 2010;30:15616-15627.

Ounap K, Uibo O, Zordania R, Kiho L, Ilus T, Oiglane-Shlik E *et al*. Three patients with 9p deletions including DMRT1 and DMRT2: a girl with XY complement, bilateral ovotestes, and extreme growth retardation, and two XX females with normal pubertal development. *Am J Med Genet A* 2004;130A:415-423.

Owen MJ, Williams HJ, O'Donovan MC. Schizophrenia genetics: advancing on two fronts. *Curr Opin Genet Dev* 2009;19:266-270.

Oya H, Yokoyama A, Yamaoka I, Fujiki R, Yonezawa M, Youn MY *et al*. Phosphorylation of Williams Syndrome transcription factor by MAPK induces a switching between two distinct chromatin remodeling complexes. *J Biol Chem* 2009;284:32472-32482.

Ozonoff S, Young GS, Carter A, Messinger D, Yirmiya N, Zwaigenbaum L *et al*. Recurrence risk for autism spectrum disorders: a baby siblings research consortium study. *Pediatrics* 2011;128:e488-e495.

Pagnamenta AT, Khan H, Walker S, Gerrelli D, Wing K, Bonaglia MC *et al*. Rare familial 16q21 microdeletions under a linkage peak implicate cadherin 8 (CDH8) in susceptibility to autism and learning disability. *J Med Genet* 2011;48:48-54.

Pagnamenta AT, Wing K, Sadighi Akha E, Knight SJ, Bolte S, Schmotzer G *et al*. A 15q13.3 microdeletion segregating with autism. *Eur J Hum Genet* 2009;17:687-692.

Palmieri F. The mitochondrial transporter family (SLC25): physiological and pathological implications. *Pflugers Arch* 2004;447:689-709.

Palmieri L, Persico AM. Mitochondrial dysfunction in autism spectrum disorders: cause or effect? *Biochim Biophys Acta* 2010;1797:1130-1137.

Pampanos A, Volaki K, Kanavakis E, Papandreou O, Youroukos S, Thomaidis L *et al*. A substitution involving the *NLGN4* gene associated with autistic behavior in the Greek population. *Genet Test Mol Biomarkers* 2009;13:611-615.

Pan PY, Cai Q, Lin L, Lu PH, Duan S, Sheng ZH. SNAP-29-mediated modulation of synaptic transmission in cultured hippocampal neurons. *J Biol Chem* 2005; 280:25769-25779.

Papadimitriou GN, Dikeos D, Daskalopoulou E, Karadima G, Avramopoulos D, Contis C *et al*. Association between GABA-A receptor alpha 5 subunit gene locus and schizophrenia of a later age of onset. *Neuropsychobiology* 2001;43:141-144.

Papadimitriou GN, Dikeos DG, Karadima G, Avramopoulos D, Daskalopoulou EG, Vassilopoulos D *et al*. Association between the GABA(A) receptor alpha5 subunit gene locus (GABRA5) and bipolar affective disorder. *Am J Med Genet* 1998;81:73-80.

Pardo CA, Eberhart CG. The neurobiology of autism. *Brain Pathology* 2007;17:434-447.

Parisi MA, Dinulos MB, Leppig KA, Sybert VP, Eng C, Hudgins L. The spectrum and evolution of phenotypic findings in *PTEN* mutation positive cases of Bannayan-Riley-Ruvalcaba syndrome. *J Med Genet* 2001;38:52-58.

Park SH, Zhu P, Parker RL, Blackstone C. Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network. *J Clin Invest* 2010;120:1097-1110.

Parkkila S, Parkkila AK, Rajaniemi H, Shah GN, Grubb JH, Waheed A *et al*. Expression of membrane-associated carbonic anhydrase XIV on neurons and axons in mouse and human brain. *Proc Natl Acad Sci USA* 2001;98:1918-1923.

Paspalas CD, Perley CC, Venkitaramani DV, Goebel-Goody SM, Zhang Y, Kurup P *et al*. Major vault protein is expressed along the nucleus-neurite axis and associates with mRNAs in cortical neurons. *Cerebral Cortex* 2009;19:1666-1677.

Pasteris NG, Cadle A, Logie LJ, Porteous ME, Schwartz CE, Stevenson RE *et al*. Isolation and characterization of the faciogenital dysplasia (Aarskog-Scott syndrome) gene: a putative Rho/Rac guanine nucleotide exchange factor. *Cell* 1994;79:669-678.

Pastorcic M, Das HK. Analysis of transcriptional modulation of the presenilin 1 gene promoter by ZNF237, a candidate binding partner of the Ets transcription factor ERM. *Brain Res* 2007;1128:21-32.

Patterson PH. Immune involvement in schizophrenia and autism: etiology, pathology and animal models. *Behav Brain Res* 2009;204:313-321.

Paul R, Cohen DJ, Volkmar FR. Autistic behaviors in a boy with Noonan syndrome. *J Autism Dev Disord* 1983;13:433-434.

Paul S, Lombroso PJ. Receptor and nonreceptor protein tyrosine phosphatases in the nervous system. *Cell Mol Life Sci* 2003;60:2465-2482.

Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen JA *et al*. Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. *Hum Mol Genet* 2001;10:3037-3048.

Pawlisz AS, Mutch C, Wynshaw-Boris A, Chenn A, Walsh CA, Feng Y. Lis1–Nde1-dependent neuronal fate control determines cerebral cortical size and lamination. *Hum Mol Genet* 2008;17:2441-2455.

Paylor R, Glaser B, Mupo A, Ataliotis P, Spencer C, Sobotka A *et al*. *Tbx1* haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. *Proc Natl Acad Sci USA* 2006;103:7729-7734.

Pebrel-Richard C, Kemeny S, Gouas L, Eymard-Pierre E, Blanc N, Francannet C *et al*. An atypical 0.8 Mb inherited duplication of 22q11.2 associated with psychomotor impairment. *Eur J Med Genet* 2012;55:650-655.

Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN *et al*. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* 2011;472:437-442.

Peça J, Feng G. Cellular and synaptic network defects in autism. *Curr Opin Neurobiol* 2012;22:866-872.

Pedrosa E, Shah A, Tenore C, Capogna M, Villa C, Guo X *et al.* β-catenin promoter ChIP-chip reveals potential schizophrenia and bipolar disorder gene network. *J Neurogenet* 2010;24:182-193.

Pelka GJ, Watson CM, Radziewic T, Hayward M, Lahooti H, Christodoulou J *et al*. Mecp2 deficiency is associated with learning and cognitive deficits and altered gene activity in the hippocampal region of mice. *Brain* 2006;129:887-898.

Pellicano F, Inglis-Broadgate SL, Pante G, Ansorge W, Iwata T. Expression of coiled-coil protein 1, a novel gene downstream of FGF2, in the developing brain. *Gene Expr Patterns* 2006;6:285-293.

Pellicano F, Thomson RE, Inman GJ, Iwata T. Regulation of cell proliferation and apoptosis in neuroblastoma cells by *ccp1*, a FGF2 downstream gene. *BMC Cancer* 2010;10:657.

Perry GH, Ben-Dor A, Tsalenko A, Sampas N, Rodriguez-Revenga L, Tran CW *et al*. The fine-scale and complex architecture of human copy-number variation. *Am J Hum Genet* 2008;82:685-695.

Persico AM, Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. *Trends Neurosci* 2006;29:349-358.

Persico AM, D'Agruma L, Maiorano N, Totaro A, Militerni R, Bravaccio C *et al*. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Mol Psychiatry* 2001;6:150-159.

Pertz OC, Wang Y, Yang F, Wang W, Gay LJ, Gristenko MA *et al*. Spatial mapping of the neurite and soma proteomes reveals a functional Cdc42/Rac regulatory network. *Proc Natl Acad Sci USA* 2008;105(6):1931-1936.

Pfeifhofer-Obermair C, Thuille N, Baier G. Involvement of distinct PKC gene products in T cell functions. *Front Immunol* 2012;3:220.

Philippe A, Guilloud-Bataille M, Martinez M, Gillberg C, Råstam M, Sponheim E *et al*. Analysis of ten candidate genes in autism by association and linkage. *Am J Med Genet* 2002;114:125-128.

Philippe A, Martinez M, Guilloud-Bataille M, Gillberg C, Rastam M, Sponheim E *et al*. Genome-wide scan for autism susceptibility genes. *Hum Mol Genet* 1999;8:805-812.

Philippe O, Rio M, Malan V, Van Esch H, Baujat G, Bahi-Buisson N *et al*. NF-κB signaling requirement for brain myelin formation is shown by genotype/MRI phenotype correlations in patients with Xq28 duplications. *Eur J Hum Genet* 2012 Jul 18. [Epub ahead of print].

Philippi A, Roschmann E, Tores F, Lindenbaum P, Benajou A, Germain-Leclerc L *et al*. Haplotypes in the gene encoding protein kinase c-beta (*PRKCB1*) on chromosome 16 are associated with autism. *Mol Psychiatry* 2005;10:950-960.

Philippi A, Tores F, Carayol J, Rousseau F, Letexier M, Roschmann E *et al*. Association of autism with polymorphisms in the paired-like homeodomain transcription factor 1 (*PITX1*) on chromosome 5q31: a candidate gene analysis. *BMC Med Genet* 2007;8:74.

Philpot BD, Thompson CE, Franco L, Williams CA. Angelman syndrome: advancing the research frontier of neurodevelopmental disorders. *J Neurodev Disord* 2010;3:50-56.

Pierpont EI, Pierpont ME, Mendelsohn NJ, Roberts AE, Tworog-Dube E, Seidenberg MS. Genotype differences in cognitive functioning in Noonan syndrome. *Genes Brain Behav* 2009;8:275-282.

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R *et al*. Functional impact of global rare copy number variation in autism spectrum disorders. *Nature* 2010;466:368-372.

Piton A, Gauthier J, Hamdan FF, Lafrenière RG, Yang Y, Henrion E *et al*. Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. *Mol Psychiatry* 2011;16:867-880.

Piton A, Michaud JL, Peng H, Aradhya S, Gauthier J, Mottron L *et al*. Mutations in the calcium-related gene *IL1RAPL1* are associated with autism. *Hum Mol Genet* 2008;17:3965-3974.

Pittman AM, Myers AJ, Abou-Sleiman P, Fung HC, Kaleem M, Marlowe L *et al*. Linkage disequilibrium fine mapping and haplotype association analysis of the tau gene in progressive supranuclear palsy and corticobasal degeneration. *J Med Genet* 2005;42:837-846.

Pizzarelli R, Cherubini E. Alterations of GABAergic signaling in autism spectrum disorders. *Neural Plast* 2011;2011:297153.

Poelmans G, Engelen JJ, Van Lent-Albrechts J, Smeets HJ, Schoenmakers E, Franke B *et al*. Identification of novel dyslexia candidate genes through the analysis of a chromosomal deletion. *Am J Med Genet B Neuropsychiatr Genet* 2009;150B:140-147.

Polzin A, Shipitsin M, Goi T, Feig LA, Turner TJ. Ral-GTPase influences the regulation of the readily releasable pool of synaptic vesicles. *Mol Cell Biol* 2002;22:1714-1722.

Potokar M, Lacovich V, Chowdhury HH, Kreft M, Zorec R. Rab4 and Rab5 GTPase are required for directional mobility of endocytic vesicles in astrocytes. *Glia* 2012;60:594-604.

Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL *et al*. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry* 2004;9:684-697.

Prandini P, Pasquali A, Malerba G, Marostica A, Zusi C, Xumerle L *et al*. The association of rs4307059 and rs35678 markers with autism spectrum disorders is replicated in Italian families. *Psychiatr Genet* 2012;22:177-181.

Prasad C, Prasad AN, Chodirker BN, Lee C, Dawson AK, Jocelyn LJ *et al*. Genetic evaluation of pervasive developmental disorders: the terminal 22q13 deletion syndrome may represent a recognizable phenotype. *Clin Genet* 2000;57:103-109.

Pratt T, Conway CD, Tian NM, Price DJ, Mason JO. Heparan sulphation patterns generated by specific heparan sulfotransferase enzymes direct distinct aspects of retinal axon guidance at the optic chiasm. *J Neurosci* 2006;26:6911-6923.

Proulx E, Young EJ, Osborne LR, Lambe EK. Enhanced prefrontal serotonin 5-HT(1A) currents in a mouse model of Williams-Beuren syndrome with low innate anxiety. *J Neurodev Disord* 2010;2:99-108.

Purohit A, Tynan SH, Vallee R, Doxsey SJ. Direct interaction of pericentrin with cytoplasmic dynein light intermediate chain contributes to mitotic spindle organization. *J Cell Biol* 1999;147:481-491.

Qiao Y, Riendeau N, Koochek M, Liu X, Harvard C, Hildebrand MJ *et al*. Phenomic determinants of genomic variation in autism spectrum disorders. *J Med Genet* 2009;46:680-688.

Qin M, Kang J, Burlin TV, Jiang C, Smith CB. Post-adolescent changes in regional cerebral protein synthesis: an in vivo study in the *Fmr1* null mouse. *J Neurosci* 2005;25:5087-5095.

Quast T, Tappertzhofen B, Schild C, Grell J, Czeloth N, Förster R *et al*. Cytohesin-1 controls the activation of RhoA and modulates integrin-dependent adhesion and migration of dendritic cells. *Blood* 2009;113:5801-5810.

Rabionet R, Jaworski JM, Ashley-Koch AE, Martin ER, Sutcliffe JS, Haines JL *et al*. Analysis of the autism chromosome 2 linkage region: *GAD1* and other candidate genes. *Neurosci Lett* 2004;372:209-214.

Rademakers R, Cruts M, van Broeckhoven C. The role of tau (MAPT) in frontotemporal dementia and related tauopathies. *Hum Mutat* 2004;24:277-295.

Ragusa L, Elia M, Scifo R. Growth hormone deficit in autism. *J Autism Dev Disord* 1993;23:421-422.

Ramelli GP, Silacci C, Ferrarini A, Cattaneo C, Visconti P *et al*. Microduplication 22q11.2 in a child with autism spectrum disorder: clinical and genetic study. *Dev Med Child Neurol* 2008;50:953-955.

Ramocki MB, Peters SU, Tavyev YJ, Zhang F, Carvalho CM, Schaaf CP *et al*. Autism and other neuropsychiatric symptoms are prevalent in individuals with *MeCP2* duplication syndrome. *Ann Neurol* 2009;66:771-782.

Ramocki MB, Tavyev YJ, Peters SU. The *MECP2* duplication syndrome. *Am J Med Genet A* 2010;152A:1079-1088.

Ramsey SA, Klemm SL, Zak DE, Kennedy KA, Thorsson V, Li B *et al*. Uncovering a macrophage transcriptional program by integrating evidence from motif scanning and expression dynamics. *Plos Comput Biol* 2008;4:e1000021.

Ranheim EA, Kwan HC, Reya T, Wang YK, Weissman IL, Francke U. Frizzled 9 knockout mice have abnormal B cell development. *Blood* 2005;105:2487-2494.

Rauch A, Thiel CT, Schindler D, Wick U, Crow YJ, Ekici AB *et al*. Mutations in the pericentrin (*PCNT*) gene cause primordial dwarfism. *Science* 2008;319:816-819.

Ray MA, Graham AJ, Lee M, Perry RH, Court JA, Perry EK. Neuronal nicotinic acetylcholine receptor subunits in autism: an immunohistochemical investigation in the thalamus. *Neurobiol Dis* 2005;19:366-377.

Raymond GV, Bauman ML, Kemper TL. Hippocampus in autism: a Golgi analysis. *Acta Neuropathol* 1996;91:117-119.

Reddy KS. Cytogenetic abnormalities and fragile-X syndrome in Autism Spectrum Disorder. *BMC Med Genet* 2005;6:3.

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD *et al*. Global variation in copy number in the human genome. *Nature* 2006;444:444-454.

Refojo D, Schweizer M, Kuehne C, Ehrenberg S, Thoeringer C, Vogl AM *et al*. Glutamatergic and dopaminergic neurons mediate anxiogenic and anxiolytic effects of CRHR1. *Science* 2011;333:1903-1907.

Reichelt AC, Rodgers RJ, Clapcote SJ. The role of neurexins in schizophrenia and autistic spectrum disorder. *Neuropharmacology* 2011;62:1519-1526.

Reiss AL, Feinstein C, Rosenbaum KN, Borengasser-Caruso MA, Goldsmith BM. Autism associated with Williams syndrome. *J Pediatr* 1985;106:247-249.

Ressler KJ, Mercer KB, Bradley B, Jovanovic T, Mahan A, Kerley K *et al*. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* 2011;470:492-497.

Ressler KJ, Paschall G, Zhou XL, Davis M. Regulation of synaptic plasticity genes during consolidation of fear conditioning. *J Neurosci* 2002;22:7892-7902.

Rhinn M, Lun K, Ahrendt R, Geffarth M, Brand M. Zebrafish *gbx1* refines the midbrain-hindbrain boundary border and mediates the Wnt8 posteriorization signal. *Neural Dev* 2009;4:12.

Rhinn M, Lun K, Luz M, Werner M, Brand M. Positioning of the midbrain-hindbrain boundary organizer through global posteriorization of the neuroectoderm mediated by Wnt8 signaling. *Development* 2005;132:1261-1272.

Risch N, Spiker D, Lotspeich L, Nouri N, Hinds D, Hallmayer J *et al*. A genomic screen of autism: evidence for a multilocus etiology. *Am J Hum Genet* 1999;65:493-507.

Risinger C, Bennett MK. Differential phosphorylation of syntaxin and synaptosome-associated protein of 25 kDa (SNAP-25) isoforms. *J Neurochem* 1999;72:614-624.

Ritvo ER, Freeman BJ, Scheibel AB, Duong T, Robinson H, Guthrie D *et al*. Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC Autopsy Research Report. *Am J Psychiatry* 1986;143:862-866.

Robertson KD. DNA methylation and human disease. *Nat Rev Genet* 2005;6:597-610.

Robertson SP. Filamin A: phenotypic diversity. *Curr Opin Genet Dev* 2005;15:301-307.

Rocak S, Linder P. DEAD-box proteins: the driving forces behind RNA metabolism. *Nat Rev Mol Cell Biol* 2004;5:232-241.

Rodríguez-Seguel E, Alarcón P, Gómez-Skarmeta JL. The Xenopus Irx genes are essential for neural patterning and define the border between prethalamus and thalamus through mutual antagonism with the anterior repressors Fezf and Arx. *Dev Biol* 2009;15;329:258-68

Roessler E, Du Y, Mullor JL, Casas E, Allen WP, Gillessen-Kaesbach G *et al*. Loss-of-function mutations in the human *GLI2* gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proc Natl Acad Sci USA* 2003;100:13424-13429.

Röll S, Seul J, Paulsson M, Hartmann U. Testican-1 is dispensable for mouse development. *Matrix Biol* 2006;25:373-381.

Roohi J, Montagna C, Tegay DH, Palmer LE, DeVincent C, Pomeroy JC *et al*. Disruption of contactin 4 in three subjects with autism spectrum disorder. *J Med Genet* 2009;46:176-182.

Rosenberg RE, Law JK, Yenokyan G, McGready J, Kaufmann WE, Law PA. Characteristics and concordance of autism spectrum disorders among 277 twin pairs. *Arch Pediatr Adolesc Med* 2009;163:907-914.

Rosenfeld JA, Ballif BC, Torchia BS, Sahoo T, Ravnan JB, Schultz R *et al*. Copy number variations associated with Autism spectrum disorders contribute to a spectrum of neurodevelopmental disorders. *Genet Med* 2010;12:694-702.

Rosenfeld JA, Coppinger J, Bejjani BA, Girirajan S, Eichler EE, Shaffer LG *et al*. Speech delays and behavioral problems are the predominant features in individuals with developmental delays and 16p11.2 microdeletions and microduplications. *J Neurodev Disord* 2010;2:26-38.

Rosso SB, Sussman D, Wynshaw-Boris A, Salinas PC. Wnt signaling through Dishevelled, Rac and JNK regulates dendritic development. *Nat Neurosci* 2005;8:34-42

Roussignol G, Ango F, Romorini S, Tu JC, Sala C, Worley PF *et al*. Shank expression is sufficient to induce functional dendritic spine synapses in aspiny neurons. *J Neurosci* 2005;25:3560-3570.

Ruusala A, Aspenström P. Isolation and characterisation of DOCK8, a member of the DOCK180-related regulators of cell morphology. *FEBS Lett* 2004;572:159-166.

Rytych JL, Elmore MR, Burton MD, Conrad MS, Donovan SM, Dilger RN *et al*. Early life iron deficiency impairs spatial cognition in neonatal piglets. *J Nutr* 2012;142:2050-2056.

Sahay A, Kim CH, Sepkuty JP, Cho E, Huganir RL, Ginty DD *et al*. Secreted semaphorins modulate synaptic transmission in the adult hippocampus. *J Neurosci* 2005;25:3613-3620.

Sahoo T, Peters SU, Madduri NS, Glaze DG, German JR, Bird LM *et al*. Microarray based comparative genomic hybridization testing in deletion bearing patients with Angelman syndrome:genotype–phenotype correlations. *J Med Genet* 2006;43:512-516.

Saito S, Sakagami H, Tonosaki A, Kondo H. Localization of mRNAs for CDP-diacylglycerol synthase and phosphatidylinositol synthase in the brain and retina of developing and adult rats. *Brain Res Dev Brain Res* 1998;110:21-30.

Saito T, Guan F, Papolos DF, Rajouria N, Fann CS, Lachman HM. Polymorphism in *SNAP29* gene promoter region associated with schizophrenia. *Mol Psychiatry* 2001;6:193-201.

Sakaguchi G, Manabe T, Kobayashi K, Orita S, Sasaki T, Naito A *et al*. Doc2alpha is an activity-dependent modulator of excitatory synaptic transmission. *Eur J Neurosci* 1999;11:4262-4268.

Sakai K, Tada M, Yonemochi Y, Nakajima T, Onodera O, Takahashi H *et al*. Marinesco-Sjögren syndrome with atrophy of the brain stem tegmentum and dysplastic cytoarchitecture in the cerebral cortex. *Neuropathology* 2008;28:541-546.

Sakai Y, Shaw CA, Dawson BC, Dugas DV, Al-Mohtaseb Z, Hill DE *et al*. Protein interactome reveals converging molecular pathways among autism disorders. *Sci Transl Med* 2011;3:86ra49.

Sakurai T, Dorr NP, Takahashi N, McInnes LA, Elder GA, Buxbaum JD. Haploinsufficiency of *Gtf2i*, a gene deleted in Williams Syndrome, leads to increases in social interactions. *Autism Res* 2011;4:28-39.

Sala C, Piech V, Wilson NR, Passafaro M, Liu G, Sheng M. Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron* 2001;31:115-130.

Salin PA, Malenka RC, Nicoll RA. Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. *Neuron* 1996;16:797-803.

Salinas PC, Fletcher C, Copeland NG, Jenkins NA, Nusse R. Maintenance of Wnt-3 expression in Purkinje cells of the mouse cerebellum depends on interactions with granule cells. *Development* 1994;120:1277-1286.

Salinas PC, Zou Y. Wnt signaling in neural circuit assembly. *Ann Rev Neurosci* 2008;31:339-358.

Salman ED, Kadlubar SA, Falany CN. Expression and localization of cytosolic sulfotransferase (SULT) 1A1 and SULT1A3 in normal human brain. *Drug Metab Dispos* 2009;37:706-709.

Samaco RC, Hogart A, LaSalle JM. Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. *Hum Mol Genet* 2005;14:483-492.

Samaco RC, Nagarajan RP, Braunschweig D, LaSalle JM. Multiple pathways regulate MeCP2 expression in normal brain development and exhibit defects in autism-spectrum disorders. *Hum Mol Genet* 2004;13:629-639.

Sanders AR, Rusu I, Duan J, Vander Molen JE, Hou C, Schwab SG *et al*. Haplotypic association spanning the 22q11.21 genes *COMT* and *ARVCF* with schizophrenia. *Mol Psychiatry* 2005;10:353-365.

Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D *et al*. Multiple recurrent *de novo* CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. *Neuron* 2011;70:863-885.

Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ *et al*. *De novo* mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature* 2012;485:237-241.

Sandhu HK, Hollenbeck N, Wassink TH, Philibert RA. An association study of *PCQAP* polymorphisms and schizophrenia. *Psychiatr Genet* 2004;14:169-172.

Sano K, Nakamura H, Mabuchi S, Tanaka T, Nakagawara A, Takai Y. Expression of the smg p25A (a ras p21-like GTP-binding protein) gene in human neuroblastoma cell lines and tumor tissues. *Cancer Res* 1990;50:7242-7245.

Schaaf CP, Sabo A, Sakai Y, Crosby J, Muzny D, Hawes A *et al*. Oligogenic heterozygosity in individuals with high-functioning autism spectrum disorders. *Hum Mol Genet* 2011;20:3366-3375.

Schaaf CP, Zoghbi HY. Solving the autism puzzle a few pieces at a time. *Neuron* 2011;70:806-808.

Schaefer GB, Lutz RE. Diagnostic yield in the clinical genetic evaluation of autism spectrum disorders. *Genet Med* 2006;8:549-556.

Schanen NC. Epigenetics of autism spectrum disorders. *Hum Mol Genet* 2006;15:138-150.

Schaniel C, Ang YS, Ratnakumar K, Cormier C, James T, Bernstein E *et al*. Smarcc1/Baf155 couples self-renewal gene repression with changes in chromatin structure in mouse embryonic stem cells. *Stem Cells* 2009;27:2979-2991.

Schenck A, Bardoni B, Langmann C, Harden N, Mandel JL, Giangrande A. CYFIP/Sra-1 controls neuronal connectivity in Drosophila and links the Rac1 GTPase pathway to the fragile X protein. *Neuron* 2003;38:887-898.

Scheuerle A, Wilson K. *PARK2* copy number aberrations in two children presenting with autism spectrum disorder: further support of an association and possible evidence for a new microdeletion/microduplication syndrome. *Am J Med Genet B Neuropsychiatr Genet* 2011;156B:413-420.

Schindler KM, Richter MA, Kennedy JL, Pato MT, Pato CN. Association between homozygosity at the *COMT* gene locus and obsessive compulsive disorder. *Am J Med Genet* 2000;96:721-724.

Schlüter OM, Basu J, Südhof TC, Rosenmund C. Rab3 superprimes synaptic vesicles for release: Implications for short-term synaptic plasticity. *J Neurosci* 2006;26:1239-1246.

Schlüter OM, Schmitz F, Jahn R, Rosenmund C, Südhof TC. A complete genetic analysis of neuronal Rab3 function. *J Neurosci* 2004;24:6629-6637.

Schmidt F, van den Eijnden M, Pescini Gobert R, Saborio GP, Carboni S, Alliod C *et al*. Identification of VHY/Dusp15 as a regulator of oligodendrocyte differentiation through a systematic genomics approach. *Plos One* 2012;7:e40457

Schmidt T, Bartelt-Kirbach B, Golenhofen N. Phosphorylation-dependent subcellular localization of the small heat shock proteins HspB1/Hsp25 and HspB5/αB-crystallin in cultured hippocampal neurons. *Histochem Cell Biol* 2012;138:407-418.

Schmitz N, Daly E, Murphy D. Frontal anatomy and reaction time in Autism. *Neurosci Lett* 2007;412:12-17.

Schneider T, Skitt Z, Liu Y, Deacon RM, Flint J, Karmiloff-Smith A *et al*. Anxious, hypoactive phenotype combined with motor deficits in Gtf2ird1 null mouse model relevant to Williams syndrome. *Behav Brain Res* 2012;233:458-473.

Schofield CM, Hsu R, Barker AJ, Gertz CC, Blelloch R, Ullian EM. Monoallelic deletion of the microRNA biogenesis gene Dgcr8 produces deficits in the development of excitatory synaptic transmission in the prefrontal cortex. *Neural Dev* 2011;6:11.

Schroer RJ, Phelan MC, Michaelis RC, Crawford EC, Skinner SA, Cuccaro M *et al*. Autism and maternally derived aberrations of chromosome 15q. *Am J Med Genet* 1998;76:327-336.

Schroeter EH, Kisslinger JA, Kopan R. Notch-1 signaling requires ligand-induced proteolytic release of intracellular domain. *Nature* 1998;393:382-386.

Schumann CM, Bloss CS, Barnes CC, Wideman GM, Carper RA, Akshoomoff N *et al*. Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. *J Neurosci* 2010;30:4419-4427.

Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci* 2012;13:465-477.

Schwarcz R, Du F, Schmidt W, Turski WA, Gramsbergen JB, Okuno E *et al*. Kynurenic acid: a potential pathogen in brain disorders. *Ann N Y Acad Sci* 1992;648:140-153.

Scott-Van Zeeland AA, Abrahams BS, Alvarez-Retuerto AI, Sonnenblick LI, Rudie JD, Ghahremani D *et al*. Altered functional connectivity in frontal lobe circuits is associated with variation in the autism risk gene *CNTNAP2*. *Sci Transl Med* 2010;2:56ra80.

Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T *et al*. Strong association of *de novo* copy number mutations with Autism. *Science* 2007;316:445-449.

Sedita J, Izvolsky K, Cardoso WV. Differential expression of heparan sulfate 6-O-sulfotransferase isoforms in the mouse embryo suggests distinctive roles during organogenesis. *Dev Dyn* 2004;231:782-94.

Segref A, Hoppe T. Think locally: control of ubiquitin-dependent protein degradation in neurons. *EMBO Rep* 2009;10:44-50.

Seki N, Hattori A, Sugano S, Suzuki Y, Nakagawara A, Muramatsu M *et al*. A novel human gene whose product shares significant homology with the bovine brain specific protein p25 on chromosome 5p15.3. *J Hum Genet* 1999;44:121-122.

Sempere Pérez A, Manchón Trives I, Palazón Azorín I, Alcaraz Más L, Pérez Lledó E, Galán Sánchez F. 15Q11.2 (BP1-BP2) microdeletion, a new syndrome with variable expressivity. *An Pediatr (Barc)*. 2011;75:58-62.

Sen B, Singh AS, Sinha S, Chatterjee A, Ahmed S, Ghosh S *et al*. Family-based studies indicate association of Engrailed 2 gene with autism in an Indian population. *Genes Brain Behav* 2010;9:248-255.

Serajee FJ, Nabi R, Zhong H, Mahbubul Huq AHM. Association of *INPP1*, *PIK3CG*, and *TSC2* gene variants with autistic disorder: implications for phosphatidylinositol signaling in autism. *J Med Genet* 2003;40:119.

Serajee FJ, Zhong H, Nabi R, Huq AH. The metabotropic glutamate receptor 8 gene at 7q31: partial duplication and possible association with autism. *J Med Genet* 2003;40:e42.

Serizawa S, Miyamichi K, Sakano H. One neuron-one receptor rule in the mouse olfactory system. *Trends Genet* 2004;20:648–653.

Shah GN, Ulmasov B, Waheed A, Becker T, Makani S, Svichar N *et al*. Carbonic anhydrase IV and XIV knockout mice: roles of the respective carbonic anhydrases in buffering the extracellular space in brain. *Proc Natl Acad Sci USA* 2005;102:16771-16776.

Shahbazian M, Young J, Yuva-Paylor L, Spencer C, Antalffy B, Noebels J *et al*. Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron* 2002a;35:243-254.

Shahbazian MD, Antalffy B, Armstrong DL, Zoghbi HY. Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. *Hum Mol Genet* 2002b;11:115-124.

Sharp AJ, Hansen S, Selzer RR, Cheng Z, Regan R, Hurst JA *et al*. Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. *Nat Genet* 2006;38:1038-1042.

Sharp AJ, Locke DP, McGrath SD, Cheng Z, Bailey JA, Vallente RU *et al*. Segmental duplications and copy-number variation in the human genome. *Am J Hum Genet* 2005;77:78-88.

Sharp AJ, Mefford HC, Li K, Baker C, Skinner C, Stevenson RE *et al*. A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nat Genet* 2008;40:322-328.

Sheitman BB, Knable MB, Jarskog LF, Chakos M, Boyce LH, Early J *et al*. Secretin for refractory schizophrenia. *Schizophr Res* 2004;66:177-181.

Shen Y, Schneider G, Cloutier JF, Veillette A, Schaller MD. Direct association of protein-tyrosine phosphatase PTP-PEST with Paxillin. *J Biol Chem* 1998;273:6474-6481.

Shimmura C, Suda S, Tsuchiya KJ, Hashimoto K, Ohno K, Matsuzaki H *et al*. Alteration of plasma glutamate and glutamine levels in children with high-functioning autism. *Plos One* 2011;6:e25340.

Shimogori T, VanSant J, Paik E, Grove EA. Members of the Wnt, Fz, and Frp gene families expressed in postnatal mouse cerebral cortex. *J Comp Neurol* 2004;473:496-510.

Shimoyama Y, Morikawa Y, Ichihara M, Kodama Y, Fukuda N, Hayashi H *et al*. Identification of human SEP1 as a glial cell line-derived neurotrophic factor-inducible protein and its expression in the nervous system. *Neuroscience* 2003;121:899-906.

Shin OH, Han W, Wang Y, Südhof TC. Evolutionarily conserved multiple C2 domain proteins with two transmembrane regions (MCTPs) and unusual Ca2+ binding properties. *J Biol Chem* 2005;280:1641-1651.

Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, Scott DA *et al*. Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *J Med Genet* 2010;47:332-341.

Shinawi M, Patel A, Panichkul P, Zascavage R, Peters SU, Scaglia F. The Xp contiguous deletion syndrome and autism. *Am J Med Genet A* 2009a;149A:1138-1148.

Shinawi M, Schaaf CP, Bhatt SS, Xia Z, Patel A, Cheung SW *et al*. A small recurrent deletion within 15q13.3 is associated with a range of neurodevelopmental phenotypes. *Nat Genet* 2009b;41:1269-1271.

Shinohara T, Nagashima K, Major E O. Propagation of the human polyomavirus, JCV, in human neuroblastoma cell lines. *Virology* 1997;228:269-277.

Shiow LR, Parisd K, Akanac MC, Cysterb JG, Sorensend RU, Pucka JM *et al*. Severe combined immunodeficiency (SCID) and attention deficit hyperactivity disorder (ADHD) associated with a *coronin-1a* mutation and a chromosome 16p11.2 deletion *Clin Immunol* 2009;131:24-30.

Shiow LR, Roadcap DW, Paris K, Watson SR, Grigorova IL, Lebet T *et al*. The actin regulator coronin 1A is mutant in a thymic egress-deficient mouse strain and in a patient with severe combined immunodeficiency. *Nat Immunol* 2008;9:1307-1315.

Shirakawa R, Fukai S, Kawato M, Higashi T, Kondo H, Ikeda T *et al*. Tuberous sclerosis tumor suppressor complex-like complexes act as GTPase-activating proteins for Ral GTPases. *J Biol Chem* 2009;284:21580-21588.

Shirane S, Sasaki M, Kogure D, Matsuda H, Hashimoto T. Increased ictal perfusion of the thalamus in paroxysmal kinesigenic dyskinesia. *J Neurol Neurosurg Psychiatry* 2001;71:408-410.

Shoffner J, Hyams L, Langley GN, Cossette S, Mylacraine L, Dale J *et al*. Fever plus mitochondrial disease could be risk factors for autistic regression. *J Child Neurol* 2010;25:429-434.

Shohamy D, Adcock RA. Dopamine and adaptive memory. *Trends Cogn Sci* 2010;14:464-472.

Shoukier M, Klein N, Auber B, Wickert J, Schröder J, Zoll B *et al*. Array CGH in patients with developmental delay or intellectual disability: are there phenotypic clues to pathogenic copy number variants? *Clin Genet* 2012;Jan 27. [Epub ahead of print].

Shumay E, Chen J, Fowler JS, Volkow ND. Genotype and ancestry modulate brain's DAT availability in healthy humans. *Plos One* 2011;6:e22754.

Sigurdsson T, Stark KL, Karayiorgou M, Gogos JA, Gordon JA. Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. *Nature* 2010;464:763-767.

Silverman E, Edwalds-Gilbert G, Lin RJ. DExD/H-box proteins and their partners: helping RNA helicases unwind. *Gene* 2003;312:1-16.

Sim K, Chan WY, Woon PS, Low HQ, Lim L, Yang GL *et al*. ARVCF genetic influences on neurocognitive and neuroanatomical intermediate phenotypes in Chinese patients with schizophrenia. *J Clin Psychiatry* 2012;73:320-326.

Simon-Areces J, Dopazo A, Dettenhofer M, Rodriguez-Tebar A, Garcia-Segura LM, Arevalo MA. Formin1 mediates the induction of dendritogenesis and synaptogenesis by Neurogenin3 in mouse hippocampal neurons. *Plos One* 2011;6:e21825.

Simonati A, Boaretto F, Vettori A, Dabrilli P, Criscuolo L, Rizzuto N *et al*. A novel missense mutation in the *L1CAM* gene in a boy with L1 disease. *Neurol Sci* 2006; 27:114-117.

Simonin C, Devos D, de Seze J, Charpentier P, Vaiva G, Goudemand M *et al*. Systemic lupus erythematosus presenting with recurrent psychiatric disturbances. *Rev Neurol* 2004;160:811-816.

Singer HS, Morris C, Gause C, Pollard M, Zimmerman AW, Pletnikov M. Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations: a pregnant dam mouse model. *J Neuroimmunol* 2009;211:39-48.

Singer HS, Morris CM, Williams PN, Yoon DY, Hong JJ, Zimmerman AW. Antibrain antibodies in children with autism and their unaffected siblings. *J Neuroimmunol* 2006;178:149-155.

Singh VK, Singh EA, Warren RP. Hyperserotoninemia and serotonin receptor antibodies in children with autism but not mental retardation. *Biol Psychiatry* 1997a;41:753-755.

Singh VK, Warren R, Averett R, Ghaziuddin M. Circulating autoantibodies to neuronal and glial filament proteins in autism. *Pediatr Neurol* 1997b;17:88-90.

Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immun* 1993;7:97-103.

Singh VK. Phenotypic expression of autoimmune autistic disorder (AAD): a major subset of autism. *Ann Clin Psychiatry* 2009;21:148-161.

Skjoerringe T, Lundvig DMS, Jensen PH, Moos T. P25 alpha/tubulin polymerization promoting protein expression by myelinating oligodendrocytes of the developing rat brain. *J Neurochem* 2006;99:333-342.

Sklar P, Pato MT, Kirby A, Petryshen TL, Medeiros H, Carvalho C *et al*. Genome-wide scan in Portuguese island families identifies 5q31-5q35 as a susceptibility locus for schizophrenia and psychosis. *Mol Psychiatry* 2004;9:213-218.

Skuse DH. Imprinting, the X-chromosome, and the male brain: explaining sex differences in the liability to autism. *Pediatr Res* 2000;47:9-16.

Slater SJ, Seiz JL, Stagliano BA, Stubbs CD. Interaction of protein kinase C isozymes with Rho GTPases. *Biochemistry* 2001;40:4437-4445.

Sly WS, Sato S, Zhu XL. Evaluation of carbonic anhydrase isozymes in disorders involving osteopetrosis and/or renal tubular acidosis. Clin Biochem 1991;24:311-318.

Smart TG, Hosie AM, Miller PS. Zn^{2+} ions: modulators of excitatory and inhibitory synaptic activity. *Neuroscientist* 2004;10:432-442.

Smith EN, Bloss CS, Badner JA, Barrett T, Belmonte PL, Berrettini W *et al*. Genome-wide association study of bipolar disorder in European American and African American individuals. *Mol Psychiatry* 2009;14:755-763.

Smith SEP, Zhou YD, Zhang G, Jin Z, Stoppel DC, Anderson MP. Increased gene dosage of *Ube3a* results in autism traits and decreased glutamate synaptic transmission in mice. *Sci Transl Med* 2011;3:103ra97.

Soleimani L, Roder JC, Dennis JW, Lipina T. Beta N-acetylglucosaminyltransferase V (Mgat5) deficiency reduces the depression-like phenotype in mice. *Genes Brain Behav* 2008;7:334-343.

Soto MC, Qadota H, Kasuya K, Inoue M, Tsuboi D, Mello CC *et al*. The GEX-2 and GEX-3 proteins are required for tissue morphogenesis and cell migrations in C. elegans. *Genes Dev* 2002;16:620-632.

Spencer JP, Murphy KP. Activation of cyclic AMP-dependent protein kinase is required for long-term enhancement at corticostriatal synapses in rats. *Neurosci Lett* 2002;329:217-221.

Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R *et al*. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 2004;119:19-31.

Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R *et al*. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 2004;119:19-31.

Splawski I, Yoo DS, Stotz SC, Cherry A, Clapham DE, Keating MT. *CACNA1H* Mutations in Autism Spectrum Disorders. *J Biol Chem* 2006;281:22085-22091.

Sprecher E, Ishida-Yamamoto A, Mizrahi-Koren M, Rapaport D, Goldsher D, Indelman M *et al*. Mutation in SNAP29, coding for a SNARE protein involved in intracellular trafficking, causes a novel neurocutaneous syndrome characterized by cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma. *Am J Hum Genet* 2005;77:242-251.

Stack A, Carrier N, Dietz D, Hollis F, Sorenson J, Kabbaj M. Sex differences in social interaction in rats: role of the immediate-early gene *zif268*. *Neuropsychopharmacology* 2010;35:570-580.

Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM. Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. *Eur Psychiatry* 2007;23:289-299.

Stankiewicz P, Lupski JR. Structural variation in the human genome and its role in disease. *Ann Rev Med* 2010;61:437-455.

State MW. The genetics of child psychiatric disorders: focus on autism and Tourette syndrome. *Neuron* 2010;68:254-269.

Stefansson H, Rujescu D, Cichon S, Pietiläinen OP, Ingason A, Steinberg S *et al*. Large recurrent microdeletions associated with schizophrenia. *Nature* 2008;455:232-236.

Steffenburg S, Gillberg CL, Steffenburg U, Kyllerman M. Autism in Angelman syndrome: a population-based study. *Pediatr Neurol* 1996;14:131-136.

Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF-alpha. *Nature* 2006;440:1054-1059.

Stelzl U, Worm U, Lalowski M, Haenig C, Brembeck FH, Goehler H *et al*. A human protein-protein interaction network: a resource for annotating the proteome. *Cell* 2005;122:957-968.

Stengel D, Parma J, Gannagé MH, Roeckel N, Mattei MG, Barouki R *et al*. Different chromosomal localization of two adenylyl cyclase genes expressed in human brain. *Hum Genet* 1992;90:126-130.

Stephenson DT, O'Neill SM, Narayan S, Tiwari A, Arnold E, Samaroo HD *et al*. Histopathologic characterization of the BTBR mouse model of autistic-like behavior reveals selective changes in neurodevelopmental proteins and adult hippocampal neurogenesis. *Mol Autism* 2011;2:7.

Stergiakouli E, Langley K, Williams H, Walters J, Williams NM, Suren S *et al*. Steroid sulfatase is a potential modifier of cognition in attention deficit hyperactivity disorder. *Genes Brain Behav* 2011;10:334-344.

Stielke S, Keilhoff G, Kirches E, Mertens PR, Neumann KH, Tsokos GC *et al*. Adhesion molecule expression precedes brain damages of lupus-prone mice and correlates with kidney pathology. *J Neuroimmunol* 2012;252:24-32.

Stigler KA, McDonald BC, Anand A, Saykin AJ, McDougle CJ. Structural and functional magnetic resonance imaging of autism spectrum disorders. *Brain Res* 2011;1380:146-161.

Stradal TE, Rottner K, Disanza A, Confalonieri S, Innocenti M, Scita G. Regulation of actin dynamics by WASP and WAVE family proteins. *Trends Cell Biol* 2004;14:303-311.

Stroth N, Holighaus Y, Ait-Ali D, Eiden LE. PACAP: a master regulator of neuroendocrine stress circuits and the cellular stress response. *Ann N Y Acad Sci* 2011;1220:49-59.

Su Q, Mochida S, Tian JH, Mehta R, Sheng ZH. SNAP-29: A general SNARE protein that inhibits SNARE disassembly and is implicated in synaptic transmission. *Proc Natl Acad Sci USA* 2001;98:14038-14043.

Suda S, Iwata K, Shimmura C, Kameno Y, Anitha A, Thanseem I *et al*. Decreased expression of axon-guidance receptors in the anterior cingulate cortex in autism. *Mol Autism* 2011;2:14.

Sudhof TC. Neuroligins and neurexins link synaptic function to cognitive disease. *Nature* 2008;455:903-911.

Südhof TC. The synaptic vesicle cycle. *Annu Rev Neurosci* 2004;27:509-547.

Sun W, Kesavan K, Schaefer BC, Garrington TP, Ware M, Johnson NL *et al*. MEKK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway. *J Biol Chem* 2001;276:5093-5100.

Sun W, Kesavan K, Schaefer BC, Garrington TP, Ware M, Johnson NL *et al*. MEKK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway. *J Biol Chem* 2001;276:5093-5100.

Sun ZY, Wei J, Xie L, Shen Y, Liu SZ, Ju GZ *et al*. The *CLDN5* locus may be involved in the vulnerability to schizophrenia. *Eur Psychiatry* 2004;19:354-357.

Suzuki G, Harper KM, Hiramoto T, Funke B, Lee M, Kang G *et al*. Over-expression of a human chromosome 22q11.2 segment including *TXNRD2*, *COMT* and *ARVCF* developmentally affects incentive learning and working memory in mice. *Hum Mol Genet* 2009;18:3914-3925.

Suzuki K, Sugihara G, Ouchi Y, Nakamura K, Tsujii M, Futatsubashi M *et al*. Reduced acetylcholinesterase activity in the fusiform gyrus in adults with autism spectrum disorders. *Arch Gen Psychiatry* 2011;68:306-313.

Suzuki T, Nishiyama K, Yamamoto A, Inazawa J, Iwaki T, Yamada T *et al*. Molecular cloning of a novel apoptosis-related gene, human Nap1 (NCKAP1), and its possible relatin to Alzheimer disease. *Genomics* 2000;63:246-254.

Swanson JM, Kinsbourne M, Nigg J, Lanphear B, Stefanatos GA, Volkow N *et al*. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychol Rev* 2007;17:39-59.

Swarr DT, Bloom D, Lewis RA, Elenberg E, Friedman EM, Glotzbach C *et al*. Potocki-Shaffer syndrome: Comprehensive clinical assessment, review of the literature, and proposals for medical management. *Am J Med Genet A* 2010;152A:565-572.

Sweeten TL, Posey DJ, McDougle CJ. High blood monocyte counts and neopterin levels in children with autistic disorder. *Am J Psychiatry* 2003;160:1691-1693.

Swillen A, Hellemans H, Steyaert J, Fryns JP. Autism and genetics: high incidence of specific genetic syndromes in 21 autistic adolescents and adults living in two residential homes in Belgium. *Am J Med Genet* 1996;67:315-316.

Szafranski P, Schaaf CP, Person RE, Gibson IB, Xia Z, Mahadevan S *et al*. Structures and molecular mechanisms for common 15q13.3 microduplications involving CHRNA7: benign or pathological? *Hum Mutat* 2010;31:840-850.

Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ *et al*. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nat Genet* 2007;39:319-328.

Tabet AC, Pilorge M, Delorme R, Amsellem F, Pinard JM, Leboyer M *et al*. Autism multiplex family with 16p11.2p12.2 microduplication syndrome in monozygotic twins and distal 16p11.2 deletion in their brother. *Eur J Hum Genet* 2012;20:540-546.

Tai HC, Schuman EM. Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction. *Nat Rev Neurosci* 2008;9:826-838.

Tai HC, Schuman EM. Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction. *Nat Rev Neurosci* 2008;9:826-838.

Takanaga H, Tsuchida-Straeten N, Nishide K, Watanabe A, Aburatani H, Kondo T. Gli2 is a novel regulator of Sox2 expression in telencephalic neuroepithelial cells. *Stem Cells* 2009;27:165-174.

Takase K, Ohtsuki T, Migita O, Toru M, Inada T, Yamakawa-Kobayashi K *et al*. Association of *ZNF74* gene genotypes with age-at-onset of schizophrenia. *Schizophr Res* 2001;52:161-165.

Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, Heilbut A *et al*. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell* 2012;149:525-537.

Tamamaki N, Fujimori K, Nojyo Y, Kaneko T, Takauji R. Evidence that Sema3A and Sema3F regulate the migration of GABAergic neurons in the developing neocortex. *J Comp Neurol* 2003;455:238-248.

Tanaka M, Olsen RW, Medina MT, Schwartz E, Alonso ME, Duron RM *et al*. Hyperglycosylation and reduced GABA currents of mutated GABRB3 polypeptide in remitting childhood absence epilepsy. *Am J Hum Genet* 2008;82:1249-1261.

Tarabeux J, Kebir O, Gauthier J, Hamdan FF, Xiong L, Piton A *et al*. Rare mutations in N-methyl-D-aspartate glutamate receptors in autism spectrum disorders and schizophrenia. *Transl Psychiatry* 2011;1:e55.

Task Force on DSM-IV. Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR. edn 4. Washington, DC. American Psychiatric Association; 2000.

Tavazoie SF, Alvarez VA, Ridenour DA, Kwiatkowski DJ, Sabatini BL. Regulation of neuronal morphology and function by the tumor suppressors Tsc1 and Tsc2. *Nat Neurosci* 2005;8:1727-1734

Tay J, Goulet M, Rusche J, Boismenu R. Age-related and regional differences in secretin and secretin receptor mRNA levels in the rat brain. *Neurosci Lett* 2004;366:176-181.

Tep C, Kim ML, Opincariu LI, Limpert AS, Chan JR, Appel B *et al*. Brain-derived neurotrophic factor (BDNF) induces polarized signaling of small GTPase (Rac1) protein at the onset of Schwann cell myelination through partitioning-defective 3 (Par3) protein. *J Biol Chem* 2012;287:1600-1608.

Tepass U. FERM proteins in animal morphogenesis. *Curr Opin Genet Dev* 2009;19:357-367.

Thoeringer CK, Binder EB, Salyakina D, Erhardt A, Ising M, Unschuld PG *et al*. Association of a Met88Val diazepam binding inhibitor (DBI) gene polymorphism and anxiety disorders with panic attacks. *J Psychiatr Res* 2007;41:579-584.

Tichauer J, Saud K, von Bernhardi R. Modulation by astrocytes of microglial cell-mediated neuroinflammation: effect on the activation of microglial signaling pathways. *Neuroimmunomodulation* 2007;14:168-174.

Tirian L, Hlavanda E, Olah J, Horvath I, Orosz F, Szabó B *et al*. TPPP/p25 promotes tubulin assemblies and blocks mitotic spindle formation. *Proc Natl Acad Sci* 2003;100:13976-13981.

Tommerup N, Vissing H. Isolation and fine mapping of 16 novel human zinc finger-encoding cDNAs identify putative candidate genes for developmental and malignant disorders. *Genomics* 1995;27:259-264.

Tong C, Brion LP, Suarez C, Chesler M. Interstitial carbonic anhydrase (CA) activity in brain is attributable to membrane-bound CA type IV. *J Neurosci* 2000;20:8247-8253.

Toro R, Konyukh M, Delorme R, Leblond C, Chaste P, Fauchereau F *et al*. Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. *Trends Genet* 2010;26:363-372.

Torres AR, Maciulis A, Stubbs EG, Cutler A, Odell D. The transmission disequilibrium test suggests that HLA-DR4 and DR13 are linked to autism spectrum disorder. *Hum Immunol* 2002;63:311-316.

Tran TS, Rubio ME, Clem RL, Johnson D, Case L, Tessier-Lavigne M *et al*. Secreted Semaphorins control spine distribution and morphogenesis in the postnatal CNS. *Nature* 2009;462:1065-1069.

Trent S, Cassano T, Bedse G, Ojarikre OA, Humby T, Davies W. Altered serotonergic function may partially account for behavioral endophenotypes in steroid sulfatase-deficient mice. *Neuropsychopharmacology* 2012;37:1267-1274.

Trillingsgaard A, Østergaard J. Autism in Angelman syndrome: an exploration of comorbidity. *Autism* 2004;8;163-174.

Trioulier Y, Torch S, Blot B, Cristina N, Chatellard-Causse C, Verna JM *et al*. Alix, a protein regulating endosomal trafficking, is involved in neuronal death. *J Biol Chem* 2004;279:2046-2052.

Tsao CY, Mendell JR. Autistic disorder in 2 children with mitochondrial disorders. *J Child Neurol* 2007;22:1121-1123.

Tsetsenis T, Younts TJ, Chiub CQ, Kaesera PS, Castillob PE, Südhofa TC. Rab3B protein is required for long-term depression of hippocampal inhibitory synapses and for normal reversal learning. *Proc Natl Acad Sci USA* 2011;108:14300-14305.

Tsuchiya KJ, Hashimoto K, Iwata Y, Tsujii M, Sekine Y, Sugihara G *et al*. Decreased serum levels of platelet-endothelial adhesion molecule (PECAM-1) in subjects with high-functioning autism: a negative correlation with head circumference at birth. *Biol Psychiatry* 2007;62:1056-1058.

Tu JC, Xiao B, Naisbitt S, Yuan JP, Petralia RS, Brakeman P *et al*. Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* 1999;23:583-592.

Tu JC, Xiao B, Yuan JP, Lanahan AA, Leoffert K, Li M *et al*. Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. *Neuron* 1998;21:717-726.

Tuchman R, Rapin I. Epilepsy in autism. *Lancet Neurol* 2002;1:352-358.

Tudor M, Akbarian S, Chen RZ, Jaenisch R. Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. *Proc Natl Acad Sci* 2002;99:15536-15541.

Tunbridge EM, Harrison PJ, Weinberger DR. Catechol-O-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol Psychiatry* 2006;60:141-151.

Tuzun E, Sharp AJ, Bailey JA, Kaul R, Morrison VA, Pertz LM *et al*. Fine-scale structural variation of the human genome. *Nat Genet* 2005;37:727-732.

Uemura T, Mori H, Mishina M. Direct interaction of GluRdelta2 with Shank scaffold proteins in cerebellar Purkinje cells. *Mol Cell Neurosci* 2004;26:330-341.

Uetrecht AC, Bear JE. Coronins: the return of the crown. *Trends Cell Biol* 2006;16:421-426.

Ullmann R, Turner G, Kirchhoff M, Chen W, Tonge B, Rosenberg C *et al*. Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. *Hum Mutat* 2007;28:674-682.

Ulvestad E, Williams K, Bjerkvig R, Tiekotter K, Antel J, Matre R. Human microglial cells have phenotypic and functional characteristics in common with both macrophages and dendritic antigen-presenting cells. *J Leukoc Biol* 1994;56:732-740.

Utine GE, Kiper PO, Alanay Y, Haliloğlu G, Aktaş D, Boduroğlu K *et al*. Searching for copy number changes in nonsyndromic X-linked intellectual disability. *Mol Syndromol*. 2012;2:64-71

Vaags AK, Lionel AC, Sato D, Goodenberger M, Stein QP, Curran S *et al*. Rare deletions at the neurexin 3 locus in Autism spectrum disorder. *Am J Hum Genet* 2012;90:133-141.

Vadasz C, Saito M, Gyetvai BM, Oros M, Szakall I, Kovacs KM *et al*. Glutamate receptor metabotropic 7 is cis-regulated in the mouse brain and modulates alcohol drinking. *Genomics* 2007;90:690-702.

Vadasz C, Smiley JF, Figarsky K, Saito M, Toth R, Gyetvai BM *et al*. Mesencephalic dopamine neuron number and tyrosine hydroxylase content: Genetic control and candidate genes. *Neurosci* 2007;149:561-572.

Valjent E, Aubier B, Corbille AG, Brami-Cherrier K, Caboche J, Topilko P *et al*. Plasticity-associated gene *Krox24*/*Zif268* is required for long-lasting behavioral effectsofcocaine. *J Neurosci* 2006;26:4956-4960.

Valnegri P, Montrasio C, Brambilla D, Ko J, Passafaro M, Sala C. The X-linked intellectual disability protein IL1RAPL1 regulates excitatory synapse formation by binding PTPd and RhoGAP2. *Hum Mol Genet* 2011;20:4797-4809.

van Bon BW, Mefford HC, Menten B, Koolen DA, Sharp AJ, Nillesen WM *et al*. Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome. *J Med Genet* 2009;46:511-523.

van Bon BWM, Koolen DA, Brueton L, McMullan D, Lichtenbelt KD, Ades LC *et al*. Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome. *J Med Genet* 2009;46:511-523.

Van der Aa N, Rooms L, Vandeweyer G, van den Ende J, Reyniers E, Fichera M *et al*. Fourteen new cases contribute to the characterization of the 7q11.23 microduplication syndrome. *Eur J Med Genet* 2009;52:94-100.

van der Zwaag B, Staal WG, Hochstenbach R, Poot M, Spierenburg HA, de Jonge MV *et al*. A co-segregating microduplication of chromosome 15q11.2 pinpoints two risk genes for autism spectrum disorder. *Am J Med Genet B Neuropsychiatr Genet* 2010;153B:960-966.

van Kuilenburg AB, Meijer J, Mul AN, Hennekam RC, Hoovers JM, de Die-Smulders CE *et al*. Analysis of severely affected patients with dihydropyrimidine dehydrogenase deficiency reveals large intragenic rearrangements of DPYD and a *de novo* interstitial deletion del(1)(p13.3p21.3). *Hum Genet* 2009;125:581-590.

Vandewalle J, Van Esch H, Govaerts K, Verbeeck J, Zweier C, Madrigal I *et al*. Dosage-dependent severity of the phenotype in patients with mental retardation due to a recurrent copy-number gain at Xq28 mediated by an unusual recombination. *Am J Hum Genet* 2009;85:809-822.

Vandeweyer G, Van der Aa N, Reyniers E, Kooy RF. The contribution of *CLIP2* haploinsufficiency to the clinical manifestations of the Williams-Beuren syndrome. *Am J Hum Genet* 2012;90:1071-1078.

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol* 2005;57:67-81.

Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K *et al*. Neuroligins determine synapse maturation and function. *Neuron* 2006;51:741-754.

Vawter MP, Evans S, Choudary P, Tomita H, Meador-Woodruff J, Molnar M *et al*. Gender-specific gene expression in post-mortem human brain: localization to sex chromosomes. *Neuropsychopharmacology* 2004;29:373-384.

Veltman MW, Craig EE, Bolton PF. Autism spectrum disorders in Prader–Willi and Angelman syndromes: a systematic review. *Psychiatr Genet* 2005;15:243-254.

Venkateswarlu K. Interaction protein for Cytohesin Exchange Factors 1 (IPCEF1) binds Cytohesin 2 and modifies its activity. *J Biol Chem* 2003;278:43460-43469.

Veres TZ, Rochlitzer S, Braun A. The role of neuro-immune cross-talk in the regulation of inflammation and remodeling in asthma. *Pharmacol Ther*; 2009;122:203-14

Verhoeven K, De Jonghe P, Van de Putte T, Nelis E, Zwijsen A, Verpoorten N *et al*. Slowed conduction and thin myelination of peripheral nerves associated with mutant Rho guanine-nucleotide exchange factor 10. *Am J Hum Genet* 2003;73:926-932.

Vernier P, Moret F, Callier S, Snapyan M, Wersinger C, Sidhu A. The degeneration of dopamine neurons in Parkinson's disease: insights from embryology and evolution of the mesostriatocortical system. *Ann N Y Acad Sci* 2004;1035:231-249.

Vidal CN, Nicolson R, DeVito TJ, Hayashi KM, Geaga JA, Drost DJ *et al*. Mapping corpus callosum deficits in autism: an index of aberrant cortical connectivity. *Biol Psychiatry* 2006;60:218-225.

Vinci G, Chantot-Bastaraud S, El Houate B, Lortat-Jacob S, Brauner R, McElreavey K. Association of deletion 9p, 46,XY gonadal dysgenesis and autistic spectrum disorder. *Mol Hum Reprod* 2007;13:685-689.

Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S *et al*. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 2011;474:380-384.

Vojdani A, Campbell AW, Anyanwu E, Kashanian A, Bock K, Vojdani E. Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, Chlamydia pneumoniae and Streptococcus group A. *J Neuroimmunol* 2002;129:168-177.

Volkow ND, Fowler JS, Wang GJ, Swanson JM, Telang F. Dopamine in drug abuse and addiction: results of imaging studies and treatment implications. *Arch Neurol* 2007;64:1575-1579.

Volonte MA, Moresco RM, Gobbo C, Messa C, Carpinelli A, Rizzo G *et al*. A PET study with [11-C]raclopride in Parkinson's disease: preliminary results on the effect of amantadine on the dopaminergic system. *Neurol Sci* 2001;22:107-108.

von der Brelie C, Waltereit R, Zhang L, Beck H, Kirschstein T. Impaired synaptic plasticity in a rat model of tuberous sclerosis. *Eur J Neurosci* 2006;23:686-692.

Vorstman JA, Morcus ME, Duijff SN, Klaassen PW, Heineman-de Boer JA, Beemer FA *et al*. The 22q11.2 deletion in children: high rate of autistic disorders and early onset of psychotic symptoms. *J Am Acad Child Adolesc Psychiatry* 2006;45:1104-1113.

Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol Psychiatry* 2006;11:18-28.

Vrijenhoek T, Buizer-Voskamp JE, van der Stelt I, Strengman E; Genetic Risk and Outcome in Psychosis (GROUP) Consortium, Sabatti C *et al*. Recurrent CNVs disrupt three candidate genes in schizophrenia patients. *Am J Hum Genet* 2008;83:504-510.

Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM *et al*. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* 2008;320:539-543.

Wang H, Duan S, Du J, Li X, Xu Y, Zhang Z *et al*. Transmission disequilibrium test provides evidence of association between promoter polymorphisms in 22q11 gene *DGCR14* and schizophrenia. *J Neural Transm* 2006;113:1551-1161.

Wang J, Cao L, Li X, Hu Z, Li J, Zhang J *et al*. Identification of *PRRT2* as the causative gene of paroxysmal kinesigenic dyskinesias. *Brain* 2011;134:3490-3498.

Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS *et al*. Common genetic variants on 5p14.1 associate with autism spectrum disorders. *Nature* 2009;459:528-533.

Wang LW, Berry-Kravis E, Hagerman RJ. Fragile X: leading the way for targeted treatments in autism. *Neurotherapeutics* 2010;7:264-274.

Wang T, Bray SM, Warren ST. New perspectives on the biology of fragile X syndrome. *Curr Opin Genet Dev* 2012;22:256-263.

Wang T, Chiu H, Chang Y, Hsu T, Chiu I, Chen L. The adaptor protein SH2B3 (Lnk) negatively regulates neurite outgrowth of PC12 cells and cortical neurons. *Plos One* 2011;6:e26433.

Wang X, Wang T, Yuan MX, Liu MG, Wang Q. Advances in the studies on the molecular and genetic aspects of epilepsy. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao.* 2005;27:388-393.

Wang Y, Kakinuma N, Zhu Y, Kiyama R. Nucleo-cytoplasmic shuttling of human Kank protein accompanies intracellular translocation of beta-catenin. *J Cell Sci* 2006;119:4002-4010.

Wang Y, Su B, Xia Z. Brain-derived neurotrophic factor activates ERK5 in cortical neurons via a Rap1-MEKK2 signaling cascade. *J Biol Chem* 2006;281:35965-35974.

Wang YF, Du XZ. Hira gene and development: from yeast to human. *Yi Chuan* 2005;27:989-994.

Wang Z, Xu L, Zhu X, Cui W, Sun Y, Nishijo H *et al*. Demethylation of specific Wnt/β-catenin pathway genes and its upregulation in rat brain induced by prenatal valproate exposure. *Anat Rec* 2010;293:1947-1953.

Washizuka S, Iwamoto K, Kazuno AA, Kakiuchi C, Mori K, Kametani M *et al*. Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with bipolar disorder in Japanese and the National Institute of Mental Health pedigrees. *Biol Psychiatry* 2004;56:483-489.

Washizuka S, Kametani M, Sasaki T, Tochigi M, Umekage T, Kohda K *et al*. Association of mitochondrial complex I subunit gene *NDUFV2* at 18p11 with schizophrenia in the Japanese population. *Am J Med Genet B Neuropsychiatr Genet* 2006;141B:301-304.

Wassink TH, Epping EA, Rudd D, Axelsen M, Ziebell S, Fleming FW *et al*. Influence of ZNF804a on brain structure volumes and symptom severity in individuals with schizophrenia. *Arch Gen Psychiatry* 2012;69:885-892.

Wassink TH, Piven J, Patil SR. Chromosomal abnormalities in a clinic sample of individuals with autistic disorder. *Psychiatric Genet* 2001;11:57-63.

Watanabe T, Noritake J, Kakeno M, Matsui T, Harada T, Wang S *et al*. Phosphorylation of CLASP2 by GSK-3b regulates its interaction with IQGAP1, EB1 and microtubules. *J Cell Sci* 2009;122:2969-2979.

Wawrzak D, Métioui M, Willems E, Hendrickx M, de Genst E, Leyns L. Wnt3a binds to several sFRPs in the nanomolar range. *Biochem Biophys Res Commun* 2007;357:1119-1123.

Wei H, Malik M, Sheikh AM, Merz G, Ted Brown W, Li X. Abnormal cell properties and down-regulated FAK-Src complex signaling in B lymphoblasts of autistic subjects. *Am J Pathol* 2011;179:66-74.

Weiss LA, Arking DE, Gene Discovery Project of Johns Hopkins & the Autism Consortium, Daly MJ, Chakravarti A. A genome-wide linkage and association scan reveals novel loci for autism. *Nature* 2009;461:802-808.

Weiss LA, Escayg A, Kearney JA, Trudeau M, MacDonald BT, Mori M *et al*. Sodium channels SCN1A, SCN2A and SCN3A in familial autism. *Mol Psychiatry* 2003;8:186-194.

Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R *et al*. Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med* 2008;358:667-675.

Weitzdörfer R, Höger H, Shim KS, Cekici L, Pollak A, Lubec G. Changes of hippocampal signaling protein levels during postnatal brain development in the rat. *Hippocampus* 2008;18:807-813.

Wendeler MW, Paccaud JP, Hauri HP. Role of Sec24 isoforms in selective export of membrane proteins from the endoplasmic reticulum. *EMBO Rep* 2007;8:258-264.

Weng MK, Zimmer B, Pöltl D, Broeg MP, Ivanova V, Gaspar JA *et al*. Extensive transcriptional regulation of chromatin modifiers during human neurodevelopment. *Plos One* 2012;7:e36708.

Widera D, Mikenberg I, Elvers M, Kaltschmidt C, Kaltschmidt B. Tumor necrosis factor alpha triggers proliferation of adult neural stem cells via IKK/NFkappaB signaling. *BMC Neurosci* 2006;7:64.

Wieczorek L, Maas JW Jr, Muglia LM, Vogt SK, Muglia LJ. Temporal and regional regulation of gene expression by calcium-stimulated adenylyl cyclase activity during fear memory. *Plos One* 2010;5:e13385.

Wigge P, McMahon HT. The amphiphysin family of proteins and their role in endocytosis at the synapse. *Trends Neurosci* 1998;21:339-344.

Wilkinson MB, Dias C, Magida J, Mazei-Robison M, Lobo MK, Kennedy P *et al*. A novel role of the WNT-Dishevelled-GSK3 signaling cascade in the mouse nucleus accumbens in a social defeat model of depression. *J Neurosci* 2011;31:9084-9092.

Wilkinson MB, Xiao G, Kumar A, LaPlant Q, Renthal W, Sikder D *et al*. Imipramine treatment and resiliency exhibit similar chromatin regulation in the mouse nucleus accumbens in depression models. *J Neurosci* 2009;29:7820-7832.

Willatt L, Cox J, Barber J, Cabanas ED, Collins A, Donnai D *et al*. 3q29 microdeletion syndrome: clinical and molecular characterization of a new syndrome. *Am J Hum Genet* 2005,77:154-160.

Williams JM, Beck TF, Pearson DM, Proud MB, Cheung SW, Scott DA. A 1q42 deletion involving *DISC1*, *DISC2*, and *TSNAX* in an autism spectrum disorder. *Am J Med Genet A* 2009;149A:1758-1762.

Williams NM, Franke B, Mick E, Anney RJ, Freitag CM, Gill M *et al*. Genome-wide analysis of copy number variants in attention deficit hyperactivity disorder: the role of rare variants and duplications at 15q13.3. *Am J Psychiatry* 2012;169:195-204.

Williams NM, Glaser B, Norton N, Williams H, Pierce T, Moskvina V *et al*. Strong evidence that *GNB1L* is associated with schizophrenia. *Hum Mol Genet* 2008;17:555-566.

Willis-Owen SA, Shifman S, Copley RR, Flint J. *DCNP1*: a novel candidate gene for major depression. *Mol Psychiatry* 2006;11:121-122.

Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral DG, Van de Water J. Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders. *Brain Behav Immun* 2009;23:64-74.

Wills S, Rossi CC, Bennett J, Martinez-Cerdeno V, Ashwood P, Amaral DG *et al*. Further characterization of autoantibodies to GABAergic neurons in the central nervous system produced by a subset of children with autism. *Mol Autism* 2011;2:5.

Wills ZP, Mandel-Brehm C, Mardinly AR, McCord AE, Giger RJ, Greenberg ME. The nogo receptor family restricts synapse number in the developing hippocampus. *Neuron* 2012;73:466-481.

Wishart TM, Rooney TM, Lamont DJ, Wright AK, Morton AJ, Jackson M *et al*. Combining comparative proteomics and molecular genetics uncovers regulators of synaptic and axonal stability and degeneration in vivo. *Plos Genet* 2012;8:e1002936.

Wisniowiecka-Kowalnik B, Nesteruk M, Peters SU, Xia Z, Cooper ML, Savage S *et al*. Intragenic rearrangements in *NRXN1* in three families with autism spectrum disorder, developmental delay, and speech delay. *Am J Med Genet B Neuropsychiatr Genet* 2010;153B:983-993.

Wissmann M, Yin N, Müller JM, Greschik H, Fodor BD, Jenuwein T *et al*. Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. *Nat Cell Biol* 2007;9:347-353.

Wittmann T, Waterman-Storer CM. Spatial regulation of CLASP affinity for microtubules by Rac1 and GSK3b in migrating epithelial cells. *J Cell Biol* 2005;169:929-939.

Wiznitzer M. Autism and tuberous sclerosis. *J Child Neurol* 2004;19:675-679.

Wolburg H, Wolburg-Buchholz K, Kraus J, Rascher-Eggstein G, Liebner S, Hamm S *et al*. Localization of claudin-3 in tight junctions of the blood-brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme. *Acta Neuropathol* 2003;105:586-592.

Wong EV, Schaefer AW, Landreth G, Lemmon V. Casein kinase II phosphorylates the neural cell adhesion molecule L1. *J Neurochem* 1996;66:779-786.

Wong KK, deLeeuw RJ, Dosanjh NS, Kimm LR, Cheng Z, Horsman DE *et al*. A comprehensive analysis of common copy-number variations in the human genome. *Am J Hum Genet* 2007;80:91-104.

Wong ML, Whelan F, Deloukas P, Whittaker P, Delgado M, Cantor RM *et al*. Phosphodiesterase genes are associated with susceptibility to major depression and antidepressant treatment response. *Proc Natl Acad Sci USA* 2006;103:15124-15129.

Wong V. Study of the relationship between tuberous sclerosis complex and autistic disorder. *J Child Neurol* 2006;21:199-204.

Wu N, Zhang X, Jin S, Liu S, Ju G, Wang Z *et al*. A weak association of the CLDN5 locus with schizophrenia in Chinese case-control samples. *Psychiatry Res* 2010;178:223.

Wu Q, Tommerup N, Ming Wang S, Hansen L. A novel primate specific gene, *CEI*, is located in the homeobox gene IRXA2 promoter in Homo sapiens. *Gene* 2006;371:167-173.

Wuyts W, Roland D, Lüdecke HJ, Wauters J, Foulon M, Van Hul W *et al*. Multiple exostoses, mental retardation, hypertrichosis, and brain abnormalities in a boy with a *de novo* 8q24 submicroscopic interstitial deletion. *Am J Med Genet A* 2002;113A:326-332.

Xu B, Roos JL, Dexheimer P, Boone B, Plummer B, Levy S *et al*. Exome sequencing supports a *de novo* mutational paradigm for schizophrenia. *Nat Genet* 2011;43:864-868.

Xu J, Burgoyne PS, Arnold PA. Sex differences in sex chromosome gene expression in mouse brain. *Hum Mol Genet* 2002;11:1409-1419.

Yamamoto T, Yamashita A, Yamada K, Hata R. Immunohistochemical localization of chemokine CXCL14 in rat hypothalamic neurons. *Neurosci Lett* 2011;487:335-340.

Yan J, Noltner K, Feng J, Li W, Schroer R, Skinner C *et al*. Neurexin 1alpha structural variants associated with autism. *Neurosci Lett* 2008;438:368-370.

Yang K, Cao F, Sheikh AM, Malik M, Wen G, Wei H *et al*. Up-regulation of Ras/Raf/ERK1/2 signaling impairs cultured neuronal cell migration, neurogenesis, synapse formation, and dendritic spine development. *Brain Struct Funct* 2012 May 5. [Epub ahead of print]

Yang K, Sheikh AM, Malik M, Wen G, Zou H, Brown WT *et al*. Upregulation of Ras/Raf/ERK1/2 signaling and ERK5 in the brain of autistic subjects. *Genes Brain Behav* 2011;10:834-843.

Yang P, Shu BC, Hallmayer JF, Lung FW. Intronic single nucleotide polymorphisms of engrailed homeobox 2 modulate the disease vulnerability of autism in a han chinese population. *Neuropsychobiology* 2010;62:104-115.

Yang Y, Chung EK, Wu YL, Savelli SL, Nagaraja HN, Zhou B *et al*. Gene copy-number variation and associated polymorphisms of complement component C4 in human systemic lupus erythematosus (SLE): low copy number is a risk factor for and high copy number is a protective factor against SLE susceptibility in European Americans. *Am J Hum Genet* 2007;80:1037-1054.

Yang Y, Hwang CK, D'Souza UM, Lee SH, Junn E, Mouradian MM. Three-amino acid extension loop homeodomain proteins Meis2 and TGIF differentially regulate transcription. *J Biol Chem* 2000;275:20734-20741.

Yasui DH, Peddada S, Bieda MC, Vallero RO, Hogart A, Nagarajan RP *et al*. Integrated epigenomic analyses of neuronal MeCP2 reveal a role for long-range interaction with active genes. *Proc Natl Acad Sci* 2007;104:19416-19421.

Yatsenko SA, Hixson P, Roney EK, Scott DA, Schaaf CP, Ng YT *et al*. Human subtelomeric copy number gains suggest a DNA replication mechanism for formation: beyond breakage-fusion-bridge for telomere stabilization. *Hum Genet* 2012;131:1895-1910.

Yi JJ, Ehlers MD. Emerging roles for ubiquitin and protein degradation in neuronal function. *Pharmacol Rev* 2007;59:14-39.

Yokota Y, Ring C, Cheung R, Pevny L, Anton ES. Nap1-regulated neuronal cytoskeletal dynamics is essential for the final differentiation of neurons in cerebral cortex. *Neuron* 2007;54:429-445.

Yonan AL, Alarcón M, Cheng R, Magnusson PK, Spence SJ, Palmer AA *et al*. A genomewide screen of 345 families for autism-susceptibility loci. *Am J Hum Genet* 2003;73:886-897.

Yoo HJ, Cho IH, Park M, Yang SY, Kim SA. Family based association of *GRIN2A* and *GRIN2B* with Korean autism spectrum disorders. *Neurosci Lett* 2012;512:89-93.

Yoshida K, Watanabe M, Kato H, Dutta A, Sugano S. BH-protocadherin-c, a member of the cadherin superfamily, interacts with protein phosphatase 1 alpha through its intracellular domain. *FEBS Lett* 1999;460:93-98.

Yoshida T, Yasumura M, Uemura T, Lee SJ, Ra M, Taguchi R *et al*. IL-1 receptor accessory protein-like 1 associated with mental retardation and autism mediates synapse formation by trans-synaptic interaction with protein tyrosine phosphatase δ. *J Neurosci* 2011;31:13485-13499.

Yoshimura K, Kitagawa H, Fujiki R, Tanabe M, Takezawa S, Takada I *et al*. Distinct function of 2 chromatin remodeling complexes that share a common subunit, Williams syndrome transcription factor (WSTF). *Proc Natl Acad Sci USA* 2009;106:9280-9285.

Young EJ, Lipina T, Tam E, Mandel A, Clapcote SJ, Bechard AR *et al*. Reduced fear and aggression and altered serotonin metabolism in Gtf2ird1-targeted mice. *Genes Brain Behav* 2008;7:224-234.

Young FB, Butland SL, Sanders SS, Sutton LM, Hayden MR. Putting proteins in their place: palmitoylation in Huntington disease and other neuropsychiatric diseases. *Prog Neurobiol* 2012;97:220-238.

Yu JH, Crevenna AH, Bettenbühl M, Freisinger T, Wedlich-Söldner R. Cortical actin dynamics driven by formins and myosin V. *J Cell Sci* 2011;124:1533-1541.

Yuan X, Yao J, Norris D, Tran DD, Bram RJ, Chen G *et al*. Calcium-modulating cyclophilin ligand regulates membrane trafficking of postsynaptic GABA(A) receptors. *Mol Cell Neurosci* 2008;38:277-289.

Yung WH, Leung PS, Ng SS, Zhang J, Chan SC, Chow BK. Secretin facilitates GABA transmission in the cerebellum. *J Neurosci* 2001;21:7063-7068.

Yuzaki M. Synapse formation and maintenance by C1q family proteins: a new class of secreted synapse organizers. *Eur J Neurosci* 2010;32:191-197.

Zaheer S, Wu Y, Bassett J, Yang B, Zaheer A. Glia maturation factor regulation of STAT expression: a novel mechanism in experimental autoimmune encephalomyelitis. *Neurochem Res* 2007;32:2123-2131.

Zappella M, Meloni I, Longo I, Canitano R, Hayek G, Rosaia L *et al*. Study of *MECP2* gene in Rett syndrome variants and autistic girls. *Am J Med Genet B Neuropsychiatr Genet* 2003;119:102-107.

Zearfoss NR, Alarcon JM, Trifilieff P, Kandel E, Richter JD. A molecular circuit composed of CPEB-1 and c-Jun controls growth hormone-mediated synaptic plasticity in the mouse hippocampus. *J Neurosci* 2008;28:8502-8509.

Zeis T, Graumann U, Reynolds R, Schaeren-Wiemers N. Normal-appearing white matter in multiple sclerosis is in a subtle balance between inflammation and neuroprotection. *Brain* 2008;131:288-303.

Zhang C, Milunsky JM, Newton S, Ko J, Zhao G, Maher TA *et al*. A neuroligin-4 missense mutation associated with autism impairs neuroligin-4 folding and endoplasmic reticulum export. *J Neurosci* 2009;29:10843-10854.

Zhang D, Yoon HG, Wong J. JMJD2A is a novel N-CoR-interacting protein and is involved in repression of the human transcription factor achaete scute-like homologue 2 (ASCL2/Hash2). *Mol Cell Biol* 2005;25:6404-6414.

Zhang K, Smouse D, Perrimon N. The crooked neck gene of Drosophila contains a motif found in a family of yeast cell cycle genes. *Genes Dev* 1991;5:1080-1091.

Zhang W, Savelieva KV, Suwanichkul A, Small DL, Kirkpatrick LL, Xu N *et al*. Transmembrane and ubiquitin-like domain containing 1 (Tmub1) regulates locomotor activity and wakefulness in mice and interacts with CAMLG. *Plos One* 2010;5:e11261.

Zhang Y, Liu W, Ma C, Geng J, Li Y, Li S *et al*. Endoplasmic reticulum stress contributes to CRH-induced hippocampal neuron apoptosis. *Exp Cell Res* 2012;318:732-740.

Zhang Y, Sun Y, Wang F, Wang Z, Peng Y, Li R. Downregulating the canonical Wnt/β-catenin signaling pathway attenuates the susceptibility to autism-like phenotypes by decreasing oxidative stress. *Neurochem Res* 2012;37:1409-1419.

Zhao C, Aviles C, Abel RA, Almli CR, McQuillen P, Pleasure SJ. Hippocampal and visuospatial learning defects in mice with a deletion of frizzled 9, a gene in the Williams syndrome deletion interval. *Development* 2005;132:2917-2927.

Zhao Q, Li T, Zhao X, Huang K, Wang T, Li Z *et al*. Rare CNVs and Tag SNPs at 15q11.2 are associated with schizophrenia in the Han Chinese population. *Schizophr Bull* 2012 Feb 8. [Epub ahead of print].

Zhao X, Leotta A, Kustanovich V, Lajonchere C, Geschwind DH, Law K *et al*. A unified genetic theory for sporadic and inherited autism. *Proc Natl Acad Sci USA* 2007;104:12831-12836.

Zhao X, Ren W, Yang W, Wang Y, Kong H, Wang L *et al*. Wnt pathway is involved in pleomorphic adenomas induced by overexpression of PLAG1 in transgenic mice. *Int J Cancer* 2006;118:643-648.

Zhao X, Ueba T, Christie BR, Barkho B, McConnell MJ, Nakashima K *et al*. Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. *Proc Natl Acad Sci USA* 2003;100:6777-6782.

Zheng F, Wang L, Jia M, Yue W, Ruan Y, Lu T *et al*. Evidence for association between disrupted-inschizophrenia 1 (*DISC1*) gene polymorphisms and autism in Chinese Han population: a family-based association study. *Behav Brain Funct* 2011,7:14.

Zhou RM, Jing YY, Guo Y, Gao C, Zhang BY, Chen C *et al*. Molecular interaction of TPPP with PrP antagonized the CytoPrP-induced disruption of microtubule structures and cytotoxicity. *Plos One* 2011;6:e23079.

Zhou T, Wang S, Ren H, Qi XR, Luchetti S, Kamphuis W *et al*. Dendritic cell nuclear protein-1, a novel depression-related protein, upregulates corticotropin-releasing hormone expression. *Brain* 2010;133:3069-3079.

Zhou Y, Sonobe Y, Akahori T, Jin S, Kawanokuchi J, Noda M *et al*. IL-9 promotes Th17 cell migration into the central nervous system via CC chemokine ligand-20 produced by astrocytes. *J Immunol* 2011;186:4415-4421.

Zimmerman AW, Connors SL, Matteson KJ, Lee LC, Singer HS, Castaneda JA *et al*. Maternal antibrain antibodies in autism. *Brain Behav Immun* 2007;21:351-357.

Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N *et al*. Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat Neurosci* 2006;9:268-275.

Ziv Y, Schwartz M. Immune-based regulation of adult neurogenesis: implications for learning and memory. *Brain Behav Immun* 2008;22:167-176.

Zoghbi HY, Bear MF. Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. *Cold Spring Harb Perspect Biol* 2012;4(3).

Zori RT, Marsh DJ, Graham GE, Marliss EB, Eng C. Germline *PTEN* mutation in a family with Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. *Am J Med Genet* 1998;80:399-402.

Zou H, Yu Y, Sheikh AM, Malik M, Yang K, Wen G *et al*. Association of upregulated Ras/Raf/ERK1/2 signaling with autism. *Genes Brain Behav* 2011;10:615-624.

Zschocke J, Manthey D, Bayatti N, van der Burg B, Goodenough S, Behl C. Estrogen receptor alpha-mediated silencing of caveolin gene expression in neuronal cells. *J Biol Chem* 2002;277:38772-38780.

Zunner D, Deschermeier C, Kornau HC. GABA(B) receptor subunit 1 binds to proteins affected in 22q11 deletion syndrome. *Biochem Biophys Res Commun* 2010;393:185-189.

Zweier C, de Jong EK, Zweier M, Orrico A, Ousager LB, Collins AL *et al*. *CNTNAP2* and *NRXN1* are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in Drosophila. *Am J Hum Genet* 2009;85:655-666.

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