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Abstract

Context

Comparative genomic hybridization (array-CGH) studies have suggested that rare copy number variations (CNVs) at numerous loci are involved in the etiology of mental retardation (MR), autism spectrum disorders (ASD) and schizophrenia.

Objective

The goal of the present paper was (i) to provide an estimate of the collective frequency of a set of recurrent/overlapping CNVs in three different groups of patients as compared with healthy controls and (ii) to assess whether each CNV is present in more than one clinical category.

Design, setting and population

We have investigated 28 candidate loci previously identified by array-CGH studies for gene dosage alteration in 247 subjects with MR, 260 with ASD, 236 with schizophrenia or schizoaffective disorder and 236 healthy controls.

Main outcome measures

Collective and individual frequency of the analyzed CNVs in patients as compared with controls.

Results

Recurrent or overlapping CNVs were found in patients at 40% of the selected loci. We show that the collective frequency of CNVs at these loci is significantly increased in autistic patients, patients with schizophrenia and patients with MR as compared with controls (p= 0.005, p< 0.001 and p= 0.001 respectively, Fisher exact test). Individual significance (p= 0.02) was reached for association between autism and a 350 kb deletion located in 22q11 and spanning the PRODH gene.

Conclusions

These results support the hypothesis that weakly to moderately recurrent CNVs, either transmitted or occurring de novo , are causing or contributory factors for these diseases. Second, we show that most of these CNVs, which contain genes involved in neurotransmission or synapse formation and maintenance, are present in the 3 pathological conditions, supporting the existence of shared biological pathways between these neurodevelopmental disorders.

MESH Keywords Adolescent ; Adult ; Autistic Disorder ; diagnosis ; genetics ; Case-Control Studies ; Chromosome Mapping ; statistics & numerical data ; Comparative Genomic Hybridization ; statistics & numerical data ; Female ; Gene Dosage ; genetics ; Gene Frequency ; Genotype ; Humans ; In Situ Hybridization, Fluorescence ; statistics & numerical data ; Male ; Mental Retardation ; diagnosis ; genetics ; Neurogenesis ; Oligonucleotide Array Sequence Analysis ; Proline ; blood ; Psychotic Disorders ; diagnosis ; genetics ; Schizophrenia ; diagnosis ; genetics

The development of microarray based technologies for comparative genomic hybridization (array-CGH) analysis has enabled the detection of submicroscopic microdeletions or microduplications also referred as copy number variations (CNVs). In the past months, this approach has been widely used in neurological and psychiatric disorders including mental retardation (MR)1 –3 , autism spectrum disorders (ASD)4 –7 and schizophrenia8 –11 . These studies have suggested that several genes involved in similar neurodevelopmental pathways may be associated with these conditions. However, so far only rare structural variants, sometimes present in a single patient have been identified. It is therefore difficult to decipher which of these variations are causative, which are risk factors and which are only rare polymorphisms unrelated to any pathological phenotype. It is usually considered that de novo rearrangements are likely to be pathogenic, but this argument which is acceptable for rare large rearrangements detectable by conventional cytogenetics, should be considered with caution for smaller CNVs, for which a very high mutation rate is expected. Indeed, it has been estimated that one de novo segmental deletion occurs in one per eight newborns and one segmental duplication in one per 50 newborns, most of these rearrangements being benign polymorphic variants12 . Therefore, the disease association of CNVs has to be tested systematically by comparing the frequency of each candidate CNV in patients and controls. Given the very low frequency of each CNV, this would require the study of huge series, only achievable in the context of forthcoming meta-analyses. Another problem arises from the fact that the ascertainment of most of the published samples, initially recruited for linkage studies, is biased toward multiplex cases and that control samples, when present, are generally composed of subjects not screened for the studied pathologies. The goal of the present paper was (i) to provide an estimate of the collective frequency of a set of recurrent/overlapping CNVs in three different groups of patients as compared with healthy controls (ii) to assess whether each CNV is present in more than one clinical category.

Material and Methods

Ascertainment and diagnoses

Patients with schizophrenia and subjects with mental retardation were ascertained at Rouen Hospital from consecutive hospitalizations in patients with schizophrenia, or from consecutive referral for phenotypic and genetic investigations in subjects with intellectual disability. The ASD sample included patients ascertained from consecutive consultations in four units specialized in autism diagnosis and evaluation located at Rouen, Dijon, Tours (France) and Messina (Italy) and patients directly referred by the French Autism Foundation. Controls, all ascertained at Rouen, were screened with a standardized data sheet derived from the SADS-LA 13 and were required to be free of any psychotic disorder or MR in themselves or in their first degree relatives. All psychiatric diagnoses were established according to DSM4 criteria following review of case notes and direct examination of subjects. The schedule for affective disorder and schizophrenia (SADS-LA) 13 was used for the clinical assessment of all patients with schizophrenia or schizoaffective disorder, the Autism Diagnostic Interview-Revised (ADI-R) 14 , the Autism Diagnostic Observation Schedule-general (ADOS-G) 15 or the Childhood Autism Rating Scale (CARS)16 were used for 83% of ASD patients (100% of ASD patients with CNVs). IQs were evaluated using standardized neuropsychological tests i.e. validated mental age-appropriate Weschler scales (WPPSI, WISC or WAIS).

The schizophrenia group included 189 subjects with a diagnosis of schizophrenia and 47 with a diagnosis of schizoaffective disorder. Post morbid IQs were available for 2/3 of patients with schizophrenia; 18% of these subjects had an IQ< 70. The ASD group included 257 patients with autism and 3 with Asperger syndrome. The MR group included 12 patients with developmental language disorder. All MR patients and 2/3 of ASD patients were examined by an experienced clinical geneticist and were also screened for fragile X mutation and karyotype abnormalities. Subjects with large chromosomal anomalies, fragile X syndrome or other established syndromes were not included. Subjects with common environmental etiologies of MR such as fetal alcohol syndrome or birth complications were also excluded. Additional clinical features including intrauterine or postnatal growth retardation, dysmorphic features or malformations were present in 8.5 % of ASD patients and 62 % of subjects with MR. Demographic characteristics of the sample, including a total of 979 Caucasian subjects from France or Italy, are summarized in Table 1 .

Blood samples were drawn, after written informed consent, from all included subjects and whenever possible from parents and affected relatives of patients. Ethics committee approval was obtained from all regions where families were recruited.

Candidate genes and QMPSF analysis

A medline search with the words CNV, schizophrenia, autism and mental retardation allowed us to select non exhaustively a set of 28 loci with microrearrangements characterized by prior array-CGH analyses, often in a single patient. This set included major candidate CNV loci identified in ASD and SZ before April 2008 as well as 8 functionally related CNV loci identified in MR (Table 2). Each locus contained generally a single disease-associated CNV, but in some cases overlapping CNVs with different boundaries had been described in patients. The gene content of these loci ranged from 1 to 28. At each locus at least one candidate gene had been previously suggested in the seminal reports and was retained for the present analysis. Functionally, most of these candidate genes can be classified in two main categories related to synapse formation and maintenance or neurotransmission.

Copy number variation at each locus was assessed by quantitative multiplex PCR of short fluorescent fragments (QMPSF), a method based on the simultaneous amplification of several short genomic fragments under quantitative conditions 17 . For each locus, amplicons were designed in the coding sequence of selected candidate genes. All assays were grouped in three multiplex PCR experiments including 10 short genomic fragments (range 101–301bp) each. Primer sequences and PCR conditions are summarized in supplementary Table S1. DNA fragments generated by QMPSF were separated on an ABI Prism 3100 sequencer (Applied Biosystem) and the resulting fluorescence profiles were analyzed using the Gene Scan 3.7 Software (PE Applied Biosystems). For each patient, the QMPSF profile was superimposed to that generated from a reference subject by adjusting to the same level the peak obtained for a control amplicon corresponding to a short exonic fragment of the PBGD gene. When a copy number variation was detected, further analyses aiming to confirm and delineate the size of the rearrangements were performed using additional dedicated QMPSF assays (supplementary figure S1), array-CGH and/or fluorescence in situ hybridization (FISH) analyses.

Agilent 44K array

Oligonucleotide array-CGH was performed using the Agilent Human Genome CGH microarray 4x44K (Agilent Technologies, Santa Clara, CA, USA). This array contains 44 290 60-mer oligonucleotide probes covering the whole genome with an average spatial resolution of ≈ 30–35 kb. 84% of the probes reside in intragenic regions, and over 30 000 genes are each represented by at least one probe. All experiments were performed according to version 4.0 (June 2006) of the protocol provided by Agilent (“Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis”).

Fluorescence in situ hybridization (FISH)

FISH analyses were performed on metaphase spreads obtained from peripheral lymphocytes from the patients. Selected Human genomic BAC clones were obtained from the BACPAC resources center (http://bacpac.chori.org).

DNA sequencing and paternity checking

Sequence analysis of the coding exons of the PRODH gene was performed using primers and PCR conditions previously described 18 on an Applied Biosystem model 3100 automated sequencer (PE Applied Biosystem). Paternity was checked by microsatellite typing.

Determination of plasma proline level

In patients, plasma proline levels were determined after overnight fasting. All samples were analyzed using ion exchange chromatography on a BIOTRONIK LC 3000 system.

Statistical analysis

Categorical variables were compared by Fisher's exact test. Two hypotheses were tested. First, the distribution of the collective set of recurrent/overlapping CNVs found in each group of patients was compared with that found in controls (3 tests). Second, the distribution of each recurrent/overlapping CNV present in our population was compared between each disease group and controls (33 tests). P-values were reported without Bonferroni's correction.

Results

CNVs in the control group

Among the 28 loci selected for this study (Table 2), only one CNV identical to a previously described disease-associated one (i.e. a 22q11 350kb deletion spanning the PRODH and DGCR6 genes)19 was detected in the control group. This deletion, present in a single control subject, had a low frequency (1/236)similar to that previously reported in Japanese 20 and Canadian 21 populations. A common CNV reciprocal to the expected one (i.e. a 350 kb duplication) was also found at the same locus in 6/236 controls. This CNV, also present in 7/236 patients with schizophrenia, 4/260 ASD patients and 9/247 MR patients was clearly a benign polymorphism. Another overlapping reciprocal CNV (i.e. a 490 kb duplication) was detected at the CHRNA7 locus located on chromosome 15q13.3. This CNV present in 2/236 controls, 1/236 patients with schizophrenia, 1/260 ASD patients and 1/247 MR was equally unrelated to any pathological condition. (Supplementary Table S2)

Diseases associated CNVs

The proportion of recurrent/overlapping CNVs identified among the 28 selected loci in the present sample totaling 743 affected subjects was 11/28 (40 %). Their collective frequency was 10/236 (4 %) in patients with schizophrenia, 16/260 (6 %) in ASD patients and 13/247 (5.3 %) in MR patients vs 1/236 (0.4 %) in controls, therefore demonstrating a significant excess of these CNVs in each disease group as compared with controls ($p= 0.005$, $p< 0.001$ and $p= 0.001$ respectively, Fisher exact test) (Table 3a). Individual significance for association with ASD was reached for the PRODH/DGCR6 deletion (9/260 in ASD patients vs 1/236 in controls, $p= 0.02$). None of the cases had more than one of these 28 CNVs. Among the four most prevalent CNVs, three, located in 22q11, 16p11 and 15q13, were flanked by known regions of segmental duplication and resulted most likely from a non allelic homologous recombination mechanism (NAHR). At the 2p16 locus, the NRXN1 gene was recurrently disrupted by a set of partially overlapping deletions spanning either the promoter and first exons of neurexin1 alpha or the exons coding for the middle section of this protein as well as for the proximal region of neurexin1 beta. These rearrangements occurred in a region devoid of any segmental duplication and resulted from another mechanism distinct from NAHR.

Transmission and cosegregation in multiplex sibships

Among 27 families in which transmission was tested (69% of the total sample), it was found that only eight CNVs (located in 8p23, 15q11-q13, 15q13, 16p11 and 22q13) had occurred de novo (Table 3a). Paternal age was not significantly different between families with de novo and inherited CNVs (27.2 ± 4.7 y vs 30.7 ± 4.6 y, NS, Mann Whitney U test). In most families, CNVs were transmitted from an apparently unaffected (although not clinically nor neuropsychologically assessed) parent. This includes a partial duplication of the X linked GRIA3 gene, present in a young male autistic patient, which was inherited from the unaffected mother. The 22q11 350 kb deletion spanning the PRODH/DGCR6 locus was also transmitted in 11/11 tested cases. PRODH encodes for the proline dehydrogenase and PRODH deficiency is responsible for type 1 hyperprolinemia, a condition often associated with both cognitive impairment and psychotic symptoms 18 . However, hemizygous deletion of the PRODH gene is not sufficient per se to result in hyperprolinemia since only 35–50 % of Velo-cardio-facial syndrome (VCFS) patients, all bearing a single copy of PRODH , exhibit hyperprolinemia 18 , 22 . Indeed, a reduction of more than 50% of the enzymatic activity is generally required to produce hyperprolinemia 18 . The presence of a mutation affecting enzyme activity 23 on the second allele is therefore necessary. To examine this issue, the remaining PRODH allele was sequenced in all affected subjects bearing the 350 kb deletion, and, when possible, plasma proline level was assessed. As shown in Table 4 , 14/15 patients harbored a genotype predicted to result in a reduction of at least 70% of enzymatic activity. Among the 10 subjects in whom plasma proline level was assessed, 7 had mild to severe hyperprolinemia and 3 had plasma proline level at the upper boundary of normal values.

Cosegregation of the CNV with pathological conditions was examined in 4 multiplex families in which DNA from affected siblings was available. In family 144, the two sibs with schizophrenia both harbored the PRODH/DGCR6 deletion. In family 11695, the two MR sibs both harbored the 2p16.3 deletion. In family 14390, the 16p11 deletion was present in the proband with developmental language disorder but not in his mentally retarded sib. In family 33, the 16p11 duplication was present in two sibs with schizophrenia as well as in a non affected sibling, but not in a third sibling with schizophrenia.

Disease specificity

Combining the results of the present study and those of previous reports, we found that, with the possible exception of the maternally derived 15q13 duplication associated with ASD, none of the observed rearrangement was disease-specific. The 22q11 19 and 2p16 1 deletions were found in the three conditions, whereas the 22q13 deletion found in two ASD patients had already been described in ASD and MR 4 , 24 –26 . The 16p11 and 8p23 rearrangements previously described in ASD 4 , 5 , were both found in SZ and also in MR patients for the 16p11 rearrangements. The Xp11.4 duplication spanning the TSPAN7 gene, previously described in MR and ASD 4 , 27 , was found in a patient with SZ, as well as the 17q21 duplication previously described in a patient with MR 28 . Two different sized 15q13 duplications encompassing APBA2 were found in one ASD and one SZ patient. A partial duplication of the GRIA 3 gene, encompassing the promoter region and the exons coding for the proximal region of GRIA3 was detected in a single autistic patient. Although slightly different by its size and by the number of duplicated exons, this partial duplication is reminiscent from that recently reported in a patient with MR 29 . At the 8p23 locus, both gain and loss of material were found, as well as at the 16p11 and 17q21 loci, as recently described 28 , 30 , 31 . This suggests that dosage-sensitive genes, whose expression is finely tuned, are located within these rearranged segments.

Comorbidity

From a phenotypic point of view, it is noteworthy that 3 out of 9 patients with schizophrenia bearing a candidate CNV had mild MR in an IQ assessment obtained after the onset of their psychotic symptoms (Table 3b). Although postmorbid IQ is likely to constitute an underestimate of the premorbid level of cognitive functioning in patients with schizophrenia, cognitive deficits testified by severe learning disorder were already noted in these 3 patients during childhood before the onset of their psychotic symptoms, thus supporting the diagnosis of comorbidity between MR and SZ. With the exception of two subjects who had normal cognitive functioning

(high-functioning autism), all tested CNV bearing autistic patients had IQs falling in the range of mental retardation, albeit one of them had only mild cognitive dysfunction (Table 3b).

Comments

After a first wave of CNV discovery by array-CGH analyses in neuropsychiatric disorders, this study for the first time examines the involvement of a limited number of candidate loci in large samples of patients with different clinical diagnoses. Two strengths of our study design are the inclusion of controls carefully screened for the studied pathologies and of series of patients mostly ascertained through consecutive admissions or consultations and therefore including subjects belonging either to simplex or multiplex families. Given the expected rarity of each variant, our first goal was not to test the association of every individual CNV with SZ, ASD or MR but to determine whether these variants were collectively more frequent in patients with these diseases than among healthy controls. This aim was successfully achieved and, in addition, we were able to obtain suggestive statistical significance for the association between the 22q11 350 kb deletion and ASD. This deleted segment, located within the chromosomal region deleted in VCFS, a contiguous gene syndrome known to be associated with a high frequency of MR, ASD and psychosis, contained two genes PRODH and DGCR6 . Although we cannot exclude an involvement of DGCR6 in the neuropsychiatric phenotype of the subjects bearing this CNV, previous work from our group strongly suggests that PRODH is the prime candidate. We had previously shown that hyperprolinemia, resulting from the partial or total inactivation of this enzyme (i) may lead to MR and autism in patients with type 1 hyperprolinemia 18 (ii) is a risk factor for schizoaffective disorder 32 (iii) is inversely correlated with IQ in the VCFS 18 . Here, we show that all but one patients harboring this deletion were in fact compound heterozygotes, also bearing mutations affecting enzymatic activity on the second allele. It results in a loss of at least 70% of the predicted PRODH residual activity in 14/15 subjects and in hyperprolinemia in 7/10 assessed subjects.

Second, we show that both de novo CNVs and CNVs inherited from an apparently healthy parent can be found in patients. For transmitted CNVs, the mode of inheritance of the disease was, in some cases recessive (e.g. hyperprolinemia related to the 22q11 deletion) or implied the transmission of an X linked gene by a woman to her son (GRIA3). Consistent with previous reports 4 , 8 , 33 , the 16p11 rearrangements were inherited from an apparently non affected parent in three families. These CNVs, whose estimated frequency in Icelandic population was 5/18,834 (0.03%) for the duplication and 2/18,834 (0.01%) for the deletion 31 , should therefore be considered as risk factors rather than fully causative variations. The presence of affected siblings that do not share the CNV, already noted in a previous report 33 , does not necessarily rule out the causative implication of these CNVs, but raises the question of intra-familial genetic heterogeneity. This hypothesis, which is not un- plausible for these frequent disorders often characterized by assortative mating, remains speculative since the parents and their relatives were not psychiatrically nor cognitively assessed in these families.

Third and more importantly, our study confirms and extends the conclusions of several recent reports suggesting that a large number of candidate CNVs are not disease-specific but are involved in the expression of different behavioral phenotypes including MR, ASD and SZ. This implies the existence of shared biological pathways between these three neurodevelopmental conditions. These pathways affect chiefly synapse formation and maintenance as well as neurotransmission with a special emphasis on glutamate and GABA. The dysfunction of specific neuronal networks underlying the peculiar symptomatology of each clinical condition most likely depends on additional genetics, epigenetics and environmental factors which remain to be characterized. From a clinical point of view, it should be stressed that despite the diversity of categorical diagnoses, many subjects harboring these CNVs shared some clinical features: 1/3 of patients with schizophrenia and 80% of autistic patients with CNVs had a level of cognitive functioning which falls in the range of mental retardation. This is in accordance with previous reports showing that that point prevalence of schizophrenia is increased by a factor of 3 in subjects with intellectual disabilities 34 and that 3/4 of autistic patients have mental retardation 35 . However, it should be remembered that (i) because attention and communication are markedly impaired in children with autism, assessment of their IQs (even performance IQs in non verbal subjects) are notoriously unreliable and (ii) these results were not obtained in a single community based population, but in three disease groups ascertained according to different schemes, a factor whose exact impact is difficult to appreciate but which is likely to have implications related to the phenotypic severity of these subject.

Finally, we would like to point out the utility of targeted methods for CNV analysis, such as the QMPSF method, as a cost effective alternative to a-CGH for the screening of candidate loci in large case-control cohorts. We plan to conduct extensive resequencing of these candidate genes in order to further validate their role in these conditions.

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Footnotes:

Author Contributions: Dr Campion had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design : Audrey Guilmatre, PhD; Christèle Dubourg, PhD, Anne Laure Mosca, MD; Alice Goldenberg, MD; Valérie Drouin-Garraud, MD; Valérie Layet, MD; Antoine Rosier, MD; Sylvain Briault, MD; Frédérique Bonnet-Brilhault, MD, PhD; Frédéric Laumonnier, PhD; Sylvie Odent, MD; Gabriella Di Rosa, MD; Christian Andres, MD; Laurence Faivre, MD; Thierry Frébourg, MD, PhD; Pascale Saugier Veber, PhD; Dominique Campion, MD, PhD. Acquisition of data : All authors Analysis and interpretation of data : Audrey Guilmatre, PhD; Christèle Dubourg, PhD; Anne Laure Mosca, MD; Solenn Legallic, BSc; Géraldine Joly-Helas, MD; Pascale Saugier Veber, PhD; Dominique Campion, MD, PhD Drafting of the manuscript : Dominique Campion, MD, PhD Critical revision of the manuscript for important intellectual content : Audrey Guilmatre, PhD; Pascale Saugier Veber, PhD; Dominique Campion, MD, PhD Statistical analysis : Gael Le Vacon, MD Obtained funding : Thierry Frébourg, MD, PhD; Pascale Saugier Veber, PhD; Dominique Campion, MD Study supervision : Dominique Campion, MD, PhD

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Note added in proof Since the submission of this manuscript additional reports documenting shared CNVs between MR, ASD and/or schizophrenia have been published 9 , 36 , 37 .

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Table 1
Demographic and clinical features of the sample

	age (y)	% Male	% Familial	% syndromic	Geographic ancestry
ASD (n= 260)	11.8 ± 7.6	80	9.6	8.5	France and Italy (n=29)
MR (n= 247)	13.2 ± 11.6	64.8	34	62	North western France
SZ (n= 236)	38 ± 11	66.8	23.7	/	North western France
Controls (n= 236)	39.5 ± 16.8	43.8	/	/	North western France

ASD: autism spectrum disorder, SZ: schizophrenia and schizoaffective disorder, MR: mental retardation

Table 2
Candidate regions and genes selected for QMPSF analysis

Chromosomal location	Type	Candidate Genes	Pathways	Function	Evidence	Disorder	Selected references
2p16.3	loss	NRXN1	synapse formation and maintenance	cell adhesion molecule	CR	ASD, SZ, MR	1, 4, 7, 8, 10, 38, 39
2q12.3-q14.2	loss/gain	DPP10	neurotransmission	dipeptidyl peptidase	CR	ASD	4
2q33.3-q34	loss	ERBB4	synapse formation and maintenance	NRG1 receptor	CR, M	SZ	8
3p26-p25	loss/gain	CNTN4	synapse formation and maintenance	Axon-associated cell adhesion molecule	CR, M	ASD, MR	40, 41
3p26.1-p25.2	loss	GRM7	neurotransmission	glutamate receptor	CR	SZ	8
3q26.31	gain	NLGN1	synapse formation and maintenance	cell adhesion molecule	CR	MR	42
4p14-q21.1	gain	GABRG1, GABRA4, GABRA2	neurotransmission	GABA receptor subunits	CR	ASD	4, 43
5p13	loss	SLC1A3	neurotransmission	excitatory amino acid transporter	CR, M	SZ	8
7q31.1	loss	ST7	other	tumor suppressor	CR, M	ASD	4
7q35-q36	loss	CNTNAP2	synapse formation and maintenance	contactin associated protein	CR, M	ASD, SZ, MR, TS	44
8p22-p11	loss	NRG1	synapse formation and maintenance	signaling protein	CR	MR	45
8p23	gain	DLGAP2	neurotransmission	associated to NMDA receptor and K+ channels	CR	ASD	4
8q24-qter	gain	PTK2	neuronal migration and growth	focal adhesion kinase	CR	SZ	8
11q21	loss	DLG2	synapse formation and maintenance	synaptic scaffolding protein	CR	SZ	8
12q14.3	loss	GRIP1	neurotransmission	glutamate receptor interacting protein	CR	MR	1, 3
15q11-q14	gain	GABRA5, GABRB3, GABRG3	neurotransmission	GABA receptor subunits	CR	ASD, MR	46, 47
15q13	gain	APBA2	neurotransmission	synaptic exocytosis	CR	ASD, SZ	6, 10
15q13.3	loss	CHRNA7	neurotransmission	acetylcholine receptor	CR	MR	48
16p11.2	loss/gain	DOC2A	neurotransmission	neurotransmitter release regulation	CR	ASD, SZ	4, 6, 8, 31, 33
17q21	loss/gain	MAPT	other	microtubule associated protein	CR	MR	28, 30
22q11	loss	PRODH	neuromodulation	proline dehydrogenase	CR, M	ASD, SZ, MR	19
22q13	loss	SHANK3	synapse formation and maintenance	synaptic scaffolding protein	CR, M	ASD, MR	4, 24_26
Xp22.3	loss/gain	NLGN4	synapse formation and maintenance	cell adhesion molecule	CR, M	ASD, TS, MR	4, 49_52
Xp11.4	gain	TSPAN7	neuronal migration and growth	transmembrane component	CR, M	ASD, MR	4, 27
Xp11.4	loss	CASK	synapse formation and maintenance	synaptic scaffolding protein	CR	MR	2
Xp22.1-p21.3	loss	IL1RAPL1	other	interleukin receptor	CR, M	ASD, MR	4, 53
Xq25	gain	GRIA3	neurotransmission	Kainate receptor subunit	CR	MR	29
Xq28	gain	MECP2	other	methylated CpG binding protein	CR, M	MR	42, 54

CR: Chromosomal rearrangement, M: mutation, TS: Tourette syndrome

Table 3
Recurrent CNVs and clinical features in ASD, SZ and MR patients

Table 3a

Patients ID	CNV diagnosis	CNV chromosomal location	CNV Size	Last outer margin (bp)	First inner margin (bp)	Last inner margin (bp)	First outer margin (bp)	genes involved	type	Confirmation	transmission
113	SZ	Xp11.4	56 kb	38,326,267	38,376,483	38,432,836	38,549,143	TSPAN7	dup	QMPSF, a-CGH	unknown
313	SZ	8p23	110 kb	1,436,299	1,481,035	1,590,831	1,642,837	DLGAP2	dup	QMPSF, a-CGH	unknown
185	SZ	15q13	994 kb	26,999,744	27,000,694	27,994,706	28,109,371	APBA2, TJP1, NDNL2	dup	QMPSF, a-CCH	unknown
144.1	SZ	AFF	22q11	350 kb				PRODH, DGCR6	del	QMPSF	unknown*
223	SZ	2p16.3	107 kb	50,704,195	51,006,556	51,114,057	51,433,167	NRXN1alpha exons 1,2	del	QMPSF, a-CGH	unknown
220	SZ	2p16.3	<532 kb	50,172,024	50,420,164	50,420,164	50,704,195	NRXN1alpha/beta	del	QMPSF, a-CGH	unknown
33	SZ	16p11	500 kb					DOC2A and 24 genes	dup	QMPSF	maternally inherited**
136	SZ	16p11	500 kb					DOC2A and 24 genes	dup	QMPSF	de novo
146	SZ AFF	16p11	500 kb					DOC2A and 24 genes	dup	QMPSF	unknown
151	SZ	17q21	627 kb	40,869,151	41,073,486	41,700,762	42,143,048	MAPT and 2 genes	dup	QMPSF, a-CGH	unknown
T 35	autism	2p16.3	<427 kb	51,006,556	51,114,057	51,114,057	51,433,167	NRXN1alpha exons 1,2	del	QMPSF, a-CGH, FISH	paternally inherited
45431	autism	2p16.3	107 kb	50,704,195	51,006,556	51,114,057	51,433,167	NRXN1alpha exons 1,2	del	QMPSF, a-CGH	maternally inherited
47604	autism	22q13	2,26 Mb	47,124,905	47,265,476	49,525,071	----------	SHANK3 and 28 genes	del	QMPSF, a-CGH	de novo
Si22	autism	22q13	ND					SHANK3	del	QMPSF, MLPA	de novo
60478	autism	15q11-q13	4 Mb					GABRA5, GABRB3, GABRG3 and 17 genes	dup	QMPSF, FISH	de novo¶
T 34	autism	Xq25	1,42 Mb	120,669,107	120,746,477	122,166,142	122,316,448	GRIA3 exons 1-4	dup	QMPSF, a-CGH, FISH	maternally inherited
12746	HFA	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
12452	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
13899	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	unknown
44737	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
45435	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	unknown
45856	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited

46261	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
47766	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
Si30	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
44813	HFA	15q13	3,8 Mb	26,198,996	26,999,744	30,796,716	30,812,300	APBA2, CHRNA7 and 16 genes	dup	QMPSF, a-CGH	maternally inherited
12363	MR	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
11780	MR	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
9680	MR	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
14684	MR	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
11695	MR	2p16.3	<427 kb	51,006,556	51,114,057	51,114,057	51,433,167	NRXN1alpha exon 1	del	QMPSF, a-CGH	maternally inherited****
14921	MR	16p11	500 kb					DOC2A and 24 genes	del	QMPSF, FISH	de novo
10417	MR	16p11	500 kb					DOC2A and 24 genes	del	QMPSF, FISH	maternally inherited
13165	MR	16p11	500 kb					DOC2A and 24 genes	dup	QMPSF, FISH	maternally inherited
14390	MR	16p11	500 kb					DOC2A and 24 genes	del	QMPSF, FISH	de novo***
13907	MR	15q13	1,57 Mb	28,109,371	28,725,507	30,298,096	30,701,373	CHRNA7 and 5 genes	del	QMPSF, a-CGH	unknown
11919	MR	15q13	1,57 Mb	28,109,371	28,725,507	30,298,096	30,701,373	CHRNA7 and 5 genes	del	QMPSF, a-CGH	unknown
9930	MR	15q13	1,57 Mb	28,109,371	28,725,507	30,298,096	30,701,373	CHRNA7 and 5 genes	del	QMPSF, a-CGH	de novo
12988	MR	8p23	3 Mb					DLGAP2 and 23 genes	del	QMPSF, HRK	de novo

Table 3b

						Clinical features	
Patients ID	**diagnosis**	**sex**	**age (y)**	**family history**	**CNV**	**Cognitive features**	**Associated features**
113	SZ	M	52	sporadic	dup(Xp11.4)	IQ, 105	
313	SZ	M	32	familial	dup(8p23)	IQ, 67	D
185	SZ	M	48	sporadic	dup(15q13)	IQ, 73	
144.1	SZ AFF	F	42	familial	del(22q11)	IQ, 48	
223	SZ	M	38	sporadic	del(2p16.3)	IQ, 94	
220	SZ	M	25	unknown	del(2p16.3)	NA	
33	SZ	F	53	familial	dup(16p11)	IQ, 56	
136	SZ	F	41	sporadic	dup(16p11)	IQ, 75	
146	SZ AFF	M	32	familial	dup(16p11)	IQ, 95	

151	SZ	M	53	sporadic	dup(17q21)	IQ, 90	
T 35	autism	F	38	sporadic	del(2p16.3)	IQ, 66	
45431	autism	M	10	sporadic	del(2p16.3)	NA	
47604	autism	M	8	sporadic	del(22q13)	IQ<40	
Si22	autism	M	9	sporadic	del(22q13)	IQ<40	D, E
60478	autism	M	15	sporadic	dup(15q11-q13)	IQ<40	E
T 34	autism	M	10	sporadic	dup(Xq25)	NA	
12746	HFA	M	6	sporadic	del(22q11)	IQ, 74	
12452	autism	F	8	sporadic	del(22q11)	IQ<40	
13899	autism	M	11	sporadic	del(22q11)	IQ<40	
44737	autism	M	7	sporadic	del(22q11)	IQ<40	
45435	autism	F	31	sporadic	del(22q11)	IQ<40	
45856	autism	M	38	sporadic	del(22q11)	IQ<40	
46261	autism	F	11	sporadic	del(22q11)	NA	
47766	autism	M	8	sporadic	del(22q11)	NA	
Si30	autism	F	5	sporadic	del(22q11)	IQ<40	D, E
44813	HFA	M	8	familial	dup(15q13)	NA	
12363	MR	M	11	sporadic	del(22q11)	Mild MR	D
11780	MR	M	3	sporadic	del(22q11)	Mod MR	D
9680	MR	F	11	familial	del(22q11)	Mod MR	D
14684	MR	M	6	familial	del(22q11)	DLD	
11695	MR	F	10	familial	del(2p16.3)	Mod MR	
14921	MR	M	4	familial	del(16p11)	Mild MR	D
10417	MR	M	14	familial	del(16p11)	Mild MR	IUGR, D
13165	MR	F	4	sporadic	dup(16p11)	Mild MR	D
14390	MR	F	9	familial	del(16p11)	DLD	
13907	MR	F	9	sporadic	del(15q13)	Mild MR	M, D
11919	MR	F	7	familial	del(15q13)	Mild MR	
9930	MR	M	12	familial	del(15q13)	Mild MR	D
12988	MR	M	8	sporadic	del(8p23)	Mild MR	M, D, E

a: diseases associated CNVs, b: clinical features of patients with CNVs, M: male; F: female, HFA: High-functioning autism, SZ: schizophrenia, SZ AFF: schizoaffective disorder, MR: mental retardation, Mod: moderate, DLD: developmental learning disorder, Del: deletion; dup: duplication, HRK: High resolution karyotype, D: dysmorphism; E: epilepsy; M: microcephaly; IUGR: intrauterine growth retardation, NA: not assessed

* deletion also present in a sibling with schizophrenia

** duplication present in a schizophrenic and a healthy sibling, and not present in a schizoaffective sibling

*** deletion not present in a sibling with mental retardation

**** deletion present in the proband and an affected sibling

¶ maternally derived

Table 4

Genotype and plasma proline level of the PRODH deletion bearing patients

Predicted PRODH residual activity was evaluated for each genotype according to the functional data published by Bender et al.[23] . Mutations with severe effect on PRODH activity appear in bold, mutations with moderate effect in normal character and mutations with unknown effect in italic. Abnormal plasma proline values appear in bold. Fasting abnormal plasma proline values: age >18 years: males > 377 μmol/L, females > 316 μmol/L; 5 years>age <18 years: > 270 μmol/L; age < 5 years: > 235 μmol/L.

ID	diagnosis	sex	age	plasma proline (μmol/L)	genotype	predicted PRODH residual activity
144.1	SZAFF	F	42	**538**	DEL/**R453C** + R185W	2%
144.2	SZ	F	39	**338**	DEL/Q19P	30%
12 452	Autism	M	6	**287**	DEL/R185W	25%
12 746	HFA	F	8	214	DEL/Q19P+ A58T	≤30%
13 899	Autism	M	11	**312**	DEL/Q19P	≤25%
44 737	Autism	M	7	ND	DEL/T275N + V427M	≤20%
45 435	Autism	F	31	ND	DEL/R185W+ Q19P	≤25%
45 856	Autism	M	38	ND	DEL/Q19P+ P30S	≤30%
46 261	Autism	F	11	243-**283**	DEL/R185W+ Q19P	≤25%
47 766	Autism	M	8	ND	DEL/WT	50%
Si30	Autism	F	5	**422-1883**	DEL/**R453C**+ Q19P+ A58T + V427M	≤2%
12363	MR	M	11	**512**	DEL/R185W	25%
11780	MR	M	3	ND	DEL/R185W+ Q19P	≤25%
9680	MR	F	11	**299**	DEL/Q19P+ P30S	≤30%
14684	MR	M	6	238	DEL/R185W+ Q19P	≤25%

ND: not determined. WT: wild type. HFA: High-functioning autism