

## COPY NUMBER VARIATIONS IN THE ETIOLOGY OF AUTISM SPECTRUM DISORDERS

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*[Le CNVs nell'eziologia dei disturbi dello spettro autistico]*

### ABSTRACT

*Autism Spectrum Disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders, characterized by qualitative impairment in social interaction and communication and restricted, repetitive and stereotyped patterns of behavior, interests and activities. They have a multifactorial etiology, but today different studies are showing the central role of genetics. Different genetic alterations were detected: chromosomal abnormalities, mutations, trinucleotide repeats and copy number variations (CNVs).*

*Several studies identified many CNVs associated with ASDs and possible candidate genes, whose loss or gain could have a key role in the etiopathogenesis of these disorders. In particular, they seem to be involved in neurogenesis, neuronal migration, differentiation and degeneration.*

*We want emphasize that the final phenotype is variable, related not only to the genetic background but also to environmental factors.*

**Key words:** *Autism spectrum disorders, a-CGH, genomic variants.*

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### Introduction

When we talk about Autism Spectrum Disorders (ASDs), we refer to a heterogeneous group of neurodevelopmental disorders, that in the past were just called "autism". More than a single disease, today the term ASDs is referred to a group of clinical conditions characterized by qualitative impairment in social interaction and communication and restricted, repetitive and stereotyped patterns of behavior, interests and activities<sup>(1)</sup>. These disorders have a high prevalence in the population. Recent data suggest a prevalence of 1:150 in the pediatric population, with a M:F ratio of 4:1<sup>(2)</sup>.

In the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) Autism Spectrum Disorders are included in the category of Pervasive Developmental Disorders (PDD) and are divided into five subtypes: autistic disorder, Rett syn-

drome, childhood disintegrative disorder (CDD), Asperger syndrome, pervasive developmental disorder not otherwise specified (PDD-NOS)<sup>(3)</sup>.

Moreover, we can distinguish two major categories of autism: "essential" autism and "complex" or "syndromic" autism. Essential autism represents about 75% of cases and is characterized by the absence of dysmorphic features, M:F ratio of 6:1, high risk of recurrence (up to 35%) and family history (20% of cases). A complete and deep anamnestic evaluation of patient history with special refer to neonatal age when many risk factors (prematurity, nosocomial infection, chemical mediators<sup>(4,5,6,7,8,9,10,11,12,13,14)</sup> is important for outcome.

Conversely, syndromic autism is generally characterized by recognizable dysmorphic features. It is important to distinguish these two categories, because they have different prognosis and different risk of recurrence for family members 15.

As revealed by multiple independent studies of families, the concordance rate between monozygotic twins is 90%, compared to 10% among dizygotic twins<sup>(16)</sup>.

These studies also highlighted the existence of significant genetic heterogeneity. Genetic alterations can be inherited according to Mendelian schemes with variable penetrance (autosomal or X-linked) or can be de novo<sup>(17,18,19)</sup>. Different genetic alterations were detected: chromosomal abnormalities, mutations, trinucleotide repeats and, as shown by recent studies, copy number variations (CNVs)<sup>(20,21,22,23,24,25)</sup>.

Furthermore, a large number of genetic syndromes<sup>(26,27,28,29,30)</sup> can occur with ASDs. Modern research in genetics has focused attention on the role in the etiology of ASDs of copy number variations, that are submicroscopic alterations of the genome in excess or defect (microdeletions and microduplications).

## Discussion

Recent studies are revealing the possible involvement of copy number variations as susceptibility factors for complex diseases of unknown etiology, such as schizophrenia or autism. Although some CNVs were already known to cause global development disorders, today array-CGH (comparative genomic hybridization) allow the identification of other CNVs associated with mental retardation, Autism Spectrum Disorders and schizophrenia. Array-CGH revealed that about 15% of patients with "chromosomal" phenotype and normal karyotype are carriers of cryptic chromosomal imbalance responsible for their condition<sup>(31)</sup>.

These rearrangements are mediated by non-allelic homologous recombination (NAHR), that occurs during meiosis; it is usually flanked by duplicons, or low copy repeats (LCRs). They are blocks of a few repeated sequences, with very high mutual homology (from 90% to 100%) and several hundreds of kilobases (200-400 Kb). They are throughout the genome, representing up to 5% of the human genome, and are located in subtelomeric and pericentromeric regions. Duplicons give the DNA region high instability and susceptibility to the onset of genomic rearrangements due to the creation of breaking points. So they can lead to errors in recombination during meiotic crossing over resulting in inversions, translocations, duplications or deletions.

Generally microdeletions are more frequent than microduplications. Microdeletions can be the result of different recombination mechanisms (intra-chromatid, inter-chromatid or intrachromosomal), while microduplications are due to inter-chromatid or intrachromosomal recombination. Microdeletions give specific and more severe signs and symptoms, compared to the mild clinical features due to microduplications. CNVs are characterized by complex inheritance, given by the interaction with other alleles and with environmental factors. Several studies associate the same CNVs to different phenotypes, including autism, atypical autism, dyslexia, mental retardation and attention deficit-hyperactivity disorder (ADHD).

These findings suggest that CNVs represent predisposing genetic alterations leading to a variable final phenotype, related with the genetic background and the environment<sup>(32)</sup>. It is also important to note that CNVs can be detected even in normal population. Moreover, recent studies have identified CNVs that contain genes whose products are involved in cellular processes that begin in the early stages of central nervous system development. In particular, they seem to be involved in neurogenesis, neuronal migration, differentiation and degeneration<sup>(33)</sup>. An interesting CNV has recently been identified in the 16p11.2 region and it seems to occur in more than 1% of individuals with ASDs<sup>(34,35)</sup>. Further studies have associated 16p11.2 microdeletion syndrome with language delay and facial dysmorphism, without necessarily presenting a diagnosis of autism spectrum disorder<sup>(36,37,38,39,40)</sup>. This CNV has recurrent breakpoints defined by low copy repeats and includes 27 genes. Different studies suggest a possible role of CNVs that contain genes belonging to the family of ubiquitin genes (like UBE3A), as susceptibility factors for Autism Spectrum Disorders.

An analysis of 427 families with a diagnosis of Autism Spectrum Disorders described other CNVs containing genes, such as DPP6 (7q36.2), DPP10 (2q14.1) and PCDH9 (13q21.32). DPP6 and DPP10 seem important regulators of neuronal excitability, integration of signals through the dendrites and synaptic plasticity. Table 1 reports a summary of CNVs with the corresponding phenotypes.

### *Candidate genes in ASDs susceptibility*

In addition to the identification of CNVs, in the last few years several studies have been undertaken in order to identify possible candidate genes

CNVs	ASDs	Intellectual disability	Seizures	Psychiatric disorders	Dysmorphic features	Language delay
CNTNAP2	+	-	+	-	-	-
SHANK3	+	+	-	+	-	-
UBE3A	+	-	-	-	-	-
PARK2	+	-	-	-	-	-
del16p11.2	++	+	+/-	-	+	++
dup16p11.2	+	+	+/-	+/-	+	+
RFWD2	+	-	-	-	-	-
FBXO40	+	-	-	-	-	-
DPP6	+	-	-	-	-	-
DPP10	+	-	-	-	-	-
PCDH9	+	-	-	-	-	-
PTCHD1	+	++	-	-	-	-

**Table 1:** CNVs associated with their phenotypic features.

Candidate genes in ASDs susceptibility	ASDs	Intellectual disability	Seizures	Psychiatric disorders	Dysmorphic features	Language delay
NRNX1	+	-	-	+	-	-
CNTN4	+	-	-	-	-	-
NLGN3/4	+	-	-	-	-	-
PCDH10	+	+	-	-	-	-
DAI1	+	+	-	-	-	-
NHE9	+	+	-	-	-	-
PTEN	+	-	-	+	-	-
FOXP2	+	-	-	-	-	+/-
RELN	+	-	-	-	-	-
LAMB1	+	-	-	-	-	-
OXTR	+	-	-	-	-	-
AUTS2	+	-	-	-	-	-

**Table 1:** Candidate genes in ASDs susceptibility.

with a key role in the etiopathogenesis of autistic disorders. More genes are being identified, located both on the autosomes and the sex chromosomes (Table 2). CNTN4 is a gene located on chromosome 3 (3p26.3) encoding the CAM contactin 4, a protein involved in the formation, maintenance and plasticity of neuronal networks. Its break or its loss cause cognitive delay, characteristic of 3p deletion syndrome.

Even the long arm of chromosome 3 could be involved in the etiology of ASDs; moreover recent studies suggest an association between susceptibility to ASDs and mutations of the gene NRXN1, located in the 2p16.3 region, encoding for the neuroxine 1, a protein with a role in the synaptogenesis.

4q28 deletions, involving the locus PCDH10, have also been associated with ASDs. Proteins essential for axonal growth appear to be encoded in this region<sup>(41)</sup>. PTEN is a tumor suppressor gene located on chromosome 10 (10q23.3), that acts as a negative regulator of the PI3 kinase pathway. Heterozygous mutations in PTEN have been found in a group of patients with autism, macrocephaly and/or developmental delay<sup>(42)</sup>. This gene is also associated with Cowden syndrome, Zonana-Ruvalcaba syndrome, Proteus syndrome and Lhermitte-Duclos disease. Many candidate genes for ASDs are located on chromosome 7.

On the long arm of this chromosome a susceptibility locus for autism spectrum disorders, called AUTS1, was found. Among these, candidate genes the most studied are: FOXP2 (involved in a severe monogenic form of language delay)<sup>(43)</sup>, RELN and LAMB1. On chromosome 7 were also identified CNVs containing the gene for the contactin-associated protein-like 2, involved in neuronal migration and cell adhesion.

In conclusion, even oxytocin plays an important role in behavior and social interaction. OXTR alleles with different single nucleotide polymorphisms (3p24-26) and haplotypes were associated with ASDs.

## Conclusions

The knowledge obtained so far emphasizes the role of neurogenetics in the determining Autism Spectrum Disorders. Further studies are required to find and understand how these genetic abnormalities contribute to the development of the disease.

One important finding is that both structural and quantitative alterations can lead to different

effects in cellular processes.

Genomic rearrangements could also be responsible for alterations in transcription patterns. Moreover, they could involve regulatory elements and influence the expression of the closest genes. Finally, we want to stress the importance of environmental factors, which can act as triggers modifying the phenotype.

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