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 \textit{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; Iha 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].](image-url)
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).
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-1β, IL-6, IL-12p40, TNF-α, 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-1β, 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 Th2 phenotype. An increased production of the pro-inflammatory cytokine TNF-a was also found, that is consistent with an activated Th2 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 methylmercury 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
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].

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Function</th>
<th>Evidence</th>
<th>Inheritance</th>
<th>Diagnosis</th>
</tr>
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<tr>
<td>FMR1</td>
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<td>Mutations</td>
<td>De novo</td>
<td>ASD, Fragile X syndrome</td>
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<td>Xq26</td>
<td>Chromatin remodeling</td>
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<td>TSC1</td>
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<td>mTOR/PI3K pathway</td>
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<td>16p13.3</td>
<td>mTOR/PI3K pathway</td>
<td>CNV, mutations</td>
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<td>CNV</td>
<td>De novo, inherited</td>
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<td>CNV, mutations</td>
<td>De novo</td>
<td>MR, seizures</td>
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<td>De novo</td>
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<td>CNV, rare variants</td>
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<td>ASD</td>
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<td>ASD, MR, SCZ</td>
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<td>(del 22q13)</td>
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<td>Mutation</td>
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<td>ASD</td>
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<td>ASD, MR</td>
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<td>Synaptic receptor</td>
<td>CNV</td>
<td>Inherited</td>
<td>ASD</td>
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</table>

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. *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].

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome Function</th>
<th>Evidence</th>
<th>Diagnosis</th>
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<td>Inherited CNV, SNPs, mutations</td>
<td>ASD</td>
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<td>Axonal growth</td>
<td>Inherited CNV</td>
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<td>1q42.2 Cell differentiation</td>
<td>Inherited CNV</td>
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<tr>
<td>DPP10</td>
<td>2q14.1 Dipeptidyl-peptidase activity</td>
<td>Inherited CNV</td>
<td>ASD</td>
</tr>
<tr>
<td>CNTN3</td>
<td>3p12.3 Synaptic CAM</td>
<td>Inherited CNV</td>
<td>ASD</td>
</tr>
<tr>
<td>FBXO40</td>
<td>3q13.3 Unknown function</td>
<td>Inherited CNV, P = 3.3 x 10^-3</td>
<td>ASD</td>
</tr>
<tr>
<td>SLC9A9</td>
<td>3q24 Transporter</td>
<td>Inherited CNV, mutations</td>
<td>ASD, ADHD, MR</td>
</tr>
<tr>
<td>PCDH10</td>
<td>4q28 Synaptic CAM</td>
<td>Inherited CNV</td>
<td>ASD</td>
</tr>
<tr>
<td>PARK2</td>
<td>6q26 Ubiquitination</td>
<td>Inherited CNV, P = 3.3 x 10^-3</td>
<td>ASD, PD</td>
</tr>
<tr>
<td>IMP2L</td>
<td>7q31.1 Mitochondrial protease</td>
<td>Inherited CNV</td>
<td>ASD, TS, ADHD</td>
</tr>
<tr>
<td>PCDH9</td>
<td>13q21 Synaptic CAM</td>
<td>Inherited CNV</td>
<td>ASD</td>
</tr>
<tr>
<td>MDGA2</td>
<td>14q21.3 Gpi anchor protein</td>
<td>Inherited CNV, P = 1.2 x 10^-4</td>
<td>ASD</td>
</tr>
<tr>
<td>BZRAP1</td>
<td>17q22 Benzodiazepine receptor binding</td>
<td>Inherited CNV, P = 2.3 x 10^-3</td>
<td>ASD</td>
</tr>
<tr>
<td>PLD6</td>
<td>1q43 Phospholipase D</td>
<td>SNP rs2198268, P = 1.1 x 10^-8</td>
<td>ASD</td>
</tr>
<tr>
<td>SLC25A12</td>
<td>2q31.1 Synaptic receptor</td>
<td>SNP rs2056202, P = 1 x 10^-3</td>
<td>ASD</td>
</tr>
<tr>
<td>CNTN4/CNTN</td>
<td>5q14.1 Synaptic CAM</td>
<td>SNP rs4070099, P = 3.4 x 10^-4</td>
<td>ASD</td>
</tr>
<tr>
<td>SEMA5A</td>
<td>5q15.2 Axonal guidance</td>
<td>SNP rs10513025, P = 2 x 10^-7</td>
<td>ASD</td>
</tr>
<tr>
<td>TAS2R1</td>
<td>5q15.2 Receptor</td>
<td>SNP rs10513025, P = 2 x 10^-7</td>
<td>ASD</td>
</tr>
<tr>
<td>GRIN2</td>
<td>6q16.3 Synaptic receptor</td>
<td>SNP rs2193607, P = 0.02</td>
<td>ASD, SCZ, OCD, MR</td>
</tr>
<tr>
<td>POU5F1</td>
<td>7q14.1 Transcription factor</td>
<td>SNP rs10258862, P = 4.4 x 10^-4</td>
<td>ASD</td>
</tr>
<tr>
<td>RELN</td>
<td>7q22.1 Axonal guidance</td>
<td>GGC repeat in the 5' UTR, P &lt; 0.05</td>
<td>ASD, BP</td>
</tr>
<tr>
<td>NRCAM</td>
<td>7q31.1 Synaptic receptor</td>
<td>SNP rs2003045, P = 0.017</td>
<td>ASD</td>
</tr>
<tr>
<td>MET</td>
<td>7q31.2 Tyrosine kinase</td>
<td>SNP rs1858830, P = 2 x 10^-3</td>
<td>ASD</td>
</tr>
<tr>
<td>EN2</td>
<td>7q36.3 Transcription factor</td>
<td>SNP rs1869173, P = 3 x 10^-6</td>
<td>ASD</td>
</tr>
<tr>
<td>ST8SIA2</td>
<td>15q26.1 N-glycan processing</td>
<td>SNP rs1874730, P = 4 x 10^-7</td>
<td>ASD</td>
</tr>
<tr>
<td>GRIN2A</td>
<td>16p13.2 Synaptic receptor</td>
<td>SNP rs104631, P = 2.9 x 10^-7</td>
<td>ASD, SCZ</td>
</tr>
<tr>
<td>ABAT</td>
<td>16p13.2 Enzyme</td>
<td>SNP rs1731017, P = 1 x 10^-3</td>
<td>ASD, GABA aminotransferase deficiency</td>
</tr>
<tr>
<td>SLC6A4</td>
<td>17q11.2 Serotonin transporter</td>
<td>SNP rs1731017, P = 1 x 10^-3</td>
<td>ASD, OCD</td>
</tr>
<tr>
<td>ITGB3</td>
<td>17q21.3 Cell-matrix adhesion</td>
<td>SNP rs863030, P = 2 x 10^-7</td>
<td>ASD</td>
</tr>
<tr>
<td>TLE2/TLE6</td>
<td>19p13 WNT receptor signaling pathway</td>
<td>SNP rs4006693, P = 7.8 x 10^-9</td>
<td>ASD, FHM2, AHC</td>
</tr>
<tr>
<td>MACROD2</td>
<td>20p12 Unknown function</td>
<td>SNP rs141463, P = 2 x 10^-8</td>
<td>ASD</td>
</tr>
</tbody>
</table>

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 “synaptopathies” 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].
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\textsuperscript{2+} channel function is also compromised based on decreased presynaptic Ca\textsuperscript{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; Maeyama and Jin 2010; Maeyama 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).
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 dissociation 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.

![Pie charts showing distribution of sample by sex and phenotype](image)

**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 142,494 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.
<table>
<thead>
<tr>
<th>DNA (µg)</th>
<th>44K array</th>
<th>60K array</th>
<th>array 244K</th>
</tr>
</thead>
</table>

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 (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 (Cy3 and Cy5) are used so the reaction must be performed in the dark.

**For 44K and 244K arrays:**
- 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.

**For 60K array:**
- 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 1 minute 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:
- 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).

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;

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.
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.

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).
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.

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| Tab 3. Continued. |
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|                  | 13q31.1  | Loss            | 77 kb   | /               |
| **14** M S-AU     | 9q34.3   | Gain            | 442.5 kb| CACNA1B, EHMT1 |
|                  | Xq22.3   | Gain            | 24 kb   | IL1RAPL2        |
| **15** M AU, ID   | 15q26.2  | Loss            | 38 kb   | MCTP2           |
| **16** M PDD-NOS  | 17q23.1  | Gain            | 44 kb   | CA4             |
|                  | 12p13.1  | Gain            | 69 kb   | /               |
|                  | 12p12.2p12.1 | Loss     | 387 kb  | /               |
|                  | 14q13.1  | Gain            | 131 kb  | CFL2, SNX6      |
| **18** M PDD-NOS  | 6q21     | Loss            | 47 kb   | PREP            |
| **19** M AS       | Yq11.21  | Gain            | 449 kb  | USP9Y           |
| **20** M HF-AU    | 8q12.1   | Gain            | 45.5 kb | PLAG1           |
| **21** M HF-AU    | 5q31.3   | Gain            | 76 kb   | ARHGAP26        |
| **22** M AU, ID   | 3p14.1   | Gain            | 94 kb   | ADAMTS9         |
|                  | 6q25.2   | Loss            | 15 kb   | IPCEN1          |
| **23** M PDD-NOS  | 17q21.31 | Loss            | 748 kb  | ACBD4, CRHR1, FMNL1 |
|                  | 17q24.2  | Loss            | 93 kb   | PRKCA           |
| **24** M AU, ID   | 9p24.2   | Loss            | 191 kb  | RFX3            |
|                  | 13q12.11 | Gain            | 363 kb  | MPHOSPH8, PSC1, ZMYM5 |
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|                  | 10p11.21 | Gain            | 223 kb  | CCNY, CREM     |
|                  | 15q13.3  | Gain            | 377 kb  | CHRNA7          |
|                  |          |                 |         | ALDOA, ASPHD1, CDIPT, C16orf53, DOC2A, FAM57B, GDPD3, HIRIP3, INO80E, KCDT13, KIF22, MAPK3, MAZ, MVP, QPRT, PPP4C, PRTT2, SEZ6L2, SPN, TAOK2, YPEL3 |
|                  |          |                 |         | VAT1            |

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Array CGH 44K

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Tab 3. Continued.
### Tab 3. Continued.

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*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 (18.5%), 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)§.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Function and expression</th>
<th>Interactors and possible role in brain</th>
<th>Findings in ASDs or other neuropsychiatric disorders</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADAMTS9(-)</strong></td>
<td>This gene encodes the ADAM metalloprotease 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.</td>
<td>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.</td>
<td></td>
<td>Bret et al., 2011 Jungers et al., 2005</td>
</tr>
<tr>
<td><strong>AP2M1(+)</strong></td>
<td>This gene encodes the adaptor-related protein complex 2, mu 1 subunit, which is a subunit of the heterotrimeric 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.</td>
<td>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.</td>
<td></td>
<td>Borck et al., 2008 Borginov et al., 2012</td>
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<tr>
<td><strong>ARHGAP26(-)</strong></td>
<td>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 Tuberosous Sclerosis 1 or 2. Both the TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates Cdc42, thus regulating cell adhesion and migration.</td>
<td></td>
<td>Fombonne et al., 1997 Lewis et al., 2004 Muzykewicz et al., 2007 Wiznitzer, 2004 Wong, 2006</td>
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</table>
### ARHGEF10(+) Intracellul ar 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 particular in prefrontal cortex, amygdala 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 GTase that allows its interaction with downstream effector proteins, thus playing a central role in defining the temporal and spatial activation of the corresponding GTase 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 associated 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 facioscapulohumeral dystrophy, 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 SCZ and, possibly, ASD.

### 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 amygdala, suggesting that the protein is involved in limbic-striatal function.

In mouse a similar protein is enriched in the central extended amygdala. 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

### ATP6API(+) 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 ATP6API have never been reported in patients with ASD.

Jansen and Martens, 2012
Vanlaere et al., 2009
<table>
<thead>
<tr>
<th>Gene</th>
<th>Regulation of microtubule cytoskeleton dynamics</th>
<th>Branched-chain amino acid catabolism</th>
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</table>
| **BCKDHA(-)**| This nuclear gene encodes the branched chain keto acid dehydrogenase E1, alpha polypeptide, which is a subunit of an inner 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 amino acids (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) isoenzymes. 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 muscle tone, 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). | Mutations and/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). |
| **B9D2(+)**  | This gene encodes the B9 protein domain 2, which is exclusively found in ciliated organisms. The gene is upregulated during microciliary 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. |
### CA4(+)*

**Excitatory 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 neuronal activity.

The principal isoforms of carbonic anhydrases in the brain are CA4 and CA14, and both enzymes catalyze the buffering of activity-dependent pH 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 have been reported in a few autistic patients.

### 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 amygdala.

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, amygdala, 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
Himata-Wabantsu et al., 2005
Kallman, 2012
Siplawski et al., 2004, 2006

### CAMK2G(-)*

**Intracellular Wnt/Ca**

**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/β-catenin signaling pathway 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.

Chang et al., 2011
De Ferrari and Moon, 2006
Liu and Murray, 2012
Olerud and Cheyette, 2011
Wang et al., 2010
Zhang et al., 2012
<table>
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<th>Genes</th>
<th>Function</th>
<th>Expression</th>
<th>Other remarks</th>
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<td><strong>CCNY</strong></td>
<td>Intracellular Wnt signaling pathway</td>
<td>This gene encodes the cyclin Y protein. Cyclins, such as CCNY, control cell division cycles and regulate cyclin-dependent kinases (e.g., CDK2). 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.</td>
<td>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. Deregression of Wnt/beta-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as other neuropsychiatric disorders. Ching et al., 2011 Davidson and Niehrs, 2010 De Ferrari and Moon, 2006 Jiang et al., 2009 Gherland and Chetty, 2011 Wang et al., 2010 Zhang et al., 2012</td>
</tr>
<tr>
<td><strong>CFDP1</strong></td>
<td>Embryonal development</td>
<td>This gene encodes the craniofacial development protein 1, which may play a role during embryogenesis. Good expression in fetal brain and in postnatal CNS, in particular in temporal lobe, prefrontal cortex, amygdala, thalamus, and hypothalamus. Good expression in immune cell types.</td>
<td>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 LICAM has been reported in an adult male patient with L1 disease and autism. Iwashita et al., 1999 Luan and Derbishech, 2002 Nakamura et al., 2002, 2011 Risinger and Bennett, 1999 Simonait et al., 2006 Wong et al., 1996</td>
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<tr>
<td><strong>CFL2</strong></td>
<td>Intracellular signaling: regulation of actin cytoskeleton dynamics</td>
<td>This gene encodes 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.</td>
<td>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 phosphorylation by LIM kinase and is reactivated by dephosphorylation 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.</td>
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<tr>
<td><strong>CREM</strong></td>
<td>Transcriptional regulation</td>
<td>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 spermatogeneric 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 amygdala.</td>
<td>The family of CREB (cAMP response element modulator) 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. Aqaudo et al., 2009 Diaz-Ruiz et al., 2008</td>
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Tab. 4. Continued.
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**CSHI(-)**
Organogenesis, intrauterine growth
This gene encodes the chorionic somatomammotropin hormone 1, which is a member of the somatomammotropin/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 GH/IGF-1 axis may play an important role in CNS functions, including those associated with neuronal growth, development, and protection. Furthermore, the GH/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 choriid 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 hormones deficiency 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.

**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.

**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 progression.

Low expression in fetal brain, moderate expression in postnatal CNS, in particular 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.

**DIP2A(-)**
Neurogenesis: axon patterning
This gene encodes the DLP2 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, except 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.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Synaptogenesis and synaptic plasticity</th>
<th>Synaptogenesis and synaptic plasticity</th>
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<tr>
<td><strong>DLGAP2(SAPAP2)</strong> (+)</td>
<td>This gene encodes the discs, large (Drosophila) homology-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.</td>
<td>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.</td>
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<tr>
<td><strong>DLG2(-)</strong></td>
<td>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.</td>
<td>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.</td>
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<tr>
<th>Author</th>
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<tbody>
<tr>
<td>Baris et al., 2007</td>
<td><strong>A de novo CNV involving DLGAP2 has been reported in a patient with ASD.</strong></td>
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<tr>
<td>Bolliger et al., 2001</td>
<td>Mutations and/or CNVs affecting SHANK2 and NLGN4X have been previously reported in autistic patients.</td>
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<td>Cousin and Stephenson, 2012</td>
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<td>Jamain et al., 2003</td>
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<td>Kent et al., 2008b</td>
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<td>Yoo et al., 2012</td>
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### 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, RhoG, 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.

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<td>Fombonne et al., 1997</td>
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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 dysgenesis.

DOCK8 disruption 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.

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 other neuropsychiatric disorders.

**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.

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<td>Wilkinson et al., 2009, 2011</td>
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<td><strong>EIF2S3 (-)</strong>*</td>
<td>Translational regulation</td>
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<td><strong>FLNA (-)</strong>*</td>
<td>Neurodevelopment: actin cytoskeleton organization</td>
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<tr>
<td><strong>GH2 (-)</strong></td>
<td>Organogenesis, intrauterine growth</td>
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<tr>
<th>Gene</th>
<th>Metabolism/Pathway</th>
<th>Description</th>
<th>Mutations and/or CNVs affecting this gene</th>
<th>References</th>
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<tr>
<td>GLDC(+)</td>
<td>Glycine metabolism</td>
<td>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 methyamine 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, amygdala and hypothalamus.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting GLDC have never been reported in patients with ASD. Hamosh et al., 2009</td>
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<tr>
<td>GPR98(-)</td>
<td>CNS development</td>
<td>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.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting GPR98 have never been reported in patients with ASD. McMillan et al., 2002, Munoz-Yunta et al., 2008, O’Roak et al., 2011, Wang et al., 2005, Weiss et al., 2003</td>
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<tr>
<td>GRID2(-)</td>
<td>Synaptogenesis and synaptic plasticity</td>
<td>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.</td>
<td>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 have 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.</td>
<td>Rare single gene mutations affecting GRID2 have been reported in two autistic patients. Moreover, a de novo CNV at 4q22.2 including GRID2 has recently been reported in an autistic patient. Schiaf et al., 2010, 2012, Leblond et al., 2012, Pinto et al., 2010, Schaaf et al., 2011, Uemura et al., 2004</td>
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<tr>
<td>Gene</td>
<td>Synaptogenesis and synaptic plasticity</td>
<td>Intracellular signaling: neuroprotection during stress response mediated by activation of mTOR pathway</td>
<td>CNS development: myelin formation</td>
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<td><strong>GRM7</strong>&lt;sup&gt;(−)&lt;/sup&gt;*</td>
<td>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.</td>
<td>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 marine 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 HSPB1 are a cause of Charcot-Marie-Tooth disease type 2P and distal hereditary motor neuropathy.</td>
<td>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 amygdala and thalamus. Very high expression in immune cell types.</td>
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<td><strong>HSPB1</strong>&lt;sup&gt;(−)&lt;/sup&gt; de novo</td>
<td>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 cell 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 hypothalamus where the expression is good.</td>
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<tr>
<td><strong>IKBKG</strong>&lt;sup&gt;(+)&lt;/sup&gt;</td>
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**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
Sergerie et al., 2003
Vadass et al., 2007

Chapman et al., 2011
Fombonne et al., 1997
Kirbach and Golenhofen, 2011
Kunishi et al., 1997
Lewis et al., 2004
Muznyckyts et al., 2007
Schmidt et al., 2012
Wiznitzer, 2004
Wong, 2006

Malik et al., 2011
Nak et al., 2011
Philippe et al., 2012
Ramocki et al., 2009, 2010

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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.

Mutations and CNVs affecting GRM7 have never been reported in patients with ASD.

Mutations and/or CNVs affecting HSPB1 have been reported in patients with ASD.

Mutations and CNVs affecting IKBKG have never been reported in patients with ASD.

Mutations affecting GRM5 and GRM8 have been recently reported in a few autistic patients.

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-xB signaling in skin fibroblasts derived from patients with white matter anomalies, thus further supporting the role of NF-xB signaling in astroglial cells for normal myelin formation of the CNS.

NF-xB is an important gene transcriptional factor that mediates cellular responses in inflammation, immunity, development, cell proliferation and apoptosis. Elevated levels of NF-xB have been reported in autistic patients vs. controls.
## 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.

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<td>Valnegri et al., 2011</td>
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## 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 ARF$_{6,GTP}$ 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.

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<tr>
<td>Hernández-Deviez et al., 2004, 2007</td>
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<td>Venkateswarlu, 2003</td>
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## 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 05 to modulate survival of peripheral sensory neurons after injury.

Mutations and/or CNVs affecting IPO11 have never been reported in patients with ASD.

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<td>Ben-Yakob et al., 2012</td>
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<th><strong>JMJD2A(-)</strong></th>
<th>Chromatin remodeling</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(KDM4A)</strong></td>
<td>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 SUV3A 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.</td>
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<tr>
<td><strong>JMJD2C(-)</strong></td>
<td>Chromatin remodeling</td>
</tr>
<tr>
<td><strong>(KDM4C)</strong></td>
<td>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 amygdala. 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.</td>
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<tr>
<td><strong>KANK1(-)</strong></td>
<td>Intracellular signaling: regulation of actin cytoskeleton dynamics</td>
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<tr>
<td><strong>(ANKR1D5)</strong></td>
<td>This gene encodes the KANK (ANKRD15) 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-dependent 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 particular in prefrontal cortex, amygdala, 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 ANKR1D5 have been reported in a female and a male patient with ASD. The male patient presented also a gonadal dysgenesis. Deregulation of Wnt/β-catenin signaling pathway has been implicated in the pathogenesis of ASD as well as 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.</td>
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<tr>
<td><strong>KIF2A(-)</strong></td>
<td>Intracellular trafficking: regulation of microtubule cytoskeleton dynamics</td>
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<tr>
<td><strong>(KIF2A)</strong></td>
<td>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-aspartate (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.</td>
</tr>
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</table>
**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 mitochondrial 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. |
| **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, subthalaic nucleus, globus pallidus, ciliary ganglion and spinal cord. The MACROD2 haploinsufficiency 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. |
| **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 maintaining 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. Mutations and/or CNVs affecting MAPK15 have never been reported in patients with ASD. |
| **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. | 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. |

**References:**
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- Mau et al., 2007
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- Abe et al., 2002
- Grotheer and Lannigan, 2010
- Klevecic et al., 2006, 2009
- Libbey et al., 2008
- Mostafa and Al-Ayash, 2011
- Singh, 2009
- Stephenson et al., 2011
- Vogliani et al., 2002
- Yang et al., 2011, 2012
- Zimmerman et al., 2007
- Zou et al., 2011
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<tr>
<td><strong>MCTP2(-)</strong></td>
<td>This gene encodes the multiple C2 domains, transmembrane 2 protein.</td>
<td>Mild expression in postnatal temporal lobe and amygdala.</td>
<td>MCTP2 may be involved in intercellular signal transduction and synaptic function.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting MCTP2 have never been reported in patients with ASD.</td>
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<tr>
<td>Intracellular membrane trafficking</td>
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<td><strong>ME3(-)</strong></td>
<td>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.</td>
<td>High expression in fetal brain and in postnatal CNS, in particular in prefrontal cortex, thalamus, hypothalamus, amygdala, caudate nucleus, and amygdala.</td>
<td>ME3 is involved in the metabolic pathway of glycolysis. Energy metabolism is essential for neuronal growth and function.</td>
<td>Proteome analysis of the thalamus and cerebrospinal fluid performed post-mortem in patients affected by SCZ reveals glycolysis dysfunction.</td>
<td>Mutations and/or CNVs affecting ME3 have never been reported in patients with ASD.</td>
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<td>Pyruvate metabolism-glycolysis</td>
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<td><strong>MPHOSPH9(+)</strong></td>
<td>This gene encodes the M-phase phosphoprotein 9, which has a role in negative regulation of transcription through methylated DNA binding and in cell cycle regulation.</td>
<td>Good high expression in fetal brain and in postnatal CNS, in particular in occipital and parietal lobes, prefrontal cortex, amygdala, thalamus and hypothalamus.</td>
<td>Good high expression in fetal brain and in postnatal CNS, in particular in prefrontal cortex, thalamus, hypothalamus.</td>
<td>High expression in immune cell types.</td>
<td>Mutations and/or CNVs affecting MPHOSPH9 have never been reported in patients with ASD.</td>
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<tr>
<td>Transcriptional regulation</td>
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<tr>
<td><strong>MPHOSPH9(-)</strong></td>
<td>This gene encodes the M-phase phosphoprotein 9, which is involved in cell cycle regulation.</td>
<td>High expression in fetal brain and in postnatal CNS, in particular in cortex, parietal lobe, thalamus and hypothalamus.</td>
<td>High expression in immune cell types.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting MPHOSPH9 have never been reported in patients with ASD.</td>
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<td>Cell cycle regulation</td>
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<td><strong>NDST2(-)</strong></td>
<td>This gene encodes the N-deacylase/N-sulfotransferase 2 protein, which is a member of the N-deacylase/N-sulfotransferase subfamily of the sulfotransferase 1 proteins. The encoded enzyme has dual functions in processing glucosamine and heparin polymers, including N-deacylation and N-sulfation.</td>
<td>Moderate expression in fetal brain and good expression in postnatal cortex and thalamus.</td>
<td>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.</td>
<td>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 HS3ST3 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 GPC3/GPC5 gene cluster, which encodes the glypicans 5 and glypicans 6 HSPGs in tandem array, on chromosome 13q22.</td>
<td>Mutations and/or CNVs affecting NDST2 have never been reported in patients with ASD.</td>
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<tr>
<td>de novo</td>
<td>Heparan sulfate and heparin biosynthesis</td>
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Tab. 4. Continued.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression and Function</th>
<th>Mutations and/or CNVs affecting</th>
<th>Reference(s)</th>
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</table>
| **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.  
NLGNX4 directly interacts with neurexins-1β through Ca²⁺ 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. | Mutations and/or CNVs affecting NLGN4X have never been reported in patients with ASD. | 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. | Mutations and/or CNVs affecting PARD3 have never been reported in patients with ASD. | Anney et al., 2012, Buljte 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 neurogenesis. 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 PARD6G have been associated to ASD.  
Point mutations and CNVs affecting TSC1 and TSC2 have been reported in patients with ASD and Tubersclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates Cdc42, thus regulating cell adhesion and migration. | Mutations and/or CNVs affecting PARD6G have never been reported in patients with ASD. | Anney et al., 2012, Da Silva et al., 2003, Fombonne et al., 1997, Lewis et al., 2004, Liššik et al., 2010, Muzikowski et al., 2007, Perta et al., 2008, Wurtzleben, 2004, Zhang et al., 2006 |
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<tr>
<td><strong>PCDH7(+)</strong></td>
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<td>Synaptogenesis and</td>
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<td>the protocadherin</td>
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<td>protocadherin</td>
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<td>cadherin family.</td>
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<td>PCDH7 is an integral</td>
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<td>distribution of mRNAs</td>
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<td>PCDH11, PCDH17, and</td>
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<td>PCDH20) exhibited</td>
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<td>particular, PCDH7 and</td>
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<td>PCDH20 mRNAs were</td>
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<td>predominantly expressed</td>
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<td>(parietal) and visuospatial</td>
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<td>(occipital) cortices.</td>
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<td>Mutations and/or CNVs affecting PCDH7 have never</td>
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<td>been reported in patients with ASD.</td>
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<td>Homozygous deletion</td>
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<td>PCDH10 has been</td>
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<td>protocadherin PCDH17</td>
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<td>is involved in the</td>
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<td>pathogenesis of SCZ.</td>
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| **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        |
| amygdala 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 y- |
| tubulin complex to   |
| the centrosome,      |
| providing microtubule |
| nucleation sites.    |
| Thus DISC1-PCNT       |
| interaction might be  |
| involved in the       |
| pathophysiology of   |
| mental disorders     |
| owing to its 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.  |

<p>| <strong>PDE9A(-)</strong>*       |
| 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. |
| Very 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 serotoninergic 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. |</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Expression</th>
<th>Related Studies</th>
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<tr>
<td><strong>PLAG1</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Transcriptional regulation: control of cell fate and proliferation</td>
<td>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 hypothalamus.</td>
<td>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 Kini et al., 2008 Zhao et al., 2006</td>
</tr>
<tr>
<td><strong>PLD5</strong></td>
<td>Neurodevelopment</td>
<td>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 Dhani and Ferguson, 2006</td>
<td>Anney et al., 2010 Dhami and Ferguson, 2006</td>
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<tr>
<td><strong>PLXNA3</strong>&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Intracellular semaphorin signaling: regulation of actin cytoskeleton dynamics</td>
<td>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, amygdala, 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 such as PLXNA4 in the anterior cingulate cortex in autism. Gatt et al., 2009 Giger et al., 2000 Sahay et al., 2005 Suda et al., 2011 Tamamaki et al., 2003 Tran et al., 2009</td>
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<tr>
<td><strong>POLR1A</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Transcriptional regulation</td>
<td>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.</td>
<td>A duplication involving REEP1-POLR1A has been recently detected in three autistic subjects. Donati et al., 2011 Holk et al., 2012</td>
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<td>Gene</td>
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<td>Expression Patterns</td>
<td>Protein Functions/Pathways</td>
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<tr>
<td><strong>PREP(-)</strong>&lt;br&gt;de novo</td>
<td>CNS metabolism: neuropeptide maturation and degradation</td>
<td>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, amygdala 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 gene trap 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.</td>
<td>Mutations and/or CNVs affecting PREP have never been reported in patients with ASD.</td>
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<tr>
<td><strong>PRKCA(-)</strong></td>
<td>Intracellular signaling: regulation of actin cytoskeleton dynamics</td>
<td>This gene encodes 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, subthalamic 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 behavioral 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.</td>
<td>Mutations and/or CNVs affecting PRKCA have never been reported in patients with ASD.</td>
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<tr>
<td><strong>PRMT8(-)</strong>&lt;br&gt;Post-translational modifications: arginine methylation</td>
<td>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. 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.</td>
<td>Mutations and/or CNVs affecting PRMT8 have never been reported in patients with ASD.</td>
<td>Mostafa and AL-Ayadhi, 2011 D'Agostino et al., 2012 D'Agostino et al., 2012</td>
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<tr>
<td><strong>PRR16(-)</strong>&lt;br&gt;Unknown function</td>
<td>This gene encodes for the proline rich 16 protein with unknown function.</td>
<td>High expression in fetal brain and in postnatal brain, particularly in postnatal parietal lobe, cerebellum, thalamus, and caudate nucleus. No interacting proteins have been reported.</td>
<td>Mutations and/or CNVs affecting PRR16 have never been reported in patients with ASD.</td>
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<tr>
<td><strong>PSPC1(+)</strong></td>
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<td><strong>Transcriptional regulation</strong></td>
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<td>This gene encodes the paraspeckle component 1 protein, a nucleolar protein which localizes to 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.</td>
<td>PSCP1 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.</td>
<td>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 other neuropsychiatric disorders.</td>
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<td><strong>PTPN12(-)</strong>*</td>
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<td><strong>Intracellular FAK signaling: regulation of actin cytoskeleton dynamics</strong></td>
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<td>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 particular in prefrontal cortex, amygdala, and hypothalamus. High expression in immune cell types.</td>
<td>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.</td>
<td>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.</td>
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<tr>
<td><strong>RAP1GDS1(-)</strong>*</td>
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<td><strong>Intracellular signaling: regulation of actin cytoskeleton dynamics</strong></td>
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<td>This gene encodes the RAP1, GDP-GDP dissociation stimulator 1 protein, which stimulates GDP/GTP exchange reaction of a 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.</td>
<td>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.</td>
<td>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 Tuberosous 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 other neuropsychiatric disorders.</td>
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### REEP1(-)*

**Intracellular membrane trafficking: ER-shaping**

| 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 protein 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. |
| High expression in fetal brain and in postnatal CNS. |
| CNVs affecting REEP1 have recently been reported in patients with ASD. |

### RFX3(-) de novo

**Transcriptional regulation in ciliogenesis**

| 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 (DYNCL1L1, FOXJ1, and BBS4) and genes involved in ciliary motility (DNAH11, DNAH9, and DNAH5). |
| Moderate expression in fetal brain and in postnatal parietal and temporal lobes, and thalamus. |
| It has been reported that in mouse RFX3 is expressed strongly in the ciliated ependymal cells of the subcommissural organ, choroidal 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. |

### RNASEH2B(-)

**DNA replication**

| Defects in RNASEH2B are a cause of Aicardi-Goutières 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. |
| Moderate expression in fetal brain and amygdala. |
| High expression in immune cell types. |
| Mutations and/or CNVs affecting RNASEH2B have never been reported in patients with ASD. |

### RPL10(+)

**Protein synthesis**

| 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. |
| High expression in brain, in particular in hippocampus. |
| 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. |

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* Tab. 4. Continued.
| **SELE(+)** | CNS immunosurveillance | 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. Immunohistology 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. |
| **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 expression 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 neurodevelopmental 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. |
| **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. 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. |
### 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.

**Gene Information**

- **Mutations and/or CNVs affecting SMARCC1 have never been reported in patients with ASD.**
  - Choi et al., 2012
  - Mari 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 particular 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.

**Gene Information**

- **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 hypothalamus.

SNX6 is expressed in fetal brain and, recently, it has been demonstrated a role in oligodendrocyte differentiation.

SNX6 is a candidate locus for holoprosencephaly.

**Gene Information**

- **Mutations and/or CNVs affecting SNX6 have never been reported in patients with ASD.**
  - Kamnasaran et al., 2005
  - Schindl 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.

**Gene Information**

- **Mutations and/or CNVs affecting TMSB15B have never been reported in patients with ASD.**
  - Kim et al., 2008
### Tab. 4. Continued.

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<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Expression/Pathway</th>
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<tr>
<td><strong>TSPAN5(-)</strong></td>
<td>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.</td>
<td>Very high expression in fetal brain and in postnatal CNS.</td>
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<td><strong>USP9Y(-)</strong></td>
<td>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.</td>
<td>Quite high expression in fetal brain and postnatal CNS, in particular in parietal and temporal lobes, amygdala and thalamus.</td>
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<td><strong>VAT1(-)</strong></td>
<td>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.</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
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**NOTCH2** interacts with CNTN1, contactin 1, involved in brain development, specifically in synapse formation. 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. 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. Mutations and/or CNVs affecting USP9Y have never been reported in patients with ASD.

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.

A significant association of SNPs in USP9Y with susceptibility to ASD has been reported. The protein ubiquitination pathway has been previously implicated in ASD. Mutations and/or CNVs affecting VAT1 have never been reported in patients with ASD.

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<tr>
<td>XRN1*</td>
<td>RNA degradation</td>
<td>Moderate expression in fetal brain and postnatal prefrontal cortex, thalamus and amygdala.</td>
<td>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.</td>
<td>Clifford et al., 2007; Moser et al., 2007; Shatavarian et al., 2003; Wang et al., 2010</td>
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<td>ZFX*</td>
<td>Chromatin remodeling</td>
<td>High expression in immune cell types.</td>
<td>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.</td>
<td>Adegbola et al., 2008; Albarián and Huang, 2009; Azziziana et al., 2009</td>
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<tr>
<td>ZMYM5 or ZNF237*</td>
<td>Transcriptional regulation</td>
<td>Moderate expression in postnatal parietal lobe, cerebellum and cerebellum peduncles.</td>
<td>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.</td>
<td>De Ferrari and Moon, 2006; De Strooper, 2003; Kopan and Goetz, 2000; Nizzari et al., 2012; Tommerup and Vissing, 1995</td>
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<tr>
<td>ZNF138(-)</td>
<td>Transcriptional regulation</td>
<td>Moderate expression in fetal brain and postnatal CNS, high expression in cerebellum.</td>
<td>ZNF138 is probably involved in development.</td>
<td>Okerlund and Cheyette, 2007; Pastoric and Das, 2007; Tommerup and Vissing, 1995</td>
</tr>
<tr>
<td>ZNF236(-)*</td>
<td>Transcriptional regulation</td>
<td>It is ubiquitous expressed with the highest levels in skeletal muscle and brain.</td>
<td>Mutations and/or CNVs affecting ZNF236 have never been reported in patients with ASD.</td>
<td>Zhang et al., 2010; Zhang et al., 2012</td>
</tr>
</tbody>
</table>
Tab. 4. Continued.

<table>
<thead>
<tr>
<th>ZNF280A(-)</th>
<th>This gene encodes 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 amygdala.</th>
</tr>
</thead>
</table>

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 neuronal 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 reticulum; 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, Silver-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)§.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Function and expression</th>
<th>Interactors and possible role in brain</th>
<th>Findings in ASDs or in other neuropsychiatric disorders</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient 10, gain of 3.9 Mb at 2q14.2q14.3 (chr2:119130298-123004562)</strong></td>
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<tr>
<td><strong>CLASP1(+)</strong></td>
<td>Intracellular membrane trafficking: regulation of microtubule cytoskeleton dynamics</td>
<td>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.</td>
<td>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 (D LB), 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.</td>
<td>Mutations and/or CNVs affecting CLASP1 have never been reported in patients with ASD.</td>
</tr>
<tr>
<td><strong>C1QL2(+)</strong></td>
<td>CNS development</td>
<td>This gene encodes the complement component 1, q subcomponent-like 2. Highly expressed in the CNS.</td>
<td>Many member of the C1q family are secreted and play a crucial role in intercellular signaling. The gene expression of the C1q 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, Chlb, 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.</td>
<td>A de novo duplication of 4.2 Mb at 2q14.1q4.2, 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.</td>
</tr>
<tr>
<td>Gene</td>
<td>Role</td>
<td>Additional Information</td>
<td>References</td>
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</table>
| **DBI** (+) | 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 neuromodulator 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 2q14.1q4.2, 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** (+) | 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 together with β-catenin regulates transcription of β-catenin target genes in neuronal cells. | 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 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  
De Ferrari and Moon, 2006 |
| **EPB41L5** (+) | 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 2q14.1q4.2, 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 SNP in DBI has been associated with anxiety disorders with panic attacks. | Devillard et al., 2010  
Lee et al., 2007  
Trpass, 2009 |
<table>
<thead>
<tr>
<th><strong>GLI2(+)</strong></th>
<th>Transcriptional regulation</th>
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</table>
| 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 embryos, 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 of the Sonic hedgehog (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 tenecephalic 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 ptiary 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 dyskinesia in patients with chronic SCZ. |}

<table>
<thead>
<tr>
<th><strong>MARCO(+)</strong></th>
<th>Microglial cells maturation</th>
</tr>
</thead>
</table>
| 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, Jinotecis et al., 2003, Ling and Wong, 1993, Matsyak et al., 1999, Ulvestad et al., 1994 |}

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<tr>
<th><strong>PCDP1(+)</strong></th>
<th>Ciliogenesis and ciliary motility</th>
</tr>
</thead>
</table>
| 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 |}

<table>
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<tr>
<th><strong>PTPN4(+)</strong></th>
<th>Intracellular signaling</th>
</tr>
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</table>
| 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 particular in thalamus and amygdalae. High expression in immune cell types. | PTP4N 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. |}
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 multifunctional 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.

Mutations and/or CNVs affecting RNU4ATAC have never been reported in patients with ASD. 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. Mutations and/or CNVs affecting RNU4ATAC have never been reported in patients with ASD.
### Patient 14, gain of 442.5 kb at 9q34.3 (chr9:140527202-140969676)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Literature References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACNA1B(*)</td>
<td>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. 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 comorbid 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.</td>
<td>Andelka et al., 2002, Bhui et al., 2012, Davisson et al., 2002, Glessner et al., 2010, Hemara-Wahabani et al., 2005, Ikeda et al., 2004, Kleefstra et al., 2005, 2009, McMullan et al., 2009, Moskvina et al., 2009, Sahou et al., 2006, Sapienza et al., 2004, 2006</td>
</tr>
<tr>
<td>EHMT1(*)</td>
<td>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-mortem human brain of patients affected by neuropsychiatric disorders vs. controls. EHMT1 point mutations and deletions of different size include EHMT1 cause the 9q subtelomeric deletion syndrome (Kleefstra syndrome), which is comorbid with ASD. Balanced chromosomal abnormalities affecting EHMT1 have been found in autistic patients.</td>
<td>Albarran and Huang, 2009, Anderlid et al., 2002, Davisson et al., 2002, Ikeda et al., 2004, Kleefstra et al., 2005, 2009, McMullan et al., 2009, Sahou et al., 2006, Talkowski et al., 2012</td>
</tr>
<tr>
<td>ACBD4(-)</td>
<td>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 acbd4 and acbd3 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.</td>
<td>Betancur et al., 2008, Drew et al., 2012, Gisart et al., 2009, Koellen et al., 2008</td>
</tr>
<tr>
<td>CRHR1(-)</td>
<td>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.</td>
<td>Amath et al., 2012, Betancur et al., 2008, Gisart et al., 2009, Hsu et al., 2012, Koellen et al., 2008, Reljic et al., 2011, Zhang et al., 2012</td>
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</table>

### Patient 23, loss of 748 kb, at 17q21.31 (chr17:43193251-43941693)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Literature References</th>
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<tbody>
<tr>
<td>CACNA1B</td>
<td>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.</td>
<td>Bhat et al., 2012, Drew et al., 2012, Glessner et al., 2010, Ikeda et al., 2004, McMullan et al., 2009, Moskvina et al., 2009, Talkowski et al., 2012</td>
</tr>
<tr>
<td>EHMT1</td>
<td>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-mortem human brain of patients affected by neuropsychiatric disorders vs. controls. EHMT1 point mutations and deletions of different size include EHMT1 cause the 9q subtelomeric deletion syndrome (Kleefstra syndrome), which is comorbid with ASD. Balanced chromosomal abnormalities affecting EHMT1 have been found in autistic patients.</td>
<td>Albarran and Huang, 2009, Anderlid et al., 2002, Davisson et al., 2002, Ikeda et al., 2004, Kleefstra et al., 2005, 2009, McMullan et al., 2009, Sahou et al., 2006, Talkowski et al., 2012</td>
</tr>
<tr>
<td>ACBD4</td>
<td>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 acbd4 and acbd3 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.</td>
<td>Betancur et al., 2008, Drew et al., 2012, Gisart et al., 2009, Koellen et al., 2008</td>
</tr>
<tr>
<td>CRHR1</td>
<td>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.</td>
<td>Amath et al., 2012, Betancur et al., 2008, Gisart et al., 2009, Hsu et al., 2012, Koellen et al., 2008, Reljic et al., 2011, Zhang et al., 2012</td>
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<td><strong>Tab. 4.1. Continued.</strong></td>
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<tr>
<td><strong>FMNL1(-) de novo</strong></td>
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<tr>
<td>Neurodevelopment and macrophages motility and survival: actin cytoskeleton dynamics</td>
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<tr>
<td>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.</td>
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<tr>
<td>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.</td>
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<tr>
<td>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.</td>
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<tr>
<td>Mutations and/or CNVs affecting FMNL1 have never been reported in patients with ASD.</td>
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<tr>
<td><strong>CHRNA7(+)</strong> Synaptogenesis and synaptic plasticity</td>
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<td>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.</td>
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<tr>
<td>High expression in fetal brain and in postnatal CNS, in particular in cortex, amygdalae and thalami.</td>
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<tr>
<td>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.</td>
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<tr>
<td>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 encompassing CHRNA7 have been found in patients with ADHD, DD, and ID.</td>
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</table>

*Patient 25, gain of 377 kb at 15q13.3 (chr15:32085731-32462701)*
<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Expression</th>
<th>Phenotype</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDOA</td>
<td>Brain metabolism: glycolysis</td>
<td>Low expression in fetal brain and good expression in postnatal CNS.</td>
<td>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.</td>
<td>ALDOA maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
</tr>
<tr>
<td>ASPHD1</td>
<td>Neurodevelopment</td>
<td>Good expression in fetal brain and in postnatal CNS, in particular in cerebellum.</td>
<td>The zebrafish loss of function model for the ASPHD1 gene shows a weak phenotype with defective in brain morphology.</td>
<td>ASPHD1 maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
</tr>
<tr>
<td>CDIPT</td>
<td>Intracellular signaling: phosphatidylinositol signaling system</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
<td>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 been reported in the olfactory mitral cells, the cerebral cortex, the hippocampal and dentate neuronal layer and the cerebellar Purkinje and granule cells.</td>
<td>CDIPT maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
</tr>
</tbody>
</table>

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**Tab. 4.1. Continued.**

- Patient 25, gain of 538 kb at 16p11.2 (chr16:29652999-30190568)
- Patient 38, gain 659 kb at 16p11.2 (chr16:29673954-30332851)
- Patient 39, gain 659 kb at 16p11.2 (chr16:29673954-30332851)

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- Blakeslee et al., 2012
- Bjihma et al., 2009
- Christian et al., 2008
- Fernandez et al., 2010
- Hanson et al., 2010
- Kumar et al., 2008
- Marshall et al., 2008
- Meshida et al., 1998
- Pattoo et al., 2010
- Rosenfeld et al., 2010
- Shina et al., 2010
- Weiss et al., 2008
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<tr>
<th>Tab. 4.1. Continued.</th>
<th>CORO1A(+)</th>
<th>C16orf53(+)</th>
<th>DOC2A(+)</th>
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<tr>
<td><strong>CNS immunosurveillance, neurodevelopment: actin cytoskeleton dynamics</strong></td>
<td>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.</td>
<td>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.</td>
<td>This gene encodes the double C2-like domains, alpha protein. DOC2A is mainly expressed in brain and is suggested to be involved in Ca²⁺-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.</td>
</tr>
<tr>
<td><strong>Chromatin remodeling</strong></td>
<td>CORO1A maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
<td>C16orf53 maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
<td>DOC2A maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
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</table>
| **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 hematopoietic cells, are T-lymphopcytopenic 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.** | The zebrafish loss of function model for C16orf53 shows defective in brain morphology. | 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. | **References:**
- Blakes-Lee et al., 2012
- Bijlma 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
- Mechina et al., 1998
- Pinto et al., 2010
- Rosenfeld et al., 2010
- Shankar et al., 2000
- Shaw et al., 2008, 2009
- Urech and Bear, 2006
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<th>Model</th>
<th>Genomic Region</th>
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<tr>
<td><strong>FAM57B</strong> (+)</td>
<td>Neurodevelopment</td>
<td>Good expression in fetal brain and in postnatal CNS.</td>
<td>The zebrafish loss of function model for FAM57B shows defective in brain morphology.</td>
<td>FAM57RA maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
<td>Blaker-Lee et al., 2012, Bijloma et al., 2009, Fernandez et al., 2010, Hanson et al., 2010, Kumar et al., 2008, Marshall et al., 2008, Mochida et al., 1998, Pato et al., 2010, Rosenfeld et al., 2010, Shinawi et al., 2010, Weiss et al., 2008</td>
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<tr>
<td><strong>GDPD3</strong> (+)</td>
<td>Neurodevelopment</td>
<td>Moderate expression in postnatal CNS.</td>
<td>The zebrafish loss of function model for GDPD3 shows defective in brain morphology.</td>
<td>GDPD3 maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
<td>Blaker-Lee et al., 2012, Bijloma et al., 2009, Fernandez et al., 2010, Hansson et al., 2010, Kumar et al., 2008, Marshall et al., 2008, Mochida et al., 1998, Pato et al., 2010, Rosenfeld et al., 2010, Shinawi et al., 2010, Weiss et al., 2008</td>
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<tr>
<td><strong>HIRIP3</strong> (+)</td>
<td>Neurodevelopment: histone metabolism</td>
<td>Low expression in fetal brain and in postnatal CNS, except for amygdala, cerebellum and thalamus where good levels of expression have been detected.</td>
<td>The zebrafish loss of function model for HIRIP3 shows defective in brain morphology.</td>
<td>HIRIP3 maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
<td>Blaker-Lee et al., 2012, Bijloma et al., 2009, Fernandez et al., 2010, Hanson et al., 2010, Kumar et al., 2008, Marshall et al., 2008, Mochida et al., 1998, Pato et al., 2010, Rosenfeld et al., 2010, Shinawi et al., 2010, Weiss et al., 2008</td>
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<tr>
<td><strong>INO80E</strong> (+)</td>
<td>Chromatin remodeling, transcriptional regulation</td>
<td>Moderate expression in fetal brain and in postnatal whole brain.</td>
<td>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.</td>
<td>INO80E maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
<td>Blaker-Lee et al., 2012, Bijloma et al., 2009, Fernandez et al., 2010, Hanson et al., 2010, Kumar et al., 2008, Marshall et al., 2008, Mochida et al., 1998, Pato et al., 2010, Rosenfeld et al., 2010, Shinawi et al., 2010, Weiss et al., 2008</td>
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<tr>
<td><strong>KCTD13(+)</strong></td>
<td>Neurodevelopment: actin cytoskeleton dynamics</td>
<td>Good expression in fetal brain and in postnatal CNS.</td>
<td>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 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, CD1DPT, SEZ6L2, ASPHD1 and KCTD13, and a subject with a complex 16p11.2 rearrangement involving de novo structural alteration of KCTD13.</td>
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<tr>
<td><strong>KIF22(+)</strong></td>
<td>Intracellular membrane trafficking: regulation of microtubule cytoskeleton dynamics</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
<td>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.</td>
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<tr>
<td><strong>MAPK3-ERK1(+)</strong></td>
<td>Intracellular MAP kinase signaling</td>
<td>Good expression in fetal brain and in postnatal CNS.</td>
<td>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.</td>
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<td>Gene (+)</td>
<td>Description</td>
<td>Expression Patterns</td>
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<td><strong>MAZ (+)</strong></td>
<td>Neurodevelopment</td>
<td>This gene encodes the MYC-associated zinc finger protein, a purine-binding transcription factor.</td>
<td>High expression in dendritic cells, NK and thalamus. Low expression in postnatal CNS, in particular in amygdalae and thalamus.</td>
<td>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.</td>
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<tr>
<td><strong>MVP (+)</strong></td>
<td>Intracellular signaling: regulation of MAP kinase signaling</td>
<td>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 nucleocytoplasmic 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.</td>
<td>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 phallolin 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.</td>
<td>MVP maps within the genomic region involved in the 16p11.2 microdeletion/microduplication syndrome, which shows comorbidity with ASD, EP, SCZ, ID and ADHD.</td>
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<tr>
<td><strong>QPRT (+)</strong></td>
<td>Tryptophan metabolism</td>
<td>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 excitotoxin to neurons.</td>
<td>Moderate expression in fetal brain and in postnatal CNS. High expression in dendritic cells, NK-cells and monocytes. The zebrafish homolog of human QPRT is not known.</td>
<td>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 immunoeexcitotoxicity (see the ACMDS gene).</td>
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<tr>
<td>PPP4C (+)</td>
<td>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 (JNK) 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-kappa B is an important gene transcriptional factor that mediates cellular responses in inflammation, immunity, development, cell proliferation and apoptosis. Elevated levels of NF-kappa B have been reported in autistic patients vs. controls.</td>
<td>High expression in fetal brain and in postnatal CNS. High expression in cerebellum.</td>
<td>Neurodevelopment: microtubule cytoskeleton dynamics</td>
<td>Blakers-Lee et al., 2012 Bijloma et al., 2009 Bonito 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 Nak et al., 2011 Philippé et al., 2012 Pinto et al., 2010 Rosenbergfeld et al., 2010 Shiu et al., 2010 Weiss et al., 2008</td>
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<tr>
<td>PRRT2 (+)</td>
<td>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 choreothetosis (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.</td>
<td>Good expression in fetal brain and in postnatal CNS.</td>
<td>Neurodevelopment</td>
<td>Blakers-Lee et al., 2012 Bijloma et al., 2009 Christian et al., 2008 Fernandez et al., 2010 Gahm et al., 2002 Hamano et al., 1995 Hanson et al., 2010 Hayashi et al., 1997 Itai et al., 2004 Ko et al., 2001 Kumar et al., 2008 Marshall et al., 2008 Mochida et al., 1998 Pinto et al., 2010 Rosenbergfeld et al., 2010 Shiu et al., 2010 Shurase et al., 2001 Stedl et al., 2005 Volonte et al., 2001 Wang et al., 2011 Weiss et al., 2008</td>
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<tr>
<td>SEZ6L2 (+)</td>
<td>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 demonstrated 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 with ASD, EP, SCZ, ID and ADHD. A coding variant in SEZ6L2 has been significantly associated with ASD (data not replicated).</td>
<td>Very high expression in fetal brain and in postnatal CNS.</td>
<td>Neurodevelopment</td>
<td>Blakers-Lee et al., 2012 Bijloma et al., 2009 Christian et al., 2008 Fernandez et al., 2010 Hanson et al., 2010 Konyukh et al., 2011 Kumar et al., 2008 Marshall et al., 2008 Mochida et al., 1998 Pinto et al., 2010 Rosenbergfeld et al., 2010 Shiu et al., 2010 Weiss et al., 2008</td>
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<td><strong>SPN(+)</strong></td>
<td>T-cell activation</td>
<td>Moderate expression in fetal brain and low expression in postnatal CNS, except for amygdala and hypothalamus where expression is moderate. High expression in immune cell types.</td>
<td>SPN, which 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.</td>
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<td><strong>SULT1A3(+)</strong></td>
<td>CNS metabolism</td>
<td>Good expression in fetal brain and moderate expression in postnatal CNS. High expression in immune cell types.</td>
<td>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.</td>
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<tr>
<td><strong>TAOK2(+)</strong></td>
<td>Intracellular signaling: dendrite morphogenesis</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
<td>Very recently, it has been demonstrated that TAOK2 is essential for dendrite morphogenesis. TAOK2 downregulation impairs basal dendritic 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.</td>
</tr>
<tr>
<td><strong>YPEL3(+)</strong></td>
<td>Neurodevelopment</td>
<td>Good expression in fetal brain and in postnatal CNS.</td>
<td>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.</td>
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References:
- Bijlsma et al., 2009
- Christian et al., 2008
- Fernandez et al., 2010
- Hanon et al., 2010
- Kumar et al., 2008
- Marshall et al., 2008
- Mechida et al., 1998
- Pinto et al., 2010
- Rosenfeld et al., 2010
- Shinawi et al., 2010
- Weiss et al., 2008
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)

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<td>AIFM3(+)+ de novo</td>
<td>Neurodevelopment</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
<td>AIFM3 maps within the genomic region involved in the 22q11.21 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
</tr>
<tr>
<td>ARVCF(+)+ de novo</td>
<td>Neurodevelopment</td>
<td>Good expression in fetal brain and in postnatal CNS.</td>
<td>ARVCF maps within the genomic region involved in the 22q11.1 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
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<tr>
<td>CDC45(+)+ de novo</td>
<td>DNA replication</td>
<td>Expressed during neurogenesis.</td>
<td>CDC45 maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
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<tr>
<td><strong>CLDN5</strong>&lt;sup&gt;(+)&lt;/sup&gt; de novo</td>
<td>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-Caste 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 Vostrman et al., 2006 Wu et al., 2010</td>
<td><strong>CNS</strong>&lt;sup&gt;+&lt;/sup&gt; Immunosurveillance: brain blood barrier permeability</td>
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<tr>
<td><strong>CLTCL1</strong>&lt;sup&gt;(+)&lt;/sup&gt; de novo</td>
<td>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 amygdala 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 <strong>CLTCL1</strong> 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 homoyzygous, potentially pathogenic recessive mutations affecting candidate genes such as <strong>UBE3B, CLTCL1, NCKAP1L, and ZNF18</strong>, 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 Chastelet et al., 2012 Fine et al., 2005 Holmes et al., 1997 Lo-Caste 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 Vostrman et al., 2006</td>
<td><strong>Intracellular trafficking</strong>&lt;sup&gt;+&lt;/sup&gt;</td>
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<td><strong>COMT</strong>&lt;sup&gt;(+)&lt;/sup&gt; de novo</td>
<td>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 <strong>COMT</strong> have been associated over the years with many neuropsychiatric disorders such as BD, anorexia nervosa, obsessive-compulsive disorders, and aggressive behaviour in SCZ. <strong>COMT</strong> 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 <strong>COMT</strong> 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 Fush et al., 2001 Garret et al., 1993 James et al., 2006 Jones et al., 2001 Li et al., 1997 Lo-Caste et al., 2009 Marshall et al., 2008 Mukkades and Herguner, 2007 Neuvoulion, 2002 Niklasson et al., 2009 Pinto et al., 2010 Ramelli et al., 2008 Schindler et al., 2000 Siczek et al., 2009 Szatmari et al., 2007 Tushbridge et al., 2006 Vostrman et al., 2006</td>
<td><strong>Catecholamine metabolism</strong>&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td>Gene</td>
<td>Type</td>
<td>Expression</td>
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<td><strong>CRKL</strong>&lt;sup&gt;+&lt;/sup&gt;</td>
<td><strong>de novo</strong> Intracellular signaling</td>
<td>Moderate expression in fetal brain and in postnatal cerebellum and thalamus.</td>
<td>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 <strong>CRKL</strong>, <strong>ZNF74</strong>, <strong>PIK4CA</strong>, <strong>SNAP29</strong> and <strong>PCQAP</strong> known to contribute to several aspects of the DGS/VCF phenotype, has been recently described in a patient with psychomotor impairment, suggesting a role for these genes in neurodevelopment. <strong>CRKL</strong> maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
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<tr>
<td><strong>DGCR2</strong>&lt;sup&gt;+&lt;/sup&gt;</td>
<td><strong>de novo</strong> Neurodevelopment</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
<td>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. <strong>DGCR2</strong> could be involved in cell-cell or cell-matrix interactions required for normal cell differentiation and migration. The association between <strong>DGCR2</strong> and SCZ has been demonstrated through individual genotyping of 1,400 subjects. In a subsequent gene expression analysis the risk allele of a coding SNP associated with SCZ was found to be associated with a reduced expression of <strong>DGCR2</strong>. This datum was not later replicated in a German sample. A de novo potentially disruptive mutation in <strong>DGCR2</strong> has been recently detected by exome sequencing analysis in a cohort of SCZ patient. <strong>DGCR2</strong> maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
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<th>*<em>DGCR6(-)<em>de novo</em></em></th>
<th><strong>DGCR6(+)</strong> de novo</th>
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<td><strong>Synaptogenesis and synaptic plasticity</strong></td>
<td><strong>Synaptogenesis and synaptic plasticity?</strong></td>
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<td>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.</td>
<td>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.</td>
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<td>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.</td>
<td>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.</td>
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<th>DGCR8 (+) de novo</th>
<th>DGCR14 (+) de novo</th>
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<td><strong>MicroRNA biogenesis</strong></td>
<td><strong>Transcript maturation</strong></td>
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<td>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.</td>
<td>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.</td>
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<td>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 sarcoplasmic 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.</td>
<td>DGCR14 maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. DGCR14 is possibly involved pre-mRNA splicing. It has been demonstrated that the Dr16A−/− mice which carry a microdeletion including the Gscl and Dgcr14 genes, both located within the DiGeorge 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. Evidence of association between promoter polymorphisms in 22q11 gene DGCR14 and SCZ have been detected by transmission disequilibrium test.</td>
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<td>Evidence of association between promoter polymorphisms in 22q11 gene DGCR14 and SCZ have been detected by transmission disequilibrium test.</td>
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**DGCR8** maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.

**DGCR14** maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.
Tab. 4.1. Continued.

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<tr>
<th>Gene</th>
<th>Description</th>
<th>Association with Autism Spectrum Disorder</th>
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<tr>
<td>GNB1L(+)</td>
<td>de novo Intracellular signaling</td>
<td>GNB1L maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
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<td>GP1BB(+)</td>
<td>de novo Neurodevelopment</td>
<td>GP1BB maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
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<td>GSC2(GSCL)(+)</td>
<td>de novo Transcriptional regulation</td>
<td>GSCL maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
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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 (GHEWD), which may facilitate formation of heterotrimetric or multiribosomal 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 Gnb1 on prepulse inhibition, a SCZ endophenotype, in a mouse model, suggest that GNB1L is associated with SCZ phenotype observed in del22q11.2.

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.

This gene encodes the glycoprotein Ib (platelet), beta polypeptide, which is a heterodimeric transmembrane protein consisting of a disulfide-linked 140 kDa alpha chain and 22 kDa beta chain. It is part of the GPIb-IX-V complex 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 GP1BB gene have been associated with Bernard-Soulier syndrome (BSS), velo-cardio-facial 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.

Neither GP1BB nor SEPT5 have been previously suggested as autism candidate genes by molecular cytogenetic analysis and in silico studies.

This gene encodes the glycosidase homeobox 2 protein. Goosecoidlike (GSCL), a homeodomain-containing gene, resides in the critical region for VCFS/DGS on 22q11.21 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 D16HΔ/+ mice which carry a microdeletion including the Gocl and Dgcr14 genes, both located within the DiGeorge 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 Gocl 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 microdeletions/ microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.
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<th><strong>HIRA(+)</strong></th>
<th>de novo</th>
<th>Embryonic development</th>
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<td>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 heterochromatin. 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 haplinsufficiency 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.</td>
<td>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.</td>
<td>Antshel et al., 2007 Bucan et al., 2009 Fine et al., 2005 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 Versițian et al., 2006 Wang and Du, 2005</td>
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<th><strong>KLHL22(+)</strong></th>
<th>de novo</th>
<th>Intracellular signaling: protein ubiquitination pathway</th>
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<td>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.</td>
<td>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.</td>
<td>Antshel et al., 2007 Bucan et al., 2009 Fine et al., 2005 Lin et al., 2011 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 Segref and Hupper, 2009 Szatmari et al., 2007 Tai and Schuman, 2008 Versițian et al., 2006 Yi and Ehlers, 2007</td>
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<th><strong>LZTR1(+)</strong></th>
<th>de novo</th>
<th>Neurogenesis: Golgi complex stabilizer</th>
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<td>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.</td>
<td>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.</td>
<td>Antshel et al., 2007 Bucan et al., 2009 Fine et al., 2005 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 Versițian et al., 2006</td>
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<td>Gene</td>
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<td><strong>MED15</strong>&lt;sup&gt; (+)&lt;/sup&gt;</td>
<td>de novo Transcriptional regulation</td>
<td>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. <strong>MED15</strong> maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder. SNPs in <strong>MED12</strong> have been associated with ASD. Antshel et al., 2007 Beyer et al., 2012 Bucan et al., 2009 Carter, 2009 De Luca et al., 2003 Fite et al., 2005 Lo-Castro et al., 2009 Marshall et al., 2008 Mikkades and Hergerer, 2007 Niklasson et al., 2009 Pinto et al., 2010 Ramelli et al., 2008 Sandhu et al., 2004 Statmari et al., 2007 Vorstman et al., 2006</td>
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<td><strong>MRPL40</strong>&lt;sup&gt; (+)&lt;/sup&gt;</td>
<td>de novo Neurodevelopment and maintenance: mitochondrial protein synthesis</td>
<td>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 projection neurons in the bulbar 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. <strong>MRPL40</strong> 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 Mikkades and Hergerer, 2007 Niklasson et al., 2009 Pinto et al., 2010 Ramelli et al., 2008 Statmari et al., 2007 Vorstman et al., 2006</td>
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<td>Gene</td>
<td>Expression/Protein Function</td>
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<td><strong>PI4KA (+)</strong>&lt;br&gt;de novo&lt;br&gt;Phosphatidylinositol signaling system</td>
<td>Good expression in fetal brain and in postnatal CNS.&lt;br&gt;Good expression in immune cell types.</td>
<td>This gene encodes the phosphatidylinositol 4-kinase catalytic, alpha protein, which catalyzes the first committed step in the biosynthesis of phosphatidylinositol 4,5-bisphosphate. 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, PI4KCA, 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. <strong>Antshel et al., 2007</strong>&lt;br&gt;<strong>Bucan et al., 2009</strong>&lt;br&gt;<strong>Clayton et al., 2012</strong>&lt;br&gt;<strong>Fine et al., 2005</strong>&lt;br&gt;<strong>Iuvone et al., 2010</strong>&lt;br&gt;<strong>Lo-Castro et al., 2009</strong>&lt;br&gt;<strong>Marshall et al., 2008</strong>&lt;br&gt;<strong>Mukkades and Herguner, 2007</strong>&lt;br&gt;<strong>Niklasson et al., 2009</strong>&lt;br&gt;<strong>Peter et al., 2010</strong>&lt;br&gt;<strong>Ramelli et al., 2008</strong>&lt;br&gt;<strong>Stattin et al., 2007</strong>&lt;br&gt;<strong>Vorstman et al., 2006</strong></td>
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<td><strong>PRODH (+)</strong>&lt;br&gt;de novo&lt;br&gt;Proline metabolism</td>
<td>High expression in the fetal brain and in postnatal CNS.</td>
<td>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. <strong>Antshel et al., 2007</strong>&lt;br&gt;<strong>Bucan et al., 2009</strong>&lt;br&gt;<strong>Fine et al., 2005</strong>&lt;br&gt;<strong>Lo-Castro et al., 2009</strong>&lt;br&gt;<strong>Marshall et al., 2008</strong>&lt;br&gt;<strong>Mukkades and Herguner, 2007</strong>&lt;br&gt;<strong>Niklasson et al., 2009</strong>&lt;br&gt;<strong>Pinto et al., 2010</strong>&lt;br&gt;<strong>Ramelli et al., 2008</strong>&lt;br&gt;<strong>Stattin et al., 2007</strong>&lt;br&gt;<strong>Vorstman et al., 2006</strong></td>
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<td><strong>RANBP1 (+)</strong>&lt;br&gt;de novo&lt;br&gt;Intracellular RAN-GTP signaling</td>
<td><strong>Antshel et al., 2007</strong>&lt;br&gt;<strong>Bucan et al., 2009</strong>&lt;br&gt;<strong>Fine et al., 2005</strong>&lt;br&gt;<strong>Lo-Castro et al., 2009</strong>&lt;br&gt;<strong>Marshall et al., 2008</strong>&lt;br&gt;<strong>Mukkades and Herguner, 2007</strong>&lt;br&gt;<strong>Niklasson et al., 2009</strong>&lt;br&gt;<strong>Pinto et al., 2010</strong>&lt;br&gt;<strong>Ramelli et al., 2008</strong>&lt;br&gt;<strong>Stattin et al., 2007</strong>&lt;br&gt;<strong>Vorstman et al., 2006</strong></td>
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</table>
### Tab. 4.1. Continued.

<p>| <strong>RTN4R(+) de novo</strong> | 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 RhøA, 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; Guilmâtre et al., 2008; Lo-Castro et al., 2009; Marshall et al., 2008; Mikkades and Herguner, 2007; Niklasson et al., 2009; Pato et al., 2010; Ramelli et al., 2008; Szatmari et al., 2007; Wills et al., 2012; Vorsiman et al., 2006 |
| <strong>SEPT5(+) de novo</strong> | This gene encodes the septin 5 protein, which 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 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. | Both GP1BB and SEPT5 have been previously suggested as autism candidate genes by molecular cytogenetic analysis and in silico studies. | Antshel et al., 2007; Bartisch et al., 2011; Bucan et al., 2009; Fine et al., 2005; Innis et al., 2010; Lo-Castro et al., 2009; Marshall et al., 2008; Mikkades and Herguner, 2007; Niklasson et al., 2009; Pato et al., 2010; Ramelli et al., 2008; Szatmari et al., 2007; Vorsiman et al., 2006 |
| <strong>SLC7A4(+) de novo</strong> | 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 detected in astrocytes, oligodendrocytes, plexus choroides 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; Jahan et al., 2012; Lo-Castro et al., 2009; Marshall et al., 2008; Mikkades and Herguner, 2007; Niklasson et al., 2009; Pato et al., 2010; Ramelli et al., 2008; Szatmari et al., 2007; Vorsiman et al., 2006 |</p>
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<th>Gene</th>
<th>Description</th>
<th>References</th>
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<tr>
<td><strong>SLC25A1 (Solute Carrier Family 25, Member 1)</strong></td>
<td>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 mitochondrial metabolism.</td>
<td>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; Saito et al., 2001; Sprecher et al., 2005; Su et al., 2001; Statmari et al., 2007; Vorstman et al., 2006</td>
</tr>
<tr>
<td><strong>SNAP29 (Synaptosomal-Associated Protein, 29kDa)</strong></td>
<td>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 hippocampal 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/VCF syndrome phenotype, has been recently described in a patient with psychomotor impairment, suggesting a role for these genes in neurodevelopment.</td>
<td>Antshel et al., 2007; Bucan et al., 2009; de Queiroz Soares et al., 2012; Fine et al., 2005; Góldbrot 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; Pedousa 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; Statmari et al., 2007; Vorstman et al., 2006</td>
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Tab. 4.1. Continued.

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<th>Gene</th>
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<td><strong>TBX1 (+)</strong>&lt;br&gt;de novo</td>
<td>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. 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 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. 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.</td>
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<tr>
<td><strong>TRMT2A</strong>&lt;br&gt;(HTF9C)+&lt;br&gt;de novo</td>
<td>This gene encodes the TRM2 rRNA 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. 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. TRMT2A maps within the genomic region involved in the 22q11 microdeletion/microduplication syndrome, which shows comorbidity with ASD, SCZ, ADHD and mood disorder.</td>
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<tr>
<td><strong>TXNRD2 (+)</strong>&lt;br&gt;de novo</td>
<td>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 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.</td>
</tr>
<tr>
<td><strong>Neurodevelopment?</strong></td>
<td>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. TXB1 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.</td>
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<tr>
<td><strong>Mitochondrial neuronal metabolism</strong></td>
<td>It has been reported that Tb1 heterozygous mice are impaired in social interaction, ultrasonic vocalization, memory-based behavioral alternation, working memory and thigmotaxis, compared with wild-type mice. Furthermore, Tb1 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, Tb1 levels are higher during proliferation than during differentiation, and expressed in neural progenitor cells, immature and matured neurons and glial cells. 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. TX 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 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.</td>
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<td><strong>Genes</strong></td>
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<td><strong>UFD1L</strong></td>
<td>De novo protein ubiquitination pathway</td>
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<tr>
<td><strong>ZDHHC8</strong></td>
<td>De novo Synaptic transmission: post-translational modification (palmitoylation)</td>
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<td><strong>ZNF74</strong></td>
<td>De novo Transcriptional regulation</td>
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<td>Tab. 4.1. Continued.</td>
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<td>Patient 29, gain of 8.0 Mb at 15q11.1q13.1 (chr15:20575646-28535051)</td>
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<tr>
<td>Patient 50, loss of 211 kb at 15q11.2 (chr15:22873688-23085096)</td>
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<tr>
<td>Patient 55, gain of 203 kb at 15q11.2 (chr15:22873688-23076420)</td>
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**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.

**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. 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 ATP10A have been previously associated with ASD.

Bolton, 2004  
Bonati et al., 2007  
Cook et al., 1997  
Depienne et al., 2009  
Drescher-Hamaker et al., 2006  
Dubourg et al., 2009  
Hogart et al., 2008, 2010  
Nurmi et al., 2003  
Pato et al., 2010  
Sahoo et al., 2006  
Schroer et al., 1998  
Steinberg et al., 1996  
Szatmari et al., 2007  
Trillingsgaard and Østergaard, 2004  
Veltman et al., 2005
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<tr>
<th>Gene Symbol</th>
<th>Description</th>
<th>Synaptic Function and Plasticity</th>
<th>Translational Repression</th>
<th>Notes</th>
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<tr>
<td>CYFIP1 (+/+)</td>
<td>De novo</td>
<td>Translational repression</td>
<td>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 through its actin remodeling activity. Moderate expression in fetal brain and in postnatal CNS, particularly in amygdalae.</td>
<td>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.</td>
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<tr>
<td>GABRA5 (+)</td>
<td>De novo</td>
<td>Synaptic function and plasticity</td>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td>GABRB3 (+)</td>
<td>De novo</td>
<td>Synaptic function and plasticity</td>
<td>This gene encodes 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.</td>
<td>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.</td>
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<tr>
<td>Gene</td>
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<tr>
<td>GABRG3</td>
<td>Synaptic function and plasticity</td>
<td>1q13.1</td>
<td>An altered expression of gamma-aminobutyric acid A (GABA) A 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.</td>
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<tr>
<td>MAGEL2</td>
<td>Neurodevelopment</td>
<td>15q11.2-q13.1</td>
<td>MAGEL2 is expressed in various brain regions, most notably the hypothalamus. Mice with a targeted deletion of Mage2 display hypoactivity, blunted circadian rhythm, decreased fertility, and increased adiposity. It has been reported that in Mage2-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. Mage2-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.</td>
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<tr>
<td>MKRN3</td>
<td>Protein ubiquitination pathway</td>
<td>11p13.1</td>
<td>MKRN3 encodes the zinc 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.</td>
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References:
- Bolton, 2004
- Bonati et al., 2007
- Cook et al., 1997
- Depienne et al., 2009
- Dschumacher et al., 2006
- Hogart et al., 2010
- Mirmohseni et al., 2001
- Pato et al., 2010
- Sabo et al., 2006
- Schroier et al., 1998
- Steffenburg et al., 1996
- Sutcliffe et al., 2007
- Trillingsgaard and Østergaard, 2004
- Veltman et al., 2005

*Note: The table contains a continuation of the information provided in Tab. 4.1.*
<table>
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<th>Gene</th>
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| **NDN** | This intronless gene encodes the neclin 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 mice 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. | Bolson, 2004  
Boniati et al., 2007  
Cook et al., 1997  
Depienne et al., 2009  
Drischmeefer et al., 2006  
Higgit et al., 2010  
Pato et al., 2006  
Sahoo et al., 2006  
Schoen et al., 1998  
Steffenbug et al., 1996  
Stattini et al., 2007  
Trillingsgaard and Østergaard, 2004  
Vellman et al., 2005 |
| **NIPA1** | 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. | Doothen et al., 2009  
Sempere Perez et al., 2011  
van der Zwaag et al., 2012  
Zhao et al., 2012 |
| **NIPA2** | 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 amygdala. | Doothen 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** | This gene encodes the small nuclear ribonucleoprotein polypeptide N, which belongs to the snRNP SMN/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' untranscribed region. Good expression in fetal brain and high expression in postnatal CNS. Good-high expression in immune cell types. | Bolson, 2004  
Boniati et al., 2007  
Cook et al., 1997  
Depienne et al., 2009  
Drischmeefer et al., 2006  
Higgit et al., 2010  
Pato et al., 2006  
Sahoo et al., 2006  
Schoen et al., 1998  
Simonin et al., 2004  
Steffenbug et al., 1996  
Stattini et al., 2007  
Talkowski et al., 2012  
Trillingsgaard and Østergaard, 2004  
Vellman et al., 2005 |
Tab. 4.1. Continued.

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<th>Gene</th>
<th>Description</th>
<th>Significance</th>
<th>References</th>
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<tr>
<td><strong>SNURF</strong>&lt;sup&gt;+&lt;/sup&gt;/de novo</td>
<td>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.</td>
<td></td>
<td>Bolton, 2004; Bonati et al., 2007; Cook et al., 1997; Depienne et al., 2009; Deschrenemarkers et al., 2006; Hogart et al., 2010; Pati et al., 2010; Sahoo et al., 2006; Schon et al., 1998; Steffenburg et al., 1996; Sztatmai et al., 2007; Takaoko et al., 2012; Trillinggaard and Østergaard, 2004; Veltman et al., 2005</td>
</tr>
<tr>
<td><strong>TUBGCP5</strong>&lt;sup&gt;+&lt;/sup&gt;</td>
<td>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.</td>
<td>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. Biochemical 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 GCP9 and both proteins participate in the proper formation of the mitotic spindles.</td>
<td>Doerrhos et al., 2009; Inzani et al., 2008; Murphy et al., 2001; Spreade et al., 2011; van der Zwaag et al., 2010; Zhao et al., 2012</td>
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<tr>
<td><strong>cluster snoRNAs</strong>&lt;sup&gt;+&lt;/sup&gt;/de novo</td>
<td>These genes encode different classes of small nucleolar RNAs. High expression in the CNS.</td>
<td>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 15q11-13 duplication syndrome, which is the most frequently chromosomal anomaly associated with ASD. A few small deletions of different class of snoRNA genes</td>
<td>Bolton, 2004; Bonati et al., 2007; Cook et al., 1997; Depienne et al., 2009; Deschrenemarkers et al., 2006; Hogart et al., 2010; Pati et al., 2010; Sahoo et al., 2006; Schon et al., 1998; Steffenburg et al., 1996; Sztatmai et al., 2007; Trillinggaard and Østergaard, 2004; Veltman et al., 2005</td>
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</table>
This gene encodes the ubiquitin protein ligase E3A, which is part of the ubiquitin protein degradation system. This protein is maternally expressed in brain and biallelically expressed in other tissues.

Good expression in fetal brain and moderate expression in postnatal CNS.

UBE3A (+) de novo

Intracellular signaling: protein ubiquitination pathway

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 mice with mat dic15 (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, glutamatergetic, but not GABergic 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.

**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 maps within the genomic region involved in the 15q11.2-q13.1 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.

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

**ACMSD (-) de novo**

Tryptophan metabolism

This gene encodes the aminoacyl-tRNA synthetase acyltransferase. Quinolinic acid is derived from alpha-amino-beta-carboxy-muconate-episome-semialdehyde (ACMS). ACMSD can divert the side chain of the enzyme to a benzenoid catabolite and thus prevent the accumulation of quinolinic acid 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 particular, the quinoline 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 immunoeexcitotoxicity. 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.

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**Trillinggaard and Østergaard, 2004**

**Veltman et al., 2005**
### 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 GTase that allows its interaction with downstream effector proteins, thus playing a central role in defining the temporal and spatial activation of the corresponding GTase within neuronal cells.

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-evoked release of dopamine 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-Abs 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.

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, EF 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 harbor five genes: GPR148, FAM123C, ARHGEF4, FAM16SB and PLEXHB2.

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 ARHGEF5 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.

### 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 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-evoked release of dopamine 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-Abs 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
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Neurological Deficit</th>
<th>Mutations and/or CNVs affecting Gene have never been reported in patients with ASD</th>
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</thead>
<tbody>
<tr>
<td><strong>CCDC115</strong></td>
<td>This gene encodes the coiled-coil domain containing protein. Expressed throughout the brain.</td>
<td>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.</td>
<td>McConnell and Kaznowski, 1991 Pellicano et al., 2006, 2010</td>
</tr>
<tr>
<td><strong>ERCC3</strong></td>
<td>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 amygdala, thalamus and hypothalamus.</td>
<td>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 pigmentation 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 XPCS, 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 pleiotropically 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.</td>
<td>Hubank and Mayne, 1994 Oh et al., 2006</td>
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<tr>
<td>Gene/Study</td>
<td>Description</td>
<td>Expression Patterns</td>
<td>Observations</td>
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<tr>
<td><strong>FAM123C</strong> (-) de novo</td>
<td>This gene encodes the family with sequence similarity 123C protein, which belongs to the FAM123 family.</td>
<td>High expression in cerebellum.</td>
<td>Recently, the characterization 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 may have been related to the development of the nervous system.</td>
</tr>
<tr>
<td><strong>FAM168B (MANI)</strong> (-) de novo</td>
<td>This gene encodes the family with sequence similarity 168, member B protein, also known as MANI, myelin-associated neurite-outgrowth inhibitor.</td>
<td>Good expression in fetal brain and high expression in postnatal CNS, in particular in the cortex.</td>
<td>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.</td>
</tr>
<tr>
<td><strong>GPR17</strong> (-) de novo</td>
<td>This gene encodes the G protein-coupled receptor 17.</td>
<td>Moderate expression in fetal brain and high expression in postnatal CNS, in particular in amygdala, thalamus and hypothalamus.</td>
<td>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.</td>
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**Tab. 4.1. Continued.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type</th>
<th>Description</th>
<th>Associated Phenotypes</th>
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<tbody>
<tr>
<td><strong>GPR39(-) de novo</strong></td>
<td>Intracellular signaling</td>
<td>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 motility, hormone secretion and cell death. Zn$^{2+}$ acts as an agonist. Good expression in fetal brain and in postnatal thalamus and hypothalamus.</td>
<td>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 long-sought 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.</td>
</tr>
<tr>
<td><strong>GPR148(-) de novo</strong></td>
<td>Neurodevelopment?</td>
<td>This gene encodes the G protein-coupled receptor 148, which is an orphan receptor. Expression is restricted to nervous system and testis.</td>
<td>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, FAM165B and PLEKHB2. Dhamadhikari et al., 2012</td>
</tr>
<tr>
<td><strong>HS6ST1(-) de novo</strong></td>
<td>Heparan sulfate biosynthesis</td>
<td>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.</td>
<td>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 Hs6st 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.</td>
</tr>
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<th>Gene</th>
<th>Description</th>
<th>Function</th>
<th>Mutations/Genetic Variation</th>
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<tbody>
<tr>
<td><strong>LYPD1</strong></td>
<td><em>de novo</em></td>
<td>Neurodevelopment</td>
<td>High expression in fetal brain and in postnatal CNS.</td>
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<td>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 amygdala 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 of GPR39 expression must be still clarified.</td>
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<td>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</td>
</tr>
<tr>
<td><strong>MAP3K2</strong></td>
<td><em>de novo</em></td>
<td>Intracellular signaling: MAP kinase and NF-kappa B signaling pathways</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
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<td>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 (MEIK2), a MEK5 kinase. MAP3K2 directly binds to SH2D2A, which is involved in the control of T-cell activation.</td>
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<td>Mutations and/or CNVs affecting MAP3K2 have never been reported in patients with ASD. Malik et al., 2011, Philippe et al., 2012, Sun et al., 2001, Wang et al., 2006</td>
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<tr>
<td><strong>MGAT5</strong></td>
<td><em>de novo</em></td>
<td>Glycoprotein oligosaccharide biosynthesis</td>
<td>Moderate expression in fetal brain and in postnatal temporal lobe, amygdala and thalamus.</td>
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<td>As known, the CNS is rich in glycoconjugates, located on cell surface and in extracellular matrix. The products of Golgi UDP-GlcNAc-N-acetylgalactosaminyltransferases (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 littersmates 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.</td>
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<td>Mutations and/or CNVs affecting MGAT5 have never been reported in patients with ASD. Luciano et al., 2012, Soleiman et al., 2008</td>
</tr>
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</table>

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 MGA5 and NCKAP5 has been recently replicated in two independent samples.

Recessive mutations affecting NCKAP5L have been recently identified in an autistic patient.

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### PLEKHB2(-) de novo

**Intracellular signaling and trafficking**

This gene encodes the pleckstrin homology domain containing, family B (evecins) member 2 protein. High expression in fetal brain and in postnatal CNS.

PLEKHB2 (EVT-2) works as a coupling factor 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 through 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, EF 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.

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### 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.

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<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Function</th>
<th>Expression</th>
<th>Mutations and CNVs</th>
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<tbody>
<tr>
<td><strong>RAB3GAP1(-)</strong>&lt;sup&gt;de novo&lt;/sup&gt;</td>
<td>Intracellular membrane trafficking</td>
<td>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.</td>
<td>Mutations in this gene are associated with Warburg microsyndrome, 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, corticostratial 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.</td>
<td>Mutations and/or CNVs affecting RAB3GAP1 have never been reported in patients with ASD.</td>
</tr>
<tr>
<td><strong>TUBA3D(-)</strong>&lt;sup&gt;de novo&lt;/sup&gt;</td>
<td>Neurodevelopment: organization of microtubule cytoskeleton</td>
<td>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.</td>
<td>It has been demonstrated that prenatal exposure to cocain affects CNS development altering, for example, cytoskeleton organization through a down-regulation of TUBA3D expression.</td>
<td>Mutations and/or CNVs affecting TUBA3D have never been reported in patients with ASD.</td>
</tr>
<tr>
<td><strong>TUBA3E(-)</strong>&lt;sup&gt;de novo&lt;/sup&gt;</td>
<td>Neurodevelopment: organization of microtubule cytoskeleton</td>
<td>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.</td>
<td>By analogy with other members of the same family, TUBA3E may be involved in neurodevelopment.</td>
<td>Mutations and/or CNVs affecting TUBA3E have never been reported in patients with ASD.</td>
</tr>
</tbody>
</table>
Patient 52, gain of 1.4 Mb at 7q11.23 (chr7:72726578-74139390)

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 (HAXY142ph). HAXY142ph 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 neurula 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.

BAZ1B is a component of the chromatin remodeling complex containing ING proteins and the ISWI/CHD family proteins. It is involved in the regulation of transcription at sites of DNA damage.

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 amygdala.

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.

This gene encodes the B-cell CLL lymphoma 7B protein and a component of tight junction strands. Tight junctions present 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 amygdala 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 plasmalemna 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.

Veicovic et al., 2006
Berg et al., 2007
Challman et al., 2003
Cus et al., 2006
Deppienne et al., 2007
Gilberg and Rausmusen, 1994
Gosch and Pankau, 1994
Hergerer and Mikaldes, 2006
Kirchhoff et al., 2007
Klein-Tasman et al., 2009
Lincoln et al., 2007
Osborne, 2010
Qiao et al., 2009
Qiao et al., 2009
Rein et al., 1985
Van der Aa et al., 2009
Yoshimura et al., 2009
<table>
<thead>
<tr>
<th>Gene</th>
<th>De Novo</th>
<th>Expression/Development/Function</th>
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<tbody>
<tr>
<td><strong>CLDN4</strong></td>
<td><strong>De Novo</strong></td>
<td>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. <strong>CLDN4</strong> is a tight junction protein involved in the blood-brain barrier integrity. It has been reported that polymorphisms in <strong>CLDN4</strong> 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. <strong>CLDN4</strong> maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.</td>
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<tr>
<td><strong>CLIP2</strong></td>
<td><strong>De Novo</strong></td>
<td>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. <strong>CLIP2</strong> may operate in the control of brain-specific organelle translocations. High expression in fetal brain and in postnatal CNS. <strong>CLIP2</strong> protein enable neuronal polarization by controlling the stabilization of microtubules and growth cone dynamics. In particular, <strong>CLIP2</strong> regulates the cytoskeleton through the microtubule network, having a role in cytoskeleton remodeling. It has been previously reported that mouse models of <strong>CLIP2</strong> exhibited some degree of hippocampal dysfunction as evidenced by deficits in contextual fear conditioning and altered synaptic plasticity. However, the recently reported Clip2 heterozygotes and homozygotes show impaired motor coordination on some tasks, but no differences in anxiety or amygdala function, suggesting that <strong>CLIP2</strong> may contribute to coordination problems in WS but not to the characteristic behavioral profile. <strong>CLIP2</strong> maps within the genomic region involved in the Williams syndrome (7q11.23 deletion)/7q11.23 duplication syndrome, which show comorbidity with ASD.</td>
</tr>
<tr>
<td><strong>EIF4H</strong></td>
<td><strong>De Novo</strong></td>
<td>This gene encodes the eukaryotic translation initiation factor 4H, which functions to stimulate the initiation of protein synthesis at the level of mRNA utilization. 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 <strong>EIF4H</strong> 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 <strong>EIF4H</strong> 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 <strong>EIF4H</strong> might contribute to certain deficits associated with Williams-Beuren syndrome. <strong>EIF4H</strong> 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 <strong>EIF4H</strong> have been previously reported in a few autistic patients and their unaffected fathers.</td>
</tr>
</tbody>
</table>

### References

- Berg et al., 2007
- Challman et al., 2003
- Depienne et al., 2007
- Gillberg and Rasmussen, 1994
- Gosh and Pankau, 1994
- Haines et al., 1996
- Herger and Mukaddes, 2006
- Kirchhoff et al., 2007
- Klein-Tasman et al., 2009
- Lincoln et al., 2007
- Qiao et al., 2007
- Reiss et al., 1985
- Van der Auwera et al., 2009
- Weltzien et al., 2003
- Van der Auwera et al., 2009
- Weltzien et al., 2012
- Van der Auwera et al., 2009
- Weltzien et al., 2003
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<th>Dysregulation</th>
<th>Reference(s)</th>
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<tr>
<td><strong>FZD9 (+)</strong></td>
<td>de novo Intracellular Wnt signaling pathway</td>
<td>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.</td>
<td>Good expression in fetal brain and in postnatal CNS.</td>
<td>Berg et al., 2007; Chalman et al., 2003; Depienne et al., 2007; Gilberg and Rasmussen, 1994; Gosh and Parka, 1994; Herzner and Makiadde, 2006; Kirchhoff et al., 2007; Klein-Tasman et al., 2009; Linol, 2007; Qiao et al., 2009; Ranheim et al., 2005; Reiss et al., 1985; Van der Aa et al., 2009; Zhao et al., 2005; Reiss et al., 1985; Van der Aa et al., 2009; Zhao et al., 2005.</td>
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<tr>
<td><strong>GTF2I (-)</strong></td>
<td>de novo Transcriptional regulation</td>
<td>This gene encodes the general transcription factor II, which is a multifunctional phosphoprotein with roles in transcription and signal transduction.</td>
<td>High expression in fetal brain and in postnatal CNS.</td>
<td>Berg et al., 2007; Chalman et al., 2003; Depienne et al., 2007; Gilberg and Rasmussen, 1994; Herzner and Makiadde, 2006; Klein-Tasman et al., 2009; Linol, 2007; Qiao et al., 2009; Ranheim et al., 2005; Reiss et al., 1985; Van der Aa et al., 2009; Zhao et al., 2005.</td>
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<tr>
<td><strong>GTF2IRD1 (+)</strong></td>
<td>de novo Transcriptional regulation</td>
<td>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 amygdala-based learning and memory, which correlates with the high sociability, lack of social anxiety and distraction 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.</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
<td>Berg et al., 2007; Chalman et al., 2003; Depienne et al., 2007; Gilberg and Rasmussen, 1994; Herzner and Makiadde, 2006; Klein-Tasman et al., 2009; Kirchhoff et al., 2007; Linol, 2007; Qiao et al., 2009; Ranheim et al., 2005; Reiss et al., 1985; Van der Aa et al., 2009; Zhao et al., 2005.</td>
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### LAT2<sup>(+)</sup> de novo

**T-cell activation**

This gene encodes the linker for activation of T cell family, member 2 protein.

- **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**
- **Depienne et al., 2007**
- **Gilberg and RAumsen, 1994**
- **Herzoginer and Mukaddes, 2006**
- **Klein-Tasman et al., 2009**
- **Kirchhoff et al., 2007**
- **Licoln et al., 2007**
- **Qiao et al., 2009**
- **Reiss et al., 1985**
- **Van der Aa et al., 2009**

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### LIMK1<sup>(+)</sup> de novo

**Neurodevelopment: axon growth through 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.

- **LIMK1**
  - (+)
  - *de novo*
  - **Neurodevelopment:** axon growth through 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 promoting 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 been 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**
- **Gilberg and RAumsen, 1994**
- **Herzoginer and Mukaddes, 2006**
- **Kaufmann and Moser, 2000**
- **Klein-Tasman et al., 2009**
- **Kirchhoff et al., 2007**
- **Licoln et al., 2007**
- **Meng et al., 2002**
- **Oxbone, 2010**
- **Qiao et al., 2009**
- **Reiss et al., 1985**
- **Van der Aa et al., 2009**
**STX1A (+)**

**de novo**

Intracellular signaling: 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 synaptophysin 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.

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.

**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 ubiquitinates, 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 haplosufficiency in the gastrointestinal pathologies and/or the cognitive profile of WBS patients.

**Clinical relevance**

The protein ubiquitination pathway has been previously implicated in ASD.

**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, cingulate gyrus, 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 laminar patterns in each of the major subdivisions of the cerebral cortex: the olfactory bulb, the hippocampal formation, and the neocortex. In particular, FRZB (sSFRP3) 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 metastasis 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 2q13.
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 glutamate 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-keto glutarate. In addition, it increases cellular antioxidant 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.

| **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.

| **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, amygdala, thalamus, corpus callosum, and spinal cord.

| | | Recent research in ASD has aroused interest in anterior cingulate cortex and in the neurotransmitter glutamine. 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 2q13q33.

| | | 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 2q13q33.

| | | 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 2q13q33.

Shimizu et al., 2011

Bejani et al., 2012

IMGSAC, 2001

Rabionet et al., 2004

Rabionet et al., 2004
**Tab. 4.1. Continued.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Regulation</th>
<th>Description</th>
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<tr>
<td><strong>INPP1</strong>&lt;sup&gt;(de novo)&lt;/sup&gt;</td>
<td>Intracellular phosphatidylinositol signaling</td>
<td>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 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate. Good expression in fetal brain and in postnatal CNS. Mutations and/or CNVs affecting INPP1 have never been reported in patients with ASD. SNPs in genes implicated in phosphatidylinositol signaling pathway, such 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.</td>
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<tr>
<td><strong>ITGA4</strong>&lt;sup&gt;(de novo)&lt;/sup&gt;</td>
<td>Neuroinflammation: focal adhesion, leukocyte transendothelial migration</td>
<td>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. 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.</td>
</tr>
<tr>
<td><strong>NAB1</strong>&lt;sup&gt;(de novo)&lt;/sup&gt;</td>
<td>Transcriptional regulation</td>
<td>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 gene 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 neuroligin-1 (NLGN1) 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. SNPs in genes associated to BD in a Korean sample. Mutations and/or CNVs affecting NAB1 have never been reported in patients with ASD. SNPs in genes associated to BD in a Korean sample. Mutations and/or CNVs affecting NAB1 have never been reported in patients with ASD. SNPs in genes associated to BD in a Korean sample. 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.</td>
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<tr>
<td><strong>NCKAP1(-)</strong> de novo</td>
<td>Neuronal differentiation: actin cytoskeleton organization</td>
<td>This gene encodes the NCK-associated protein 1. Very high expression in fetal brain and in postnatal CNS.</td>
</tr>
<tr>
<td><strong>NEUROD1(-)</strong> de novo</td>
<td>Transcriptional regulation</td>
<td>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.</td>
</tr>
<tr>
<td><strong>ORMDL1(-)</strong> de novo</td>
<td>Neurodevelopment?</td>
<td>This gene encodes the ORM1-like 1 (S. cerevisiae) protein, which is a negative regulator of sphingolipid synthesis. Expressed in brain.</td>
</tr>
<tr>
<td><strong>PDE1A(-)</strong> de novo</td>
<td>Intracellular cAMP signaling</td>
<td>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.</td>
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Tab. 4.1. Continued.
### STAT1(-) de novo

**Modulation of neuroinflammation: intracellular STAT signaling**

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<td>This gene encodes the signal transducer and activator of transcription 1/9kDa 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 (O2·). 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-linked), 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.</td>
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IMGSAC, 2001
Rabionet *et al.*, 2004
Tichauer *et al.*, 2007
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.

**STAT4(-) de novo**

Modulation of neuroinflammation: intracellular STAT signaling

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT4</td>
<td>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.</td>
</tr>
</tbody>
</table>

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, AKT1, 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.

IMSAC. 2003
Rabionet et al., 2004
Zaheer et al., 2007
Zeis et al., 2008
<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Expression</th>
<th>Mutations and/or CNVs Affecting Gene</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMEFF2-de novo</td>
<td><strong>Neurodevelopment</strong></td>
<td>This gene encodes the transmembrane 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.</td>
<td>Mutations and/or CNVs affecting TMEFF2 have never been reported in patients with ASD.</td>
<td>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.</td>
</tr>
<tr>
<td>UBE2E3-de novo</td>
<td><strong>Intracellular signaling: protein ubiquitination pathway</strong></td>
<td>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.</td>
<td>Mutations and/or CNVs involving other E3 ligase genes have been reported in patients with ASD.</td>
<td>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.</td>
</tr>
<tr>
<td>ZNF804A-de novo</td>
<td><strong>Transcriptional regulation</strong></td>
<td>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.</td>
<td>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 connectivity.</td>
<td>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.</td>
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</table>
### Tab. 4.1. Continued.

- **Patient 57, gain of 3.8 Mb at 5q31.1q31.2 (chr5:133871536-137708167)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Expression</th>
<th>Mutations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAMLG(+)</strong>&lt;br&gt;<strong>de novo</strong></td>
<td>Synaptogenesis and synaptic plasticity&lt;br&gt;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.&lt;br&gt;High expression in fetal brain and in postnatal CNS, in particular in caudate nucleus and amygdala.</td>
<td>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.&lt;br&gt;CAMLG is reported to be involved in recycling and endocytic processing of GABA receptors. In a cortical culture it has been demonstrated that the reduction of CAMLG translated into current remained unaltered in these neurons.&lt;br&gt;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.</td>
<td>Mutations and/or CNVs affecting CAMLG have never been reported in patients with ASD.</td>
<td>Beam and Crabtree, 1994&lt;br&gt;Yuan et al., 2008&lt;br&gt;Zhang et al., 2010</td>
</tr>
<tr>
<td><strong>C5orf20</strong>&lt;br&gt;(DCNP1)(+)&lt;br&gt;<strong>de novo</strong></td>
<td>T-cell activation, regulation of the corticotropin-releasing hormone in the brain&lt;br&gt;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.&lt;br&gt;Moderate expression in fetal brain and good expression in postnatal CNS, in particular in thalamus and hypothalamus. Good expression in dendritic cells.</td>
<td>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.&lt;br&gt;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.</td>
<td>Mutations and/or CNVs affecting C5orf20 have never been reported in patients with ASD.</td>
<td>Bosker et al., 2011&lt;br&gt;Philippi et al., 2005&lt;br&gt;Willis-Owen et al., 2006&lt;br&gt;Zhou et al., 2010</td>
</tr>
<tr>
<td><strong>CDC23(+)</strong>&lt;br&gt;<strong>de novo</strong></td>
<td>Intracellular signaling: protein ubiquitination pathway&lt;br&gt;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.&lt;br&gt;Moderate expression in fetal brain and low expression in postnatal CNS. High expression in immune cell types.</td>
<td>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.&lt;br&gt;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.</td>
<td>Mutations and/or CNVs affecting CDC23 have never been reported in patients with ASD.&lt;br&gt;The protein ubiquitination pathway has been previously implicated in ASD.</td>
<td>Glassner et al., 2009&lt;br&gt;Kishino et al., 1997&lt;br&gt;Matsuura et al., 1997&lt;br&gt;Zhang et al., 1991</td>
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Tab. 4.1. Continued.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Description</th>
<th>Expression Pattern</th>
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<tbody>
<tr>
<td><strong>CDC25C (+)</strong> de novo</td>
<td>Intracellular signaling: regulation of cell cycle progression</td>
<td>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.</td>
</tr>
<tr>
<td><strong>CXCL14 (+)</strong> de novo</td>
<td>Homeostasis of monocyte-derived macrophages, synaptic plasticity mediated by chemokine signaling</td>
<td>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.</td>
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<td><strong>Tab. 4.1. Continued.</strong></td>
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<tr>
<td><strong>DDX46</strong>(+) de novo</td>
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<td>RNA metabolism</td>
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<td>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.</td>
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<tr>
<td><strong>FAM13B</strong>(+) de novo</td>
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<tr>
<td>Unknown function</td>
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<tr>
<td>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, amygdala, and thalamus. High expression in immune cell types.</td>
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<tr>
<td><strong>FAM53C</strong>(+) de novo</td>
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<tr>
<td>Unknown function</td>
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<tr>
<td>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.</td>
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<tr>
<td><strong>FBXL21</strong>(+) de novo</td>
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<tr>
<td>Intracellular signaling: protein ubiquitination pathway</td>
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<td>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, amygdala, and spinal cord.</td>
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<tr>
<td>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 FBXL2 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.</td>
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</table>

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.

**FAM13B** has been reported in a patient with ASD.

Mutations and/or CNVs affecting **FAM53C** have never been reported in patients with ASD.

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.

**FBXL21** has been associated with SCZ in two Irish samples.

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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
Matsumura et al., 1997
Noda et al., 2005
Pato et al., 2010
Philipp et al., 2005
Silverman et al., 2003
Sikic et al., 2004
<table>
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<tr>
<th><strong>H2AFY(+) de novo</strong></th>
<th><strong>IL9(+) de novo</strong></th>
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<tbody>
<tr>
<td><strong>Chromatin remodeling</strong></td>
<td><strong>Cytokine-mediated neurodevelopment and neuroinflammation</strong></td>
</tr>
<tr>
<td>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 immune cell types.</td>
<td>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.</td>
</tr>
<tr>
<td>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.</td>
<td><em>IL9</em> 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, in the experimental autoimmune encephalomyelitis mouse, 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.</td>
</tr>
<tr>
<td>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. Nevertheless, no association was found between a few SNPs in H2AFY and ASD.</td>
<td>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.</td>
</tr>
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Tab. 4.1. Continued.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Expression and Function</th>
</tr>
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</table>
| **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 5K-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 Rabkinin-6 (KIF20A) suggests 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., 1996  
Shiota 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. | Mutations and/or CNVs affecting KLHL3 have never been reported in patients with 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-Ciuto et al., 2009  
Marshall et al., 2008  
Matsumi et al., 1997  
Mulliken and Berguer, 2007  
Niklasson et al., 2009  
Patto et al., 2010  
Ramelli et al., 2008  
Segref and Hoppe, 2009  
Sztulmi 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 (LECT2), 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 (TrkB, TrkA, 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 Ohno, 2010 |
<table>
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<tr>
<th>Gene</th>
<th>Description</th>
<th>Associated Phenotypes</th>
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<tr>
<td><strong>NEUROGI(+)</strong>&lt;br&gt;de novo</td>
<td>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.</td>
<td>A 1.2 Mb region of chromosome 5q31, including NEUROGI, 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 other neuropsychiatric disorders. SNP's in NEUROGI have been previously associated with SCZ.</td>
</tr>
<tr>
<td><strong>PITX1(+)</strong>&lt;br&gt;de novo</td>
<td>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.</td>
<td>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 SNP's in PITX1 have been associated with ASD.</td>
</tr>
<tr>
<td><strong>SARIB(+)</strong>&lt;br&gt;de novo</td>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td><strong>SEC24A(+)</strong>&lt;br&gt;de novo</td>
<td>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.</td>
<td>SEC24A interacts with SAR1B to form the COPII complex. Mutations and/or CNVs affecting SAR1B have never been reported in patients with ASD.</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Mutations and/or CNVs affecting gene</td>
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</tbody>
</table>
| **SLC25A48**<sup>(+)</sup> **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. | Mutations and/or CNVs affecting SLC25A48 have never been reported in patients with ASD. | Hattin et al., 2006  
Liu et al., 2011  
Palmieri, 2004 |
| **SPOCK1**<sup>(+)</sup> **de novo** | Neurogenesis: regulation of neuronal migration and axonal outgrowth  
This gene encodes the sparc/osteonectin, cwcw and kazal-like family, member 1 protein.  
Core of a seminal plasma proteoglycan containing chondroitin- and heparan-sulfate chains. The protein's function is unknown, although similarity to thryrin-type cysteine protease-inhibitors suggests its function may be related to protease inhibition.  
Very high expression in fetal brain and in postnatal CNS. | 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  
Vulazia et al., 2007 |
| **WNT8A**<sup>(+)</sup> **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). | Mutations and/or CNVs affecting WNT8A have never been reported in patients with ASD. | 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.

<p>| Patient 60, loss of 7.8 Mb at 5p15.33p15.31 (chr5:95243-7859564) |
|---|---|---|
| <strong>ADCY2</strong>&lt;sup&gt;-&lt;/sup&gt;&lt;sub&gt;-&lt;/sub&gt;&lt;sup&gt;de novo&lt;/sup&gt; | Intracellular signaling | This gene encodes the adenylyl cyclase 2 protein, which is a member of the family of adenylyl cyclases. They are membrane-associated enzymes that catalyze the formation of the secondary messenger cyclic adenosine monophosphate (cAMP). This enzyme is insensitive to Ca&lt;sup&gt;2+&lt;/sup&gt;/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. |
| <strong>AHRR</strong>&lt;sup&gt;-&lt;/sup&gt;&lt;sub&gt;-&lt;/sub&gt;&lt;sup&gt;de novo&lt;/sup&gt; | 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. |
| <strong>CEP72</strong>&lt;sup&gt;-&lt;/sup&gt;&lt;sub&gt;-&lt;/sub&gt;&lt;sup&gt;de novo&lt;/sup&gt; | 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-membranous 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. |</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>De Novo</th>
<th>Neurogenesis</th>
<th>Expression Profile</th>
<th>Mutations and/or CNVs Affecting C5orf38 Have Never Been Reported in Patients with ASD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5orf38(-)</td>
<td>De novo</td>
<td>This gene encodes the chromosome 5 open reading frame 38.</td>
<td>Moderate expression in fetal brain and in postnatal CNS. Good expression in thalamus.</td>
<td>Mutations and/or CNVs affecting C5orf38 have never been reported in patients with ASD</td>
<td>Wu et al., 2006</td>
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<tr>
<td>EXOC3(SEC6)(-)</td>
<td>De novo</td>
<td>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.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting EXOC3 have never been reported in patients with ASD</td>
<td>Hanika et al., 1999, Hsu et al., 1996, 1999, Polzin et al., 2002</td>
</tr>
<tr>
<td>IRX1(-)</td>
<td>De novo</td>
<td>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.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting IRX1 have never been reported in patients with ASD.</td>
<td>Cheng et al., 2001, Rodríguez-Seguel et al., 2009</td>
</tr>
<tr>
<td>IRX2(-)</td>
<td>De novo</td>
<td>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.</td>
<td>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 (Irx1-3) 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 extent, spinal cord.</td>
<td>Mutations and/or CNVs affecting IRX2 have never been reported in patients with ASD.</td>
<td>Rodríguez-Seguel et al., 2009</td>
</tr>
</tbody>
</table>

Tab. 4.1. Continued.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Expression Patterns</th>
<th>Function</th>
<th>Mutations</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>IRX4(-)</strong>&lt;br&gt;de novo</td>
<td>Neurogenesis</td>
<td>This gene encodes the iroquois homeobox 4 protein. Moderate expression in postnatal parietal lobe, cerebellum, thalamus hypothalamus, subthalamic nucleus, and spinal cord. High expression in prostate and heart.</td>
<td>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 extent, spinal cord. Moreover, several findings support a role for IRX4 in heart development.</td>
<td>Mutations and/or CNVs affecting IRX4 have never been reported in patients with ASD.</td>
<td>Garriock et al., 2001&lt;br&gt;Rodríguez-Seguel et al., 2009</td>
</tr>
<tr>
<td><strong>LPCAT1(-)</strong>&lt;br&gt;de novo</td>
<td>CNS homeostasis: lipid metabolism</td>
<td>This gene encodes the lysophosphatidylcholine acyltransferase 1 protein, which catalyzes the conversion of LPC to phosphatidylcholine (PC) in the remodeling pathway of PC biosynthesis.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting LPCAT1 have never been reported in patients with ASD.</td>
<td>Cheng et al., 2009&lt;br&gt;Kihara et al., 2008&lt;br&gt;Matsumoto et al., 2007&lt;br&gt;Nakanishi et al., 2006</td>
</tr>
</tbody>
</table>
This gene encodes the NOP2/Sun domain family, member 2 protein, which is a methyltransferase that catalyzes the methylation of cytosine to 5-methylcytosine (5mC) 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. 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 amygdala, thalamus and hypothalamus. High expression in immune cell types. 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.

This gene encodes the ALG-2 protein, which is 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 amygdala, 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.
Tab. 4.1. Continued.

<table>
<thead>
<tr>
<th><strong>SDHA</strong>(-) de novo</th>
<th>CNS metabolism: mitochondrial oxidative phosphorylation</th>
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<tbody>
<tr>
<td><strong>SDHA</strong>(-) de novo</td>
<td>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.</td>
</tr>
<tr>
<td><strong>SDHA</strong>(-) de novo</td>
<td>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.</td>
</tr>
<tr>
<td><strong>SDHA</strong>(-) de novo</td>
<td>Mutations and/or CNVs affecting SDHA have never been reported in patients with ASD.</td>
</tr>
<tr>
<td><strong>SDHA</strong>(-) de novo</td>
<td>Alston et al., 2012 Horvath et al., 2006</td>
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<tr>
<th><strong>SLC6A3</strong>(-) de novo</th>
<th>Trafficking: dopamine transport</th>
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<tbody>
<tr>
<td><strong>SLC6A3</strong>(-) de novo</td>
<td>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 amygdala.</td>
</tr>
<tr>
<td><strong>SLC6A3</strong>(-) de novo</td>
<td>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 reported 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.</td>
</tr>
<tr>
<td><strong>SLC6A3</strong>(-) de novo</td>
<td>VNTR polymorphisms in SLC6A3 have been associated with idiopathic epilepsy, ADHD, PD, addiction disorders. Moreover, specific SLC6A3 alleles have been associated with more severe social anxiety and tic symptoms in ASD patients.</td>
</tr>
<tr>
<td><strong>SLC6A3</strong>(-) de novo</td>
<td>CNVs involving PARK2 have been reported in ASD patients.</td>
</tr>
<tr>
<td><strong>SLC6A3</strong>(-) de novo</td>
<td>Arias-Carrión et al., 2010 Gadow et al., 2008 Glessner et al., 2009 Jiang et al., 2004 Labate 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</td>
</tr>
</tbody>
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### SRD5A1(-) de novo

**CNS metabolism: neurosteroid biosynthesis**

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.

**Description:** 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 enzymes 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:** 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.

**Description:** 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:** TPPP has been previously suggested as autism candidate gene by molecular cytogenetic analysis and in silico studies.

*Colletta et al., 2002
Huvianda et al., 2002
Ilanov et al., 2010
Otsa et al., 2008
Seki et al., 1999
Skwerrings 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.

**Description:** 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:** Mutations and/or CNVs affecting ZDHHC11 have never been reported in patients with ASD.

*Fukata and Fukata, 2010*
### Tab. 4.1. Continued.

<table>
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<th>Patient 60, gain of 9.1 Mb at 18p11.32p11.22 (chr18:180229-9281969)</th>
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<tbody>
<tr>
<td><strong>ADCYAPI(+) de novo</strong></td>
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<tr>
<td>Synaptic transmission</td>
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<tr>
<td>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 (PAC1). Recent studies reveal that genetic variants of the PACAP and PAC1 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 ADCYAPI1 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, MDD and post-traumatic stress disorder.</td>
</tr>
<tr>
<td><strong>ARHGAP28(+) de novo</strong></td>
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<tr>
<td>Intracellular signaling: regulation of actin cytoskeleton dynamics</td>
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<tr>
<td>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 Tubersus Sclerosis 1 or 2. Both TSC1 and TSC2 proteins activate RhoA whereas TSC2 activates Cdc42, thus regulating cell adhesion and migration.</td>
</tr>
<tr>
<td><strong>DLGAPI(+) de novo</strong></td>
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<tr>
<td>Synaptogenesis and synaptic plasticity</td>
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<tr>
<td>This gene encodes the discs, large (Drosophila) homolog-associated protein 1. High expression in fetal brain an in postnatal CNS, in particular 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 DLGAPI have never been reported in patients with ASD. Mutations and/or CNVs affecting several genes encoding proteins 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.</td>
</tr>
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**Hashimoto et al., 2007, 2010**

**Hatton et al., 2012**

**Ishiguro et al., 2001**

**Koga et al., 2010**

**Luboff et al., 2008**

**McInnes et al., 2001**

**Resier et al., 2011**

**Stroth et al., 2011**
<table>
<thead>
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<th>Gene</th>
<th>Mutations and/or CNVs affecting</th>
<th>Reference(s)</th>
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<tr>
<td><strong>EPB41L3(+)</strong>&lt;br&gt; de novo</td>
<td></td>
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<tr>
<td>Neurodevelopment: nerve conduction</td>
<td>Very high expression in fetal brain and in postnatal CNS and PNS.</td>
<td>Einheber et al., 2012</td>
</tr>
<tr>
<td>This gene encodes the erythrocyte membrane protein band 4.1-like 3 (4.1B protein).</td>
<td>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.</td>
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<tr>
<td><strong>LAMA1(+)</strong>&lt;br&gt; de novo</td>
<td></td>
<td>Anney et al., 2012</td>
</tr>
<tr>
<td>Neurogenesis</td>
<td>Moderate expression in fetal brain and in postnatal CNS.</td>
<td></td>
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<tr>
<td>This gene encodes the laminin, alpha 1 protein, which is thought to mediate the attachment, migration and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components.</td>
<td>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.</td>
<td>Heng et al., 2011</td>
</tr>
<tr>
<td><strong>NDUFV2(+)</strong>&lt;br&gt; de novo</td>
<td></td>
<td>Mukherjee et al., 2006</td>
</tr>
<tr>
<td>CNS metabolism: mitochondrial oxidative phosphorylation</td>
<td>Low expression in fetal brain and moderate expression in postnatal CNS. High expression in immune cell types.</td>
<td>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.</td>
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</table>
Tab. 4.1. Continued.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression and Function</th>
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<tr>
<td><strong>PTPRM(+) de novo</strong></td>
<td>Intracellular signaling</td>
</tr>
<tr>
<td>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/GNB2LI, 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.</td>
<td>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 Ugt9), and six genes are involved in growth factor signaling (Gfif1, Fzd3, Erbb3, Igfbp4, Igfbp6, and Ptpnm). All of these genes have prior evidence of differential expression in human postmortem brains from mood disorder subjects.</td>
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<tr>
<td><strong>RAB12(+) de novo</strong></td>
<td>Intracellular membrane trafficking</td>
</tr>
<tr>
<td>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.</td>
<td>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.</td>
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<tr>
<td><strong>TGIF1(+) de novo</strong></td>
<td>Transcriptional regulation</td>
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<tr>
<td>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 retinoïd-binding protein II promoter. In addition, TGIF1 is an active transcriptional co-repressor of SMAD2 and may participate in the transission 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.</td>
<td>TGF is an atypical homeo-domain protein. In vitro studies have shown that TGF can repress transcription mediated by either of two signaling pathways: TGF-beta and retinoïd acid signaling. In vivo overexpression of TGF in the developing chick neural tube demonstrated that TGF 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 Dl(1A) gene, which encodes the predominant dopamine receptor in the striatum. The two proteins have opposite roles: while MEIS2 activates Dl(1A) transcription, TGIF1 represses the transcription of this gene. Recently, it has been suggested that TGIF1 may have a role in macrophage activation.</td>
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</table>

Mutations and/or CNVs affecting **PTPRM** have never been reported in patients with ASD. A rare CNV affecting **PTPT1** has been reported in a patient with ASD.

Mutations and/or CNVs affecting **RAB12** have never been reported in patients with ASD.

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 3 (HPE-3), 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. |
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Expression Patterns</th>
<th>Pathological Effects</th>
<th>Mutations and/or CNVs</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>THOC1(+)</td>
<td>This gene encodes the THO complex 1 protein, which is part of the TREX (transcription/export) complex, which includes TEX1, THO2, ALY, and UAP56.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting THOC1 have never been reported in patients with ASD.</td>
<td>Matsui and Fukuda, 2011 Rytych et al., 2012</td>
<td></td>
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<tr>
<td>USP14(-)*</td>
<td>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 ubiquitinated proteins.</td>
<td>High expression in fetal brain and in postnatal CNS. High expression in immune cell types.</td>
<td>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.</td>
<td>Mutations and/or CNVs affecting USP14 have never been reported in patients with ASD. The protein ubiquitination pathway has been previously implicated in ASD.</td>
<td>Bhattacharyya et al., 2012</td>
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<tr>
<td>PNPLA4(+)</td>
<td>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.</td>
<td>PNPLA4 has a role in the metabolism of structural phospholipids involved in the formation and repair of the neuronal membrane.</td>
<td>Mutations or CNVs affecting PNPLA4 have never been reported in patients with ASD.</td>
<td>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.</td>
<td>Carrascosa-Romero et al., 2012</td>
</tr>
</tbody>
</table>

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)
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
- STS
- This gene encodes the steroid sulfatase isozyme S, which catalyzes the conversion of sulfated steroid precursors to estrogens during pregnancy.
- 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 non-sulfate steroids metabolism in CNS. Indeed, in the brain sulphated and non-sulfated steroids can influence the function of GABA 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.

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| Genes implicated in CNS metabolism | Genes implicated in synaptogenesis and synaptic plasticity. | Genes implicated in CNS-IR 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 reticulum; 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 \textit{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 \textit{et al}., 2012; Ishiguro \textit{et al}., 2001; Kuss \textit{et al}., 2011; Lohoff \textit{et al}., 2008; McInnes \textit{et al}., 2001], thus supporting the existence of shared neurological pathways among different neuropsychiatric diseases, as previously reported [Bateman and Gull; 2011; Elia \textit{et al}., 2011a and b; Jarick \textit{et al}., 2012; Kirov \textit{et al}., 2009; Lachman, 2008; Lionel \textit{et al}., 2011; Shoukier \textit{et al}., 2012; Stefansson \textit{et al}., 2008; Utine \textit{et al}., 2012; Vrijenhoek \textit{et al}., 2008; Walsh \textit{et al}., 2008; Williams \textit{et al}., 2012].

Interestingly, in the ASD cohort reported here, the finding of rare \textit{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 \textit{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 \textit{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 \textit{et al}., recently reported the finding of point mutations with a mild effect in “syndromic” genes (i.e., missense mutations), such as 
\textit{TSC1}, \textit{TSC2}, \textit{PTEN}, and \textit{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 \textit{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 \textit{EHMT1} gene, encoding a histone-methyltransferase, and, often, of the \textit{CACNA1B} gene, which is located more distally and encodes a voltage-dependent calcium channel implicated in neurotransmitter release (Tab. 4.1) [Anderlid \textit{et al}., 2002; Dawson \textit{et al}.,
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 \textit{EHMT1}, thus suggesting that increased dosage of \textit{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 \textit{EHMT1} and \textit{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 \textit{EHMT1} and \textit{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 \textit{IL1RAPL2} gene, encoding an interleukin receptor (Tabs. 3 and 4.1). By analogy with the function of another member of the same family, \textit{IL1RAPL1}, \textit{IL1RAPL2} is likely involved in presynaptic calcium-dependent neurotransmitter release and differentiation of dendritic spines. Since mutations and CNVs affecting \textit{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 \textit{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.

![Diagram of Neurogenesis and Development](image)

**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, LIMKI (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 coflin. 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 NCKAPI seen in patient 53 may have resulted in an impairment of functional connectivity in the cerebral cortex (Tab. 3). NCKAPI 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
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 \textit{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 \textit{et al}., 2012; El-Zein \textit{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 \textit{et al}., 2012; Matise \textit{et al}., 1998; Takanaga \textit{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 face, 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., BAZIB, 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 [Marci et al., 2012; Schaniel et al., 2009]. Finally, BAZIB 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 CACNAIB (potentially disrupted in patient 14, Tab. 3) and CACNAIC (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), STXIA (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 ADCYAPI 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].

ADCYAPI 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 hypothalomo-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/G3/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 \textit{CA4} gene, which is fully duplicated in patient 16 as a result of a \textit{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 \textit{et al.}, 2000]. To date, defects in \textit{CA4} have only been associated with retinitis pigmentosa type 17. However, rare single-gene mutations affecting \textit{CA6}, another member of the same family, have been reported in a few autistic patients [Bucan \textit{et al.}, 2009].

CNS metabolism and homeostasis (the genes affected by \textit{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., \textit{ACMSD}, \textit{BCHDHA}, \textit{GLDC}, \textit{GLS}, \textit{HIBCH}, \textit{PRODH}, and \textit{QPRT}); neurotransmitter and neuropeptide maturation and degradation (e.g., \textit{COMT}, \textit{PREP}, and \textit{SULT1A3}); glycolysis, Krebs cycle, and mitochondrial metabolism (e.g., \textit{ALDOA}, \textit{ME3}, \textit{NDUFV2}, \textit{SDHA}, \textit{SLC25A48}, and \textit{TXNRD2}); and biosynthesis of heparan sulfate molecules, phospholipids and lipoproteins (\textit{ABCD4}, \textit{HS6ST1}, \textit{LCLAT1}, \textit{LPCAT1}, and \textit{NDST2}) (Tabs. 4 and 4.1, Fig. 23).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{cns_metabolism_diagram.png}
\caption{Schematic view of the principal reactions implicated in CNS metabolism that are potentially dysregulated in the present ASD cohort.}
\end{figure}
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 \textit{HS6ST1} and \textit{NDST2}, are affected by \textit{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 \textit{LPCAT1}, which is included in a large \textit{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, \textit{PREP}, which was found deleted in patient 18 due to a \textit{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 an NMDA receptor agonist.
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, presynaptically, 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.

![Diagram of Wnt signaling pathways](image.png)

**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].
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.

CNS development and homeostasis mediate by the immune system, immunosurveillance and modulation of inflammation (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, however 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

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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 astrogial 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.


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