CHAPTER

34

Autisms

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OUTLINE

Neural Circuit Development and Function in the Brain: Comprehensive Developmental Neuroscience, Volume 3 http://dx.doi.org/10.1016/B978-0-12-397267-5.00045-5

34.1 INTRODUCTION

34.1.1 The History of Autism

The term *autism* was coined in 1911 by Swiss psychiatrist Eugen Bleuler to designate one of the hallmarks of schizophrenia, namely the social withdrawal resulting in enclosure in one's self (self = αʹυτός autòs, in ancient Greek) (Bleuler, 1911). During the following three decades, this term reached an ever broader audience in psychiatry, mainly through the work of Eugène Minkowski (1927), who addressed schizophrenic autism in great detail in his famous text 'La Schizophrénie'. However, schizophrenic autism must not be confused with autism spectrum disorder (ASD), which defines an independent nosological entity and not a mere symptom. This disorder was first described in 1943 by Leo Kanner in a cohort of 11 children, who essentially shared an 'enclosure in one's self' as their distinctive trait (Kanner, 1943). Only 1 year later, in 1944, the Austrian pediatrician Hans Asperger described four boys displaying some, but not all, of the behavioral symptoms present in Kanner's patients (Asperger, 1944a). Asperger's work, written in German, reached a wider audience after it was publicized in 1981 by Lorna Wing, who described 34 individuals, ranging from 5 to 35 years of age, whose clinical picture was closer to Asperger's cases than to Kanner's (Wing, 1981). Thereafter, it was translated into English by Uta Frith in 1991 (Asperger, 1944b). Hence, the existence of clinical heterogeneity in autism is by no means a recent acquisition; it was recognized from the beginning that autistic patients do indeed share some common features, primarily an enclosure in one's self, and display an impressive variability in symptom patterns, developmental trajectories, disease course, and severity of impairment, spread along a dimensional continuum that was later designated as the 'autism spectrum' (Piven et al., 1997). This impressive clinical variability is underscored by an equally impressive degree of etiological heterogeneity, which has led the term *autisms* to designate a set of neurodevelopmental disorders with early onset in life that share autism as a common feature but that are produced through distinct processes. This chapter will summarize the current state of knowledge regarding these 'autisms'; readers interested in 'schizophrenic autism' are referred to the excellent review by Parnas et al. (2002).

34.1.2 Definition and Epidemiology of Autism Spectrum Disorder

ASD is characterized by deficits in social interaction and communication, as well as by stereotyped behaviors and insistence on sameness (i.e., restricted patterns of interest and activities) (American Psychiatric Association, 1994). Its onset occurs in early childhood, before 3 years of age (American Psychiatric Association, 1994). ASD essentially encompasses three different pervasive developmental disorders listed in the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) (American Psychiatric Association, 1994), namely autistic disorder, Asperger disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS). These separate diagnostic categories likely will merge into a single comprehensive ASD category in the upcoming DSM-V (American Psychiatric Association, 2012) following the ever-swinging logic behind categorical diagnosis in psychiatry, which historically alternates between analysis and synthesis.

The incidence of ASD has risen dramatically during the last two decades, from 2–5 in 10 000 to approximately 1–2 in 1000 children; broader diagnostic criteria and increased awareness in the medical community certainly have contributed to this trend, but a real increase in incidence, possibly due to gene–environment interactions, is also likely (Fombonne, 2005; Persico and Bourgeron, 2006; Rutter, 2005). Males are particularly susceptible, with male-to-female ratios ranging from approximately 4:1 to 8:1, depending on disease severity and recruiting context (Fombonne, 2005; Rutter, 2005). An additional layer of complexity stems from comorbidity with seizures and mental retardation (MR) present in up to 30% and 65% of cases, respectively (Fombonne, 2005; Tuchman and Rapin, 2002).

34.1.3 Neuropathological and Systemic Abnormalities in Autism Spectrum Disorder

Altered neurodevelopment occurring during the first and second trimesters of prenatal life is now widely recognized as the underlying neuropathological cause of ASD (DiCicco-Bloom et al., 2006). Postmortem studies of autistic brains have uncovered important neuroanatomical abnormalities in the central nervous system (CNS) of ASD patients, generally resulting from reduced programmed cell death and/or increased cell proliferation, altered cell migration, and abnormal cell differentiation with reduced neuronal size and abnormal wiring (Bauman and Kemper, 2005). These neuropathological anomalies, especially the patchy cytoarchitectonic abnormalities present in the cerebral and cerebellar cortex, would seemingly explain the imbalance in local vs long-distance connectivity on the one hand and excitatory vs inhibitory connectivity on the other currently believed to underlie disrupted sensory integration, altered social information processing, and frequent comorbidity with epilepsy (Courchesne and Pierce, 2005; Geschwind and Levitt, 2007; Rubenstein and Merzenich, 2003). All these neurodevelopmental processes physiologically occur

during the first and second trimesters of pregnancy (Rice and Barone, 2000). Hence, despite some methodological limitations and predictable brain-to-brain variability, neuropathological studies collectively have been instrumental in indicating a prenatal origin for autism. Further support comes from behavioral analyses demonstrating a delayed appearance or inhibition of specific motor reflexes already on the day of birth or early on in neonates later diagnosed with an ASD (Teitelbaum et al., 2004). An additional confirmation of the existence of a prenatal time window for autism vulnerability comes from studies of teratogenic drugs and congenital infections known to cause autism in some cases (see Section 34.4). This temporal framework ought to be considered when attempting to incorporate potential environmental factors into realistic pathogenetic models, which should not necessarily exclude modulatory roles for early postnatal exposures but *must* incorporate this crucial prenatal component.

Viewing autism exclusively as a brain disease would be an oversimplification; ASD patients also display variable degrees of systemic involvement, with signs and symptoms frequently including macrosomy (Sacco et al., 2007a), gastrointestinal disorders (Buie et al., 2010), and immune dysreactivity (Ashwood et al., 2006; Jyonouchi et al., 2005). In summary, autism should be viewed as a multiorgan systemic disorder, primarily involving but not restricted to the nervous system, with prenatal onset and postnatal clinical expression.

34.1.4 Toward a Classification of the Autisms

To address the great heterogeneity present in ASD, investigators have aimed at identifying subgroups of patients who at least partly share common pathophysiological underpinnings. These attempts essentially have followed two complementary strategies, namely the study of endophenotypes and the use of genetic approaches:

1. An endophenotype can be best described as a familial and heritable quantitative trait associated with a complex disease (Gottesman and Gould, 2003). The most important endophenotypes reported to date in autism research are summarized in Table 34.1. A detailed discussion of endophenotypes will be provided elsewhere (Persico and Sacco, 2013). The study of endophenotypes in complex disorders, such as autism, provides several advantages: (a) the lesser complexity of an endophenotype and its greater proximity to the genetic level, as compared with clinical affection status and behavioral symptoms, facilitates the interpretation of the results; (b) a continuous measure reflects more faithfully the existence of a continuum of signs and symptoms in the autism spectrum compared with a categorical 'case versus control' distinction; and (c) standardized and automated procedures are used to measure biological parameters (Sacco et al., 2010).
2. For more than two decades, autism has been identified as 'the most genetic' neuropsychiatric disorder because of the monozygotic twin concordance rate as high as 73–95%, impressive heritability (>90%, as estimated by twin studies), and

TABLE 34.1 Endophenotypes in the Autism Spectrum

Behavioral/neurodevelopmental

- Delayed expressive speech (Alarcón et al., 2008; Spence et al., 2006)
- ADI-R domains: social interaction domain; restricted and repetitive behaviors (Liu et al., 2008; Sakurai et al., 2006)
- Savant skills: absolute pitch, calendar calculations, etc. (Wallace et al., 2009)
- Social Responsiveness Scale scores (Duvall et al., 2007)

Neuropsychological

- Pattern of face processing (Adolphs et al., 2008; Hernandez et al., 2009; Klin et al., 2002)
- Executive functions (Delorme et al., 2007)

Neurophysiological

- Reduced cingulate self-response in a visual imagery task, when playing with a human partner (Chiu et al., 2008)
- Abnormal patterns of cortical auditory activation (Boddaert et al., 2003; Bonnel et al., 2010; Bruneau et al., 2003; Gomot et al., 2008)
- Dysfunctional mirror neuron systems (Cattaneo et al., 2007; Dapretto et al., 2006; Martineau et al., 2010)
- Centroparietal and temporal EEG related to autistic behaviors and intellectual impairment (Roux et al., 1997)
- Blunted or delayed frontal activation during visual attention tasks (Belmonte et al., 2010)

Morphological

- Macrocephaly (Fombonne et al., 1999; Lainhart et al., 1997; Miles et al., 2000; Sacco et al., 2007a; Stevenson et al., 1997; Woodhouse et al., 1996)
- Macrosomy (Bigler et al., 2010; Dissanayake et al., 2006; Lainhart et al., 2006; Sacco et al., 2007a; van Daalen et al., 2007)
- Minor physical anomalies (Hammond et al., 2008; Miles et al., 2008; Tripi et al., 2008)

Biochemical

- Hyperserotoninemia (Hérault et al., 1996; McBride et al., 1998; Mulder et al., 2004; Piven et al., 1991)
- Oligopeptiduria (Reichelt et al., 1981; Sacco et al., 2010)
- Urinary dopamine and HVA levels (Hameury et al., 1995)
- Decreased plasma fatty acids (Vancassel et al., 2001)

Endocrine

- Decreased melatonin plasma levels (Melke et al., 2008)
- Decreased oxytocin plasma levels (Modahl et al., 1998)

Immunological

- Increased proinflammatory and IL-10-producing immune cells, decreased CD4+ T lymphocytes, increased naive and effector memory CD8+ T lymphocytes (Saresella et al., 2009)

a noticeable sibling recurrence risk (5–6% for full-blown autistic disorder, approximately 15% for broad ASD) (for reviews of autism genetics, see Abrahams and Geschwind, 2008; Freitag, 2007; Geschwind, 2011; Muhle et al., 2004; Persico and Bourgeron, 2006). These heritability estimates, obtained primarily in the UK and in Northern Europe in the early 1990s, were not replicated by a more recent California-based twin study that supported a larger proportion of variance explained by shared environmental factors as opposed to genetic heritability (55% vs. 37% for strict autism, respectively) (Hallmayer et al., 2011). Conceivably, the relative weight of genetic and environmental factors may be region-specific and change over time. Nonetheless, the parallel increase in sibling recurrence risk, estimated by recent baby sibling studies at 18.7% (26.2% for males and 9.1% for females) (Ozonoff et al., 2011), and the presence of mild autistic traits in many first-degree relatives of autistic patients (Piven et al., 1997) still point toward a strong genetic component in ASD playing a sizable permissive role at a minimum. Linkage and association studies have identified numerous susceptibility genes located on various chromosomes, especially 2q, 7q, 15q, and on the X chromosome. The clinical heterogeneity of ASD is believed at least partly to reflect the complexity of its genetic underpinnings, the general underlying mechanisms of which are summarized in Table 34.2.

34.2 'CLASSIC' SYNDROMIC AUTISMS

34.2.1 General Description

In approximately 10% of ASD cases, autistic symptoms are part of a broader syndrome due to a known medical cause. These syndromes can stem from (a) genomic DNA mutations, triplet repeat expansions, or cytogenetic abnormalities visible by classical G band karyotyping, conditions summarized in Table 34.3; (b) mitochondrial DNA (mtDNA) mutations or gene dosage abnormalities, which are listed in Table 34.4; or (c) copy number variants (CNVs), genomic DNA microdeletions/microduplications detectable only using microarray technologies. Genetic and genomic forms have been reviewed by Gillberg (1998), Cohen et al. (2005), Feinstein and Singh (2007), Zafeiriou et al. (2007), and Benvenuto et al. (2009); autism linked to mitochondrial disease and mtDNA abnormalities has been reviewed recently by Palmieri and Persico (2010) and by Rossignol and Frye (2011); CNVs have been reviewed by Merikangas et al. (2009), Guilmatre et al. (2009), Weiss (2009), and Carvalho et al. (2010).

In general, malformations and/or facial dysmorphisms, moderate-to-profound mental retardation, severe epilepsy, neurological signs, and symptoms are largely more frequent in syndromic autism than in idiopathic forms. Overall, the M:F gender ratio is close to 1, although males are particularly prone to suffer from specific syndromes. Abnormal growth in the form

TABLE 34.2 Mechanisms Underlying the Complexity of Autism Genetics

1. Genetic heterogeneity	Different contributing genes cause the disease in distinct patients, who may display similar clinical phenotypes
2. Different modes of inheritance	
(a) Polygenic or oligogenic	Several functional polymorphisms located in different genes and widely distributed in the general population ('common variants'), each conferring a small risk, are collectively required for an individual to develop the disease
(b) Monogenic	Genetic mutations or genomic rearrangements affecting a single gene cause the disease, typically in a single or in very few patients ('private' or 'rare variants,' respectively)
(c) Combined genetic and genomic quasi-recessive mode	Convergence onto the same individual of one allele carrying a null mutation inherited from one parent and the other allele carrying a genomic rearrangement (typically a microdeletion) inherited from the other parent. Both mutation and microdeletion are recessive, and neither by itself is pathogenic in either parent; they may even be present at low frequency in the general population
Phenocopies	Cases exclusively due to environmental factors and clinically indistinguishable from genetic cases
Variable penetrance and expressivity	Variable degrees of phenotypic expression of genetic variants: generally high level of pathology caused by rare variants, lower degree of expression for common variants
Epistasis	Gene–gene interactions, with permissive and blunting effects exerted by common variants ('modifier genes'). Phenotypic expression is complex, and not a mere summation of single-gene effects. By this mechanism, an identical mutation can produce different phenotypes in different individuals or mouse inbred strains
Gene–environment interactions	Genes may confer vulnerability to or protection from the disease by lowering or raising the threshold of sensitivity to pathogenic environmental factors

TABLE 34.3 Syndromic Autisms Due to Known Mutations, Triplet Repeat Expansions, or Cytogenenetic Abnormalities Visible by G Band Karyotyping

	Gene/ch. region	Prevalence	Percentage of autistic patients with the syndrome	Percentage of patients with the syndrome who are autistic	Signs and symptoms
Fragile X syndrome	FMR1	1/3500–1/9000	2.1	25–33	Facial dysmorphisms, macroorchidism, poor eye contact, social anxiety, language impairment, stereotypies, hyperactivity, sensory hyper-reactivity
Tuberous sclerosis	TSC1 TSC2	1–1.7/10000	1–4% (8–14% if seizures present)	16–65	Hamartomas in skin, CNS, kidney, heart, lungs, retina; autism, mental retardation, learning disability, epilepsy (infantile spasms)
Neurofibromatosis type 1	NF1	1/3000–1/4000	≤1.4	?	Café-au-lait macules, neurofibromas, axillary or groin frecklings, optic pathway tumors, bone dysplasias
Untreated phenylketonuria	PAH	1/10000–1/15000	–	5.7	Microcephaly, hypertonia, mental retardation, language impairment, psychomotor agitation, autism, seizures
Adenylosuccinate lyase deficiency	ADSL	?	≤1	80–100	Mental retardation and severe autism, seizures, psychomotor regression
Smith–Lemli–Opitz syndrome	DHCR7	1/10000–1/60000	≤1	46–53	Microcephaly, facial dysmorphism, malformations (sometimes lethal, usually cleft palate, cardiac m., hypospadia), short stature, variable mental retardation, sensory hyper-reactivity, language impairment, self-injurious behavior, sleep disturbance, opisthokinesis and other stereotypies
Cohen syndrome	COH-1 ?	1/105000	≤1	48	Microcephaly, facial dysmorphism, truncal obesity, hematologic and eye abnormalities, mental retardation, motor clumsiness, hypotonia, language impairment, autism
Cornelia de Lange syndrome	NIPBL SMC1A SMC3 ?	1/10000	≤1	35–50	Facial dysmorphism, growth deficiency with short stature, major malformations (especially cardiac, gastrointestinal, musculoskeletal), developmental delay, mental retardation, feeding difficulties, extreme shyness, self-injurious behavior, hyperactivity with attention deficit, aggression, obsessive-compulsive behavior, depression
Sotos syndrome	NSD1	1/10000–1/50000 (?)	≤1	?	Macrocephaly, pre- and postnatal overgrowth, facial dysmorphism, developmental delay
Cole–Hughes macrocephaly	?	?	≤1	?	Macrocephaly, mental retardation, attention deficit and hyperactivity, developmental delay, autism, language impairment, obesity, delayed bone age, facial dysmorphism
Lujan–Fryns syndrome	UPF3B MED12	?	≤1	80	X-linked mental retardation with marfanoid habitus (tall stature, facial dysmorphism), hypotonia, mild-to-moderate mental retardation, ascending aortic aneurysm, autism, aggression, hyperactivity, emotional instability
San Filippo syndromes: A	SGSH	0.3–1.6/100000	≤1	?	Prominent regression or developmental delay, autism, motor and verbal stereotypies, hyperactivity, aggression, sleep disturbance,

Continued

TABLE 34.3 Syndromic Autisms Due to Known Mutations, Triplet Repeat Expansions, or Cytogenenetic Abnormalities Visible by G Band Karyotyping—cont'd

	Gene/ch. region	Prevalence	Percentage of autistic patients with the syndrome	Percentage of patients with the syndrome who are autistic	Signs and symptoms
B	NAGLU				inappropriate effect, variable malformations (visceromegaly, facial, skeletal, etc.). Onset, usually (but not always) beyond age 3, qualifies for DSMIV disintegrative disorder
C	HGSNAT				
D	GNS				
ARX syndrome	ARX	?	≤1	?	X-linked mental retardation with or without autism, and X-linked infantile spasms for insertion/missense mutations; X-linked lissencephaly with agenesis of the corpus callosum and ambiguous genitalia for truncating mutations (death due to neurodevelopmental delay and intractable seizures)
Ch 2q37 deletion syndrome	2q37	?	≤1	?	Brachymetaphalangism, mental retardation, autism
Williams–Beuren syndrome	7q11.23 del	2–5/100000	≤1	?	Elfin face, stenosis of the aorta and other arteries, short stature, dental malformations, hypercalcemia, loquaciousness, sociability, autism (rare), attention deficit, hyperactivity, anxiety, visuocognitive deficits
Williams–Beuren region duplication syndrome	7q11.23 dup	?	≤1	?	Growth delay, facial and dental dysmorphisms, autism, mental retardation, developmental delay, impaired expressive language, seizures
Ch 13 deletion syndrome	13q	?	≤1	?	Mental retardation, language impairment, retinoblastoma, growth retardation, various malformations (cardiac, craniofacial, gastrointestinal, renal, limbs and digits)
15Q CHROMOSOMAL SYNDROMES					
Angelman syndrome	Del or mutation in maternal UBE3A	1/10000–1/12000	≤1	42	Facial dysmorphism, developmental delay, speech impairment, stereotypies, mental retardation, gait ataxia, 'happy puppet' attitude, hyperactivity with attention deficit, temper tantrums, frequently microcephaly and seizures
Prader–Willi syndrome	Del of paternal allele at 15q11–q13	1/10000–1/15000	?	25.3	Developmental delay, short stature, mental retardation, hyperphagia, obesity, hypotonia, hypogonadism, obsessive-compulsive behavior
Isodicentric 15q	Dup 15q11–q13, GABRB3	1/30000 (?)	≤1	70	Short stature, diabetes, anal and jejunal atresias, acanthosis nigrans, severe autism, developmental delay, mental retardation, hypotonia, seizures
Hypomelanosis of Ito	Chr dup/dels, often 15q11-q13	1/10000	≤1	10	Hypopigmented macules, neurological deficits, variable mental retardation and seizures, multiple malformations (brain, ocular, musculoskeletal)
Smith–Magenis syndrome	17p11.2 del	1/25000	≤1	93	Mental retardation, developmental delay, self-injurious behavior, facial dysmorphisms, hearing impairment; skeletal, renal, cardiac, and eye abnormalities

TABLE 34.3 Syndromic Autisms Due to Known Mutations, Triplet Repeat Expansions, or Cytogenenetic Abnormalities Visible by G Band Karyotyping—cont'd

	Gene/ch. region	Prevalence	Percentage of autistic patients with the syndrome	Percentage of patients with the syndrome who are autistic	Signs and symptoms
Potocki–Lupsky syndrome	17p11.2 dup	?	≤1	?	Hyperactivity, attention deficit, autism, mental retardation, developmental delay, short stature, hypotonia, mild dysmorphism, cardiac and dental abnormalities
Down syndrome	Trisomy of ch. 21	1/1000	≤2.5	≤10	Facial dysmorphism, cardiac and intestinal malformations, variable degree of mental retardation, severe autism (when present)
Velofaciocardial/ Di George syndrome	22q11.2 del	1/4000	≤1	20–31	Facial dysmorphism, cleft palate, cardiac malformations, hypoplasia of the thymus, hypoparathyroidism, autism, mental retardation, developmental delay, attention deficit, hyperactivity, psychosis, seizures
Ch 22q11 duplication syndrome	22q11.2 dup	?	≤1	?	Facial dysmorphism, velopharyngeal insufficiency, autism, mental retardation, developmental delay
Ch 22q13.3 deletion syndrome	22q13.3 del	?	≤1	?	Mild dysmorphisms, severe hypotonia, mental retardation, developmental delay, impaired language development

?, no data available.

TABLE 34.4 Syndromic Autisms Due to mtDNA Mutations or Rearrangements

References	Mutation	mtDNA gene	Number of patients	Signs and symptoms
Graf et al. (2000)	8363G>A	$tRNA^{Lys}$	Two siblings	Brother: autism, behavioral regression, extreme hyperactivity, lack of attention, mild fine and gross motor dyspraxia
				Sister: partial complex seizures, unsteady gait, myoclonus, swallowing dysfunction, moderate mental retardation
Fillano et al. (2002)	Large mtDNA deletions		Five ASD patients	Autism, ataxia, cardiomyopathy
Pons et al. (2004)	3243A>G	$tRNA^{Leu(UUR)}$	Two ASD and their two mothers	Highly heterogeneous: typically mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), or maternally inherited progressive external ophthalmoplegia. In these two patients, autism with developmental delay, clumsiness, attention deficit, neurologic deterioration in the presence of fever, microcephaly or macrocephaly
	?	mtDNA? genomic DNA?	One ASD with mtDNA depletion	Autism, muscle hypotonia, seizures, myoclonus, and developmental delay
Weissman et al. (2008)	3397A>G	ND1 subunit of complex I	25 ASD patients with primary mit. disorder (3/25 mtDNA mutation carriers)	Autism, excessive fatigability and/or exercise intolerance, gastrointestinal dysfunction, cardiovascular abnormalities, facial dysmorphisms, microcephaly or macrocephaly, developmental gross motor delays, growth retardation
	4295A>G	$tRNA^{Ileu}$		
	11984T>C	ND4 subunit of complex I		

of microsomy, or less frequently macrosomy, is not unusual. In many syndromes, clinical manifestations of autism can be highly heterogeneous, even in the presence of the same well-characterized mutation or genomic rearrangement.

34.2.2 Mitochondrial Autisms

Biochemical parameters linked to mitochondrial function are frequently abnormal in ASD (Giulivi et al., 2010; Palmieri and Persico, 2010; Rossignol and Frye, 2011).

As many as 5% of autistic children even satisfy diagnostic criteria for a full-blown mitochondrial disease (Rossignol and Frye, 2011). Yet, mutations or chromosomal rearrangements in mtDNA or nuclear DNA (nDNA) are detected only in approximately 20% of children with ASD and mitochondrial disease (i.e., ≤1% of all ASD children), and each mtDNA mutation or chromosomal rearrangement listed in Table 34.4 is detected in ≤0.1% of all cases. Hence, mitochondrial dysfunction appears to be secondary in the vast majority of patients, that is, downstream of other pathophysiological abnormalities such as excessive oxidative stress (Palmieri and Persico, 2010). Importantly, since mitochondrial function requires approximately 1500 nuclear genes and oxidative phosphorylation involves at least 80 proteins, only 13 encoded by mtDNA, mutations, and chromosomal rearrangements should be sought both in nDNA and in mtDNA (Shadel, 2008; Zeviani and Di Donato, 2004). Indeed, chromosomal rearrangements, which could affect mitochondrial functions, include deletions in 15q11–q13 (cytochrome *C* oxidase subunit 5A, COX5A), 13q13–q14.1 (mitochondrial ribosomal protein 31, MRPS31), 4q32–q34.68 (electron-transferring-flavoprotein dehydrogenase, ETFDH), and 2q37.3 (NADH dehydrogenase ubiquinone 1 alpha subcomplex 10, NDUFA10), as recently reviewed by Smith et al. (2009).

Mitochondrial autism, despite an even more prominent clinical heterogeneity, often displays some peculiarities which should prompt clinicians to request molecular investigations (Palmieri and Persico, 2010; Rossignol and Frye, 2011). Its neurological signs and symptoms, such as oculomotor abnormalities, dysarthria, ptosis, hearing deficits, hypertonia, and movement disorders, are generally atypical for autism. Behavioral regression, especially in concomitance with fever, is frequently reported by parents (Shoffner et al., 2010; Weissman et al., 2008). Except in the case of mitochondrial depletion, family history is generally positive for mitochondrial diseases in the maternal lineage. At least one biochemical parameter among several typically assessed to screen for mitochondrial disorders is usually abnormal in children. The incidence of microcephaly and microsomy is unusually high, reaching approximately 20% of all cases. Neuroanatomical abnormalities are relatively frequent, although highly variable in nature (Nissenkorn et al., 2000; Shoffner et al., 2010; Weissman et al., 2008). 'Ragged red fibers,' characterized by a segmental proliferation and accumulation of abnormal mitochondria under the sarcolemmal membrane, are usually visible in muscle biopsies of adults, but in most affected children muscle tissue histology will be negative.

34.2.3 Copy Number Variants

The recent advent of microarray-based high-resolution genome analysis has dramatically increased our ability to detect genomic deletions and duplications. CNVs are deletions and duplications of at least 1 kb in size, undetectable by standard chromosomal banding and karyotyping techniques. They are, however, discernible using microarray-based approaches, such as array-comparative genome hybridization (CGH) techniques employing either bacterial artificial chromosome (BAC) or single nucleotide polymorphism (SNP) arrays, whereby signal intensity is used to estimate the number of alleles. Initial genome-wide studies reported enhanced frequencies of CNVs in autistic patients compared to controls (on average 6–10% vs. 1–3%, respectively). In particular, Jacquemont et al. (2006) found 8 of 29 (27.5%) autistic patients carrying deletions or duplications between 1.4 and 16.0 Mb in size, including six *de novo* chromosomal rearrangements. Sebat et al. (2007) found *de novo* CNVs in 12/118 (10%) autistic children from simplex families (i.e., families with only one autistic child) and in 2/196 (2%) normal trios. Marshall et al. (2008) found 27/427 (6.3%) autistic patients carrying *de novo* CNVs, which were more common in simplex (4/56 = 7.1%) than in multiplex (1/49 = 2.0%) families. Christian et al. (2008) reported the presence of seven *de novo* and 44 inherited CNVs in 397 ASD patients. Collectively, these results were compatible with the existence of genomic instability in a sizable subgroup of autistic patients. However, later studies have not replicated genome-wide differences in CNV frequency between ASD patients and controls using genomic DNA extracted from leukocytes or lymphoblastoid cell lines (Bucan et al., 2009; Glessner et al., 2009; Pinto et al., 2010). We have recently performed a small-scale study using genomic DNA extracted from neocortical post-mortem specimens, finding increased genomic instability in only one out of ten autistic brains compared to ten matched controls (Roberto Sacco, Antonio M., Persico, Shawn Levy, and colleagues, unpublished observation). Therefore, excessive genomic instability may characterize some families with autistic patients, but it does not represent a widespread hallmark of autism either in the CNS or in peripheral tissues. CNV location may instead play a more relevant role compared to CNV frequency and mean size. Rare or even private CNVs seemingly affect the coding region of functionally important genes more often among ASD patients than in controls: disrupted loci belong to gene families involved in synaptogenesis, cell proliferation and migration, ubiquitination, and GTPase/Ras signaling (Bucan et al., 2009; Glessner et al., 2009; Pinto et al., 2010). This conclusion has been further strengthened by two large data sets that have recently uncovered highly heterogeneous *de novo* copy-number variants which collectively affect several hundred loci and presumably account for 5–8% of cases of simplex forms of ASD (Levy et al., 2011; Sanders et al., 2011; for comment see Schaaf and Zoghbi, 2011). Network-based functional analysis of these rare CNVs confirms the involvement of these loci in synapse development, axon targeting, and neuron

motility (Gilman et al., 2011). We shall encounter again many of these genes in surveying monogenic forms of autism (Section 34.3).

Most CNVs are unique to any given patient, both in size and genomic distribution. However, recurrent microdeletion syndromes have also been identified: their chromosomal location and associated clinical features are listed in Table 34.5 (Fernandez et al., 2010; Kumar et al., 2008; Liang et al., 2009; Rajcan-Separovic et al., 2007; Weiss et al., 2008). In general, CNVs can be associated with a variety of clinical features, including major or minor malformations, facial dysmorphisms, severe neurological symptoms, full-blown autism, milder autism-spectrum traits, or even behavioral disorders outside of the autism spectrum (frequently seen in siblings carrying the same CNV as their autistic sib). Variable penetrance and great phenotypic heterogeneity thus characterize CNV expressivity to the same extent as we have seen occur in many 'classical' syndromic forms listed in Table 34.3. This is true to the point that it is often difficult to determine whether in a given patient a CNV is the sole cause of autism, confers vulnerability to the disease, or represents a chance finding. Indeed, the majority of CNVs are inherited from either one of the parents, who may show some autism spectrum traits, but certainly do not satisfy criteria for autistic disorder. Also, a sizable percentage of population controls carries CNVs, available in public databases (Iafrate et al., 2004). Finally, many CNVs found in ASD patients are not autism specific, but are found also in patients with mental retardation, schizophrenia, or other psychopathologies.

34.3 NOVEL SYNDROMIC FORMS OF MONOGENIC AUTISMS

34.3.1 General Description

In recent years, several monogenic forms of autism have been uncovered (see review by Lintas and Persico, 2009). Each is present in a small number of patients (i.e., <1%) and can result from mutations or cytogenetic anomalies proved to be absent from large pools of control chromosomes. These findings have led to the proposal that most autisms may represent a collection of syndromes due to rare, if not even, private mutations or CNVs (Buxbaum, 2009). However, causal mutations and chromosomal rearrangements should ideally appear *de novo*, but they are more often segregating in the family, which again underscores their variable degree of penetrance and heterogeneous expressivity. We shall now review the characteristics and neurobiological bases of the most important monogenic forms recently discovered, which are listed in Table 34.6.

34.3.2 Synaptic Genes

Several genes involved in monogenic autisms are known to play a role in synapse formation, maturation, and stabilization. This functional role in the establishment and fine-tuning of neuronal connections is pathophysiologically appealing, when considering autism as a 'dysconnection syndrome' (Courchesne and Pierce, 2005; Geschwind and Levitt, 2007; Rubenstein

TABLE 34.5 Syndromic Autisms Due to Recurrent CNVs

Ch region	Del/Dup	Neurodevelopmental signs and symptoms	Other signs and symptoms
1q21	Del	Autism, attention deficit, hyperactivity, antisocial behavior, anxiety, epilepsy, mental retardation, developmental delay, depression, hallucinations, schizophrenia	Minor dysmorphisms, cardiac defects, cataracts, multiple congenital malformations
	Dup	Autism, attention deficit, hyperactivity, epilepsy, mental retardation, developmental delay, impaired language, learning disability	Minor dysmorphisms, multiple congenital malformations
2p15–2p16.1	Del	Autism, developmental delay	Microsomy, microcephaly, dysmorphic features
15q13	Del	Autism, attention deficit, hyperactivity, aggression, anxiety, epilepsy, mental retardation, developmental delay, impaired language, schizophrenia	Minor dysmorphisms, cardiac defects
	Dup	Autism, anxiety, bipolar disorder, mental retardation, developmental delay, obsessive-compulsive disorder, language delay	Minor dysmorphisms, hypotonia, obesity, recurrent ear infections
16p11.2	Del	Autism, Asperger syndrome, attention deficit, hyperactivity, dyslexia, bipolar disorder, anxiety, epilepsy, mental retardation, developmental delay, language impairment, schizophrenia	Minor dysmorphisms, hypotonia, multiple congenital malformations
	Dup	Autism, attention deficit, hyperactivity, anxiety, epilepsy, mental retardation, developmental delay, obsessive-compulsive disorder	

and Merzenich, 2003). However, there are also issues raising caution in interpreting autistic signs and symptoms as merely due to reduced synaptogenesis, as briefly addressed in Section 34.6.

34.3.2.1 The Neuroligin Genes (NLGN3, NLGN4, and NLGN4Y)

The *NLGN3*, *NLGN4*, and possibly *NLGN4Y* genes, located in human ch Xq13, Xq22.33, and Yq11.2, respectively, have been found to host mutations seemingly responsible for behavioral phenotypes, including autism (Table 34.6). Neuroligin genes encode for cell adhesion molecules located postsynaptically in glutamatergic (*NLGN1*, *NLGN3*, *NLGN4*, *NLGN4Y*) and GABAergic (NLGN2) synapses (for reviews, see Betancur et al., 2009; Craig and Kang, 2007; Südhof, 2008). At the extracellular level, postsynaptic neuroligins interact with presynaptic α- or β-neurexins (see Section 34.3.2.3); at the intracellular level, neuroligins associate with postsynaptic scaffolding proteins, such as SHANK3 (see Section 34.3.2.2). This network of synaptic proteins appears to play a critical role in synapse generation,

TABLE 34.6 Mutations and Cytogenetic Abnormalities, Either *de novo* or Segregating, Affecting NLGN, SHANK3, NRXN1, MECP2, HOXA1, PTEN, and Calcium-Related Genes, their Incidence in Samples of ASD Individuals, and Associated Clinical Phenotypes

Gene	References	Mutations/del	Incidence	Clinical phenotype
NLGN3	Jamain et al. (2003)	R451C	1/158 (0.6%)	Autism, Asperger's syndrome
NLGN4	Jamain et al. (2003)	D396X	1/158 (0.6%)	Autism, Asperger's syndrome
	Laumonnier et al. (2004)	D429X	One family with 13 affected males	Autism, MR, PDD-NOS
	Yan et al. (2005)	G99S	1/148 (0.7%)	Severe autism, MR, language disability
		K378R	1/148 (0.7%)	Autism
		V403M	1/148 (0.7%)	PDD-NOS
		R704C	1/148 (0.7%)	Autism
	Lawson-Yuen et al. (2008)	del exons 4,5,6	One family with one affected male[c]	Autism with motor tics
	Daoud et al. (2009)	−355G>A	1/96 (1.0%)	Autism with severe MR
	Pampanos et al. (2009)	K378R	1/169 (0.6%)	Mild autism with normal IQ
NLGN4Y	Yan et al. (2008a)	I679V	1/290 (0.3%)	Autism
SHANK3	Durand et al. (2007)	142 kb del	1/227 (0.4%)	Autism with severe language deficits and MR
		E409X	1/227 (0.4%)	Autism with severe language deficits and MR
		800 kb del	1/227 (0.4%)	Autism with severe language deficits and MR (The trisomic brother has Asperger's syndrome)
	Moessner et al. (2007)	277 kb del, 3.2 Mb del, 4.36 Mb del	1/400 (0.25%) each, 3/400 (0.75%) total	Autism with severe language deficits and MR
		Q321R	1/400 (0.25%)	PDD-NOS with regression of spoken words
	Gauthier et al. (2009)	L68P	1/427 (0.23%)	PDDNOS with regression of spoken words
		c. 2265C +1delG	1/427 (0.23%)	Autism
NRXN1	Feng et al. (2006)	S14L	3/264 (1.1%)	Autism, MR, seizures, mild facial dysmorphism
		T40S	1/264 (0.4%)	Autism, MR, mild facial dysmorphism
	The Autism Genome Project Consortium (2007)	300 kb del at 2p16	2/196 (0.5%)	Autism, MR, mild to severe spoken language deficits
	Kim et al. (2008)	ins(16;2)(q22.1; p16.1p16.3)[f]	case report	Autism, MR

TABLE 34.6 Mutations and Cytogenetic Abnormalities, Either *de novo* or Segregating, Affecting NLGN, SHANK3, NRXN1, MECP2, HOXA1, PTEN, and Calcium-Related Genes, their Incidence in Samples of ASD Individuals, and Associated Clinical Phenotypes—cont'd

Gene	References	Mutations/del	Incidence	Clinical phenotype
		t(1;2)(q31.3;p16.3)	case report	PDD-NOS, ADHD, conduct disorder, intermittent explosive disorder
		L18Q	1/57+0/53 (0.9%)	Autism (?)
		L748I	1/57+2/53 (2.7%)	Autism (?) with incomplete penetrance
	Yan et al. (2008a,b)	R8P, L13F, c1024 +1 G>A, T665I, E715K	1/116 (0.9%) each 5/116 (4.3%) total	Autism (?)
	Zweier et al. (2009)	180 kb del + p. S979X	1/179 (0.6%)	Autism, severe MR, lack of spoken language
MECP2	Lam et al. (2000)	IVS2+2delTAAG	1/21F (4.8%)	Autism, MR. No regression, epilepsy, or microcephaly
	Vourc'h et al. (2001)	–	0/59 (42M,17F)	
	Beyer et al. (2002)	–	0/202 (154M,48F)	
	Carney et al. (2003)	1157del41, R294X	2/69F (2.9%)	Autism, MR, history of regression. No stereotypies, epilepsy, or microcephaly
	Zappella et al. (2003)	R133C, R453X	2/19F (4.7%)	Preserved speech variant of Rett syndrome
	Shibayama et al. (2004)	c.1638 G>C, c. 6809 T>C, P376R	1/24 (4.1%) each 3/24 (12.5%) total	Autism (?)
	Lobo-Menendez et al. (2004)	–	0/99 (58M,41F)	
	Li et al. (2005)	–	0/65 (49M,16F)	
	Xi et al. (2007)	c.1461 G>A	1/31M (3.2%)	Autism (?)
	Harvey et al. (2007)	–	0/401 (266M,135F)	
	Coutinho et al. (2007a,b)	G206A	1/172 (0.6%) (141M, 31F)	Autistic male with severe MR and lack of spoken language
		Twelve 3′UTR variants, c.27-55G>A, c.377+18C>G	1/172 (0.6%) each	Autism (?)
HoxA1	Tischfield et al. (2005) and Bosley et al. (2007)	c.84 C>G (Y28X)	One patient from a Turkish consanguineous family	BSAS with variable degrees of horizontal gaze abnormalities, deafness, focal weakness, hypoventilation, vascular malformations of the internal carotid arteries and cardiac outflow tract, MR and autism (present in 3/9 Saudi Arabian patients)
		175-176insG	Nine patients from 5 Saudi Arabian consanguineous fam.	
PTEN	Goffin et al. (2001)	Y178X	Case report	Cowden syndrome with autism and progressive macrocephaly
	Butler et al. (2005)	H93R, D252G, F241S	3/18 (16.6%) all macrocephalic	Extreme macrocephaly and macrosomy
	Boccone et al. (2006)	I135R	1 (case report)	Bannayan–Riley–Ruvalcaba syndrome with reactive nuclear lymphoid hyperplasia and autism
	Buxbaum et al. (2007)	D326N	1/88 (1.1%) all macrocephalic	Macrocephaly (+9.6 SD), polydactily at both feet, autism, MR, language delay
	Orrico et al. (2009)	Y176C, N276S	1/40 (2.5%) each, all macrocephalic	Autism

Continued

TABLE 34.6 Mutations and Cytogenetic Abnormalities, Either *de novo* or Segregating, Affecting NLGN, SHANK3, NRXN1, MECP2, HOXA1, PTEN, and Calcium-Related Genes, their Incidence in Samples of ASD Individuals, and Associated Clinical Phenotypes—cont'd

Gene	References	Mutations/del	Incidence	Clinical phenotype
		H118P	1/40 (2.5%), all macrocephalic	Developmental delay without autism
	Herman et al. (2007a) and Varga et al. (2009)	520insT, R130X, E157G, L139X, IVS6-3C>G	5/60 (8.3%) total, 5/27 (18.5%) macrocephalic	Macrocephaly, autism or PDD-NOS, developmental delay, MR, language delay
EIF4E	Neves-Pereira et al. (2009)	46,XY,t(4,5)(q23;q31.3)	Case report	Autism with regression (loss of spoken words and social interaction at age 2)
		C_8-4EBE	2/120 (1.6%) multiplex families ($N=4$ subjects)	Severe autism, language delay
CACNA1C	Splawski et al. (2004)	G406R	13 patients with Timothy syndrome	Timothy syndrome: lethal arrhythmias, webbing of fingers and toes, congenital heart disease, immune deficiency, intermittent hypoglycemia, autism and MR
CACNA1F	Hemara-Wahanui et al. (2005), Hope et al. (2005)	I745T	One pedigree with 3 ASD males out of 10 mutation carriers	5/10 mutation carriers have MR, and 3 of these 5 individuals has MR+autism
CACNA1H	Splawski et al. (2006)	R212C, R902W, W962C	1/491 (0.2%) each, 3/491 (0.6%) total	Autism (?)
		R1871Q+A1874V	3/491 (0.6%)	Autism (?)
BKCa	Laumonnier et al. (2006)	46,XY, t(9,10)(q23,q22)	Case report	Autism, lack of spoken language
		Ala138Val	1/116 (0.9%)	Autism
SCN2A	Weiss et al. (2003)	R1902C	1/229 (0.4%) families	Autism (AGRE family AU0247, only in one of two affected children)

Variants are not listed if also present in control samples.
MR, mental retardation; PDD-NOS, pervasive developmental disorder not otherwise specified; (?), clinical phenotype not described.

maturation, stabilization, and maintenance (Betancur et al., 2009; Craig and Kang, 2007; Südhof, 2008). *In vitro* assays initially suggested that the interaction between neuroligins and neurexins may trigger the formation of functional presynaptic boutons in contacting neurites, both in neuronal and even in non-neuronal cells (Dean et al., 2003; Scheiffele et al., 2000). Later studies of triple knockout mice lacking neuroligins 1, 2, and 3 showed that their absolute numbers of synapses are unchanged, whereas synaptic transmission is severely hampered, leading to respiratory failure and death on the day of birth (Varoqueaux et al., 2006). These results underscore the role of neuroligins as critical to synaptic function, more than to synaptogenesis *per se*.

Jamain et al. (2003) reported one frameshift (D396X in *NLGN4*) and one missense (R451C in *NLGN3*) mutation in two unrelated Swedish families, both inherited from apparently unaffected mothers. Mouse mutants carrying the human R451C mutation show a mild behavioral phenotype, described by Tabuchi et al. (2007) and by Chadman et al. (2008). Reduced ultrasonic vocalizations in males represent the most consistent behavioral abnormality, followed by impaired social novelty preference (Tabuchi et al., 2007, but see Chadman et al., 2008). Surprisingly, enhanced spatial learning abilities and increased inhibitory synaptic transmission were also recorded (Tabuchi et al., 2007). Functional *in vitro* studies of the R451C mutation show defective vesicle trafficking with partial retention of NLGN3 in the endoplasmic reticulum. Reduced synapse induction properties are due to blunted NLGN3 delivery to the cell membrane, as mutated NLGN3 retains synaptogenetic properties in the minority of cells where delivery to the membrane does occur (Chih et al., 2004; Chubykin et al., 2005; Comoletti et al., 2004). These abnormalities lead to reduced glutamate-driven excitation in the neocortex, while AMPA-driven excitation, NR2B subunit delivery, and long-term potentiation are all enhanced in the hippocampus (Etherton et al., 2011a). Instead, the R704C mutation initially reported by Yan et al. (2005) causes a

major and selective decrease in AMPA receptor-mediated synaptic transmission, leaving the number of synapses unchanged (Etherton et al., 2011b).

Multiple studies collectively confirm the low frequency of *NLGN* gene mutations among idiopathic ASD patients (Table 34.6) (Lintas and Persico, 2009). The clinical phenotype of patients carrying *NLGN* mutations is highly heterogeneous, ranging from severe autistic disorder to Asperger's syndrome (the 'speech-preserved' variant of autism), PDD-NOS (the autism variant satisfying only some, but not all diagnostic criteria), nonspecific X-linked mental retardation, specific language impairment, and Tourette syndrome (Table 34.6). Disease onset may be slow and insidious or sudden and regressive (see Section 34.6). Mutation carriers typically display no dysmorphic feature and are phenotypically indistinguishable (Lintas and Persico, 2009).

34.3.2.2 The SH3 and Multiple Ankyrin Repeat Domains 3 Gene (SHANK3)

The *SHANK3* gene, located in chromosome 22q13.3, encodes for a scaffolding protein found in the postsynaptic density complex of excitatory synapses, where it binds to neuroligins and to actin, affecting actin polymerization, growth cone motility, dendritic spine morphology, and synaptic transmission (Durand et al., 2011). Several recent studies have described rare mutations or small cytogenetic rearrangements affecting *SHANK3* in patients with an autistic phenotype mainly characterized by severe expressive language impairment (Table 34.6). Similarly, 13 patients carrying deletions of the terminal 22q13 region encompassing or breaking the *SHANK3* locus all share mental retardation and severe delay in or absence of expressive speech (Dhar et al., 2010). As for neuroligins, also *SHANK3* mutations or deletions/duplications represent rare events, affecting only 9/1054 (0.85%) ASD individuals (see Table 34.6). No evidence of association was found in large samples (Qin et al., 2009; Sykes et al., 2009), demonstrating that the *SHANK3* gene hosts rare variants, but not common variants. With the possible exception of language impairment, mutations and cytogenetic abnormalities affecting *SHANK3* display highly variable phenotypic expression: (a) they are most often inherited from parents described as either healthy or epileptic; (b) in some families, they are present also in unaffected siblings of the autistic proband; (c) two *de novo* mutations, R536W and R1117X, different from those found in ASD patients, were detected in patients with borderline or moderate mental retardation and either schizoaffective disorder, attention-deficit/hyperactivity disorder (ADHD), or schizophrenia/atypical chronic psychosis (Gauthier et al., 2010). Importantly, autistic individuals carrying inherited 22q13 deletions involving *SHANK3* (800 kb in Durand et al. (2007) and 3.2 Mb at 22q13 in Moessner et al. (2007)) due to a paternal balanced translocation have siblings with partial 22q13 trisomy diagnosed with Asperger syndrome, showing early language development and ADHD. Hence, a physiological window for *SHANK3* may be functionally crucial to cognitive development in humans.

34.3.2.3 The Neurexin 1 Gene (NRXN1)

Presynaptic neurexins are able to induce postsynaptic differentiation by interacting with postsynaptic neuroligins (Betancur et al., 2009; Craig and Kang, 2007; Südhof, 2008). In addition, α neurexins are involved in neurotransmitter release, as they link calcium (Ca^{2+}) channels to synaptic vesicle exocytosis (Missler et al., 2003; Zhang et al., 2005). The three neurexin genes (*NRXN1*, *NRXN2*, and *NRXN3*, located on human ch 2p16.3, 11q13, and 14q24.3–q31.1, respectively) have two independent promoters, yielding long and short mRNAs which encode for α and β neurexins, respectively (Ichtchenko et al., 1995). Several studies have reported rare sequence variants or CNVs affecting the *NRXN1* locus, as summarized in Table 34.6. However, an exact definition of *NRXN1* roles in autism is complicated by an extreme interindividual variability in genotype–phenotype correlations. An initial *NRXN1*β screening conducted by Feng et al. (2006) identified two heterozygous missense mutations (S14L and T40S) present in 4/264 (1.5%) ASD patients and not in 729 controls (Table 34.6). These 'mutations' are actually rare segregating polymorphisms found also in first-degree relatives, who clinically range from apparently normal behavior to hyperactivity, depression, and/or learning problems. Similarly, one of two chromosomal rearrangements affecting the *NRXN1* gene was also shown to be paternally inherited in one patient (Kim et al., 2008). In another study, a *de novo* heterozygous 300 kb deletion in the coding exons of the *NRXN1* gene was found in two autistic sisters (Autism Genome Project Consortium, 2007); interestingly, one girl was reported to be nonverbal, whereas the other only had mild language regression. CNVs disrupting the *NRXN1* coding sequence can result in schizophrenia and not in ASD (Kirov et al., 2009; Rujescu et al., 2009). This phenotypic variability is further underscored by a large retrospective study involving 3540 individuals, identifying in 12 of them exonic *NRXN1* microdeletions causing very heterogeneous clinical phenotypes including ASD, mental retardation, language delay, and muscle hypotonia to a variable degree (Ching et al., 2010). Finally, a recent study by Zweier et al. (2009) concerning a girl with severe mental retardation and autism demonstrated the inheritance in compound heterozygosity of a 180 kb deletion from her unaffected mother and a stop mutation in exon 15 from her healthy father. These genetic abnormalities are predicted to deprive this patient of the longer alpha *NRXN1* isoform, whose lack cannot be functionally complemented

by the shorter beta isoform, leading to significantly decreased numbers of synapses both in alpha *NRXN1* knockout mice and in *Drosophila* (Etherton et al., 2009; Li et al., 2007a; Zeng et al., 2007). In Section 34.6, we shall return to compound heterozygosity as a viable mechanism able to explain the complexities of rare variant contributions to autism pathogenesis in some families.

34.3.3 Chromatin Architecture Genes

Rett syndrome is a peculiar PDD initially described by the Austrian pediatrician Andreas Rett in 1966. This severe neurodevelopmental disorder is characterized by regression, autism, microcephaly, stereotyped behaviors, epilepsy, and breathing problems (Chahrour and Zoghbi, 2007; Rett, 1966). The discovery that approximately 80% of females with Rett syndrome carry *de novo* mutations in the methyl-CpG-binding protein 2 (MeCP2) gene, which plays a critical role in determining chromatin structure by modulating DNA methylation (Amir et al., 1999), spurred great interest in the role of this gene in other PDDs and more broadly in the role of epigenetic mechanisms in autism.

34.3.3.1 The Methyl-CpG-Binding Protein 2 Gene

The methyl-CpG-binding protein 2 (MeCP2) binds to methylated CpG dinucleotides, recruiting histone deacetylase 1 (HDAC1) and other proteins involved in chromatin repression at specific gene promoters. It thus acts as a transcriptional repressor (Chahrour and Zoghbi, 2007), not only during development but throughout adult life (McGraw et al., 2011). *De novo* mutations of the *MECP2* gene located on chromosome Xq28, in addition to classical Rett syndrome, can also result in asymptomatic phenotypes, mild mental retardation, and verbal Rett variants, depending upon the specific mutation, the genetic background of the patient, and the X-inactivation pattern, which is highly skewed in the presence of mutations affecting X-linked genes, such as *NLGN3* and *MECP2*, albeit not being skewed in ASD families altogether (Gong et al., 2008). Instead, *MECP2* mutations are generally lethal in males (Amir et al., 1999; Chahrour and Zoghbi, 2007).

Several groups have screened the *MECP2* gene for mutations in nonsyndromic ASD patients, finding positives in 5 of the 397 females (1.3%) and none of the 741 males assessed to date (Lintas and Persico, 2009) (Table 34.6). Three *de novo MECP2* mutations have been found in two out of eleven studies assessing female ASD patients: the IVS2+2delTAAG splice variant in intron 2, the frameshift mutation 1157del41, and the nonsense mutation R294X (Carney et al., 2003; Lam et al., 2000). Two additional *de novo* mutations, R133C and R453X, were identified in two autism-spectrum girls fulfilling criteria for the 'preserved speech' variant of Rett syndrome (Zappella et al., 2003). A few other missense, intronic, or 3′-UTR variants listed in Table 34.6 either are inherited from one of the parents, or it is not specified whether they are inherited or occurring *de novo*. Importantly, young girls carrying *MECP2* mutations appear autistic and mentally retarded, but display none of the symptoms typical of Rett syndrome (epilepsy, microcephaly, stereotypies, and breathing problems). Also, regression is not consistently reported by parents (Carney et al., 2003). Signs and symptoms more typical of Rett syndrome may appear when they grow older (Young et al., 2008). There is thus a rationale for *MECP2* gene screenings in female autistic patients and for follow-up programs to monitor these patients clinically over time (Lintas and Persico, 2009).

34.3.4 Morphogenetic and Growth-Regulating Genes

Many syndromic patients display facial dysmorphisms, minor or major malformations, microcephaly or macrocephaly either in isolation or as part of a broader microsomy or macrosomy, respectively (Tables 34.3 and 34.5). Also, idiopathic autistic children sometimes display minor facial dysmorphisms, which are beginning to be characterized using standardized or even automated methods for consistency (Hammond et al., 2004, 2008; Miles et al., 2008; Tripi et al., 2008). In addition, head and body growth rates are often abnormal. Macrocephaly has been consistently found in approximately 20% of autistic patients (Fombonne et al., 1999; Lainhart et al., 1997; Miles et al., 2000; Sacco et al., 2007a; Stevenson et al., 1997; Woodhouse et al., 1996). Head circumference in these ASD patients is typically normal at birth, and an overgrowth seemingly develops over the first few years of life (Courchesne et al., 2007). This macrocephaly is part of a broader macrosomy in most, though not all, patients (Bigler et al., 2010; Dissanayake et al., 2006; Lainhart et al., 2006; Sacco et al., 2007a). On the contrary, a small subset of idiopathic autistic patients is instead microcephalic and usually also microsomic (see Figures 1 and 2 in Sacco et al., 2007a).

34.3.4.1 The Homeobox A1 Gene (HOXA1)

HOXA1 is a homeobox gene located on chromosome 7p15.3. It is critically involved in the development of head and neck structures directly or indirectly derived from the distal part of rhombomere 4 and from rhombomere 5 during embryogenesis (Chisaka et al., 1992; Mark et al., 1993; Rossell and Capecchi, 1999): these include brainstem, cerebellum, several cranial nerves, medium and internal ear, and occipital and hyoid bones. Both common and rare *HOXA1* gene variants have been

sought. The common A218G polymorphism exerts a sizable effect on head growth rates both in autistic and in typically developing children, with the G allele yielding faster head growth and smaller cerebellar volumes (Canu et al., 2009; Conciatori et al., 2004; Muscarella et al., 2007, 2010). This measurable effect on the growth of regions known to be involved in autism, such as the cerebellum (Courchesne, 1997), is intriguing, despite the nonreplication of an initial report suggesting that this common *HOXA1* variant could possibly contribute to autism (Ingram et al., 2000b; but see Gallagher et al., 2004; Li et al., 2002; Romano et al., 2003; Sen et al., 2007; Talebizadeh et al., 2002). The G allele leads to the substitution of the second of ten contiguous histidines by an arginine (His73Arg). This stretch of ten histidines underlies protein–protein interactions, which could be modulated by this gene variant, although direct experimental evidence is still lacking.

In reference to rare *HOXA1* gene variants possibly causing autism, the study of five consaguineous families from Saudi Arabia and of one from Turkey disclosed homozygous stop codon mutations in ten affected individuals (Bosley et al., 2007; Tischfield et al., 2005). Two different mutations were identified: a c.84 C>G mutation, which results in the introduction of a stop codon (Y28X) in the Turkish patient, and a 175–176insG, which causes a reading frame shift and a premature protein truncation in nine Saudi Arabian patients (Bosley et al., 2007; Tischfield et al., 2005). Mutation carriers show some phenotypic similarities, including horizontal gaze abnormalities, deafness, focal weakness, hypoventilation, vascular malformations of the internal carotid arteries and cardiac outflow tract, mental retardation, and autism: this set of clinical symptoms and malformations was named Bosley–Salih–Alorainy syndrome (BSAS) (Bosley et al., 2007). Importantly, all signs and symptoms (even vascular malformations) display a significant degree of interindividual variability in both presence and severity, with developmental delay and autism reported only in a subset of patients (see Section 34.6). Many signs and symptoms of BSAS overlap with those present in the Athabascan brainstem dysgenesis syndrome found in Native American children carrying a distinct *HOXA1* R26X mutation (Bosley et al., 2008). Since these causal mutations are recessive, similar phenotypes should be sought only in areas where inbreeding is substantial. In other geographical areas, oligogenic heterozygosity also involving rare *HOXA1* variants may play a role in idiopathic autism (Schaaf et al., 2011).

34.3.4.2 The Phosphatease and Tensin Homolog Gene (PTEN)

PTEN is a tumor suppressor gene located on human chromosome 10q23, which favors cell-cycle arrest in G1 and apoptosis. In conjunction with other tumor-suppressor genes, such as *TSC1*, *TSC2*, and *NF1*, it balances the stimulation physiologically exerted on cell proliferation and body growth by nutrient availability (sugars and proteins), insulin release, and pro-inflammatory cytokines, through the ERK/PI3K/mTOR pathway (Figure 34.1) (Ma and Blenis, 2009). Mutations inactivating these tumor-suppressor genes cause diseases often associated with syndromic autisms (see Table 34.3). *PTEN* knockout mice indeed display macrosomy, macrocephaly, CNS overgrowth with thickening of the neocortex and cytoarchitectonic abnormalities in the hippocampus, excessive dendritic and axonal growth, and increased numbers of synapses (Kwon et al., 2006). Furthermore, in humans, germline mutations resulting in *PTEN* haploinsufficiency facilitate cell-cycle progression and oncogenesis, leading to macrocephaly/macrosomy and to cancer development, respectively (Eng, 2003). Germline *PTEN* mutations have been documented in the vast majority of patients diagnosed with Cowden syndrome, which carries enhanced risk for breast, endometrial, and thyroid cancers (Eng, 2003). Also, individuals suffering from other related hamartoma disorders, such as Bannayan–Riley–Ruvalcaba syndrome, Proteus syndrome, and Proteus-like syndromes, display germline *PTEN* mutations in 60%, 20%, and 50% of cases, respectively (Eng, 2003). Interestingly, genetic syndromes due to *PTEN* germline haploinsufficiency are also frequently associated with autism or mental retardation, as initially reported by Goffin et al. (2001) (e.g., see Boccone et al., 2006). Instead, several missense mutations affecting evolutionarily conserved amino acid residues have been detected in macrocephalic individuals affected by idiopathic autism (Table 34.6). ASD patients carrying *PTEN* mutations are invariably characterized by severe to extreme macrocephaly (i.e., cranial circumference >97th percentile or +2 SD, but most *PTEN* mutation carriers typically display ≥+3 SD). In some cases, the overgrowth starts prenatally, whereas other *PTEN* mutation carriers display a normal head circumference at birth and macrocephaly develops only postnatally, as generally occurs in macrocephalic autistic children (Courchesne et al., 2007). In addition, the majority of macrocephalic autistic patients are actually macrosomic, underscoring a systemic disruption of body growth control mechanisms (Sacco et al., 2007a). Although all mutation carriers share macrocephaly as a unifying feature, behavioral phenotypes can again differ significantly between patients, and some mutations are inherited from apparently normal fathers (Varga et al., 2009). The incidence of *PTEN de novo* mutations can be estimated at 4.7% (6/126) among macrocephalic/macrosomic ASD patients, who in turn are approximately 20% of all ASD patients (Lintas and Persico, 2009). The percentage of PTEN mutation carriers may be even higher in selected clinical populations (Butler et al.,

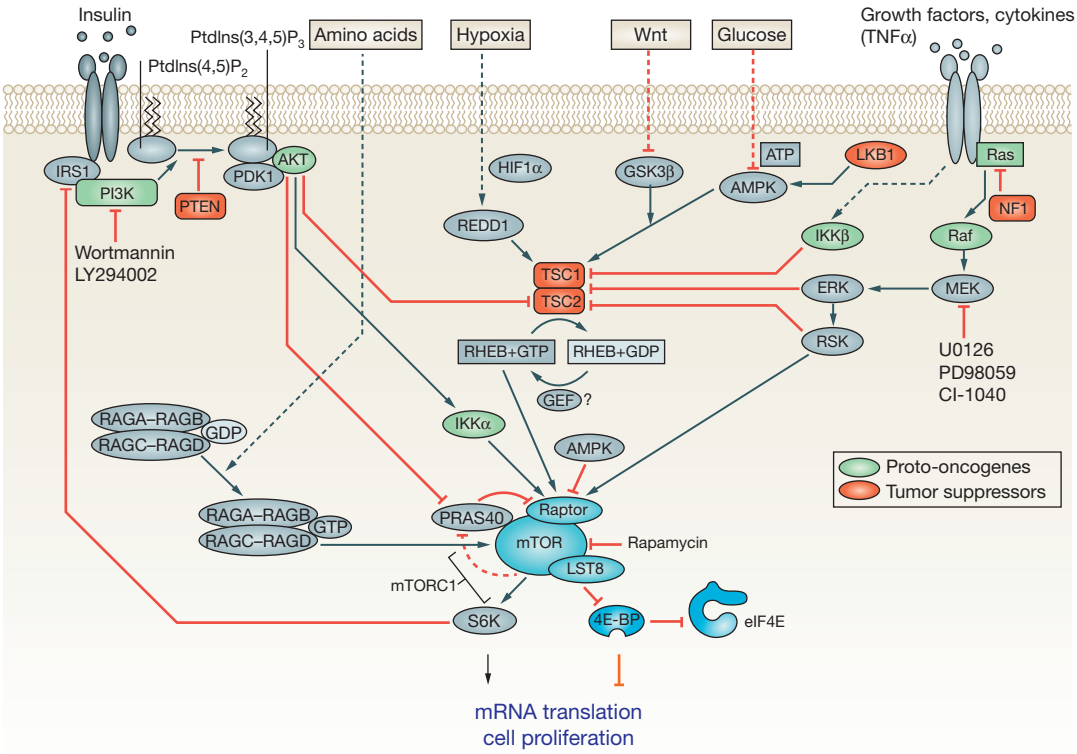

FIGURE 34.1 The ERK/PI3K/mTOR pathway (with permission from Ma and Blenis (2009), modified).

2005; Varga et al., 2009). This high genetic yield and the predisposition toward malignancies underscore the importance of screening for *PTEN* mutations in macrocephalic/macrosomic autistic children (Herman et al., 2007a,b; Lintas and Persico, 2009; McBride et al., 2010; Schaefer and Mendelson, 2008; Varga et al., 2009).

34.3.4.3 *The Eukaryotic Translation Initiation Factor 4E Gene (EIF4E)*

The *EIF4E* gene, located on human chromosome 4q21–q25, encodes the rate-limiting component of eukaryotic translation initiation and the downstream effector in the mTOR pathway (Figure 34.1). Recently, a balanced translocation disrupting the *EIF4E* locus was found in an autistic boy with loss of initial language and social interactions at 2 years of age (Neves-Pereira et al., 2009) (Table 34.6). In the same study, both affected children of 2/120 multiplex families were found to inherit a C insertion from an apparently unaffected father, extending to eight a stretch of seven cytosines located in the basal promoter element of the *EIF4E* gene (4-EBE) (Neves-Pereira et al., 2009). The C_8-4EBE allele seemingly binds with much higher affinity an abundant nuclear protein (probably hnRNPK), resulting in a twofold increase in gene expression compared to the C_7-4EBE allele (Neves-Pereira et al., 2009). Interestingly, despite carrying the same paternally inherited C_8-4EBE allele, only one of these four autistic children underwent behavioral regression, as had occurred to the proband carrying the *de novo* translocation (Neves-Pereira et al., 2009).

34.3.5 Calcium-Related Genes

Many lines of evidence indicate that excessive Ca^{2+} signaling is pivotal in the pathophysiological processes leading to autism, as reviewed by Krey and Dolmetsch (2007). Excessive intracellular Ca^{2+} spikes can modulate the aspartate/glutamate mitochondrial carrier AGC1, leading to abnormal energy metabolism and enhanced oxidative stress (for review, see Palmieri and Persico, 2010). Rare gain-of-function mutations causing autism or multisystem disorders encompassing autistic behaviors have been detected in genes encoding ion channels either directly conducting Ca^{2+} or indirectly prolonging the opening time of voltage-gated Ca^{2+} channels.

34.3.5.1 *The Ion Channel-encoding Genes* CACNA1C, CACNA1F, CACNA1H, BKCa, *and* SCN2A

Gain-of-function mutations in the gene encoding for the L-type voltage-gated Ca^{2+} channel $Ca_v1.2$ (*CACNA1C*) cause Timothy syndrome, a multisystem disorder characterized by lethal arrhythmias, webbing of fingers and toes, congenital heart disease, immune deficiency, intermittent hypoglycemia, autism, and mental retardation (Splawski et al., 2004). Similarly, mutations

in the L-type voltage-gated Ca^{2+} channel $Ca_v1.4$ (*CACNA1F*) cause an incomplete form of X-linked congenital stationary night blindness (CSNB2), frequently accompanied by cognitive impairment and autism or epilepsy, but only with gain-of-function and never with loss-of-function *CACNA1F* mutations (Hemara-Wahanui et al., 2005; Hope et al., 2005). Surprisingly, *CACNA1F* is not expressed in the CNS, except for the pineal gland (Hemara-Wahanui et al., 2005). In general, these gain-of-function mutations reduce or block voltage-dependent channel inactivation, resulting in excessive Ca^{2+} influx (Hemara-Wahanui et al., 2005; Splawski et al., 2004). Also, mutations and chromosomal abnormalities indirectly boosting cytosolic Ca^{2+} levels or amplifying intracellular Ca^{2+} signaling by hampering Ca^{2+}-activated negative feedback mechanisms have been found associated with autism. An autistic boy was found to carry a balanced translocation disrupting one copy of the *KCNMA1* gene, which encodes the α subunit of the large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel: the inactivation of one copy of this gene results in a more depolarized resting membrane potential and in a relatively less efficient control of neuronal excitability (Laumonnier et al., 2006). The R1902C variant located in the *SCN2A* gene decreases the affinity of this voltage-gated sodium channel for Ca^{2+}-bound calmodulin, which would stabilize the inactivation gate and minimize sustained channel activity during depolarization (Kim et al., 2004; Weiss et al., 2003). The situation is less clear with mutations affecting the *CACNA1H* gene, which encodes for the T-type voltage-gated Ca^{2+} channel $Ca_v3.2$ (Splawski et al., 2006). These mutations are found in ASD families and not in controls, but do not fully segregate with an autistic phenotype; furthermore, mutant channels require greater depolarizations to activate conductance and overall conduct substantially less than wild-type channels (Splawski et al., 2006). However, in the case of these autism-predisposing mutations, more depolarized potentials are also necessary to produce channel inactivation, indicating that longer-lasting calcium influx will be generated by small perturbations from the resting membrane potential (Splawski et al., 2006). Therefore, studies of patients carrying mutations in calcium-related genes collectively support excessive Ca^{2+} signaling as a critical player in the pathophysiological processes leading to autism.

34.4 NONSYNDROMIC AUTISMS: THE ROLE OF COMMON VARIANTS

34.4.1 General Description

Functional polymorphisms widely distributed in the general population can indeed confer vulnerability or protection in complex disorders, such as autism. Conceivably, a host of unfavorable common variants could even cause a disease phenotype, either directly or by lowering the sensitivity threshold to widespread environmental agents. Common genetic variants are typically sought by applying a candidate gene or, more recently, a genome-wide association approach. Although several common variants have been found associated with autism, as reviewed in detail elsewhere (Abrahams and Geschwind, 2008; Freitag, 2007; Muhle et al., 2004; Persico and Bourgeron, 2006), the evidence from independent replications and from functional studies is not equally strong for all of them (see Tables 2 and 4 in Abrahams and Geschwind, 2008). We shall now briefly summarize the results and pathophysiological bases concerning some of the most consistently replicated genes.

34.4.2 Reelin (RELN)

The *RELN* gene encodes for reelin, a critical stop signal for migrating neurons in several CNS districts, including the neocortex, the cerebellum, and the hindbrain (Herz and Chen, 2006; Rice and Curran, 2001). *RELN* maps to human chromosome 7q22, in a region linked with autism in several studies (Muhle et al., 2004; Persico and Bourgeron, 2006). Reelin acts by binding to a variety of receptors, including the VLDL receptors, APOE-R2, and α3β1 integrins, and by exerting a proteolytic activity on extracellular matrix proteins (D'Arcangelo et al., 1999; Hiesberger et al., 1999; Quattrocchi et al., 2002). *Reeler* mice lack reelin protein due to spontaneous deletions of the *RELN* gene (D'Arcangelo et al., 1995). Their brains display major cytoarchitectonic abnormalities (Goffinet, 1984), the distribution of which largely overlaps with regions of altered neuronal migration in autistic brains, as reviewed by Persico and Bourgeron (2006). Importantly, post-mortem studies have documented reductions in *RELN* and *DAB1* gene expression, as well as elevations in *VLDLR* mRNA, in the cerebral and cerebellar cortex of autistic individuals compared to controls (Fatemi et al., 2005; Lintas and Persico, 2010). Similar reductions have been found *in vivo* when measuring reelin plasma levels in ASD patients compared to controls (Fatemi et al., 2002; Lugli et al., 2003).

RELN gene mutations resulting in a lack of reelin protein yield the Norman–Roberts syndrome, a severe autosomal recessive disease characterized by lissencephaly and cerebellar hypoplasia, with severe mental retardation, abnormal neuromuscular connectivity, and congenital lymphoedema (Hong et al., 2000). *RELN* gene variants with a less dramatic functional impact have been found to confer liability to neuropsychiatric disorders, such as autism and schizophrenia (for review, see Lintas and Persico, 2008). Genetic association studies on *RELN* gene variants and autism are listed

TABLE 34.7 Genetic Association Studies on *RELN* Gene Variants and Autism

References	Polymorphisms	Experimental design	Race and ethnicity	Outcome
Persico et al. (2001)	5′UTR: GGC repeat Intron 5: rs607755 Exon 50: rs2229864	Case–control Family-based	Italians; U.S.-Caucasians	Association with GGC repeat and with haplotypes formed by GGC + rs607755 + rs2229864
Zhang et al. (2002)	5′UTR: GGC repeat	Case–control Family-based	Not specified (families from Canada and AGRE)	Association with GGC repeat
Krebs et al. (2002)	5′UTR: GGC repeat	Family-based	Mostly (94%) EU-Caucasians	No association with GGC repeat
Bonora et al. (2003)	5′UTR: GGC repeat intron 5: rs607755 exon 22: rs362691 intron 31: rs362726 exon 50: rs2229864	Family-based	EU-Caucasians: IMGSAC and German families	No association with any common variant; rare missense variants are present (see Table 34.1)
Li et al. (2004)	5′UTR: GGC repeat	Family-based	Not specified	No association with GGC repeat
Devlin et al. (2004)	5′UTR: GGC repeat	Family-based	Not specified (NIH CPEA families)	No association with GGC repeat
Skaar et al. (2005)	5′UTR: GGC repeat Intron 5: rs607755 exon 44: rs2075043 exon 45: rs362746 exon 50: rs2229864 intron 59: rs736707	Family-based	U.S.-Caucasians from Duke Univ., AGRE, and Tufts University	Association with GGC triplet and with specific haplotypes
Serajee et al. (2006)	Exon 22: rs362691[a] intron 59: rs736707[a]	Family-based	U.S.-Caucasians from AGRE	Association with rs362691 and rs736707
Dutta et al. (2007), Dutta et al. (2008)	5′UTR: GGC repeat intron 5: rs607755 intron 12: rs727531 exon 15: rs2072403 intron 15: rs2072402 exon 22: rs362691 intron 41: rs362719 exon 50: rs2229864 intron 59: rs736707	Case–control Family-based	Indian from West Bengal and Assam	No association with any common variant; possible paternal transmission of GGC 10-repeat allele
Li et al. (2008)	Intron 59: rs736707[b]	Case–control	Eastern China	Association with rs736707
Kelemenova et al. (2010)	5′UTR: GGC repeat	Case–control	Slovaks	Association with GGC triplet

[a] *Only the two SNPs found associated with autism are listed here, out of 34 SNPs assessed.*
[b] *Only one SNP found associated with autism is listed here, out of 12 SNPs assessed.*

in Table 34.7. A polymorphic GGC repeat located immediately 5′ of the AUG translation initiation codon and ranging from 4 to 23 repeats in our sample (Persico et al., 2001, 2006) has been found associated with autism in several, though not all, studies. In particular, 'long' GGC alleles (i.e., >10 GGC repeats) were shown to decrease *RELN* gene expression in neuronal (SN56 and N2A) and non-neuronal (CHO) cell lines (Persico et al., 2006). These alleles are present in approximately 20% of autistic individuals, compared with 10% of population controls. Other studies have pointed toward more 3′ regions of this large gene as possibly hosting functional variants. Some studies reporting no association with the GGC triplet repeat have nonetheless found rare variants of potential interest (Bonora et al., 2003). *RELN* variants different from those involved in autism may possibly contribute to schizophrenia: the GGC variant does not seem to confer vulnerability to schizophrenia, which has instead been found to be associated with SNP rs7341475 located in intron 4 (Shifman et al., 2008). However, this SNP does not appear to influence brain structure, working memory, or *RELN* gene expression, so the functional correlates of this association remain unclear (Tost et al., 2010). Gene–gene interactions with other schizophrenia liability genes (Hall et al., 2007) and epigenetic control of *RELN* gene expression (Grayson et al., 2006; Lintas and Persico, 2010) may perhaps play more prominent roles than single functional variants in conferring vulnerability to schizophrenia, especially after puberty.

Possible gene–gene and gene–environment interactions involving *RELN* have been previously presented, especially in reference to PON1 gene polymorphisms and prenatal exposure to organophosphate pesticides (see Section 34.4 and Persico and Bourgeron, 2006). Briefly, reelin's proteolytic activity, crucial for

neuronal migration, is inhibited by organophosphates (Quattrocchi et al., 2002). Furthermore, the *PON1* gene, encoding for the organophosphate-detoxifying enzyme present in human serum, is also associated with autism and provides evidence of gene–gene interactions with *RELN* alleles (D'Amelio et al., 2005). We thus proposed a gene–gene–environment interaction model, whereby individuals carrying genetic or epigenetic variants resulting in reduced *RELN* gene expression and in less active paraoxonase isoforms, if exposed prenatally to organophosphates during critical periods in neurodevelopment, will more likely suffer from altered neuronal migration resulting in autistic disorder (Persico and Bourgeron, 2006). We have recently measured two different *PON1* enzymatic activities in the serum of 174 ASD patients, 144 controls, and 175 first-degree relatives finding significant reductions in arylesterase, but not in diazoxonase activity, primarily due to a functional inhibition of this enzymatic activity and not due to quantitative decreases in PON1 protein levels (Gaita et al., 2010). These results were unexpected, because diazoxon is one of the most widespread organophosphates in the United States, whereas decreases in arylesterase activity have so far been recorded in the presence of enhanced oxidative stress and/or immune activation, as during viral hepatitis C (Ferré et al., 2005; Kilic et al., 2005), influenza (van Lenten et al., 2001), and HIV infections (Parra et al., 2007). Recent neuroanatomical, gene expression, and brain imaging studies strongly support an abnormal activation of the immune system in autism (see Sections 34.4.5.3 and 34.6). Within this framework, *RELN–PON1* interactions may be better explained by a joint modulation of inflammatory processes, especially monocyte recruitment and migration into the CNS (Ahmed et al., 2003; Cameron and Landreth, 2010; Gaita et al., 2010; van Lenten et al., 2002).

34.4.3 The MET Protooncogene

The MET receptor tyrosine kinase, encoded by the *MET* protooncogene located in human ch 7q31, plays an important role in modulating cell proliferation and migration, as reviewed by Levitt et al. (2004) and by Levitt and Campbell (2009). Briefly, the MET receptor binds hepatocyte growth factor (HGF), which is translated as an inactive precursor and activated by proteolytic cleavage to yield the MET receptor ligand: this cleavage is achieved by the protease plasminogen activator, urokinase-type (uPA), when uPA binds to its receptor, the urokinase plasminogen activator receptor (uPAR) (see Figure 3 in Levitt et al., 2004). The cleavage-mediated activation of HGF can instead be suppressed by the plasminogen activator inhibitor-1 (PAI-1). *MET* gene variants have been found to be associated with autism in four independent studies involving at least seven distinct family samples (Campbell et al., 2006, 2008; Jackson et al., 2009; Sousa et al., 2009). The *MET* gene variant conferring autism vulnerability in the initial study was the C allele at rs1858830, located in the *MET* gene promoter (Campbell et al., 2006). This allele dramatically reduces the binding of transcription factors SP1 and PC4, thereby decreasing *MET* transcription assessed in neuronal (SN56 and N2A) and non-neuronal (HEK) cell lines using luciferase-expressing reporter constructs (Campbell et al., 2006). The association between autism and the C allele at rs1858830 was replicated by the same group in an independent sample (Campbell et al., 2008), whereas a study from the IMGSAC Consortium found autism associated with SNP rs38845, located in intron 1 of the *MET* gene (Sousa et al., 2009). A fourth study, employing only case–control contrasts, confirmed an association with the C allele at rs1858830 in a South Carolina, and not in an Italian cohort (but the latter was deeply underpowered: South Carolina sample = 174 ASD patients vs. 369 controls; Italian sample = 65 ASD patients vs. 126 controls) (Jackson et al., 2009).

Analyses of postmortem tissue from the superior temporal gyrus (Brodmann area 41/42) confirmed an approximate twofold decrease in *MET* transcript and protein expression in ASD patients compared to matched controls (Campbell et al., 2007). Moreover, significantly lower MET protein levels were found among controls carrying the C/C, as compared to the G/G genotype at rs1858830 (Campbell et al., 2007). The same tissues unveiled increased expression of the *HGF*, *PLAUR*, and *SERPINE1* genes, which encode for HGF, uPAR, and PAI-1, respectively (Campbell et al., 2007). Conceivably, decreased *MET* gene expression at the neocortical level triggers compensatory increases in the expression of other molecules belonging to the same pathway, such as *HGF*, *PLAUR*, and *SERPINE1*. The latter two genes apparently also host common genetic variants independently contributing to autism vulnerability: a *SERPINE1* haplotype and the *PLAUR* promoter T allele at rs344781 are both associated with autism (Campbell et al., 2008). Significant gene–gene interactions have also been shown for *MET* and *PLAUR* (Campbell et al., 2008).

The functional correlates of this genetic predisposition are perhaps more interesting than its behavioral correlates, which appear rather nonspecific (Campbell et al., 2010). The MET/HGF pathway is known to play an important role in the developing CNS, in the immune system, and in gastrointestinal repair, all strongly linked to the pathophysiology of autism. Blunted MET/HGF signaling negatively affects interneuron migration from the ganglionic eminence into the cerebral cortex and granule cell proliferation in the cerebellum (Ieraci et al., 2002; Levitt et al., 2004): reduced numbers of neocortical GABAergic interneurons and a reduction in

cerebellar size, especially in the vermis, may be particularly relevant to the elevated comorbidity between autism and epilepsy, as well as to brain imaging findings of reduced cerebellar vermis size in ASD patients (Courchesne, 1997). The same also occurs in zebrafish, where *MET* is critically involved in cerebellar development and, interestingly, in the migration of cells forming the facial motor nucleus (Elsen et al., 2009; also see Section 34.4.2, Rodier et al. (1996), and Rodier (2002)). In the mouse, *MET* expression is especially pronounced in cortical projection neurons between P7 and P14, when long-range cortical connections are wired through neuronal sprouting and active synaptogenesis (Judson et al., 2009). Emx1-Cre-driven deletion of *MET* in dorsal pallially derived forebrain neurons affects dendritic development both in pyramidal cells (decreased apical and increased basal dendritic harbor length) and in medium spiny neurons (increased dendritic harbor length), the latter postsynaptic to *MET*-expressing corticostriatal afferents during development. The number of dendritic spines is unchanged, but spine head volume is significantly enlarged (Judson et al., 2010). These same animals show a twofold stronger connectivity between cortical layers 2/3 and corticostriatal, but not corticopontine, layer 5 pyramidal neurons (Qiu et al., 2011). Although human genetic variants modulate *MET* gene expression to a moderate extent, compared to these experimental manipulations, these studies clearly implicate excessive local and decreased long-range connectivity at the neocortical level as the most likely mechanism underlying the ASD risk conferred by *MET* gene alleles.

Additional evidence converging on the MET/HGF pathway also comes from *Plaur*-deficient mice, which show disrupted forebrain interneuron development, increased susceptibility to seizures, anxiety, and abnormal social behavior (Levitt et al., 2004). These same *Plaur* knockout mice also display severely impaired granulocyte and monocyte migration toward inflammatory foci (Allgayer, 2006). The MET/HGF pathway is indeed known to play both 'pro-inflammatory' roles (stimulating leukocyte adhesion and migration, migration of dendritic cells, antagonizing the effects of TGF-β) and anti-inflammatory roles (suppression of the antigen-presenting function of dendritic cells; blunting of eosinophila and airway hyperresponsiveness in animal models of asthma) (Beilman et al., 2000; Okunishi et al., 2005). Finally, the C allele at rs1858830 has been found to be associated primarily with autistic syndromes encompassing gastrointestinal symptoms (Campbell et al., 2009), which are frequently encountered in autistic patients (Buie et al., 2010). Autistic children with gastrointestinal symptoms may also display decreased serum levels of HGF (Russo et al., 2009; Sugihara et al., 2007), again pointing toward the translation of impaired MET/HGF signaling into inefficient gastrointestinal repair mechanisms.

Collectively, current evidence points toward multiple common gene variants promoting a dysregulation of the MET/HGF pathway, which represents a significant contributor to neurodevelopmental, immune, and gastrointestinal abnormalities in autism.

34.4.4 The Oxytocin Receptor Gene

The oxytocin receptor gene (OXTR) is a high-affinity G-protein-coupled receptor encoded by the *OXTR* gene located on human ch 3p26.2. It binds oxytocin (OXT), a nine-amino-acid neurohypophyseal hormone encoded by the *OXT* gene, which also encodes for neurophysin I and is located on human ch 20p13. This hormone and neuromodulator, largely distributed in limbic areas such as the nucleus accumbens and the amygdala, in addition to well-established roles in parturition and breast feeding, physiologically influences social cognition in a relatively species- and sex-specific manner (for review, see Carter, 2007; Carter et al., 2008). OXT or OXTR knockout mice display impaired social memory, while parturition is largely unaffected (Ferguson et al., 2000; Takayanagi et al., 2005). Interestingly, only male mice with a targeted forebrain OXTR knockout fail to recognize individuals of their own species, suggesting the existence of compensatory mechanisms in females (Sun et al., 2008). Heterozygous *reeler* mice display reduced neocortical *OXTR* gene expression, suggesting an intriguing crosstalk between the RELN and OXT pathways (Liu et al., 2005). Several human studies employing an intranasal administration paradigm demonstrate that OXT stimulates affiliative behaviors, subjective feelings of trustworthiness, facial recognition, and in general all social cognitive functions evolutionarily involved in the establishment of a strong emotional bond between parents and neonate (Ebstein et al., 2009).

Genetic studies of the OXT and arginine vasopressin (AVP) systems were undertaken under the assumption that a disruption of these hormonal/neurochemical systems could underlie the deficits in social cognition which characterize ASD patients (for review, see Ebstein et al., 2009). Indeed, at least six out of eight genetic studies performed to date have reported a positive association between ASD and the *OXTR* gene (Table 34.8). This consistency is surprising, especially when considering that many studies were severely underpowered. Single marker and haplotype association analyses primarily point toward a haplotype block encompassing exon 3 and the beginning of intron 3 as possibly hosting a functional variant conferring autism liability, although some evidence also points toward the 3′UTR of the gene encoded by exon 4 (Table 34.8). Findings on the *OXT* gene have been less consistent, if not entirely negative. Some studies have reported positive findings for the AVP receptor gene *AVP1a* (Kim et al.,

TABLE 34.8 Genetic Association Studies on *OXTR* Gene Variants and Autism

References	Polymorphisms	Experimental design	Race and ethnicity	Outcome
Wu et al. (2005)	Eight SNPs	Case–control Family-based	Chinese Han	Association with rs2254298 and rs53576, and with haplotypes involving rs53576
Jacob et al. (2007)	Intron 3: rs2254298 Intron 3: rs53576	Family-based	Caucasian-Americans	Association with rs2254298
Lerer et al. (2008)	16 SNPs	Family-based	Israelis	Association with rs2268494 and rs1042778, and with a 5-SNP haplotype involving rs2254298 and rs2268494
Yrigollen et al. (2008)	Intron 3: rs237885, rs2268493, rs237898	Family-based	Americans (Caucasians = 93%)	Association with rs2268493
Wermter et al. (2010)	22 SNPs	Family-based	Germans	Association with rs2270465 and with a four SNP haplotype spanning the entire locus
Tansey et al. (2010)	18 SNPs	Family-based Gene expression in LCLs and amygdala	Caucasians (Irish + Portuguese + UK)	No association with ASD; three SNPs show association with gene expression
Kelemenova et al. (2010)	Exon 3: rs2228485	Case–control	Slovaks	No association with ASD
Liu et al. (2010)	11 SNPs	Family-based Case–control	Japanese	Association with rs2254298 (case–control only), and with a 5-SNP haplotype involving rs2254298

2002; Wassink et al., 2004; Yirmiya et al., 2006) and for the gene encoding CD38, a transmembrane glycoprotein mainly expressed in immune cells (NK, T, B cells and macrophages) and responsible for triggering the release of OXT in neurons (Jin et al., 2007; Munesue et al., 2010). Interestingly, another study (Gregory et al., 2009) described a genomic deletion encompassing the *OXTR* gene in an autistic proband belonging to a multiplex family and in his mother, who displayed relevant obsessive–compulsive traits. The other affected sibling did not carry the deletion, but instead his *OXTR* promoter was hypermethylated at CpG islands located at −934, −924, and −901 bp from the translation start site. Hypermethylation of the *OXTR* promoter at several CpG islands located between −860 and −959 was also demonstrated in genomic DNA extracted from leukocytes and from post-mortem brain tissue of autistic patients, compared to matched controls (Gregory et al., 2009). As predicted, enhanced methylation was correlated with decreased *OXTR* transcript levels in temporocortical post-mortem brain tissue (Gregory et al., 2009). Hence, predisposition to autism can be apparently conferred by the *OXTR* locus through distinct genomic, genetic, and epigenetic mechanisms, all resulting in hampered OXT signaling (Gurrieri and Neri, 2009). Abnormal neuropeptide processing in autistic children, yielding reduced OXT blood levels despite enhanced concentrations of OXT precursor (Green et al., 2001; Modahl et al., 1998), may further exacerbate this deficit, bringing OXT signaling below a critical threshold necessary for the physiological development of social behavior. Based on the influence of *OXTR* gene variants on amygdalar volume, which is bilaterally smaller in healthy adults carrying the G allele at rs2254298 (Furman et al., 2011; Inoue et al., 2010), blunted OXT signaling can be predicted to have a negative impact on the development and function of specific cortical and limbic regions critical to social cognition.

34.4.5 The Contacting-Associated Protein-Like 2 Gene (CNTNAP2)

The *CNTNAP2* gene, located on human ch 7q35–q36, encodes for the contacting-associated protein (CASPR2), a member of the Neurexin family which also includes Neurexin1 (see Section 34.3.2.3). This locus was originally identified by two groups applying linkage analysis both on affection status and on language delay, used as a quantitative trait locus (QTL) (Alarcón et al., 2008; Arking et al., 2008). Follow-up association analyses carried out on potential candidate genes supported *CNTNAP2* as being solely responsible for the linkage peak (Alarcón et al., 2008; Arking et al., 2008), although negative association findings were also published (Li et al., 2010). The *CNTNAP2* allele appears to confer autism vulnerability primarily in males (Alarcón et al., 2008), and possibly if

inherited from the maternal side (Arking et al., 2008). *CNTNAP2* was shown to be highly expressed in frontal and temporal regions of the human fetus, as well as striatum, amygdala, and thalamus, all areas strongly involved in linguistic functions and emotional information processing (Abrahams et al., 2007; Alarcón et al., 2008). T1-weighted anatomical MRI scans performed in 314 healthy volunteers revealed that the autism-associated allele seemingly yields reduced gray and white matter volumes and fractional anisotropy, following sex-specific distributions involving several autism-related brain regions, such as frontal cortex, fusiform gyrus, and cerebellum (Tan et al., 2010). Further analyses by fMRI revealed that carriers of the *CNTNAP2* risk allele have widespread and bilateral connectivity distributed throughout the frontal cortex and anterior temporal poles, whereas the protective allele is associated with a left-lateralized network composed of left inferior frontal gyrus, insula, anterior temporal pole, superior temporal gyrus, and angular gyrus (Scott-Van Zeeland et al., 2010). The latter results point toward *CNTNAP2* alleles as conferring autism vulnerability by affecting the lateralization and possibly the extent of long-range connectivity. At the cellular level, the *Drosophila* orthologs of *CASPR2* and *NRXN1* have been shown to colocalize partly at synaptic active sites, and overexpression of either gene increases the density of active zones and modulates the shape of synapses (Zweier et al., 2009). These 'synaptic' roles for *CNTNAP2*, especially if applied to long-range neural pathways connecting language-related cortical regions, would indeed fit with the converging evidence on synaptic roles summarized here for several other genes (see Section 34.3.2).

Multiple lines of evidence point toward the relative nonspecificity of many 'autism' genes, which may play cognitive roles that, if deranged, can translate into different human disorders: this seems to apply even more to *CNTNAP2*. Several rare genetic variants and *de novo* cytogenetic abnormalities in *CNTNAP2* have been described in autistic probands, which oftentimes present also with seizures and regression (Bakkaloglu et al., 2008; Jackman et al., 2009; Poot et al., 2010; Rossi et al., 2008). However, *CNTNAP2* was also identified by genome-wide CNV analysis as relevant to the development of idiopathic generalized and focal epilepsies (Mefford et al., 2010). Further evidence linking *CNTNAP2* perhaps more directly with language development than with autism *per se* comes from the functional connection between *CNTNAP2* and *FOXP2*, a transcription factor critically involved in the development of expressive language (Lai et al., 2001): *FOXP2* binds to the promoter of *CNTNAP2* and dramatically downregulates its expression (Vernes et al., 2008). *CNTNAP2* gene variants have also been found to be associated with specific language impairment (Vernes et al., 2008). Moreover, common *CNTNAP2* variants have been found to confer vulnerability to schizophrenia and bipolar disorder (O'Dushlaine et al., 2010), whereas rare variants have been described in ADHD patients (Elia et al., 2010). Meanwhile, although several rare variants present in autistic probands were not found in large numbers of control chromosomes, the vast majority are inherited from an unaffected parent and many of them are transmitted to some, but not all, affected siblings in multiplex families, suggesting that they may enhance autism risk, but are not sufficient to cause the disease (Bakkaloglu et al., 2008). Hence, *CNTNAP2* may play a broader role in shaping the autistic phenotype toward language deficit and possibly epilepsy, rather than strictly conferring autism vulnerability.

34.4.6 The Engrailed 2 Gene

The Engrailed genes play an important role in the patterning of the midbrain/hindbrain region, the only CNS area where they are actively expressed during development (Davis et al., 1998). In particular, engrailed 2 (*EN2*) is expressed in cerebellum, pons, periaqueductal gray, and colliculi. *EN2* knockout mice display decreased seizure threshold to kainic acid (Tripathi et al., 2009) and a relatively subtle behavioral phenotype, with abnormalities in developmental motor, social, and memory tasks (Cheh et al., 2006). Their cerebellar size is reduced and their compartmentalization is interestingly disrupted both in the vermis and in cerebellar hemispheres (Kuemerle et al., 1997; Millen et al., 1994, 1995). In addition, 5-HT and 5-hydroxy-indolacetic acid (5-HIAA) levels are doubled in the cerebellum only (Cheh et al., 2006). The role of *EN2* in cerebellar development and the frequency of cerebellar abnormalities reported in neuroanatomical and brain imaging studies of ASD patients (Courchesne, 1997) spurred interest in genetic studies of *EN2* as early as 1995, when a significant association with autism was first reported by Petit et al. Nine studies followed this initial report, and at least seven of them replicated the initial association in various racial and ethnic groups (Table 34.9). The *EN2* gene, located in human chromosome 7q36.3, encompasses two exons and one intron. The most replicated association was found with the A–C haplotype at SNPs rs1861972 and rs1861973, embedded into intron 1. Following transfection with constructs encompassing the luciferase reporter gene, this haplotype consistently yields approximately 20% higher expression levels in neuronal PC12 cells, non-neuronal HEK293T cells, and in primary cultures of mouse cerebellar granule cells harvested on postnatal day 6 (P6) (Benayed et al., 2009). This difference in gene expression is not due to cryptic splicing, but rather to allele-specific transcription factor binding. Increased expression of *EN2* can be predicted to result in faster differentiation of the midbrain/hindbrain

region and of cerebellar circuits (Benayed et al., 2009). This should occur at the expense of the proliferating and migrating pools, thus yielding decreased Purkinje cell numbers and cytoarchitectonic abnormalities in the cerebellar cortex.

34.4.7 Gamma-Aminobutyric Acid Receptor β3 (GABRB3)

Several lines of investigation indicate the existence of abnormalities in the brain gamma-aminobutyric acid (GABA) system of autistic children. The frequent comorbidity with epilepsy and the morphogenetic roles of GABA, an inhibitory neurotransmitter in adult brain but an excitatory neurotransmitter during prenatal neurodevelopment due to high intracellular chloride concentrations in immature neurons (see review by Jentsch et al., 2002), have spurred interest into GABA receptor (GABAR) subunit genes as potential candidates for autism (Blatt et al., 2001; Hussman, 2001). In addition, the 15q11–q13 region deleted/duplicated in 1–4% of autistic patients (see Tables 34.3 and 34.5) (McCauley et al., 2004; Schroer et al., 1998) encompasses the GABAA receptor gene cluster, which consists of three GABAR genes, namely *GABRB3*, *GABRA5*, and *GABRG3*. Investigations of these genes have provided some support especially to *GABRB3*. A significant association between autism and markers located within or nearby *GABRB3* has been found in most studies (Buxbaum et al., 2002; Cook et al., 1998; Curran et al., 2005; Kim et al., 2006; Martin et al., 2000; McCauley et al., 2004; Yoo et al., 2009), although negative reports have also appeared (Ma et al., 2005; Maestrini et al., 1999; Menold et al., 2001; Salmon et al., 1999; Tochigi et al., 2007). Some have proposed that behavioral traits, such as savant skills and insistence on sameness, may be especially linked to genes located in the 15q11–q13 region (Nurmi et al., 2003; Shao et al., 2003). Maternal transmission of a *GABRB3* signal peptide variant (P11S), previously implicated in childhood absence epilepsy, is associated with autism (Delahanty et al., 2011). This rare variant, present in 17 (1.47%) of

TABLE 34.9 Genetic Association Studies on *EN2* Gene Variants and Autism

References	Polymorphisms	Experimental design	Race and ethnicity	Outcome
Petit et al. (1995)	Two RFLPs (MP4 and MP5 probes)	Case–control	French (all Caucasians)	Association with the MP4 probe and PvuII
Zhong et al. (2003)	Exon 1: rs3735653	Family-based	AGRE families (race not specified)	No association with rs3735653
Gharani et al. (2004)	Exon 1: rs3735653 intron 1: rs1861972 intron 1: rs1861973 exon 2: rs2361689	Family-based	Caucasian-Americans	Association with rs1861972 and rs1861973
Benayed et al. (2005)	Intron 1: rs1861972, intron 1: rs1861973, PvuII/MP4, and 13 other SNPs	Family-based	Two samples: AGRE and NIMH (race not specified)	Association with rs1861972 and rs1861973 (intronic haplotype)
Brune et al. (2008)	Intron 1: rs1861972	Family-based	Not specified (NIH CPEA families)	Association only with broad autism and under a recessive model
Wang et al. (2008)	Eight SNPs	Family-based	Chinese Han	Association with haplotypes involving rs3824068 (intron 1)
Yang et al. (2008)	Intron 1: rs1861972, intron 1: rs1861973	Case–control	Chinese Han	Association with single markers; 'protective' haplotype
Benayed et al. (2009)	16 SNPs	Family-based	Three samples: AGRE I, AGRE II, NIMH (Caucasian non-Hispanic subset for association)	Maximum association with rs1861972 and rs1861973 (intron 1); AC haplotype yields increased expression
Sen et al. (2010)	Exon 1: rs3735653 promoter: rs34808376 promoter: rs6150410 intron 1: rs1861972, intron 1: rs1861973	Family-based	Indian from West Bengal and Assam	Association with rs1861973
Yang et al. (2010)	Five SNPs in intron 1: rs3824068, rs3824067, rs1861972, rs1861973 and rs3830031	Case–control	Chinese Han	Association with the A–C haplotype at rs1861972 and rs1861973 (intron 1)

1152 simplex and multiplex families, yields reduced whole-cell current and decreased β3 subunit protein on the cell surface due to impaired intracellular β3 subunit processing (Delahanty et al., 2011). *GABRB3* gene variants must also be viewed within the framework of the entire set of GABAR-encoding genes, as several gene–gene interactions between them have been detected (Ashley-Koch et al., 2006; Ma et al., 2005).

In addition to genetic variants, epigenetic dysregulation of the *GABRB3* locus may also contribute to autism. *GABRB3* expression is reduced on average by as much as 50% in several neocortical and cerebellar regions (Fatemi et al., 2009). Interestingly, a sizable subset of ASD brains displays either monoallelic or abnormally downregulated *GABRB3* expression instead of the normal levels of biallelic expression present in controls (Hogart et al., 2007, 2009). Interestingly, GABRB3-deficient mice exhibit impaired social and exploratory behaviors, deficits in nonselective attention, and hypoplasia of the cerebellar vermis, all features relevant to autism (DeLorey et al., 2008). In addition, mice deficient in MeCP2 display reductions in GABRB3 protein, as MeCP2 acts as a positive regulator of *GABRB3* gene expression (Samaco et al., 2005). Collectively, these results suggest the existence of genetic and epigenetic influences leading to a behaviorally relevant downregulation of *GABRB3* in autistic brains.

34.4.8 The Serotonin Transporter (SLC6A4) and Integrin β3 Subunit Genes

Elevated whole-blood serotonin (5-HT), present in about one-third of ASD patients, represents one of the most consistent biological endophenotypes in autism research (Table 34.1). Hyperserotoninemia appears to be a genetically determined familial trait, as first-degree relatives display mean 5-HT blood levels intermediate between those of their autistic family members and of population controls (Abramson et al., 1989; Cook et al., 1990; Leventhal et al., 1990; Piven et al., 1991). In most patients, elevated 5-HT blood levels in autism seemingly stem from accumulation of 5-HT in platelets due to increased densities of functionally active serotonin transporter (5-HTT) molecules on platelet membranes, with no change in 5-HTT affinity for 5-HT and no elevation in free 5-HT plasma level (Cook et al., 1988; Katsui et al., 1986; Marazziti et al., 2000). Autism-associated hyperserotonemia has been the object of intense investigation, because either it could play a role in the etiological processes leading to the disease, or it could at least mark a relatively homogeneous subgroup of ASD patients. Genes encoding proteins involved in 5-HT metabolism and neurotransmission include, among others, the 5-HT transporter gene (*SLC6A4*) and the integrin β3 subunit gene (*ITGB3*). The serotonin transporter (5-HTT) responsible for platelet 5-HT uptake is identical in its primary sequence to the 5-HTT expressed in serotonergic neurons: both are indeed produced by a single *SLC6A4* gene, located on chromosome 17q12 (Lesch et al., 1993). In reference to functional common variants, this gene contains two variable number tandem repeats (VNTRs) affecting expression levels: (a) the 5-HTT gene-linked polymorphic region (5-HTTLPR), located in the promoter, encompasses a 'long' 16-repeat allele, yielding approximately 50% higher 5-HTT gene expression and tritiated 5-HT uptake compared with homozygosity for the 'short' 14-repeat allele or with heterozygosity (Lesch et al., 1996); (b) the serotonin transporter intron 2 (STin2) VNTR includes 9, 10, and 12 repeat alleles, with the latter acting as a transcriptional enhancer (MacKenzie and Quinn, 1999). Overall, meta-analyses of association studies between autism and these two VNTRs have been negative, although there may be some association with the 5-HTTLPR 'short' allele in North-American families only (see review Huang and Santangelo, 2008). Furthermore, contributions of these VNTRs to enhanced 5-HT blood levels appear marginal at best (Anderson et al., 2002; Betancur et al., 2002; Coutinho et al., 2004, 2007a,b; Cross et al., 2008; Persico et al., 2002). Hence, immune factors, such as TNFα and other proinflammatory cytokines, which are known to activate 5-HTT transport activity (Zhu et al., 2006), as well as common variants in other genes, such as *ITGB3* (see below), may influence 5-HT blood levels to a larger extent.

In addition to these two common VNTRs, several rare *SLC6A4* variants have been identified as significantly enhancing autism risk. In particular, four coding substitutions located at highly conserved positions and 15 other variants located in 5′ noncoding and other intronic regions are transmitted to autistic probands exhibiting rigid-compulsive behaviors (Sutcliffe et al., 2005). Two of these variants, Phe465Leu and Leu550Val, confer elevated 5-HTT surface density (V_{max}), while retaining a capacity for acute protein kinase G (PKG) and p38 mitogen-activated protein kinase (MAPK) regulation; five other variants (Thr4Ala, Gly56Ala, Glu215Lys, Lys605Asn, and Pro612Ser) demonstrate no change in V_{max}, but show a complete loss of 5-HT uptake stimulation after acute PKG and p38 MAPK activation (Prasad et al., 2005). Finally, two other variants, Gly56Ala and Ile425Leu, show markedly reduced 5-HTT association with protein phosphatase 2A (PP2A), leading to profound and long-lasting 5-HTT internalization following phosphorylation by PKC (Prasad et al., 2005, 2009). When expressed stably in CHO cells, both Gly56Ala and Ile425Leu display a striking loss of 5-HTT protein following catalytic activation (Prasad et al., 2009). Since the Gly56Ala variant is less rare than the other variants, it will be interesting to see the results of studies contrasting wild-type Gly56 versus mutated Ala56 mice (Veenstra-Vanderweele et al., 2009). Collectively, despite

showing a complex array of effects, rare *SLC6A4* variants conferring autism vulnerability (a) display enhanced 5-HT transport activity, presumably leading to decreased extracellular 5-HT levels and/or shorter exposure of 5-HT receptors to their ligand, or (b) lose the plastic regulation normally mediated by intracellular kinases and able to adapt 5-HTT activity to the functional needs of local circuits (Prasad et al., 2009).

The *ITGB3* gene was first identified as a QTL for 5-HT blood levels, initially in the Hutterites (Weiss et al., 2004, 2005a) and then in the general population (Weiss et al., 2005b). *ITGB3* maps in ch 17q21.32, under a replicated linkage peak for autism (Cantor et al., 2005; Stone et al., 2004), and *ITGB3* alleles, either alone or in interaction with *SLC6A4*, have been found at least nominally associated with autism in all five studies performed to date (Coutinho et al., 2007a,b; Ma et al., 2010; Mei et al., 2007; Weiss et al., 2006a,b). Several lines of evidence support functional interactions between *ITGB3* and *SLC6A4*: (a) the integrin receptor composed of an αIIb subunit and of the *ITGB3*-encoded β3 subunit was recently identified as a novel component of the *SLC6A4* regulatory protein complex (Carneiro et al., 2008; Weiss et al., 2006a); (b) the *ITGB3* SNP rs5918 (Leu33Pro) modulates *SLC6A4* trafficking and transport activity (Carneiro et al., 2008); (c) *ITGB3* and *SLC6A4* gene expression levels are correlated in human and mouse tissues (Weiss et al., 2006a); and finally, (d) several published studies have described significant *SLC6A4* and *ITGB3* interactions for both autism risk and 5-HT blood levels, with a male-specific effect (Coutinho et al., 2007a,b; Ma et al., 2010; Mei et al., 2007; Weiss et al., 2004, 2005b, 2006a,b). Recent results from our laboratory point toward the existence of at least two distinct functional genetic *ITGB3* variants, located at opposite ends of the *ITGB3* gene: the 5′ variants significantly influence 5-HT blood levels in ours and in other studies (Weiss et al., 2006b), whereas autism-associated SNPs cluster toward the 3′ end of the gene (Napolioni et al., 2011). Interestingly, these results closely resemble the association patterns previously reported for asthma and wheezing versus allergies and IgE levels, also associated with 5′ and 3′ markers, respectively (Thompson et al., 2007; Weiss et al., 2005c).

Finally, mice carrying a combined haploinsufficiency at *PTEN* and *SLC6A4* (PTEN+/−;SLC6A4+/−) develop larger head sizes and greater sex-specific behavioral abnormalities, compared to PTEN+/−;SLC6A4+/+ or PTEN+/+;SLC6A4+/− mice (Page et al., 2009). This influence of 5-HT on the penetrance of other morphogenetic and neurodevelopmental genes is not entirely surprising in view of the many neurotrophic roles exerted by 5-HT during development (for review, see Di Pino et al., 2004; Persico, 2009). Yet, this study underscores the urgent need to move from assessments of 5-HTT and ITGB3 contributions to the 'presence/absence' of autism, toward less reductionist and more biologically meaningful approaches, addressing 5-HT modulation of the disease phenotype in the context of gene–gene interactions.

34.5 NONSYNDROMIC AUTISMS: ENVIRONMENTAL FORMS

34.5.1 General Description

Certain environmental factors have been shown to enhance the risk of developing autism significantly, to the point that at least in some patients they can be regarded as 'the primary cause' of the disease (for review, see Landrigan, 2010). These environmental agents include teratogenic drugs, namely valproic acid, misoprostol, and thalidomide, as well as prenatal rubella and cytomegalovirus (CMV) infections. Cases conclusively shown to derive from exposure to these environmental agents are relatively rare, and the role of genetic vulnerability conferred by common variants at the individual level cannot be overlooked. Nonetheless, these cases possess high heuristic potential, as the time window of exposure is usually known and often narrow, typically occurring during early prenatal neurodevelopment.

34.5.2 The Fetal Anticonvulsant Syndrome

Prenatal exposure to phenytoin, sodium valproate, and carbamazepine, either alone or in combination, causes the 'fetal anticonvulsant syndrome.' In the largest population-based study reported to date (Rasalam et al., 2005), strict diagnostic criteria for autistic disorder were met by 5/56 (8.9%) and 2/80 (2.5%) children exposed to valproate and carbamazepine alone, respectively; including also patients treated with drug combinations, cumulative percentages rise to 9/77 (11.7%) and 5/110 (4.5%) for valproate and carbamazepine, respectively. Differently from idiopathic autism, the M:F ratio here is close to 1 (Moore et al., 2000; Rasalam et al., 2005). Clinical descriptions of these children are provided both in larger cohort studies (Moore et al., 2000; Rasalam et al., 2005) and in a number of case reports (Christianson et al., 1994; Williams and Hersh, 1997; Williams et al., 2001): patients almost invariantly display speech delay, whereas motor delay is much less prevalent; cognitive impairment is typically mild or absent, and there is no history of regression or loss of skills; major malformations are sometimes present, but minor facial dysmorphology is certainly more prevalent (notice the hypertelorism, frontal bossing, and other dysmorphic features in Figures 1 and 2 of Moore et al., 2000).

Sodium valproate is believed to exert its teratogenic effects mainly by inhibiting histone deacetylase (Phiel et al., 2001): the persistent acetylation of histones and demethylation of cytosine at the promoters of neurodevelopmentally relevant genes would then lead to a dysregulation in excess of their gene expression. This pharmacological effect impinges on epigenetic mechanisms partly overlapping with those involved in *MECP2* hemizygosity, as summarized above, yielding a broad range of neurodevelopmental and behavioral abnormalities. As predicted by its pharmacological action, valproic acid increases the expression of several neurally relevant genes, such as *HoxA1* (Stodgell et al., 2006), GATA-3 (Rout and Clausen, 2009), WNT (Wiltse, 2005), and RELN and GAD67 (Dong et al., 2007). Curiously, reduced expression has been reported for *NLGN3* (Kolozsi et al., 2009) and for genes involved in the differentiation of serotoninergic neurons, including Sonic hedgehog, its receptor Patched, and the transcription factors Gli1 and Pet-1 (Miyazaki et al., 2005). The latter effect delays differentiation, resulting in enhanced growth and abnormal distribution of serotoninergic pathways (Miyazaki et al., 2005; Tsujino et al., 2007). Neuroanatomical anomalies primarily include abnormal cerebellar cytoarchitectonics and brainstem nuclei formation (Ingram et al., 2000a; Rodier, 2002; Rodier et al., 1996). Particularly reminiscent of human autism are the local hyperconnectivity present in the neocortex and the abnormal morphology of motor cortical neurons, accompanied by delayed motor development (Rinaldi et al., 2008; Schneider and Przewłocki, 2005; Snow et al., 2008). Other neurochemical and behavioral abnormalities include increased monoamine concentrations due to enhanced expression of tyrosine hydroxylase (D'Souza et al., 2009; Narita et al., 2002), altered circadian rhythms (Tsujino et al., 2007), elevated nociceptive threshold and enkephalinergic tone (Schneider et al., 2001, 2007), abnormal fear conditioning and amygdala processing (Markram et al., 2008), increased LTP due to enhanced expression of NMDA receptors (Rinaldi et al., 2007), and a hyperactive mesocortical dopaminergic pathway (Nakasato et al., 2008). These human and animal data, in conjunction with data from Rett patients and MECP2 inactivation, strongly underscore the importance of epigenetic control over gene expression as an important player in autism pathogenesis (LaSalle, 2007; Schanen, 2006).

34.5.3 Other Teratogenic Agents: Thalidomide and Misoprostol

Thalidomide and misoprostol are two teratogenic drugs, known to induce a variety of systemic malformations. Thalidomide was commercialized as a sedative drug in the late 1950s before being withdrawn from the market in 1961. Teratogenicity is due to its angiogenesis inhibiting activity, which causes multiple systemic malformations, as well as abnormal cortical development and neuronal hyperexcitability (Hallene et al., 2006). Misoprostol is a methyl ester derivative of prostaglandin E1, used especially in Central and South America to treat gastric ulcers, but also a popular abortion inducer due to its powerful stimulatory effect on uterine contractions: the teratogenic effects of misoprostol have been studied in children born after unsuccessful abortion attempts (Bandim et al., 2003). These two drugs display several interesting parallels: both hamper fetal blood perfusion either directly (thalidomide) or indirectly (misoprostol); both produce systemic and especially ophthalmologic malformations, primarily coloboma and microphtalmos (Miller et al., 2004, 2005); both frequently cause prenatally exposed children to develop signs of Moebius sequence, including horizontal strabismus (Duane syndrome) and facial nerve palsy due to the involvement of the VI and VII cranial nerves (Bandim et al., 2003; Miller et al., 2005); both are associated with enhanced risk of autism and/or mental retardation, provided exposure occurs early in development (Miller et al., 2005; Strömland et al., 1994). The critical period for teratogenetic induction of autism has been defined in great detail for thalidomide, where it appears to be restricted to as early as 4–6 weeks into gestation (i.e., 6–8 weeks since the last menstrual cycle) (Miller et al., 2005; Strömland et al., 1994). The critical period for misoprostol has not been defined with the same precision, but it is known that maximum fetal vulnerability occurs during the first 2 months of pregnancy, and possible 5–6 weeks after fertilization (i.e., 7–8 weeks since the last menstrual cycle) (Bandim et al., 2003). Patients with idiopathic Moebius sequence and with no history of prenatal exposure to thalidomide or misoprostol have been found at enhanced risk of autism by some (Gillberg and Steffenburg, 1989), but not by others (Briegel et al., 2009). It will be important to determine conclusively whether there is a significant association between Moebius sequence and autism, because this would demonstrate that autism specificity is conferred more by a sensitive time window during development, rather than by the specific nature of prenatal insults or teratogenic mechanisms involved.

34.5.4 Environmental Pollutants as Potential Teratogens

The number of potentially teratogenic chemicals to which pregnant women may be exposed is theoretically elevated. In practice, prolonged and/or intensive exposure at critical times would be necessary to negatively influence development in any meaningful way (Rice and Barone, 2000). Such an exposure may occur in some geographical areas, primarily for ambient and

indoor air pollutants (exterminators, can sprays, and pest bombs), and for pesticides routinely used in agriculture (Landrigan, 2010; Zhang and Smith, 2003). Compounds for which preliminary evidence supports possible roles in enhancing autism risk include organochlorine pesticides, organophosphates (most clearly chlorpyrifos), heavy metals, and chlorinated solvents (Engel et al., 2007; Roberts et al., 2007; Whyatt and Barr, 2001; Whyatt et al., 2003; Windham et al., 2006). Prenatal exposure to organophosphates, such as chlorpyrifos, has been found to be associated with lower IQ, developmental delay, ADHD, and autism-spectrum traits defined as PDD-NOS (Eskenazi et al., 2008; Rauh et al., 2006). Some individuals may be genetically vulnerable to suffer from the consequences of prenatal organophosphate exposure, depending on functional genetic variants at loci such as *PON1*, the gene encoding for paraoxonase, and the HDL-associated serum enzyme responsible for organophosphate detoxification in humans (D'Amelio et al., 2005; Gaita et al., 2010). More definitive evidence linking autism, genetic vulnerability, and prenatal exposure to toxic agents is being sought through various efforts, including large epidemiological studies, such as the 'Childhood Autism Risks from Genetics and the Environment' (CHARGE) study (Hertz-Picciotto et al., 2006).

34.5.5 Congenital Viral Infections

Rubella and CMV represent the two infectious agents best known to enhance autism risk following a congenital infection (for review, see Libbey et al., 2005; van den Pol, 2006). Autistic children prenatally infected with these viruses generally present severe mental retardation and physical anomalies, such as ophthalmologic malformations, deafness, and cardiac malformations. Brain imaging findings are highly variable, ranging from cortical malformations (polymicrogyria, pachygyria, heterotopias) indicative of migration defects to abnormal intensity of the periventricular white matter suggestive of abnormal myelination in the absence of any cortical malformation. Epilepsy and cerebral palsy are also frequent.

34.5.5.1 Congenital Rubella

The largest longitudinal study involving several hundred children prenatally exposed to rubella virus estimates at 7.4% the rate of autism in this group; risk appears especially high if the infection occurs during the first 8 weeks postconception (Chess, 1971, 1977; Chess et al., 1978). Congenital rubella symptoms often change over time: some neurodevelopmental symptoms undergo remission, others are permanent, others may progressively worsen or even appear in late childhood or adolescence (Banatvala and Brown, 2004). Interestingly, mental retardation and autism do not covary over time in these children, but seemingly follow independent trajectories (Chess, 1977). The occurrence of 'late-onset' autism (i.e., onset later than 3 years of age) following congenital rubella has also been reported (Chess et al., 1978).

These data should be viewed with some caution, because: (a) the incidence of ASD among 'rubella children' was estimated well before the establishment of current standards for a clinical diagnosis of ASD and (b) these variable clinical courses should be confirmed by applying current diagnostic criteria and modern tools for clinical follow-up. However, the former limitation can be predicted to lead to an underestimation of ASD incidence following congenital rubella, since ASD diagnostic criteria have now become overinclusive, as compared to Kanner's classical criteria which would have been applied in the seventies (Berger et al., 2010). Secondly, not only psychiatric, but also physical signs and symptoms of congenital rubella change significantly over time (the 'late manifestations' can even appear during adolescence or adulthood). Furthermore, even in idiopathic ASD, a spontaneous remission by age 3 of autistic behaviors diagnosed at a younger age is not an entirely unusual event (Turner and Stone, 2007; van Daalen et al., 2009). Finally, the partial spontaneous improvement of severe autistic behaviors rapidly developed by some children following postsurgical cerebellar vermal lesions without any specific rehabilitation (Riva and Giorgi, 2000) suggests that environmental etiologies can produce clinical courses more unstable than those seen in the majority of idiopathic ASD children.

34.5.5.2 Congenital Cytomegalovirus Infection

Evidence linking prenatal CMV infection to autism is more circumstantial. Several case reports have been published (Ivarsson et al., 1990; Kawatani et al., 2010; López-Pisón et al., 2005; Markowitz, 1983; Stubbs, 1978; Stubbs et al., 1984; Sweeten et al., 2004; Yamashita et al., 2003), but risk estimates are essentially based on a small cohort of seven prenatally CMV-infected children, two of whom displayed autistic features (2/7 = 28.6%) (Yamashita et al., 2003). The presence of normal cortical gyri, indicating a substantial sparing of neuronal migration even in the presence of periventricular white matter abnormalities, led the authors to point toward the third trimester of pregnancy as the critical time window for autism-causing CMV infections (Yamashita et al., 2003). It remains to be determined to what extent autism ensues from direct viral damage, from the strong immune response driven by herpes viruses, such as CMV, or from the nature and location of cerebral malformations which are particularly frequent in congenital CMV infection (Engman et al., 2010).

34.5.5.3 *Future Perspectives: Possible Novel Roles for Congenital Viral Infections*

In addition to congenital rubella and CMV infections, our group is currently exploring vertical viral transmission as a novel mechanism potentially able to explain high 'heritability' (i.e., parent-to-offspring transmission) in the presence of relatively low rates of disease-specific genetic abnormalities (Maher, 2008). Viral genomes present in parental gametes (egg and/or sperm cells) could be passed onto the offspring already at the time of fertilization, and start being actively transcribed in permissive cells of the fetus only at a later stage during development (Lintas et al., 2010; Persico, 2010). Gamete-mediated vertical viral transmission has been well documented for several viruses, including human immunodeficiency virus, hepatitis B virus, and hepatitis C virus (Englert et al., 2004). Alternatively, seminal fluids could act as vehicles for viral transfer from father to offspring, passing horizontally through the mother. In either case, damage would be due to direct viral interference with cellular functions in permissive fetal cells, to maternal immune response prenatally, and to the patient's immune response in the late prenatal and postnatal periods.

We have recently found the genomes of polyomaviruses (BKV, JCV, and SV40) in *post-mortem* temporocortical tissue (Brodmann areas 41/42) belonging to 10/15 autistic patients and 3/13 controls ($P < 0.05$) (Lintas et al., 2010). Also, a trend toward poly-viral infections, including multiple polyoma and/or other neurotropic viruses, was recorded (40% vs. 7.7%, respectively; $P = 0.08$). Congenital polyomavirus infections, either alone or in synergy with other viruses, could conceivably explain several puzzling features of autistic disorder, as discussed in Lintas et al. (2010). Briefly, (a) converging experimental approaches have demonstrated an inappropriate and persistent activation of the innate immune system, compatible with an unresolved, early-onset viral infection accompanied by autoimmune phenomena (Garbett et al., 2008; Lintas et al., 2009; Vargas et al., 2005); (b) polyomaviruses can cause autoimmune disorders (Rekvig et al., 2006), which are also frequently encountered in first-degree relatives of autistic patients (Comi et al., 1999); (c) polyomaviruses can produce genomic instability through the activity of their early gene product large-T antigen (LTAg) (Frisque et al., 2006); (d) polyomavirus replication is more active in males, as witnessed by viruria consistently higher in males compared to females (Knowles, 2006); (e) JCV can indeed infect cultured neural progenitor cells, oligodendrocytes and astrocytes, whereas neurons are nonsusceptible to JCV infection (Hou et al., 2006); (f) following transformation of canine MDCK cells and human mesothelial cells, the LTAg of SV40 polyomaviruses has been shown to induce the production and secretion of HGF, which in turn activates by phosphorylating its receptor encoded by the *MET* gene (Cacciotti et al., 2001), which hosts some of the most consistently replicated common variants conferring vulnerability to autism (see Section 34.5.3); and (g) a recent MRI study documented for the first time the presence of temporal lobe and/or white matter abnormalities similar to those produced by viral infections, in as many as 36% of autistic children (Boddaert et al., 2009).

These preliminary results should be viewed with caution, because polyomavirus infections in autistic brains could be the consequence of immunosuppression or tissue susceptibility rather than the cause of autism (Persico, 2010). Furthermore, viral infections may have been active only prenatally and during early infancy, making it difficult to assess viral roles using biomaterials collected later in life. We are currently undertaking a thorough search of polyomaviruses in male gametes of fathers of autistic children and controls, which has already confirmed that a sizable percentage of mobile sperm samples from ASD fathers host polyomavirus genomes (Lintas and Persico, unpublished results), as previously shown by others for control samples (Martini et al., 1996). It will be very interesting to assess whether polyomavirus genomes extracted from mobile sperm cells are able to develop a cytopathic effect in permissive cells following transfection.

34.6 CONCLUSIONS: WHERE AND HOW DO COMMON VARIANTS MEET WITH RARE VARIANTS AND/OR WITH THE ENVIRONMENT?

A single pathophysiological scenario is clearly not compatible with the diversity of nonsyndromic autisms. Yet, the data summarized in this survey allow one to reach some firm conclusions and foster evidence-based speculation.

(1) Specific rare genetic variants have been convincingly shown to cause autism, at least in some cases; however, genotype–phenotype correlations are extremely labile. Not only can mutations located in the same gene result in very different clinical phenotypes, as repeatedly described in Tables 34.5–34.9 for multiple genes: the very same mutation can cause behavioral and morphological phenotypes displaying a surprising degree of variability in different patients, even in affected members of the same extended family. A clear example, introduced in Section 34.3.4.1, is provided by interindividual differences in cerebrovascular malformations seen in individuals from consanguineous families homozygous for

truncating *HOXA1* mutations, each resulting in HoxA1 protein isoforms lacking all functional domains (Bosley et al., 2007; Tischfield et al., 2005). This phenotypic variation is not at all novel in human genetics (Wolf, 1997): a similar degree of interindividual phenotypic variability occurs in syndromic forms due to well-characterized mutations, triplet repeat expansions, or genomic rearrangements, such as fragile-X syndrome or tuberous sclerosis (Table 34.3). This phenotypic variability closely mimics the impressive phenotypic variability seen when a gene inactivated by homologous recombination is backcrossed onto the genetic backgrounds of different mouse inbred strains (Doetschman, 2009). These phenotypic differences clearly emphasize the importance of common genetic variants ('genetic background,' 'modifier genes') in determining the penetrance and expressivity of rare variants.

(2) CNV studies performed to date provide several indications. Briefly: (a) estimates of the percentage of ASD patients and population controls carrying CNVs are likely to increase with the improvement of available technologies; (b) if there truly is a subgroup of ASD patients with excessive genomic instability, its size is relatively small ($\leq$10%) and thus subject to high stochastic variability in independent samples; (c) CNVs *per se* may be more immediately related to evolution than to health and disease: their presence in population controls is physiological, subject to high stochastic variability in independent human samples and may be related to increasing paternal age according to rodent models (Flatscher-Bader et al., 2011); (d) when pathogenic, CNVs seemingly act as rare variants with variable penetrance and expressivity: some *de novo* CNVs may act dominantly and even display complete penetrance, while other CNVs may follow a 'quasi-recessive' mechanism, as described by Zweier et al. (2009) for *NRXN1* (see Section 34.3.2.3) and in a recent report by Vorstman et al. (2010), who identified an autistic individual carrying a maternally inherited deletion and a paternally inherited nonsynonymous amino acid substitution, both affecting the *DIAPH3* locus in human ch. 13q21.2; (e) the genomic location of a CNV is more critical to its pathogenic potential than the total number or mean size of CNVs present in a given individual. CNVs most frequently encountered in ASD patients often encompass genes which, when mutated, are responsible for monogenic forms of autism, such as *NLGN4*, *SHANK3*, and *NRXN1*; (f) the progressive transfer of array-based approaches from the laboratory into clinical practice will indeed enhance the ability of clinicians to detect an increasing number of submicroscopic *de novo* chromosomal abnormalities; (g) deletions and duplications spanning entire genes affect expression only in a minority of 'gene dosage-sensitive loci,' as only approximately 29% of genes duplicated in trisomy 21 are actually overexpressed at or above RNA levels predicted on the basis of allele copy number (Aït Yahya-Graison et al., 2007; Lockstone et al., 2007). Homeostatic mechanisms regulating gene expression through *trans*-acting elements and noncoding RNAs can exert negative feedbacks able to establish close-to-normal gene expression levels. Hence, the conclusive definition of a given CNV as the primary cause of ASD in a given patient cannot be exclusively based on genomic data, as currently proposed (Kaminsky et al., 2011; Miller et al., 2010), but requires functional demonstration of abnormal gene expression in patient-derived cells or cell lines. Genomic evidence of *de novo* status or the absence of a patient's CNV in a very large sample of control chromosomes should be regarded as highly suggestive, but not as conclusive evidence of pathogenicity until gene expression correlates are demonstrated.

(3) Environmental factors can represent the primary cause of autism in some cases. Toxic and viral agents generally also produce major/minor malformations and neurological signs, essentially due to brainstem damage. However, no environmental teratogen or congenital infection causes autism in every single exposed subject, as summarized in Section 34.5. This again underscores the permissive role of common genetic variants, which determine the sensitivity threshold to environmental teratogens and infectious agents. Given the prenatal timing of autism-inducing teratology, more attention should be paid to common genetic variants characterizing the 'feto-maternal' unit, rather than merely the offspring.

(4) Stochastic events typically represent an overlooked nuisance to scientists, but they have been shown to provide significant contributions to deranged developmental processes (Kurnit et al., 1987). Part of the variance in affection status, symptom pattern, and disease severity currently attributed to common genetic variants may actually depend on stochastic events, possibly 'personalizing' genotype–phenotype correlations on top of differences in genetic background.

(5) A history of clinical regression, even when documented by home videos, does not necessarily imply the existence of environmental factors striking at the time when behavioral abnormalities

become manifest. Several children carrying pathogenic mutations in *NLGN* genes or in *EIF4E* ever since conception undergo apparently normal development until a severe regression occurs at approximately 2 years of age, with loss of initially acquired social and verbal milestones (see Sections 34.3.2.1 and 34.3.4.3. Regression may, thus, simply stem from a functional collapse of neural networks, occurring at the time when either they should come 'online' to support the harmonious expansion of social cognition or they are overwhelmed by pathological levels of oxidative stress or other dysfunctional processes (see below).

(6) Environmental forms indicate that pathogenic processes responsible for autism must act early on in neurodevelopment, possible as early as weeks 4–8 post-conception. An exclusively postnatal exposure of a non-genetically-vulnerable individual to prolonged psychological traumas, toxic chemicals, infectious microorganisms, and pathological reactions to vaccines, may in some cases produce psychopathology, but this is clearly distinguishable from autism (the closest example being the 'quasi-autism' of Romanian adoptees institutionalized until adoption and grown in a state of early deprivation of interpersonal contacts, which has a distinctive set of symptoms and a generally more favorable prognostic outcome, as described by Rutter et al. (2007)). In addition, autism induction may be more related to the prenatal timing of the pathogenic insult than to the nature of the insulting agent *per se*.

(7) *SHANK3*, *NRXN1*, *NLGN3*, and *NLGN4* are commonly addressed as 'synaptic genes' and their role in synaptogenesis is often depicted as critical in determining the functional disconnection of distant cortical and subcortical regions, which seemingly characterizes the CNS of autistic patients (Belmonte et al., 2010; Courchesne and Pierce, 2005; Geschwind and Levitt, 2007; Rubenstein and Merzenich, 2003). The major limit of this interpretation is that the peak of synaptogenesis in association cortices occurs late in human neurodevelopment, namely around 2 years of age. This timing is incompatible with abnormalities in cell proliferation and migration clearly documented by neuroanatomical studies of post-mortem brains and with the early prenatal timing of environmental insult in teratological forms. Furthermore, *NLGN* knockout mice and mice carrying human mutations, such as R451C, display relatively modest behavioral phenotypes overall in comparison to the severity of human autism (Chadman et al., 2008; Radyushkin et al., 2009). Finally, *NLGN* gene inactivation in *C. elegans* surprisingly yields increased sensitivity to oxidative stress as one of its main biochemical features (Hunter et al., 2010). Hence, the critical pathogenetic step may not consist in reduced synapse formation, which has never been convincingly described in ASD brains; it may also not consist in structural abnormalities of long-range neural pathways, which have been assumed to exist on the basis of neurophysiological data more than being demonstrated neuroanatomically; instead, it could consist in the excessive energy requirements imposed on brain cells by malformed and malfunctioning synapses, leading to excessive oxidative stress and consequent functional disconnections (Chauhan and Chauhan, 2006; Palmieri and Persico, 2010; Palmieri et al., 2010).

(8) Abnormal growth rates, either of the head alone or more often of the whole body, represent a frequent feature in autistic children, especially during early infancy (Courchesne et al., 2007). Converging evidence from several syndromic forms strongly points toward the ERK/PI3K/mTOR pathway as playing a pivotal role in autism (Figure 34.1) (Levitt and Campbell, 2009; Ma and Blenis, 2009). A thorough understanding of intracellular pathways involved in autism pathogenesis will be critical, as novel treatments are beginning to show promise of reversing genetically determined abnormalities even in adult mouse models of PTEN haploinsufficiency, fragile-X, and Rett syndrome (Dölen et al., 2007; Tropea et al., 2009; Zhou et al., 2009). Different autisms converging downstream on an hyperactivation of the ERK/PI3K/mTOR pathway may clinically improve using pharmacological inhibitors of this pathway, regardless of the upstream genetic background responsible for generating this biochemical imbalance.

(9) Common genetic variants are typically considered as conferring autism vulnerability. However, genetic variants conferring protection from autism could be equally important. As a concrete example, subjects VII16, 17, 47, and 48 in Hope et al. (2005), despite carrying the very same I745T mutation in the *CACNA1F* gene yielding congenital stationary night blindness type-2 in 16 members of this extended family (see Section 34.3.5.1), do not suffer from autism or profound mental retardation, presumably through the action of protective gene variants. In families with an autistic proband, protective gene variants are preferentially transmitted from parents to unaffected siblings. Electrophysiological data indicate that 'unaffected' family members suffer from disconnections between distant cortical regions

similar to those affecting their autistic siblings, but can implement compensatory circuits which are apparently not available to affected family members (Belmonte et al., 2010). Protective genetic variants may consist in the 'non-predisposing' allele at some loci, but conceivably there should be instances where one allele can be pathogenically neutral and the other can be exquisitely protective, as occurs with the sickle cell anemia allele conferring protection from malaria (Allison, 2009). *SLC25A12* and *GLOI* are two examples of genes possibly hosting common protective variants (Palmieri et al., 2010; Sacco et al., 2007b).

(10) Neuroanatomical, genome-wide expression, and brain imaging studies provide converging evidence of an abnormal activation of the immune system in autism, and particularly of its innate components (Garbett et al., 2008; Laurence and Fatemi, 2005; Petropoulos et al., 2006; Vargas et al., 2005; for review of genome-wide expression studies, see Sacco et al., 2011). This could be due to abnormal synaptic function and/or molecular processing leading to proinflammatory cytokine production, as occurs in Alzheimer disease (Meda et al., 1999). However, temporal lobe abnormalities reminiscent of virally generated lesions have been detected in 48% of autistic children in a recent brain imaging study (Boddaert et al., 2009). These results are compatible with the presence of a persistent, virally triggered immune reaction in a subgroup of genetically predisposed autistic children. Studies of vertically transmitted viruses are thus justified, as they may thus contribute to solve the mystery of the 'missing heritability' (Maher, 2008) in autism research.

(11) In a translational perspective, it may be initially easier to estimate autism risk using a limited set of the most influential common variants, than to search for rare or private variants by sequencing a large enough panel of candidate genes, if not the entire genome. A first example of a test providing combinatorial autism risk estimates from four loci, each hosting one common biallelic SNP, has recently been published (Carayol et al., 2010). As the number of common variants will increase, current 18% sensitivity will hopefully rise to match an already satisfactory 92% specificity (Carayol et al., 2010). In general, this diagnostic approach will be most useful in estimating the risk of autism in (a) newborn siblings of autistic children and (b) sporadic cases displaying initial behavioral abnormalities at 1–2 years of age and potentially evolving by age 3 toward normal behavior, or into full-blown autism, softer ASD traits, specific language impairment, ADHD, or other behavioral syndromes. However, the latter use will require testing the specificity of common variants conferring autism vulnerability, which may not reliably separate behavioral syndromes given the labile genotype–phenotype correlations presented throughout this survey. Tests of this sort will be increasingly sought, as early behavioral intervention programs, targeted to address ASD signs and symptoms much earlier than age 3, have begun showing significant efficacy in controlled trials (Dawson et al., 2010; for review, see Rogers and Vismara, 2008; Howlin et al., 2009) and are being actively pursued in many clinical centers. Interestingly, treatment response is also not a uniform dimension, as post-treatment measures always display larger dispersion compared to pretreatment, indicating the presence of treatment 'responders' and 'nonresponders' (Dawson et al., 2010). Therefore, genetic and biochemical markers may also be sought to predict treatment response. Finally, the short-term efficacy of early intervention programs is comforting, though not entirely unexpected when considering that environmental enrichment largely reverses behavioral abnormalities in an animal model as 'organic' as rats prenatally exposed to valproic acid (Schneider et al., 2006), and in *MECP2* knockout mice (Lonetti et al., 2010). Early interventions most likely yield better outcomes because they act during critical periods of greater plasticity in postnatal brain development. It will be important to see whether behavioral improvements are permanent, or whether periodic/continuous maintenance treatment will be required, most likely through parent training strategies.

Acknowledgments

The author's work is supported by the Italian Ministry for University, Scientific Research and Technology (Programmi di Ricerca di Interesse Nazionale, prot. n.2006058195), the Italian Ministry of Health (RFPS-2007-5-640174), Autism Speaks (Princeton, NJ), the Autism Research Institute (San Diego, CA), the Fondazione Gaetano e Mafalda Luce (Milan, Italy), and Autism Aids Onlus (Naples, Italy). The author is grateful to the NICHD Brain & Tissue Bank for Developmental Disorders (Baltimore, MD), the Harvard Brain Tissue Resource Center (Belmont, MA), and the Autism Tissue Program (Princeton, NJ), for coordinating and kindly providing post-mortem brain tissues essential to the author's work. The author would also like to acknowledge the members of the author's laboratory, Roberto Sacco, Carla Lintas, Laura Altieri, Federica Lombardi and Valerio Napolioni, for their commitment to autism research.

This text is dedicated to the author's father, Andrea, who passed away 1 week before its completion: "He was a great father, a devoted husband, and a proud Navy Officer: I owe to his care and to his example much of what I am today".

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