Syndromic autism: causes and pathogenetic pathways

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Background: **Autism is a severe neurodevelopmental disorder known to have many different etiologies. In the last few years, significant progresses have been made in comprehending the causes of autism and their multiple impacts on the developing brain. This article aims to review the current understanding of the etiologies and the multiple pathogenetic pathways that are likely to lead to the autistic phenotype.**

Data sources: **The PubMed database was searched with the keywords "autism" and "chromosomal abnormalities", "metabolic diseases", "susceptibility loci".**

Results: **Genetic syndromes, defined mutations, and metabolic diseases account for less than 20% of autistic patients. Alterations of the neocortical excitatory/inhibitory balance and perturbations of interneurons' development represent the most probable pathogenetic mechanisms underlying the autistic phenotype in fragile X syndrome and tuberous sclerosis complex. Chromosomal abnormalities and potential candidate genes are strongly implicated in the disruption of neural connections, brain growth and synaptic/dendritic morphology. Metabolic and mitochondrial defects may have toxic effects on the brain cells, causing neuronal loss and altered modulation of neurotransmission systems.**

Conclusions: **A wide variety of cytogenetic abnormalities have been recently described, particularly in the low functioning individuals with dysmorphic features. Routine metabolic screening studies should be performed in the presence of autistic regression or suggestive clinical findings. As etiologies of autism are progressively discovered, the number of individuals with idiopathic autism will progressively shrink. Studies of genetic and environmentally modulated epigenetic factors are beginning to provide some clues to clarify the complexities of autism pathogenesis. The role of the neuropediatrician will be to understand the neurological basis of autism, and to identify more homogenous subgroups with specific biologic markers.**

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Introduction

Autism is a severe neurodevelopmental disorder characterized by impaired language, communication and social skills as well as by repetitive and stereotypic pattern of behavior.[1] Converging links of evidence strongly point toward altered neurodevelopment during early prenatal life as crucial to autism pathogenesis.[2] The time course of brain development rather than the final product is most disturbed in autism;[3] cerebellar alterations seem to play a key role in autism, with a decreased number of Purkinje cells;[4] recently specific antibodies directed towards cerebellar cells have been shown in autistic patients.[5] Genetic factors could largely contribute to autism liability, but have proven more complex than initially anticipated due to interindividual heterogeneity, numerous contributing loci, and multiple genes and gene-environment interactions.[6] This complexity has spurred interest into morphological, biochemical, and behavioral endophenotypes, i.e., heritable traits ideally characterizing pathogenetically homogeneous subgroups of patients. Comorbidity with mental retardation and seizures occurs in up to 80% and in 30% of autistic patients, respectively.[7] Several lines of evidence strongly support a prenatal onset for developmental abnormalities, later leading to autism.[8]

Autism in its very broad spectrum of severity is a syndrome, not a disease, and it is known to have many different etiologies. The term syndromic or secondary autism is used to refer to autism with a single defined cause, such as fragile X syndrome (FXS) and tuberous sclerosis. However, none of these etiologies is specific to autism because each of them encompasses a variable proportion of individuals with and without autism.

Idiopathic or primary autism encompasses individuals whose etiology remains still unknown.[2]

In the last few years, significant progress has been made in comprehending the causes of autism and their multiple impact on the developing brain. Although a number of etiologies of autism are known, their pathogenetic role in specific subgroups of children with autism is not well understood. In this article we discuss current understanding of the pathogenesis of syndromic autism, and the multiple pathways that are likely to lead to the autistic spectrum phenotype.

Genetic diseases associated with autism

Single gene defects and chromosomal anomalies may account for approximately 10% of individuals with autism,[9] and the portion is likely to be higher when micro-array comparative genome hybridization is used.[10] Some families or individuals with autism lack genes for synaptic proteins.[11,12] Table 1 summarizes the most frequent genetic syndromes and cytogenetic abnormalities associated with autism.[13-25]

Fragile X-syndrome

Mutations in the *FMR1* gene that controls the growth and maturation of cells and synapses increase the risk for autism, but are not strict determinants of autism. Studies using comprehensive diagnostic instruments have yielded prevalence estimates for autism in the FXS population between 18% and 33%.[26,27] Dendrites are long and thin when immature, or when deprived of the fragile X mental retardation protein.[28] Abnormalities in long-term synaptic plasticity of excitatory synapses and in synaptic connectivity may be the underlying neurological substrate of autism associated with FXS.[29,30] Alterations in the neocortical excitatory/inhibitory balance as well as abnormal neural synchronization have also been reported in mouse models of FXS,[31] resulting in hyperexcitability of neocortical circuits. An immature dendritic morphology may also increase susceptibility to epilepsy and anxiety in FXS patients.[32]

Table 1. Genetic syndromes associated with autism

Syndrome	Gene(s) associated	Patients with autism that have the syndrome	Patients with the syndrome that have autism
Fragile X syndrome[13,14]	*FMR1*	2%-3%	20%-40%
Tuberous sclerosis[15]	*TSC1*, *TSC2*	3%-4%	43%-86%
15q duplication—Angelman syndrome[16]	*UBE3A GABAr* cluster	1%-2%	>40%
16p11 deletion[17,18]	*PCKB1*	1%	High
22q deletion[19,20]	*SHANK3*	1%	High
2q37 deletion[21]	*KIF1A*, *GBX2*	Unknown	50%
Joubert syndrome[22]	*AHI1*	Unknown	40%
Timothy syndrome[23]	*CACNA1C*	Unknown	60%-70%
Cortical dysplasia-focal epilepsy syndrome[24,25]	*CNTNAP2*	Rare	70%

Tuberous sclerosis complex

The affected genes are *TSC1* and *TSC2*, encoding hamartin and tuberin respectively. The hamartin-tuberin complex inhibits the mammalian-target-of-rapamycin pathway which controls cell growth and proliferation.[33] Mutations of *TSC* genes via downstream effects on neuronal and synaptic structures or neurotransmission have the potential to induce fundamental alterations in circuitry as well as an imbalance in excitation and inhibition, producing a variety of neurological manifestations including epilepsy and autism.[34] Disruption of GABAergic interneuron development may underlie part of the pathophysiological process that leads to autism and epilepsy.[35] Perturbations of interneurons development can selectively impact frontal and parietal areas.

Chromosomal abnormalities

A wide variety of cytogenetic abnormalities have been described,[36] particularly in the low functioning individuals with dysmorphic features.[37]

Chromosome 15

Chromosomal rearrangements in 15q11-15q13 region might be the most frequent cytogenetic abnormality in autism,[16] accounting for 1%–2% of patients. A "chromosome 15 phenotype" characterized by ataxia, language delay, epilepsy, mental retardation, repetitive movement disorders and facial dysmorphic features has been described in individuals with chromosome 15 duplications.[38] Within the 15q11–15q13 locus, gamma-aminobutyric acid A receptor beta 3 (GABRB3), an inhibitory neurotransmitter receptor, is currently thought to be central in the development of autism, due to its role in the neuronal inhibition and its expression in early development.[39] This finding is particularly interesting in light of the high incidence of seizures and EEG abnormalities in autistic patients, and it is supported by recent data showing a correlation between 15q13.3 microdeletion, idiopathic generalized epilepsies and a number of neuropsychiatric conditions including autism.[40]

Chromosome 7

Two of the loci most commonly associated with autism shown by genetic linkage studies (7q22 and 7q31

regions)[41,42] contain several genes implicated in the pathogenesis of autism. The *RELN* gene, found within the 7q22 region, has a pivotal role in neuronal migration and prenatal development of neural connections,[43,44] and is potently inhibited by toxic substances such as organophosphates.[45] Increased risk for autism can also be linked to a functional polymorphism in the *MET* gene, found within the 7q31 locus,[46] which plays a role in the development of the cerebral cortex and cerebellum. A functional variant in the promoter of the gene encoding the MET receptor tyrosine kinase is associated with autism. MET is a pleiotropic receptor that functions in both brain development and gastrointestinal repair. Recent evidence suggests that disrupted MET signaling may contribute to increased risk for autism spectrum disorder that includes familial gastrointestinal dysfunction.[47] The Williams-Beuren syndrome region (7q11.23) also contains several genes associated with impairment in language and social interaction,[48-50] suggesting the existence of a specific subgroup of autistic patients, characterized by dysmorphic features, mental retardation, language delay, congenital heart disease, and hypersensitivity to sound.

Chromosome 16

An association between a larger microdeletion on 16p11.2 and a syndrome that included developmental delay and distinct facial appearance has been described.[17,51,52] The chromosomal region 16p11.2 also encompasses the *PRKCB1* locus, an interesting gene associated with autism[53] and expressed in the CNS, the immune system, the digestive tract, and the kidney. A recent study has described an association between PRKCB1 and an enhanced urinary peptide excretion rate.[54]

Chromosome 2

Deletions involving 2q37 have been observed in more than 70 individuals with autism, mental retardation, and dysmorphic features.[55,56] Three different breakpoints of 2q37 (2q37.1, 2q37.2, 2q37.3) have been analyzed to clarify the genotype-phenotype relationships associated with different terminal deletions,[57] and several candidate genes for autism have been identified in 2q37.3 band.[58] Furthermore, a correlation between autism and a *de novo* cryptic deletion of chromosome 2p25.2 has been described.[59] The interaction between potential candidate genes that are expressed on these loci may explain the phenotypical heterogeneity and the spectrum of neuropsychological deficits associated with 2q37 and 2p25.2 deletion syndromes.

Other regions implicated in autism with possible candidate genes are summarized in Table 2.[60-69]

Epilepsy and regressive autism

The relationship among epilepsy, electroencephalographic (EEG) abnormalities, and regression in autistic patients is not yet well understood. Approximately 30%

Table 2. Candidate genes associated with autism

Gene	Chromosome	Functions	CNS abnormalities	Clinical phenotypes
NGL3[60,61] *NGL4*[60,61]	Xq13.1 Xp22.3	Synaptic transmission, differentiation of synaptic contacts	Synaptic or dendritic changes	Autism with motor tics, mild to severe autism, PDD-NOS
SHANK3[11,62]	22q13	Master organizer of postsynaptic density at glutamatergic synapses	Synaptic or dendritic changes	Multiple developmental delays, dysmorphic features, autism with severe language and social deficits
MAPK3[63]	16p11.2	Cell to cell signaling and postsynaptic density	Synaptic or dendritic changes	-
OXTR[64]	3p25–26	Oxytocin receptor, mediator of affiliative behavior	Abnormalities of neurotransmitters	-
CNTNAP2[24]	7q35	Contactin associated protein-like 2	Restricted pattern of expression: frontal and anterior temporal lobes, striatum, and dorsal thalamus	Seizures, developmental language delay, autism
GAD1[65]	2q31	Catalyzes the production of GABA from glutamate	-	-
CADM1[66]	11q23	Synaptic cell adhesion molecule promoting the formation of presynaptic terminals and inducing the functional synapse	Loss of cell adhesion functions on the cell surface with impairment of the synaptogenic pathway	Impairment of social behavior, ASD
MCPH1[67]	8p23.1-8p23.2	Microcephalin	-	Speech delay, learning difficulties
PTEN[68,69]	10q23	Regulation of cellular proliferation/ differentiation	Abnormalities in brain growth	Macrocephaly, autism and developmental delay

CNS: central nervous system; GABA: gamma-amino-butyric acid; PDD-NOS: pervasive developmental disorders-not otherwise specified; ASD: autism spectrum disorder.

of children with autism have epilepsy,[70] this comorbidity may be sustained by alterations in cortical-subcortical system connectivity.[71] Sometimes autistic regression is the presenting symptom in a child whose epilepsy can be documented unequivocally with the appropriate work up. An epileptic disorder must be considered in all children with a low functioning autism, especially when a history of regression and EEG paroxysmal abnormalities, such as slow spike-wave discharges during sleep and focal centrotemporal spikes, are present.[72] Severe epileptiform abnormalities may permanently alter the critical synaptogenesis by strengthening synaptic contacts that should have been naturally "pruned".[73] Cognitive functions decline in those patients who have a prolonged active phase of continuous spike-and-wave discharges during sleep.[74] Another important risk factor for a cognitive regression is an early onset epilepsy; in particular, infantile spasms have been shown to be connected with a subsequent autistic regression in a high percentage of patients.[75-77] Such children should be strictly monitored with electrophysiological, structural, and neurocognitive approaches, and can benefit from an antiepileptic treatment.[78]

Although epilepsy is not a causal factor of autism, increased understanding of common genetic and molecular mechanisms of the autism-epilepsy phenotype provided insight into the pathophysiology of autism.[71] The existence of altered Ca^{2+} signaling in autism and the bioelectrical instability resulting from mutations of the L-type voltage-gated Ca^{2+} channels may account for the high prevalence of seizures and/or EEG abnormalities among autistic individuals.[79]

Metabolic diseases

Several inborn errors of metabolism, including phenylketonuria, creatine deficiency syndromes, adenylosuccinate lyase deficiency, and metabolic purine disorders can account for less than 5% of individuals with autism.[80]

In untreated children affected by phenylketonuria, the high levels of phenylalanine may have toxic effects on the brain cells, causing reduction of myelin, neuronal loss and decreased levels of interneuronal connections.[81] Hyperphenylalaninemia also competes to absorb other amino acids, and consequent low tyrosine and tryptophan concentrations can determine a low production of dopamine and serotonin in the prefrontal cortex.[82]

In some of the most severe metabolic diseases, like adenylosuccinase deficiency or creatine deficiency syndromes, neurological and behavioral symptoms are probably not caused by deficiency of metabolites, but are more likely due to the toxic effects of the accumulating substances on the brain.[83-85] A direct role in modulation of dopaminergic and serotoninergic neurotransmission systems and axonal guidance has been hypothesized for the adenosine deaminase deficiency as a pathologic mechanism for the development of autistic symptoms.[86]

The role of mitochondrial disorders has been revitalized by the association between autism and variants of the *SLC25A12* gene, which encodes the predominant isoform of the mitochondrial aspartate (asp)/glutamate (glu) carrier (AGC) in the brain.[87,88] SLC25A12 overexpression may be involved in the pathophysiology of autism, modifying neuronal networks in specific subregions, such as the dorsolateral prefrontal cortex and fusiform gyrus, at both pre- and postnatal stages.[89] Altered Ca^{2+} homeostasis is responsible for boosting AGC activity, mitochondrial metabolism and, to a more variable degree, oxidative stress in autistic brains.[90]

Based on our clinical experience, routine metabolic screening studies should be used on a case-by-case basis, in the presence of the autistic regression, or suggestive clinical findings, such as lethargy, cyclic vomiting, early onset seizures, dysmorphic features, mental retardation with neurologic deficits, unexplained immune deficiency or unexplained hemolytic anemia, hyper- or hypotonia, self-mutilation, and muscle weakness.[91] Table 3 summarizes the main clinical features of the metabolic diseases most frequently associated with autism.[92-97]

Pathogenetic pathways

Several molecular pathways potentially involved in the disruption of neurodevelopmental trajectories during intrauterine or postnatal brain development may be associated with abnormal developmental processes, from neuronal migration and cortical organization to synaptic and dendritic conformation.[98] The Fig. illustrates many different types of potential pathogenetic mechanisms responsible for autism phenotype in the most common medical conditions associated with autism. Furthermore, environmental factors, including maternal/intrauterine infections, exposure to toxins and oxidative stress, may modify the underlying genetic substrate, leading to abnormalities in neuronal organization and cortical network development.[99] A list of etiologies observed in our series is shown in Table 4. According to our experience cerebellar malformations are often associated with an autistic symptomathology.

Defined medical syndromes, chromosomal abnormalities and *de novo* copy number variations (CNVs) account for about 10%–20% of autism cases.[100]

The appropriate use of genetic testing is relevant to

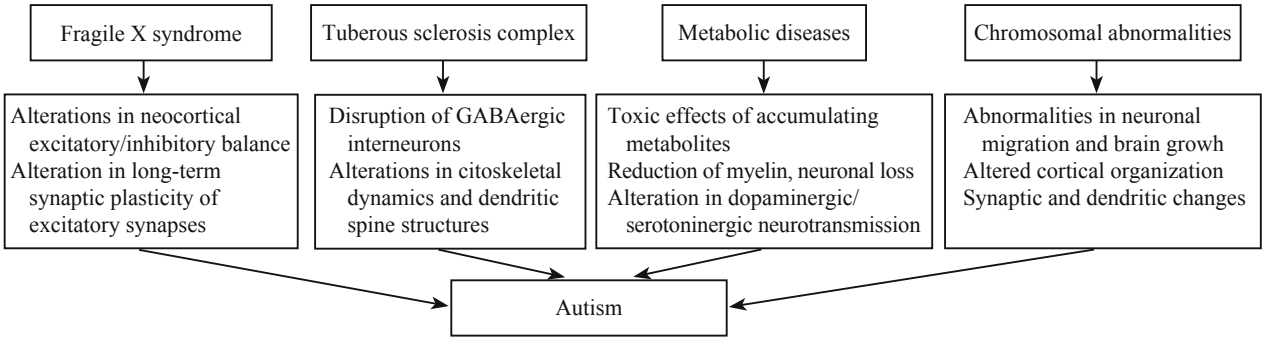

Fig. Potential pathogenetic pathways leading to autism.

Table 3. Metabolic diseases associated with autism

Metabolic diseases	Potential pathogenetic mechanisms	Clinical features	Diagnosis	Potential therapeutic options
Phenylketonuria[92,93]	Reduction of myelin; toxic effects on the brain cells; low production of DA and serotonin	Autism, seizures, severe MR	Quantitative plasma amino acids analysis	Restriction diet and aminoacids integration
Adenylosuccinase deficit[80,94]	Toxic effects of the accumulating succinyl purines on the brain	Autistic phenotype, PMR, epilepsy	Succinyl aminoimidazole, carboxamide riboside and succinyl adenosine in urine and CSF	D-ribose therapy
Smith-Lemli-Opitz syndrome[95,96]	Neurosteroid deficiency; alteration of neuroendocrine functions and behavior	Autism, psychomotor retardation, poor expressive language, behavioral abnormalities	Abnormal sterol pattern	Cholesterol replacement therapy
Creatine deficiency syndromes[85,97]	Neurotoxic effect of guanidinoacetate or other guanidine compounds	Autistic phenotype, MR, speech delay, epilepsy, extrapyramidal symptoms and signs	Blood and urinary concentration on creatine and guanidinoacetate, brain MRS	Oral creatine supplementation, dietary restriction of arginine and substitution of ornithine

DA: dopamine; MR: mental retardation; MRS: magnetic resonance spectroscopy; CSF: cerebrospinal fluid; PMR: psychomotor retardation.

Table 4. List of etiologies observed in our clinical series of 205 autistic patients observed in the years 2006-2008

Syndrome	Number of patients affected
Tuberous sclerosis complex	8
Cerebellar MRI abnormalities	7
Chromosomal abnormalities	5
Fragile X syndrome	4
Epileptic regression	3
Temporal MRI abnormalities	2
Metabolic/mitochondrial disorders	2

good clinical practice and may allow the identifications of new susceptibility variants. The advent of fluorescent *in situ* hybridization (FISH) techniques has expanded the list of chromosomal "hot spot" in autism. Individual FISH studies may be indicated in the confirmation of a clinically suspected condition,[101] and in the evaluation of low functioning patients with an IQ <50.[102] When dysmorphic features are present, it is reasonable to suspect chromosomal rearrangements, even if the karyotype appears normal. In these cases oligo array-based comparative genomic hybridization (CGH) analysis is highly advisable.[103] Whole genome-scanning by array based-technology has detected CNVs, which are copy-number changes involving a DNA fragment, and represent submicroscopic deletions or duplications that are undetectable by the routine cytogenetic analysis.[10,37,104,105] These microdeletions and microduplications cause gene dosage imbalance in several genes, many of which could be considered as candidate genes for autism.

In conclusion, analogous to broad syndromes as mental retardation, autism has many etiologies, and should be considered not as a single disorder. As etiologies of autism are progressively discovered, the number of individuals with idiopathic autism will progressively shrink. Studies of genetic and environmentally modulated epigenetic factors are beginning to provide some clues to clarify the complexities of autism pathogenesis. The role of the neuropediatrician is to identify more homogenous subgroups with specific biologic markers; selection of informative sets of autistic disorders could be very important in detecting susceptibility loci gaining a deeper understanding of the etiologies of autism.

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