GENETIC AND NON GENETIC ASPECTS OF AUTISM SPECTRUM DISORDERS

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ABSTRACT

Chromosome abnormalities have long been recognized as an important cause of learning disabilities and multiple malformation syndromes. About 0.8% of live born infants have numerical or structural chromosomal anomalies that result in an abnormal phenotype. Identification of such anomalies is important clinically and also for accurate genetic counseling. Recently, molecular cytogenetic and array-based techniques have enabled higher resolution screens for chromosome anomalies. This brief review of the etiology of autism spectrum disorders (ASD) focuses on the heritable and non heritable risk factors that underlie this major neuro-developmental disorder. Since all patients with a chromosomal imbalance are dysmorphic, the association of ASD with a facial dysmorphism seems to be a good indication for chromosomal anomaly screening.

Key words: Array CGH (microarray-based comparative genomic hybridization); Attention-deficit/hyperactivity disorder (ADHD); Autism spectrum disorders (ASD)

INTRODUCTION

Clinical features that predict an increased likelihood of finding a cytogenetic abnormality on routine testing include: congenital delay in neuro-cognitive

development, one or more major malformations, prenatal onset abnormal growth pattern, craniofacial dysmorphism, unusual behavioral phenotypes, often in the autistic spectrum, and a family history of multiple miscarriages, learning disabilities or malformations. High resolution chromosome banding has been reported to have an overall detection rate of 7.5% for anomalies in patients with mental retardation/learning disabilities [1]. Conventional cytogenetic analysis uses light microscopy to examine metaphase or prometaphase chromosomes that have been stained to produce a distinct banding pattern for each chromosome. This approach has a maximum resolution of 3-5 Mb for structural anomalies and requires mitotic cells, usually peripheral blood leucocytes, bone marrow, or fibroblasts, for analysis [2,3]. Fluorescent *in situ* hybridization (FISH) analysis uses labeled DNA probes to identify submicroscopic (or cryptic) structural chromosome anomalies: micro deletions and micro duplications [4]. Microarray-based comparative genomic hybridization (array-CGH) technology provides a diagnostic method for systematic and comprehensive analysis of human aneuploidy. Array-CGH provides much higher resolution (currently up to 100-200 kb) and has great potential for automation. Array-CGH is now being applied to human malformation syndromes and cohorts with learning disabilities, provides higher resolution definition of known structural aberrations and detects aneuploidy that was undetected by FISH or chromosome banding techniques.

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DEFINITION OF AUTISM

Autism is a complex behaviorally-defined disorder of the immature brain. It is not a disease but a syndrome with multiple non genetic and/or genetic causes. Autism spectrum disorders (ASD) are conditions which can be either isolated or syndromic, that is associated with other clinical features such as facial dysmorphism, limb or visceral malformations, and growth abnormalities.

According to the Diagnostic and Statistical Manual of Mental Disorders (4th edition) (DSM-IV) and the International Classification of Diseases (10th revision) (ICD-10) designations, autism is characterized by impairment in three behavioral domains: 1) social interaction, 2) language, communication and imaginative play, and 3) range of interests and activities. The five DSM-IV permissive developmental disorder (PDD) subtypes are: 1) autistic disorder (classic autism), 2) Asperger disorder (language development at the expected age, no mental retardation), 3) disintegrative disorder (behavioral, cognitive, and language regression between ages 2 and 10 years after entirely normal early development, including language), 4) PDD not otherwise specified (individuals who have autistic features and do not fit any of the other subtypes), and 5) Rett syndrome [5].

The highly variable cognitive manifestations of the ASD range from a non verbal child with severe mental retardation to a high-functioning college student with an above average IQ, despite impaired language use and inadequate social skills. Mental retardation is thus not a defining criterion for autism. The prevalence of ASD is ~3 to 6/1000, with a male to female ratio of 3:1 [6]. The number of children diagnosed as having ASD is increasing. This is probably attributable to heightened awareness and changing diagnostic criteria rather than to new environmental influences and the inclusion of children with disorders such as attention deficit/hyperactivity disorder (ADHD), Tourette's syndrome, or tuberous sclerosis complex (TSC) as ASD [7]. Despite the organic basis of the disorder, the diagnostic criteria have been derived through consensus, rather than being organically based; no biological "test" exists for autism. Diagnostic cut-offs have been hard to define because the manifestation of the core impairments and behaviors of autism vary

greatly from person to person. Symptoms vary in any single person with autism, and developmental change occurs over time. Currently, diagnosable medical conditions, cytogenetic abnormalities, environmental factors, and single-gene defects [*e.g.*, TSC, fragile X syndrome (FXS)] associated with autism, together account for 10-20% of cases [8].

NON GENETIC AND GENETIC CONDITIONS ASSOCIATED WITH AUTISM

Fragile X Syndrome. This is an X-linked genetic disorder that is associated with autism and is characterized by unusual facial features, macroorchidism in adulthood and cognitive impairment of variable severity. Fragile X syndrome is the most common cause of inherited mental retardation, affecting approximately 1/4,000 males and 1/8,000 females. It is caused by an increased number of trinucleotide (CGG) repeats in the FMR1 gene, which codes the fragile X mental retardation protein. Depending on the number of CGG repeats, affected alleles are classified as normal (5-40), intermediate or gray zone (~45-54), pre mutation (~55-200) or full mutation (>200). Full mutation alleles are typically associated with hypermethylation that results in gene silencing and a pathological phenotype. An important factor influencing studies of autism in FXS is that up to 90% of affected males display a variety of behavioral abnormalities that can be interpreted as atypical social interaction or autism spectrum behaviors such as perseveration, hand flapping, self-injury, avoidance of eye contact and social anxiety. Approximately 30% of individuals with FXS are within the autistic spectrum with a frequency of 7-8% FXS in populations with autism [9]. Several studies have concentrated on the differentiation between boys with both FXS and autism, and those with idiopathic autism [10,11]. The results suggest similar, although milder profiles on several measures of autistic behavior in the FXS and autism group.

Tuberous Sclerosis Complex. An autosomally dominant neurocutaneous disorder, TSC arises from genetic mutations of either *TSC1* on 9q or *TSC2* on 16p, and is characterized by ash-leaf depigmented or other cutaneous manifestations and hamartomatous lesions in multiple organs. In the brain, the lesions

or tubers are thought to cause the epilepsy seen in more than three-quarters of children with TSC [5].

Behavioral symptoms of autism are frequent in TSC. Given the frequency of epilepsy in children with TSC and the association between autism and epilepsy, it is not surprising that about 25% of patients with TSC have autism. Among patients with autism, the prevalence of TSC is 1.1-1.3%, which, although low, is 30% higher than that of TSC in the general population [5].

Rett Syndrome. This syndrome is the only ASD with a known genetic cause. It is an X-linked disorder that presents in females and is caused by mutations in the *MEC2* gene [12]. Similar to autism, RS is not diagnosed until late infancy, following a period of apparently normal development. Although *MECP2* mutations are rare in autism, defects in the level of the MeCP2 protein are found in brain samples of autism patients [13]. Multiple genetic and environmental factors could change the MeCP2 protein level in the brain and lead to a similar effect as the *MECP2* gene mutations. Research into the role of MeCP2 in the pathogenesis of RS and ASD is therefore a “Rosetta Stone” that can be used for deciphering the more complex etiology of autism in the general population [14].

OTHER GENETIC CONDITIONS ASSOCIATED WITH AUTISM

Other single-gene and micro deletion defects may be associated with autism. Neurofibromatosis, a common autosomal dominant disorder with neurological and cutaneous manifestations, is much less frequently associated with autism than is TSC or FXS [15]. Angelman syndrome (AS) and Prader-Willi syndrome (PWS) usually result from genetic deletions or uniparental disomy of the 15q11-q13 locus, with abnormal imprinting or genetic mutations being found in up to 5.1% of PWS cases and up to 15% of AS cases. Loss of paternally-derived genes result in PWS, whereas AS, more commonly associated with autism than PWS, can result from the loss or mutation of the maternally-derived ubiquitin protein ligase gene *UBE3A* or the *ATP10C* gene [16].

An unexpectedly large proportion of boys with Duchenne muscular dystrophy fall in the autistic spectrum [17]. Other rare single-gene defects that

have been associated with autism, include those found in Sotos syndrome, Williams syndrome, hypomelanosis of Ito, Cowden syndrome, and Moebius syndrome [5].

Autism may also occur in the context of abnormal cellular metabolism, such as mitochondrial disease or dysfunction. Untreated phenylketonuria is a well-documented metabolic cause of autism; however, whether this is attributable to the resulting severe mental retardation or to the specific deficit in the dopamine pathway, is uncertain [18]. A clinic-based study [19] reports high levels of uric acid secretion in up to one-quarter of patients with autism and amelioration of certain symptoms with antihyperuricosuric therapy. This represents a significant proportion of these clinical samples, but the result has not been widely replicated and the factors that are responsible for this type of “purine autism” remain to be identified.

Studies of twins [20,21] have reported 60% concordance for classic autism in monozygotic (MZ) twins vs. 0% in dizygotic (DZ) twins, the higher MZ concordance attesting to genetic inheritance as the predominant causative agent. Reevaluation for a broader autistic phenotype that included communication and social disorders, increased concordance remarkably from 60 to 92% in MZ twins and from 0 to 10% in DZ pairs. This suggests that interactions between multiple genes cause “idiopathic” autism but that epigenetic factors and exposure to environmental modifiers may contribute to variable expressions of autism-related traits. The risk for developing autism in siblings of affected individuals is 75-fold greater than in the general population [5]. Evidence from studies of twins, familial aggregation, and rare chromosomal abnormalities provide a compelling argument for some substantive heritable component in ASD etiology. However, no specific genes have been implicated.

NON GENETIC CONDITIONS ASSOCIATED WITH AUTISM

An epidemiological study [22], indicated that environmental factors such as toxic exposure, teratogens, perinatal insults, and prenatal infections such as rubella and cytomegalovirus account for some cases. Autism has been linked to a wide variety of prenatal and postnatal insults but predominantly

in individual case reports. The published literature on specific prenatal infectious pathogens known to affect the brain and ASD, including herpes simplex, rubella, syphilis, and varicella-zoster, consists mainly of occasional case reports [22].

Intrauterine exposure to the teratogenic drugs thalidomide and valproate and other anticonvulsants have been implicated as the cause of autism in a few affected children [23]. Taking thalidomide during days 20-24 of gestation was clearly correlated with an increased risk for an autistic disorder.

It has been suggested that the MMR (measles-mumps-rubella) vaccine causes autism [24,25]. The widespread use of this vaccine has reportedly coincided with an increase in the incidence of autism, and there are case reports of children in whom signs of developmental regression and gastrointestinal symptoms developed shortly after vaccination. The measles virus has been found in the terminal ileum in children with developmental disorders and gastrointestinal symptoms but not in developmentally normal children with gastrointestinal symptoms. The measles virus used in the MMR vaccine is a live attenuated virus that normally causes no symptoms or only very mild ones. However, wild-type measles can infect the central nervous system and even cause postinfectious encephalomyelitis, probably as a result of an immune-mediated response to myelin proteins. Studies designed to evaluate the suggested link between MMR vaccination and autism do not support an association, and fail to confirm that immunization with the MMR vaccine is responsible for the upsurge in autism [24,25].

CYTOGENETICS

Cytogenetic assays have long been used to uncover chromosomal defects in patients with autism. Cytogenetic abnormalities that have been described include 2q37 and 22q13.3 deletions and 45,X/46,XY mosaicism. Almost all chromosomes have been involved [26,27]. Cytogenetic abnormalities at the 15q11-q13 locus occur in 1-4% of patients with autism. Various population studies and case reports have described duplications, deletions, and inversions at this locus [28]. Although less than 10% of cases of autism are associated with chromosomal abnormalities, high-resolution cytogenetic scans in families with affected individuals help to locate

specific genes or chromosomal regions that may be potentially associated with autism.

Chromosomal Copy Number Changes in Syndromic Autism Spectrum Disorders. The introduction of array CGH has proved to be specific, sensitive and fast, and to have considerable advantages compared to other methods for the analysis of changes in DNA copy number. Array-CGH enables analysis of the whole genome in a single experiment. Although its applications have been mainly directed at detecting genomic abnormalities in cancer [29], it is suitable for analysis of DNA copy number aberrations that cause human genetic disorders [30-36]. Array-CGH combines the resolution of FISH with the whole-genome screening capacity of conventional CGH and can be applied to the detection of submicroscopic aberrations, determination of critical DNA regions of specific diseases, and elucidation of genotype-phenotype correlations [37].

Among 29 patients presenting with syndromic ASD, investigated by a DNA microarray [38], a pathogenic chromosome imbalance was detected in 27.5% of the patients. Hence, array-CGH should be considered to be an essential aspect of the genetic analysis of patients with syndromic ASD. The severity of the mental retardation does not seem to be directly related to the size of the chromosomal imbalance, as the largest rearrangement was detected in the less severely retarded patients. No recurrent abnormality was found in this cohort. These results underlie the extreme genetic heterogeneity of syndromic ASD. These data strongly support the idea that only a whole-genome high-resolution analysis such as array-CGH is able to provide an accurate diagnosis for chromosomal imbalance in patients with ASD.

Attention-Deficit/Hyperactivity Disorder.

This is a highly heritable psychiatric disorder that affects ~4-5% of children. The syndrome persists into adulthood in about one-third of the cases and affects ~0.5-2% adults. According to the criteria of DSM-IV, diagnosis of ADHD requires that a child meet six of nine criteria for inattention (inattentive subtype), or six of nine criteria for hyperactivity/impulsivity (hyperactive/impulsive subtype), or both (combined subtype). The behavioral problems must also have started before the age of 7 and must have persisted for at least 6 months in at least two different settings (*e.g.*, school and home). Boys are affect-

ed three times more often than girls in the general population and 10 times more often in clinical settings. In adults, this ratio becomes 2:1 in the general population. High levels of comorbidity with other psychiatric disorders are common in both sexes.

Attention deficit/hyperactivity disorder has an estimated heritability of 75-91%. The relative risk for siblings of affected children increases approximately 5-fold. Despite much research, the etiology of ADHD has still not been clarified.

A genome scan was performed on 164 Dutch affected sibling pairs with ADHD [39] and has located several susceptibility loci, two of which (on chromosome regions 7p and 15q) are suggestive for linkage. The 15q region is particularly interesting, since it has also been implicated in autism and reading disability. These results may provide new directions in the search for specific genetic determinants of ADHD. In another study [40], significant linkage was found on chromosome 16p13 in a region already implicated in autism. It has been suggested that ADHD and ASD, which also have a high heritability, have common genetic factors in common. According to the DSM-IV classification, a diagnosis of ASD rules out the diagnosis of ADHD; however, a substantial number of patients with ADHD have mild problems with social interactions and communication that are rather similar to the symptoms of autism. A subgroup of autistic children also showed high levels of inattention, hyperactivity, and impulsivity. Follow-up studies suggest other regions as highly likely to harbor risk genes for ADHD: 12p13, 2q24 [41], 17p11 [42].

CONCLUSIONS

Although the links between autism and certain diagnosable conditions are often convincing, the number of individuals who are within the autistic spectrum and have known genetic or non genetic conditions is only a small percentage of the whole, and an association with autism is not universal in any one of the diagnosable medical or genetic conditions mentioned. In population-based studies of children with autism, such conditions account for a small minority, probably <10%. However family studies indicate that genetics play the major causative role in most individuals with "idiopathic" autism. Since all patients with a chromosomal imbalance

are dysmorphic, the association of ASD with a facial dysmorphism seems to be a good indication for chromosomal anomaly screening. Findings such as micro- or macrocephaly, abnormal finger digit ratios, and other dysmorphic features, are associated with various developmental abnormalities of the brain and mental retardation. Because the frequency of specific diagnoses is highest in children with cognitive impairment or congenital anomalies, it is recommended that for routine clinical care, extensive testing be limited to those with a suspicious family or medical history, mental retardation, or dysmorphism and to families who wish to have additional children, as different genetic disorders have different recurrence risks.

Although prenatal diagnosis is possible for defined disorders such as FXS, there is no prenatal test to identify "idiopathic" autism. Given the recurrence rate of 2 to 8% in siblings of affected children, and that the initial diagnosis of autism is made between 1 and 4 years of age, it is especially important to offer parents information about their recurrence risks before they conceive another child.

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