

Genetics of Childhood Disorders: XLVI. Autism, Part 5: Genetics of Autism

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Autism is one of the most heritable complex genetic disorders in psychiatry. Despite this high heritability, autism has a heterogeneous etiology, with multiple genes and chromosomal regions likely to be involved. Scientists are using both indirect and direct approaches to identify autism susceptibility genes. Indirect approaches include the characterization of less complex genetic disorders that share some of the symptoms of autism, including Rett syndrome or fragile X syndrome, in the hope that these analyses will provide clues to the more complex disorder of autism (see recent reviews in this space, February and May 2000). Direct approaches include three overlapping methodologies to identify genes or regions of interest in autism: chromosomal methods, such as karyotyping and fluorescence in situ hybridization (FISH); linkage studies, such as genome

screens in affected sibling pairs; and gene association studies, including candidate gene studies. These approaches are yielding preliminary findings that are reviewed here. While no specific gene variant has been identified and confirmed that contributes to the expression of autism, it is very likely that several will be confirmed over the next decade.

Twin and sibling studies demonstrate heritability in autism. The monozygotic twin of a patient with autism, who shares nearly 100% of nuclear DNA, has an approximately 60% chance of having autism, while the concordance for an autism spectrum disorder is greater than 90%. The dizygotic twin of a patient, who shares 50% of the genes, has approximately the same risk as a sibling, about 4.5%. A member of the general population has approximately 0.2% chance of having autism.

Chromosome 2

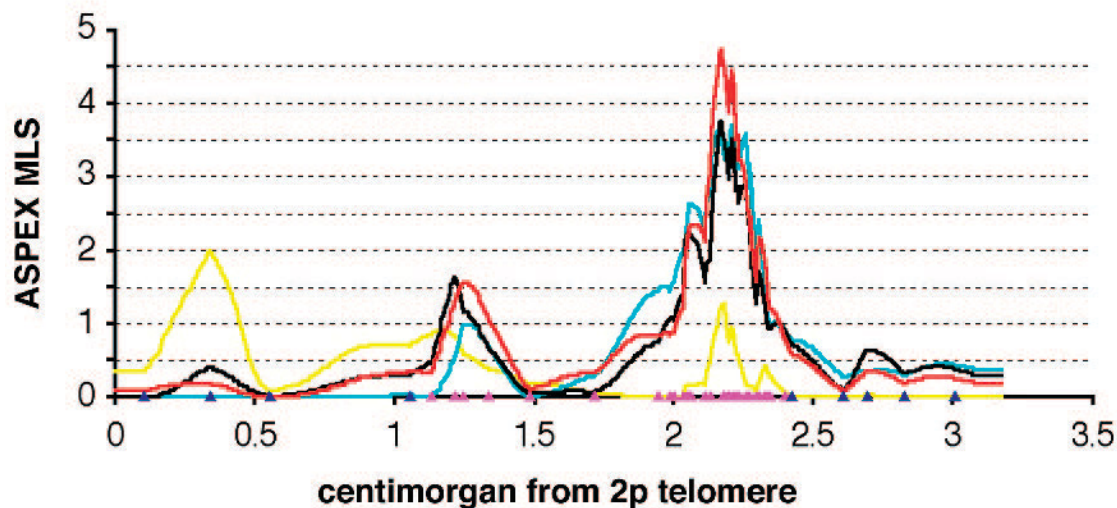


Fig. 1 Chromosome 2 linkage map generated in ASPEX for autism sibling pairs analyzed by case type. The black line represents all 153 sibling pairs. The yellow line represents pairs in which both siblings met the following criteria: clinical diagnosis of autism; Autism Diagnostic Inventory (ADI) and Autism Diagnostic Observation Schedule (ADOS) criteria for autism; IQ > 35; and language delay (44 type I-type I pairs). The blue line represents pairs in which one sibling met the above criteria and the other met at least the following criteria: clinical diagnosis of autism, Asperger's syndrome, or pervasive developmental disorder (PDD); no more than 1 point below threshold in only one behavioral domain on the ADI; at least ADOS criteria for PDD; measurable IQ; language delay not necessary (81 type I-type II pairs). The red line combines the two types of strict criteria pairs described above (125 strict criteria pairs). Dark triangles represent markers typed in the original 86 sib pairs and pink triangles markers typed in all 153 sib pairs. MLS = multipoint maximum LOD score. Adapted with permission from International Molecular Genetic Study of Autism Consortium (IMGSAC) (2001), A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. *Am J Hum Genet* 69:570-581 (published by The University of Chicago Press). © 2001 by The American Society of Human Genetics.

The ratios between these different risks provide strong evidence for heritability. The dramatically diminished risk in relatives who share 50% versus 100% of their DNA is most consistent with an oligogenic inheritance pattern, where more than 2 and as many as 100 genetic variants may contribute to susceptibility to developing autism.

Genetic heterogeneity in autism is no surprise to clinicians familiar with the varied presentation of the disorder. Recent advances in diagnosis, such as the Autism Diagnostic Inventory and the Autism Diagnostic Observation Schedule, may reduce uncertainty in diagnosis, but clinical heterogeneity remains. Symptoms and signs, rather than etiologies, currently characterize psychiatric syndromes such as autism, schizophrenia, or attention-deficit/hyperactivity disorder, as well as other medical syndromes, including diabetes and asthma. Each gene may make a different contribution to the disorder, with gene A more important for the development of social cognition and gene B more important for language acquisition.

On the other hand, different variants in the same gene may also produce different clinical pictures. When clustering of risk alleles reaches a certain threshold, an individual is at increased risk of developing the disorder. A subthreshold number of risk alleles may result in the broader autism phenotype identified in family members of patients with autism. It is also likely that several variants that contribute to susceptibility to autism (and other childhood-onset psychiatric disorders) will be relatively common in the general population and may even be advantageous (e.g., a hypothetical variant of a gene may heighten focused attention and add to risk for autism but in another context be helpful).

Language disturbance is an important characteristic of autism. A mutation in the transcription factor FOXP2 on chromosome 7q was found in affected members of a family with an autosomal dominant speech and language disturbance. However, this gene was screened in a large sample of children with autism and no evidence was found for its relationship to that disorder.

A large number of chromosomal abnormalities have been reported in autism. The study of these abnormalities serves dual purposes. First, these abnormalities may be characteristic of specific subsyndromes. Second, they point to the chromosomal location of a gene that may have other types of mutations (i.e., point mutations or deletions too small to be visible by karyotyping). In this way, researchers will get hints that a gene or gene system may be involved in patients without visible chromosomal abnormalities.

Chromosomal abnormalities have been reported on chromosomes 2q37, 7q, and 22q13, among others. However, the most common specific cause of autism appears to be maternally inherited duplications of chromosome 15q11-13, accounting for 1% to 3% of cases. Maternal duplication of this region is also found in another childhood developmental disorder, Angelman syndrome. There are several types of mutations in

this region that result in Angelman syndrome. A minority of cases have been found to be the result of a point mutation in a gene termed *UBE3A*. The majority of cases are caused by a large, visible deletion that removes several genes surrounding *UBE3A*, or after inheritance of two copies of the paternal copy of chromosome 15 (uniparental disomy) (Angelman syndrome was reviewed in this column in July 2000). In some brain areas or developmental periods, a few genes in the region, including *UBE3A* and *ATP10C*, are active only when they are inherited from the mother.

Linkage studies use highly variable polymorphisms spaced evenly throughout the genome to identify chromosomal regions shared among family members who are affected with a particular disease. The likelihood of linkage at a given point in the genome is based on the percentage of sharing between affected family members at the nearest polymorphisms. Linkage results are presented as a LOD score, which is the log (base 10) of the ratio of the likelihood of linkage relative to no linkage. Several genomewide linkage studies have been reported in autism over the past few years, primarily using affected sibling pairs. Almost all studies failed to find significant evidence for linkage. A peak on chromosome 2q achieved strict genomewide significance in a single study (LOD 4.8, $p = 1.2 \times 10^{-6}$). Meta-analysis of the first five published studies implicates two additional regions of interest on chromosome 7q and 13q. Smaller linkage peaks have been reported in numerous chromosomal regions.

Once significant linkage findings have been identified, the next step is to use more closely packed polymorphisms to pinpoint the area of linkage. Initial studies suggest that narrowing the phenotype of interest may also help in identifying a gene. While a large number of variables could be used to identify a subphenotype with greater evidence for linkage in a region, investigators have begun by narrowing the diagnostic criteria. For example, restricting the analysis to children with autism accompanied by severe language delays increased linkage findings on both 7q and 2q. Other variables that might be appropriate for consideration include head circumference, platelet serotonin, seizure disorder, and restrictive or repetitive behaviors.

Genetic association studies are often used both in the initial approach and in the final step in the dissection of complex genetic disease. Association studies look for excess sharing of alleles not just in family members with a syndrome, but among individuals with the disorder across families. Initial genetic association studies compared allele frequencies in patients in comparison with controls from the general population, but these studies were liable to population stratification bias due to undetected differences in the genetic backgrounds of the two groups of subjects. The transmission-disequilibrium test applied to parent-child trios uses family-based controls to avoid this type of bias.

The initial approach to most psychiatric disorders has considered candidate genes, thought to be involved on the basis

of pathophysiology. Some studies have merged this approach with genomic approaches, studying only those candidate genes that lie in chromosomal regions implicated by other genetic evidence, such as karyotyping analyses. Finally, following significant linkage studies, the last step in identifying complex disease genes is association mapping in regions of interest without regard to hypothetical candidate genes. When genotyping thousands of polymorphisms in each subject becomes economically feasible, genomewide searches will harness the increased power to detect gene association rather than linkage.

A number of family-based gene associations have been reported in autism. Two of these association findings have been replicated at least once, although negative association studies have also been reported. The serotonin transporter gene (*SLC6A4*) was selected as a candidate gene because of hyperserotonemia observed in approximately 25% of patients with autism and the efficacy of serotonin transporter inhibitors in treating rituals and preoccupations associated with anxiety or aggression. Most studies of *SLC6A4* have found association with a promoter variant (5-HTTLPR), but other studies have found association with opposite alleles. Following up on these findings, a linkage study found a single-point LOD score of 3.6 ($p = 2.2 \times 10^{-5}$) at a variant in the first intron of *SLC6A4*. A recent study found more significant association with other *SLC6A4* polymorphisms. Association has also been assessed at a number of genes in the chromosome 15q11-13 duplication region. A polymorphism in the GABA type A receptor $\beta 3$ subunit (*GABRB3*) has been associated with autism in two studies, but association has also been reported with a separate polymorphism near *GABRB3* and a polymorphism in the nearby Angelman disorder gene, *UBE3A*.

A number of promising association studies have not yet been replicated. Evidence for maternal transmission of alleles at three polymorphisms was identified within the glutamate receptor 6 gene (*GRIK2*) on chromosome 6q21, a region with suggestive evidence for linkage in one sample. Association has also been reported at two genes on chromosome 7q near the linkage finding: *RELN*, a gene that is mutated in recessive lissencephaly; and *WNT2*, a gene implicated in the development of the central nervous system.

Initial genetic studies in autism have been promising, but the detection of genetic variants responsible for disease has thus far been elusive. This is not unexpected in a complex genetic disease. Subgroups of patients may be identified with a simpler genetic etiology. However, most patients are likely to have at least 2 genes, and perhaps as many as 100, acting in concert to cause susceptibility to the disorder. Several initial linkage studies have yielded suggestive results in various chromosomal regions, but only a few of these regions have been implicated in more than one study, particularly at 2q and 7q. Significant association findings with a number of candidate genes are encouraging, but efforts to replicate these findings have been mixed.

Although a disadvantage in studying a complex genetic disorder is the difficulty in identifying the variants, a potential advantage is that more options for intervention may be implicated by multiple genes, and these genes may be related to a developmental neurobiological system that is not yet fully understood. Chromosomal findings such as FRAXA and maternally inherited duplications 15q11-q13 do provide additional information about recurrence risk for many families. However, it is important to recognize that for almost all cases, genetic testing is unlikely to establish a diagnosis of autism in the absence of careful clinical evaluation, since FRAXA, maternally inherited 15q11-q13 duplications, and other syndromes greatly increase the risk for autism, but do not lead to autism spectrum disorders in all cases.

WEB SITES OF INTEREST

Autism Society of America: <http://www.autism-society.org>
 Cure Autism Now: <http://www.canfoundation.org/>
 Yale Child Study Center: <http://info.med.yale.edu/chldstudy/autism/>
 TEACCH: <http://www.teacch.com>
 Centers for Disease Control and Prevention: <http://www.cdc.gov/ncbddd/dd/ddautism.htm>

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