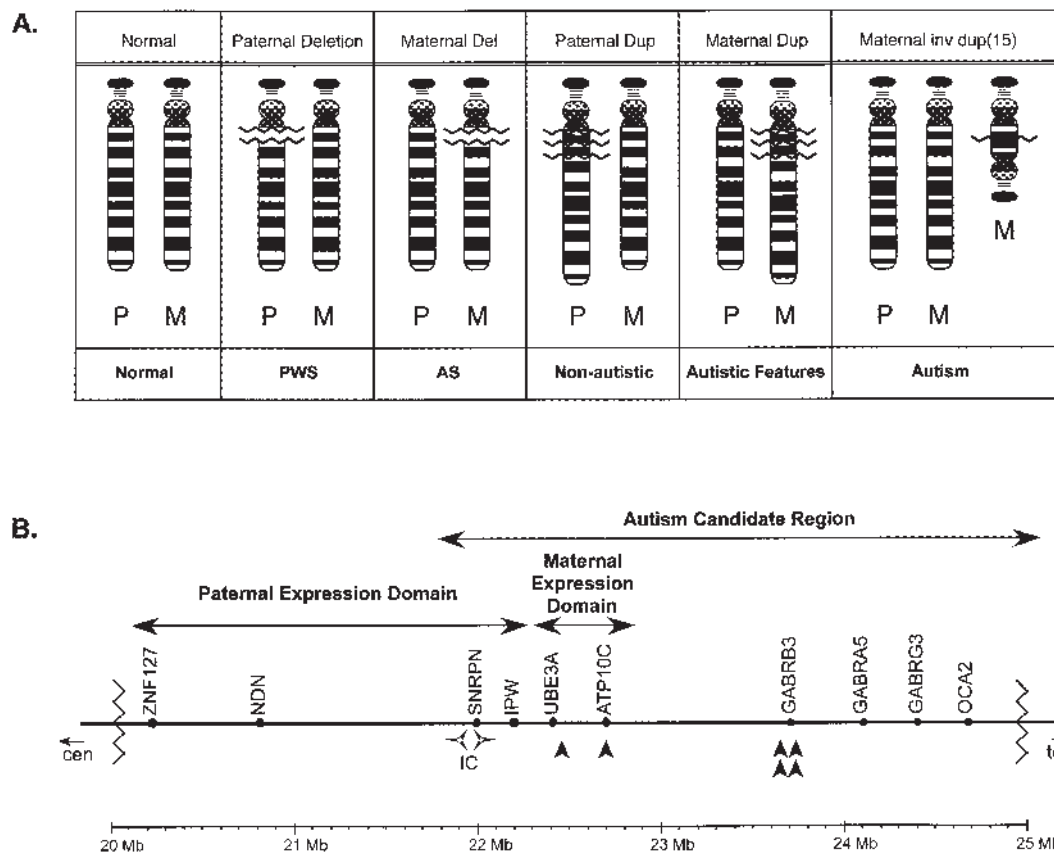


# Genetics of Childhood Disorders: XLVII. Autism, Part 6: Duplication and Inherited Susceptibility of Chromosome 15q11-q13 Genes in Autism

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Autism is a neuropsychiatric disorder that exhibits high heritability and is considered to have a complex genetic etiology. A sibling of a child with autism has a 25 to 50 times greater risk for developing autism than someone in the general population.

Autism displays both clinical and genetic heterogeneity, as reviewed in last month's column. A different set of genes may confer risk in different families or individuals (genetic heterogeneity), and different siblings in a given family may have a different clinical



**Fig. 1** A: Cytogenetic abnormalities associated with human chromosome 15q11-q13. The first panel shows a normal paternal (P) and maternal (M) chromosome 15. Deletions of 15q11-q13 arising from either the paternal or maternal chromosome result in Prader-Willi syndrome (PWS, second panel) or Angelman syndrome (AS, third panel). Paternal duplications do not produce an autistic phenotype (fourth panel). Interstitial duplications of the same common interval derived from the maternal chromosome (fifth panel), as well as a small duplication of portions of the maternal chromosome (sixth panel), are associated with autistic features. B: Schematic of the PWS/AS/autism common deletion/duplication region in chromosome 15q11-q13. The 15q11-q13 region consists of a large proximal domain of paternally expressed genes, a smaller region of maternally expressed genes, and a large distal region of apparently biallelic expression. Some of the known gene positions are shown. Imprinted gene expression throughout the region is mediated by an imprinting center (IC) near the SNRPN promoter. The autism candidate region can be narrowed to an approximately 3 megabase (Mb) interval, with a centromeric boundary defined by the IC and a telomeric border limited by the common distal deletion/duplication breakpoint (jagged lines). Regions of reported linkage and linkage disequilibrium are denoted by black arrowheads.

presentation (clinical heterogeneity). Research groups have been trying to identify susceptibility genes through genome-wide linkage studies and candidate gene analysis. The former typically identifies regions of the genome that are more frequently shared by affected sibling pairs with autism than would be predicted by chance. The latter typically takes the form of allelic association studies in which alleles of a given gene are tested for evidence of preferential transmission to affected family members (family-based) or for differences in allele frequency between autism and control populations (case-control). Despite evidence for high heritability, data emerging from genomic linkage screens suggest that there may be 10 or more major genes underlying autism susceptibility and that different sets of genes may be responsible for risk in different families.

In addition to linkage and candidate gene studies, chromosomal abnormalities may provide important clues to the identification of genes underlying autism, or any genetic disease. For example, several chromosomal translocations and other abnormalities have been identified that affect the region of 7q in virtually all genomic linkage studies of autism. Traditionally in human genetics, chromosomal abnormalities are of substantial utility in identifying a discrete region harboring a disease gene. The most frequent chromosomal abnormality seen in autism populations involves duplication of sequences in a region on the proximal part of the long arm of chromosome 15, specifically the interval 15q11-q13. These duplications typically take one of two forms: (1) tandem duplication of a 4–5 million base-pair (Mb) region corresponding to 15q11-q13, or (2) supernumerary pseudodicentric, inverted, and duplicated regions of chromosome 15 [so-called inv dup(15) or idic(15) marker chromosomes] that now contain two additional copies of a larger region. These duplications are associated with substantial risk for autism when derived from maternal but not paternal chromosomes. This parental-specific association suggests a genomic imprinting effect and makes relevant consideration of two disorders that result from interstitial deletion of the same 15q11-q13 region.

Genomic imprinting is an epigenetic phenomenon in which gene expression occurs preferentially from one or the other parentally derived homologous chromosomes. Imprinted gene expression can result in parent-of-origin effects, thus producing complicated inheritance patterns for disorders involving imprinted genes. Paternal-specific deletion of the 15q11-q13 interval results in Prader-Willi syndrome (PWS), while maternal deletion gives rise to the quite distinct Angelman syndrome (AS) phenotype. Although a common region is deleted in both disorders, PWS results from a loss of expression of multiple imprinted, paternally expressed genes, whereas AS arises from the loss of function of a single, imprinted, maternally expressed gene encoding a ubiquitin-protein ligase (*UBE3A*).

PWS presents with infantile hypotonia, failure to thrive, and feeding difficulties due to poor suck reflex. By age 2 years, indi-

viduals with PWS begin to develop hyperphagia and secondary obesity, easily the most manifest aspect of the phenotype. People with PWS display mild to moderate cognitive impairment and physical findings including decreased stature, small hands and feet, almond-shaped eyes, and hypogonadism. Persons with PWS typically have behavioral abnormalities, including aggression, self-abuse, preoccupation with ordering and arranging, resistance to change in daily routines, and food foraging. By contrast, AS is more severe, with profound mental retardation, absent speech, epilepsy, gait ataxia, hand-flapping, and inappropriate laughter. Other causes of these disorders are uniparental disomy (UPD; inheritance of both copies of a given chromosome from the same parent) for chromosome 15 and imprinting defects for both disorders, and maternal-specific point mutations of *UBE3A* for some patients with AS (see columns from June and July 2001).

Numerous cases of chromosome 15 duplication associated with autism have been described, and collectively these reports permit some conclusions. Idic(15) is typically seen affecting maternal chromosomes, is caused by errors in the normal segregation of chromosomes during meiosis, and is possibly correlated with advanced maternal age. The phenotype associated with idic(15) cases is typically more severe than that described for interstitial duplication of 15q11-q13. Many case reports of idic(15) patients describe autistic phenotypes with relatively profound cognitive impairment, learning disability, developmental delay, speech impairment, seizures, poor motor coordination, hypotonia, joint laxity, and motor stereotypies. Some of these patients have even been described as “Angelman-like,” reflecting the neurological symptoms and significant cognitive impairment. Interstitial duplications have been informative in defining the maternal specificity for autism risk. Many, but not all cases of maternal duplication meet criteria for an autism diagnosis using standard measures derived from the Autism Diagnostic Interview and Autism Diagnostic Observation Schedule. The most detailed study comparing the effect of maternal versus paternal 15q duplication showed that none of the paternal duplication cases exhibited evidence for a pervasive developmental disorder, whereas a significant minority of the maternal duplication patients met criteria for autism or related pervasive developmental disorders. A family described by Cook and colleagues was similarly informative, as two children inheriting a duplication from their mother had autism or autism-spectrum phenotypes, while the mother, carrying the duplication on her paternally derived 15, was clinically normal. Thus maternally derived duplication of 15q11-q13 confers significant risk for the development of autism.

While the idic(15) duplication spans a larger genomic interval, these cases also have a greater copy number of 15q11-q13 gene loci. Thus increasing copies of 15q11-q13 may be correlated with increasing severity of phenotype due to a gene dosage effect. Maternal specificity of duplications suggests an imprint-

ing effect, presumably related to expression of the imprinted, maternally expressed genes in the duplicated segment. In addition to *UBE3A*, another maternally expressed gene (*ATP10C*) is located in this region. The *ATP10C* gene product is believed to function as a phospholipid transporter protein that may be involved in CNS signaling. Therefore, overexpression of one or both of these genes, caused by an increase in maternal gene copy, could represent a major underlying molecular factor in the development of autism phenotypes associated with chromosome 15 duplications. However, a contiguous gene duplication effect could also be important. That is, overexpression of *multiple* genes in this region may contribute to the phenotype. This would help explain the more severe phenotype in association with *idic(15)* markers, given the greater number of duplicated genes. Furthermore, maternal UPD 15 provides a theoretically analogous situation with regard to overexpression of imprinted, maternally expressed genes; however, maternal UPD 15 differs in that nonimprinted genes in the region are presumably expressed at normal levels. Although autistic features have occasionally been reported in maternal UPD 15, autism is not a common finding. Taken together, these data support the involvement of both imprinted and nonimprinted genes in 15 duplication-derived autism.

Chromosome 15 duplications are present in only an estimated 1% to 3% of individuals with autism. An important question, therefore, is whether chromosome 15 genes play any role in the inherited risk for autism in the overwhelming majority of subjects without chromosomal abnormality. Results from genomic linkage studies with regard to chromosome 15 are mixed. Of seven linkage screens, three have supported linkage to this region. Existing genetic data converge on a cluster of three  $\gamma$ -aminobutyric acid ( $GABA_A$ ) receptor subunit genes ( $\beta 3$ ,  $\alpha 5$ , and  $\gamma 3$ ). The  $GABA$  receptor genes make attractive functional candidates given a developmental role for  $GABA$ ergic transmission in establishing neuronal connectivity and a central role in the maintenance of inhibitory tone in the adult brain. Moreover,  $GABA_A$  receptor agonists treat a number of conditions related to the autistic phenotype including seizures, anxiety, and social phobia. Candidate gene studies in this region, particularly focused on the  $GABA_A$  receptor subunit cluster, have repeatedly identified the  $\beta 3$  (*GABRB3*) gene. Several groups have reported significant evidence for allelic association at this gene using microsatellite markers. One group has observed association in the maternal expression domain, at DNA marker D15S122, located in the promoter region of *UBE3A*.

The identification and analysis of phenotypic subsets in studies of genetic linkage or allelic association will likely increase power to detect genetic effects in autism. Rather than examining chromosomal sharing in a heterogeneous population of autism families, genetic studies may be performed using only those families that exhibit, for example, significant deficits in language, score high on Autism Diagnostic Interview items

related to compulsions and rigidity, or display more (or less) social impairment. This approach is likely to identify genetically more homogeneous groups of families; analysis of these families should reveal more significant findings for gene locus variants that specifically affect a particular phenotype domain. Support for this strategy is found in efforts to identify those features of the broader autism phenotype that are significantly correlated between siblings and in the frequent observation of these traits in family members who do not have autism. Thus a susceptibility allele at a given gene locus may have a somewhat more specific impact on language or rigidity, for example, and not influence all symptom domains. Several recent reports using language as a basis for defining who is affected reveal significantly increased evidence for genetic linkage to regions on chromosomes 7q, 2q, and 13q. Preliminary studies in this regard also appear to hold promise for the detection of chromosome 15 disease-associated genes.

Phenotypic comparisons of the neurobehavioral disorders resulting from 15q11-q13 defects reveal intriguing aspects of overlap between PWS, AS, and the autism-spectrum disorders. Patients with AS have severe cognitive impairment so direct comparison is problematic, but motor stereotypies, poor motor coordination, seizures, and significant language impairment are features common to both AS and autism. Several interesting behavioral manifestations are seen in both PWS and autism, including compulsions, self-abuse, and comparatively unimpaired or even superior performance in certain discrete cognitive domains. A typical pattern of intellectual disabilities with areas of relative sparing has long been appreciated in autism and can range from performance on par with mental-age-matched controls (so-called splinter skills) to phenomenal savant abilities. These skills often include visuospatial, computational, mnemonic, and musical talents. Individuals with PWS are often regarded as gifted at solving jigsaw puzzles. It is possible, therefore, that there is a biological and genetic basis for the commonalities between these disorders. This remains to be demonstrated, but it is an area of great interest to those groups that study the genetics and psychopathology associated with defects of this chromosomal region.

To localize genetic susceptibility to autism on 15q, several groups are pursuing an assessment of allelic association for the region from *UBE3A* through the *GABR* genes using single nucleotide polymorphism (SNP) markers. SNPs are single base-pair changes in DNA sequence that occur on average every 1,000 base-pairs in the genome. The vast majority of these variations are benign changes, but some may produce physiological effects, giving rise to normal human variation but also risk for disease. SNPs are a valuable tool for mapping disease susceptibility, since they allow investigators to distinguish between the two copies or alleles of a gene. Given the frequency with which these variations occur in human DNA, they can be used to create a dense map for examining allelic association. Inasmuch

as adjacent SNPs are rarely separated by recombination events, particular alleles at nearby SNPs are most often transmitted together. This association of alleles at adjacent markers is known as linkage disequilibrium (LD). Exploiting LD relationships, investigators can detect allelic association at markers near a functional susceptibility variant, even if those markers themselves are not involved in disease. LD studies seek to identify an underlying haplotype (a preserved segment of an ancestral chromosome) that contains a susceptibility variant by detecting association at individual SNPs. An extension of this strategy examines transmission of haplotypes directly to determine whether a broader region may contain a susceptibility allele. Once a region is identified in this way, the surrounding sequence can be directly screened for functional variants that confer autism risk. In the absence of a single ancestral susceptibility allele, where many different risk alleles exist (allelic heterogeneity), this strategy is limited. A complementary approach is to directly screen genes in affected individuals to identify rare disease-associated mutations. An ever-evolving understanding of regional haplotype structure, continual advances in rapid SNP genotyping, and the implementation of methods such as phenotypic subsetting to facilitate genetic analyses, bring new insights, power, and promise to the detection and localization of a chromosome 15 autism susceptibility allele.

#### WEB SITES OF INTEREST

Several columns have appeared in this space that review concepts and techniques mentioned in this column:

Linkage analysis: <http://info.med.yale.edu/chldstudy/plomdevelop/genetics/99julgen.htm>

Association studies: <http://info.med.yale.edu/chldstudy/plomdevelop/genetics/99auggen.htm>

Angelman and Prader-Willi syndromes: <http://info.med.yale.edu/chldstudy/plomdevelop/genetics/00junggen.htm>; <http://info.med.yale.edu/chldstudy/plomdevelop/genetics/00julgen.htm>

#### ADDITIONAL WEB SITES OF INTEREST

<http://www.nimh.nih.gov/publicat/autism.cfm>

<http://www.nichd.nih.gov/autism/>

<http://www.cdc.gov/ncbddd/dd/ddautism.htm>

<http://www.nlm.nih.gov/medlineplus/autism.html>

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