THE MOLECULAR AND CELLULAR GENETICS OF AUTISM

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Autism is a behavioral syndrome that is generally associated with lifelong impairment and often confers a substantial burden on the families of affected individuals. Epidemiologic research over the past two decades has demonstrated a significant role for hereditary factors in the etiology of autism, stimulating an aggressive search for susceptibility genes. This chapter summarizes these efforts to elucidate the genetic basis of this severe neurodevelopmental disorder.

THE PHENOTYPE

The three core symptom domains of autism are excessive ritualistic and repetitive behaviors, deficits in communication, and abnormal social interaction. These domains encompass a broad spectrum of behavioral and cognitive abnormalities such as speech delay, echolalia, decreased spontaneous affection, reduced eye-to-eye gaze, motor stereotypies, rigid adherence to routine and environment, and others. The onset of autism is variable, typically manifesting at age when the normal complements, such as speech and prosocial activity, to the disturbed behaviors are expected to develop (usually by the age of 2). Symptoms change with development and generally continue throughout life. When first described in 1943, infantile autism was narrowly defined, referring to a population of children with severe manifestations of all core features and generally higher IQ levels. Kanner, for example, in his original descriptions of autism,

included elaborate stereotyped behaviors (e.g., complex rituals and marked distress in response to change in routine or environment) as an essential, pathognomonic component. The severity of this component, however, has gradually been relaxed so that current criteria require the presence of only milder behaviors in this domain.

Similarly, the concept of autism itself has been broadened and now includes the group of syndromes referred to as pervasive developmental disorders (PDDs). Specific PDDs, in addition to autistic disorder, include Asperger's syndrome, pervasive developmental disorder not otherwise specified (NOS), disintegrative disorder, and Rett syndrome. The validity of these diagnostic distinctions, however, is open to question. While the gene that causes Rett syndrome has recently been identified (1), and disintegrative disorder involves a clear loss of function that is likely to arise from a distinct mechanism, there is little evidence to support the remaining PDDs being etiologically distinct.

The existence of multiple symptom domains and the spectrum of related disorders demonstrate the complexity of the autism phenotype. The core symptom domains exist in varying combinations of severity across affected individuals, and it is unclear whether these clinically defined domains represent distinct genetic entities. Less severe symptomatology is represented as a distinct diagnostic entity, but again whether this is genetically justified is not known.

Mental Retardation

Adding to the phenotypic complexity is the wide range of IQs associated with autism. Although some individuals with autism have normal or even exceptional IQs, 70% to 80% are mentally retarded (2). Approximately one-third of those with mental retardation (MR) are in the mildly affected range, the remainder have moderate to severe deficits, and gender proportionality differs across IQ groups (see below).

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Biological Correlates

Investigators have searched for biological correlates of autism hoping to better define and categorize the phenotype. Hyperserotonemia was the first biological abnormality to be reported, found by some in up to one-fourth of autistic individuals (3). There is evidence to suggest that hyperserotonemia in autism may be familial (4,5), and that elevated platelet serotonin levels may index genetic liability for autism (6). Dysmorphic facial features have also been investigated, with one positive finding coming from a report linking autism to an early developmental abnormality in the branchial arches (7). Epilepsy occurs in 15% to 20% of individuals with autism (8), much more frequently than expected by chance, though whether the presence of epilepsy defines an etiologically meaningful autistic subgroup is unclear.

Two other biological traits that have been investigated are head size and brain morphology. Enlarged head size was noted by Kanner in seven of the first 11 children he described in 1943. Subsequent studies have revealed that approximately 20% of autistic individuals have macrocephaly (> 98th percentile for head circumference) (9,10), and the few published postmortem studies report that the brains of autistic individuals are larger and heavier (megalencephalic) than those of normal controls (11,12). Retrospective longitudinal studies of head circumference also suggest that while some enlargement may take place before birth, an increased rate of growth appears to occur during the early postnatal period (10,13). Magnetic resonance imaging (MRI) studies confirm that brain volume in autism is increased (14,15). This enlargement, rather than being generalized, may be confined to discrete structures (16). One recent report, for example, found evidence linking abnormalities in caudate volume to ritualistic-repetitive behaviors in subjects with autism, a finding that is similar to reported relationships in Tourette's syndrome and obsessive-compulsive disorder (17).

As with the core autistic symptomatology, however, these biological correlates are highly variable across individuals, and none is yet able to independently identify cases or define meaningful subgroups. As our ability to measure these correlates becomes more precise, however, their value is likely to increase. They may then serve the purpose of adding power to genetic studies by increasing phenotypic information.

EPIDEMIOLOGY AND GENETIC MECHANISMS

Prevalence

The estimated prevalence of autism has increased since the mid-1980s from 3 to 5 cases per 10,000 to a current estimate of 6 to 10 per 10,000 (2,18). This increase is most likely attributable to changing diagnostic practices and increased ascertainment. Epidemiologic studies in the 1970s and early 1980s were based primarily on Kanner's strict diagnostic criteria. With the incorporation of less stringent criteria into the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) and the International Classification of Diseases (ICD), some individuals are now diagnosed with autism who would not have been previously (18). The prevalence of the other PDDs, such as Asperger's disorder and PDD NOS, has not been studied as thoroughly as that of autism. Estimates, therefore, vary widely, though median figures from extant studies suggest a rate 1.5 to 2 times that of autism (2). Thus, taken together, the PDDs may affect 15 to 25 per 10,000 school-aged children. Given that autism is a lifelong condition, the prevalence in adults is likely to be similar to that found in children.

Family and Twin Studies

Family and twin studies help to determine the pattern and strength of the heritability of a disorder. The recurrence risk of autism for siblings of autistic probands is approximately 4% to 5% (19), translating to a sibling relative risk (sibling recurrence risk/population prevalence) of roughly 50 to 100. The risk to second- and third-degree relatives drops off dramatically to less than 1% (20). Twin studies, which compare concordance rates between monozygotic (MZ) and dizygotic (DZ) twins, estimate the heritability for autism to be greater than 90% (21,22).

Gender Differences

All epidemiologic studies of autism demonstrate a male preponderance of the disorder. The overall ratio of males to females is approximately 4:1, though this varies with IQ, approaching 6:1 in normal IQ groups and being less than 2:1 in moderate to severe MR groups (2).

Associated Medical Conditions

A host of medical conditions have been reported to cause occasional cases of autism, including neurofibromatosis, tuberous sclerosis (TS), phenylketonuria, rubella, cerebral palsy, trisomy 21, and epilepsy (23). For most of these disorders, however, whether they occur in autism more frequently than expected by chance is unclear. TS has the strongest association; its population prevalence is 1/10,000, and up to 25% of individuals with TS meet diagnostic criteria for autism or PDD (24) (discussed in more detail below). Overall, it has been estimated that approximately 5% of autistic individuals have an associated medical condition that may play an etiologic role in the development of the disorder (2).

Environmental Determinants

Investigators have repeatedly postulated that *in utero* events might predispose a fetus to the development of autism. Early twin studies, for example, suggested that obstetric complications differentiated autistic twins from nonautistic co-twins (25). Subsequent examination of these and other data, however, has shown that the obstetric complications are typically quite minor, the association between autism and complications is weak (26), and that the causality may be inverted—an impaired fetus may actually predispose to obstetric complications instead of complications having affected the fetus (27). Similarly, some studies have reported associations between viral infections [i.e., rubella (28), cytomegalovirus] during pregnancy or season of birth and the subsequent development of autism. The weight of evidence, however, either fails to support such associations or suggests that they account for only a small minority of autism cases (29,30). Thus, although perinatal factors are reasonably inferred in rare instances (e.g., encephalitis), in most cases they appear to have either a negligible effect or a minor effect of undetermined significance.

Chromosomal Abnormalities

Estimates of the frequency of chromosomal abnormalities in autism vary widely. Early studies reported rates as high as 20% (31), though recent surveys have reported lower frequencies ranging from 3% to 8% (32–34; Wassink et al., submitted), with the fragile X mutation accounting for one-third to one-half of these. These rates may increase, however, as more sophisticated molecular cytogenetic techniques are applied. Up to 10% of unexplained cases of MR, for example, have been found to be associated with cytogenetic abnormalities detectable only by recently developed subtelomeric probes, and similar abnormalities may be found in autism as well. The most common chromosomal abnormalities currently associated with autism include the fragile X mutation, other sex chromosome abnormalities, and abnormalities of 15q11-q13 (the Prader-Willi/Angelman syndrome (PW/AS) region).

Genetic Mechanisms

Thus, although a small proportion of cases of autism are due to chromosomal abnormalities or medical conditions, the vast majority are likely to be multifactorial, arising from an as yet unknown environmental component superimposed on a strong genetic predisposition. The heritability for autism of 90% exceeds that of other common psychiatric disorders such as schizophrenia, bipolar disorder, or alcoholism. The mode of heritability, like other psychiatric disorders, appears to be complex. Autism pedigrees have not been reported that demonstrate mendelian segregation (unless the broader autism phenotype is included—see below), and

the differential gender distribution across IQs suggests genetic heterogeneity. The rapidly diminishing relative risk from first- to second- to third-degree relatives, combined with the >4:1 MZ:DZ concordance ratio, indicates that autism is likely to be due to multiple genes interacting in variable combinations in additive, multiplicative, epistatic, or as yet unknown fashions (35). Estimates of the number of genes involved have ranged from at least three (36) to more than 15 (37). Furthermore, other disorders composed of isolated components of the autism phenotype (e.g., specific language impairment) are themselves considered to be due to multiple, interacting genes, making it likely that the genetics of autism will be complicated as well.

GENETIC INVESTIGATIONS OF AUTISM

Early genetic investigations of autism were hampered by a number of constraints, including small sample sizes, inconsistent diagnostic criteria, and limited molecular tools. The development of standardized diagnostic criteria and advanced molecular tools, such as high-quality, densely spaced genetic markers, FISH (fluorescent in situ hybridization) chromosomal probes, and high-throughput sequencing, is beginning to overcome these constraints. Multicenter collaborations can now gather large, consistently characterized samples, genome-wide screens are practical, sequence data are available for focused genetic investigations, and chromosomal studies are more exact and informative. These advances are reflected in the recent surge of genetic investigations of autism, which are summarized below.

Genome-Wide and Focused Linkage and Association Studies

Four genome-wide linkage studies of autism have been published to date (37–40). All these studies have examined families containing at least two affected siblings [affected sibling pair (ASP) families] and are summarized in Table 41.1. The strongest single finding to emerge from these screens is a multipoint heterogeneity logarithm of odds (LOD) score of 3.0 on chromosome 13q at 55.3 centimorgans (cM) reported by the Collaborative Linkage Study of Autism (CLSA) (39), whereas the most replicated finding consists of support for linkage to chromosome 7q.

Some caution must be taken when comparing these studies, however, because none of them report exactly the same statistic. The CLSA (39) reported a maximum multipoint LOD score (MLS), calculated using the program GENE-HUNTER (41) and based on a likelihood that allowed explicitly for heterogeneity (42). The Stanford group also reported an MLS (37), but the underlying likelihood was parameterized in terms of a multiplicative model allowing for dominance variance, and calculated using ASPEX (43, 44). The International Molecular Genetic Study of Autism

TABLE 41.1. GENOME-WIDE LINKAGE STUDIES OF AUTISM

Research Group	Sample Characteristics				Findings		
	Sample	Markers	Chrom	Markers	cM	MLS	Comments
IMGSAC (38)	87 ASPs	354	7q	D7S530	134.6	2.53	Multipoint analyses were calculated using
			4p	D4S412	4.8	1.55	ASPEX under an additive model that
			16q	D16S407	17.3	1.51	assumed no dominance variance; in a
			22q	D22S264	5.0	1.39	subsample of 56 ASPs, all from the UK,
			10q	D10S197	51.9	1.36	the MLS at D7S530 was 3.55
			14q	D14S70	32.9	0.99	
			19q	D19S49	48.2	0.99	
PARISS (40)	51 ASPs	264	6q	D6S283	132.8	2.23	The MLS was maximized over the
			19q	D19S226	24.1	1.37	"possible triangle" using
			15q	D15S118	41.1	1.10	MAPMAKER/SIBS
			7q	D7S486	135.3	0.83	
Stanford (37)	147 ASPs	519	1p	D1S1675	149.2	2.15	This study used ASPEX to calculate a
			17p	D17S1876	10.7	1.21	multiplicative model that allowed for
			7p	D7S2564	41.7	1.01	dominance variance; this study also
			18q	D18S878		1.00	found a diffuse excess of allele sharing
			7q	D7S684	147.2	0.62	across the genome, suggesting a large
			7q	D7S1804	137.0	0.93	number (≥15) of genes of small effect
			13q	D13S800	55.3	0.68	
CLSA (39)	75 ASPs	349	13q	D13S800	55.3	3.00	This study used GENEHUNTER to calculate
			13q	D13S217	17.2	2.30	an MLS that allowed for heterogeneity
			7q	D7S1813	104.0	2.20	
			4q	D4S2368	167.6	1.52	
			4q	D4S3248	72.5	1.33	
			11q	D11S968	147.8	1.22	
			16q	D16S516	100.4	1.03	
			8q	D8S1477	60.3	1.02	
			15q	D15S975	13.1	0.50	
			7q	D7S1824	149.9	0.79	

ASP, affected sibling pair; cM, centimorgan; MLS, multipoint LOD score.

Consortium (IMGSAC) also used ASPEX to calculated the MLS, but under an additive model that assumed no dominance variance (38). The Paris Autism Research International Sibpair Study (PARISS) used a related MLS, maximized over the "possible triangle" (45), using MAPMAKER/SIBS (40). While all these statistical approaches are related to one another (Huang and Vieland, in press), they may involve estimation of somewhat different numbers of parameters, or "degrees of freedom." The most appropriate use of these data in aggregate, therefore, is not to directly compare numerical results, but rather to look for regions that have either very strong support for linkage within individual studies or that have recurrent support across studies.

Focused genetic studies have examined smaller chromosomal regions chosen for one of three reasons: (a) the region showed evidence of linkage in a genome-wide screen; (b) the region contains a high rate of chromosomal abnormalities associated with autism; or (c) a "candidate" gene of interest, chosen because of its potential biological or developmental relevance to autism, is located in the region. The samples for these focused studies include both ASP families and trios (proband and both parents), and are summarized in Table 41.2.

Chromosome 7

The most replicated evidence for linkage is to chromosome 7q (Fig. 41.1). The IMGSAC, examining 87 ASPs, reported a LOD of 2.53 at D7S530 (134.6 cM), which increased to 3.55 in a subset of 66 United Kingdom ASPs (38). A recent second-stage analysis of 125 ASPs by this same group reported, in poster format, a multipoint MLS near D7S2533 (140.5 cM) of 3.63 (46). The CLSA, examining 75 ASPs, reported an MLS of 2.2 at D7S813 (104 cM) (39), whereas the PARISS (40) and Stanford (37) studies reported modestly positive results (MLS = 0.83 and 0.93, respectively).

A subsequent focused examination of nine 7q markers was performed in 76 multiplex families and 32 trios by the Duke/University of South Carolina (USC) group (47). They found a peak multipoint MLS of 1.77 at D7S2527 (129.0 cM) using an additive model calculated with ASPEX. This group also found high rates of recombination throughout 7q and evidence of linkage disequilibrium at D7S1824

TABLE 41.2. FOCUSED LINKAGE AND ASSOCIATION STUDIES OF AUTISM

Research Group	Ref	Sample	Markers Tested (Significant)	Chrom	Gene	Marker	Result	Test
			Study Characteristics				**Findings**	
IMGSAC	51	91 ASPs, 8 trios	2 (0)	17q11	HTT			Linkage
			7 (0)	15q11-q13				Linkage
Duke	47	76 ASPs, 32 trios	7	7q				Linkage
	48	63 multiplex	14			D7S2527	MLS = 1.77	Linkage
				15q11-q13		D15S217	MLS = 1.78	Linkage
	148	54 trios, 36 ASPs, 33 other	4 (1)	15q11-q13		GABRB3	$p = 0.03$	Linkage disequilibrium
Stanford	149	147 ASPs	8 (0)	15q11-q13			MLS < 0.0	Linkage
	150	97 ASPs	10 (0)	6p	HLA region		MLS < 0.0	Linkage
Other	151	125 trios, 6 ASPs	9 (1)	15q11-q13		GABRB3 155CA-2	$p = .0014$	Linkage disequilibrium
	49	86 trios	2 (1)	17q11	HTT	promoter	$p = .018$	Multiallelic TDT*
	50	117 trios	2 (1)	17q11	HTT	promoter	$p = .049$	Multiallelic TDT*
	152	53 trios	1 (0)	10q21	HTR7			TDT
	153	35 multiplex	4 (0)	Xq27	FMR-1			Linkage
	154	85 probands, 90 controls	3 (0)	17q11	NF-1			Association
	155	10 probands		15q11-q13	UBE3A	exon screening		No mutations
	156	50 probands, 50 controls	5 (1)	11p15	HRAS-1	Bam H1	$p = .008$	χ^2
	157	55 probands, 55 controls includes sample from (169)	2 (2)	11p15	HRAS-1	Bam H1	$p < .05$	χ^2
						Nsi 1	$p < .01$	χ^2
	158	48 probands, 50 controls	1 (1)	11p15	HRAS-1	Msp 1	$p = .034$	χ^2
	159	66 probands, 89 controls	1 (0)	11p15	TH			χ^2
	160	100 probands, 100 controls	2 (1)	7q36	EN2	Pvu II	$p < .01$	χ^2
	161	72 probands, 72 controls	2 (0)	11p15	Ins			χ^2
			1 (0)		IgF			χ^2
	137	19 trios, 62 controls	haplotypes	6p21	HLA-III	C4B null allele	$p = .03$	χ^2
	138	21 trios, 62 controls	haplotypes	6p21	HLA-III	DRβ1 haplotypes	$p < .001$	χ^2
	140	45 probands, 79 controls	haplotypes	6p21	HLA-III	C4B null allele	$p < .001$	χ^2
						DRβ1 haplotypes	$p < .01$	χ^2
	130	44 trios, 6 probands, 79 controls	haplotypes	6p21	HLA-III	DRβ1 HVR-3	$p < .001$	χ^2

*Significant results are for opposite alleles.
ASP, affected sibling pair; CM, centimorgan; MLS, multipoint LOD score; TDT, transmission disequilibrium test.
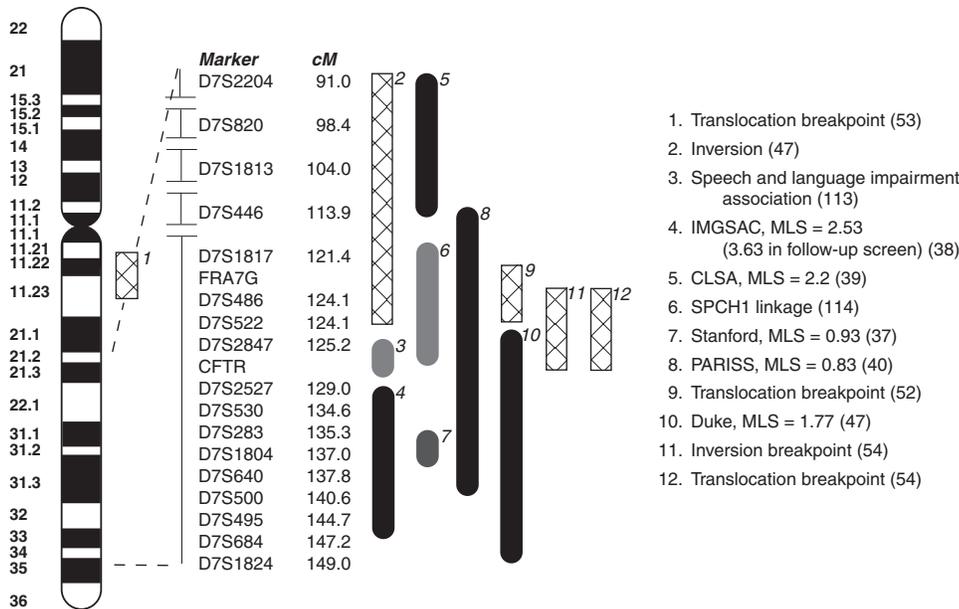
1. Translocation breakpoint (53)
2. Inversion (47)
3. Speech and language impairment association (113)
4. IMGSAC, MLS = 2.53 (3.63 in follow-up screen) (38)
5. CLSA, MLS = 2.2 (39)
6. SPCH1 linkage (114)
7. Stanford, MLS = 0.93 (37)
8. PARISS, MLS = 0.83 (40)
9. Translocation breakpoint (52)
10. Duke, MLS = 1.77 (47)
11. Inversion breakpoint (54)
12. Translocation breakpoint (54)

FIGURE 41.1. Chromosome 7 findings in autism. *Rectangles* represent chromosome abnormalities, *black ovals* indicate regions with evidence for linkage or association in autism, and *gray ovals* indicate regions with evidence for linkage or association in related disorders. MLS, maximum multipoint LOD score within the region indicated.

(149.9 cM), reporting that the linkage, excess recombination, and linkage disequilibrium appeared to be due primarily to paternal and not maternal effects.

Chromosome 15q11-Q13

Because of numerous reports of cytogenetic abnormalities (discussed below), chromosome 15q11-q13 has been intensively examined in linkage and association studies. None of the currently published genome-wide screens, which all included tightly spaced 15q11-q13 markers, identified linkage to the region. The Duke/USC group screened fourteen 15q11-q13 markers in their families and reported a maximum MLS of 1.78 at D15S217 (48). Another group has reported highly significant linkage disequilibrium with the marker GABRB3 155CA-2, a dinucleotide repeat polymorphism that was not typed in any of the genome-wide screens (Buxbaum et al., submitted). This result was obtained when the data were pooled with a number of other focused studies that included this marker.

Candidate Gene Studies

Genes tested as candidates for involvement in autism include genes involved in neurotransmission (i.e., *HTT, TH, DBH*), genes contributing to related disorders (i.e., *FMR-1*, the fragile X syndrome (FXS) gene, and *NF-1*, a neurofibromatosis gene), and genes thought to be involved in brain development (i.e., *EN2, HRAS-1*) (Table 41.2). Though positive results have occasionally been reported, replication has been limited. Cook et al. (49), for example, reported

preferential transmission of the small allele of the *HTT* promoter polymorphism to autistic probands. Klauck et al. (50), attempting replication, reported preferential transmission of the larger allele, and the IMGSAC reported no association with either allele (51). No other candidate genes tested thus far have found consistent support.

CHROMOSOMAL ABNORMALITIES

Studies of cytogenetic abnormalities can complement molecular approaches by identifying genes whose effects are either too small to be detected by linkage or are obscured by epigenetic phenomena. The X chromosome and chromosome 15q11-q13, for example, have both received scrutiny because they are frequent sites, relative to other regions, of cytogenetic abnormalities in individuals with autism. Additionally, linked regions in complex disorders are typically broad, and chromosomal abnormalities occurring in linked regions can help to pinpoint disease susceptibility genes. This can be done either by cloning the chromosomal break points and identifying disrupted genes, or by overlaying deleted regions across individuals in order to delineate a minimal deleted region that might harbor a disease susceptibility gene.

The prevalence of chromosomal abnormalities in autism has been discussed above (see Epidemiology and Genetic Mechanisms). This section, therefore, focuses on abnormalities in specific regions: 7q, 15q11-q13, the sex chromosomes, and the fragile X mutation.

Chromosome 7 Cytogenetic Abnormalities

A number of chromosome 7 cytogenetic abnormalities in close proximity to the 7q linkage findings have been identified in individuals with autism (Fig. 41.1). In one family, a paracentric inversion, inv(7)(q22q31.2), is carried by two brothers, a sister, and their mother (47). The brothers appear to have autistic disorder, the sister has expressive language disorder, and the mother has neither of these abnormalities. Another autistic individual has been described with a translocation t(7;13)(q31.2;q21) (52). The break points for these abnormalities have been cloned, and identification and screening of nearby candidate genes is in progress. Two autistic twins have been found to have translocations t(7; q20)(q11.2;p11.2) (53). The chromosome 7 break point and the gene it disrupts, named "autism-related gene 1" (*ARG1*), have been cloned; the gene is novel with an unknown function, spans an 800-kilobase (kb) genomic region, and is highly expressed in fetal and adult brain (53). Lastly, one individual with autism and another with a specific developmental disorder of speech and language (SDDSL) have been reported, both of whom have chromosomal abnormalities involving 7q31 [autism, 46,XY, inv(7)(p12.2q31.3); and SDDSL, 46,XY,t(2;7)(p23; q31.3)] (54).

Chromosome 15q11-Q13

Chromosome 15q11-13 is the most frequent site of autosomal abnormalities in autism. In a recent chromosomal survey, 6 (2.2%) of 278 autistic subjects referred for cytogenetic studies had a gross abnormality of chromosome 15 (Wassink et al., submitted). These abnormalities most commonly involve duplication of maternal DNA, typically as either interstitial duplications (55–61) or inverted duplicated isodicentric marker chromosomes [inv dup(15)] (62–65). The duplications that produce illness typically extend into the Prader-Willi syndrome (PWS) and Angelman syndrome (AS) critical region, as smaller duplications are generally asymptomatic (64,66). Complementing these data, individuals identified because of 15q11-q13 duplications frequently have autistic features (67). Deletions of 15q11-q13, though less frequent, have also been reported in autistic individuals, with the deleted material usually of paternal origin (68,69). Figure 41.2 summarizes these data, displaying the relevant genes and markers as well as a putative autistic disorder region.

In addition to these gross abnormalities, cytogenetic abnormalities at the molecular level are also being reported. Cook et al. (70), while screening autism trios across 15q11-q13, identified a nonautistic mother in whom a duplication had arisen *de novo* on her paternally derived homologue (70). The duplication was transmitted to one child who
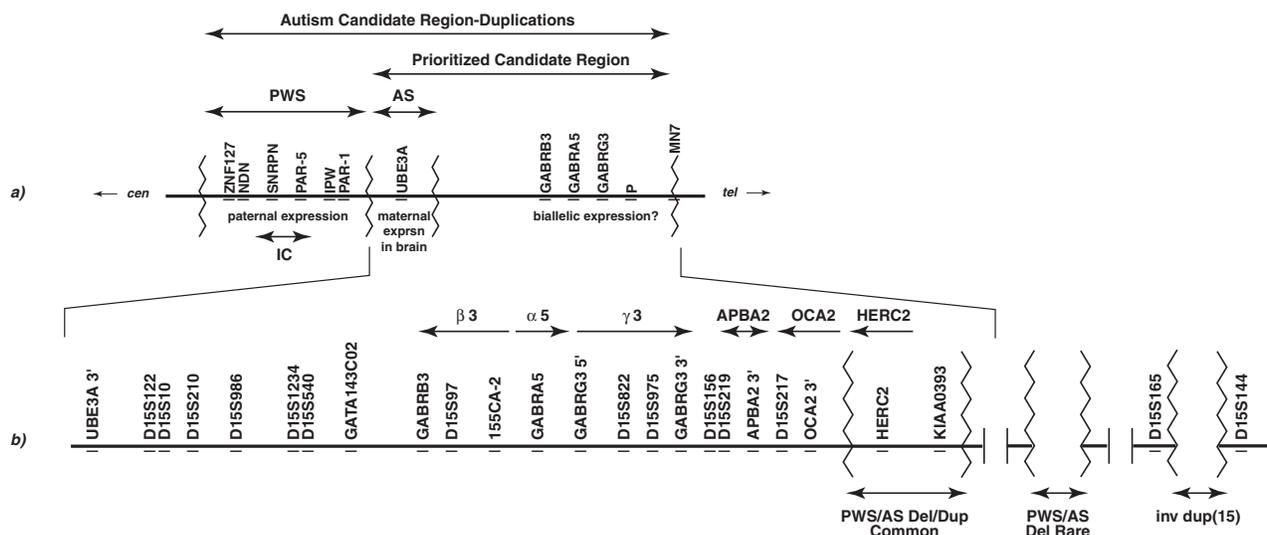

FIGURE 41.2. Schematic map of 15q11-q13 autism candidate region. The *upper part* of the figure is a low-resolution schematic representation of the 15q11-q13 interval deleted in Prader-Willi syndrome (PWS) and Angelman syndrome (AS) and duplicated in cases of autism. PWS and AS critical regions are indicated by *arrows* over the map, with relevant imprinted genes indicated in their respective regions. A prioritized autism region excludes the imprinted PWS domain based on the apparent maternal specificity of 15q11-q13 duplications [interstitial or inv dup(15) markers] in association with autism. The lower expanded region reveals gene and marker order and transcriptional orientation within the candidate region. Large break-point regions are depicted for the primary distal PWS/AS deletion break point, as well as a less common (~10%) PWS/AS break point and a break-point interval associated with inv dup(15) marker chromosomes.

developed autism and a second child with atypical autism, whereas a third child who did not receive the duplication remained unaffected. In the CLSA genomic screen, which examined 75 ASP families (152 affected individuals) and included two 15q11-q13 markers, six individuals (3.9% of probands) from four families (5.3% of families) were found to have either maternal duplications or paternal deletions at one or both of these markers (Wassink et al., submitted). Forty-five unrelated autism trios were subsequently screened using 12 polymorphic 15q markers, with three (6.1%) autistic probands found to have similar abnormalities (Wassink et al., submitted).

The apparent gender specificity of the 15q11-q13 abnormalities is presumably attributable to imprinting, an epigenetic mechanism by which only one of a gene's two inherited alleles is expressed, with expression determined by the allele's gender of origin (71). The two primary 15q11-q13 syndromes, PWS and AS, are oppositely imprinted mental retardation syndromes that bear some phenotypic similarities to autism (72). In brain tissue (but not elsewhere), *UBE3A*, the AS gene, is expressed predominantly from the maternally derived allele. Disrupted expression of the maternal *UBE3A*, therefore, produces AS, whereas disruption of the paternally derived allele produces no discernible abnormal phenotype (73). PWS genes, conversely, are paternally expressed; the predominant cause of PWS, therefore, is disrupted expression of the paternal copy of the small nuclear ribonucleoprotein polypeptide N (SNRPN) gene and other contiguous genes (74). Another recently identified element of imprinting is the presence of antisense transcripts for imprinted genes. These segments of RNA are oppositely imprinted complements to an imprinted gene's coding sequence (75). *UBE3A*, for example, has an antisense transcript that is expressed solely from the paternally derived allele (71). This antisense transcript may play a role in the suppression of the nonexpressed allele, and mutations in this transcript, therefore, could contribute to some cases of AS (76). Thus, just as imprinting plays a pivotal role in PWS and AS, it is likely to significantly influence the effect of 15q11-q13 abnormalities in autism as well.

The 15q11-q13 abnormalities themselves may be due to the presence of repeated or duplicated homologous genomic segments that exist in multiple copies throughout this region (77). Duplications of large genomic segments are associated with chromosomal abnormalities in a number of specific syndromes (78,79). One such repeated segment (duplicon) appears near each of three most common PWS/AS duplication breakpoints (80). Another, located centromeric to the PWS/AS critical region, is repeated an increased and variable number of times in PWS/AS individuals (81). Though this does not imply a traditional repeat expansion mechanism, it may be that these repeats predispose the region to recombination abnormalities or "mistakes" (77), a finding with support from data showing increased rates of recombination across 15q11-q13 in subjects with either PWS/AS (82) or autism (48).

Interestingly, one other chromosomal region that shares many of these genomic features is 22q11 (77). Chromosome 22q11 contains large repeated segments that contribute to a high rate of deletions and duplications (83). These chromosomal abnormalities are associated with a constellation of syndromes grouped under the umbrella term *CATCH-22* (84). One of these syndromes, velocardiofacial syndrome (VCFS), is associated with a high rate of schizophrenia (85), a psychiatric disorder that, like autism, is felt to arise from disturbed brain development. In addition, a small but significant percentage of individuals with schizophrenia have now been shown to have microabnormalities of 22q11 (86). Thus, schizophrenia and autism may share a common genomic mechanism for a subgroup of cases, and insights from one disorder may inform investigations into the other.

Fragile X

The association of autism with the fragile X syndrome (FXS) was first suggested nearly 20 years ago (87,88). The fragile X phenotype is frequently characterized by behaviors that can resemble the core symptom domains of autism such as language abnormalities, decreased nonverbal communication, social isolation, and repetitive motor behaviors such as rocking and hand biting (89). In support of this association, early chromosomal investigations reported a rate of the fragile X mutation [fra(X)(q27.3)] in autism that approached 20% (31,90).

Recent studies, however, have questioned the strength of the link between these two disorders. Current surveys estimate the frequency of fragile X in autism to be 2% to 4% (91–93), similar to the rate of fragile X in the general MR population (94). This is more common than other types of chromosomal abnormalities in autism, though not necessarily disproportionately so. Likewise, there are subtle but significant differences between the behavioral phenotypes of the two disorders. Autism is characterized by social indifference and deficits in the perception of emotion, whereas individuals with FXS experience social anxiety and gaze avoidance with no attendant impairment of emotional perception (95).

Genetically, FXS is a disorder of unstable DNA caused by a trinucleotide repeat that expands as it is transmitted to successive generations (96). The repeat is located at Xq27.3 in the 5′ UTR (untranslated region) of the *FMR1* gene (89). Once this expanded region crosses a threshold (approximately 200 repeats), it becomes susceptible to methylation, which inhibits transcription of *FMR1*. FMRP, the *FMR1* protein, is an RNA binding protein that appears to act as a chaperone for transport of RNA from the nucleus to the cytoplasm (97). FMRP is expressed in numerous tissues including fetal brain. Intracellularly, it is found in the nucleus near the nucleolus and in cytoplasm in associa-

tion with ribosomes. It may function, therefore, as a chaperone molecule in the transportation of messenger RNA (mRNA) from the nucleus to the cytoplasm (98). How dysfunction of this protein gives rise to FXS, however, remains unclear.

Other Sex Chromosome Abnormalities

The possibility of a sex chromosome–related genetic effect is suggested by the preponderance of males affected by autism (2). In accord with this, a sizable number of sex chromosome abnormalities, in addition to the fragile X mutation, have been reported in subjects with autism (99). In a recent survey of a clinical population, six out of 265 (2.3%) autistic individuals referred for cytogenetic testing were found to have abnormalities of the sex chromosomes other than fragile X (Wassink et al., submitted). In addition, two X-linked disorders, Turner syndrome and Rett syndrome, have phenotypic features that are similar to some of the core features of autism. Skuse et al. (100) reported that Turner syndrome (45,X) females with maternally derived X chromosomes had diminished verbal skills and social cognition compared to those with paternally derived Xs. Molecular studies implicated a paternally imprinted disease locus that escapes X-inactivation in distal Xp22.3 (101). This paternal imprinting could explain why karyotypically normal males (who have a maternally derived X) are more vulnerable to developmental disorders of language and social cognition, such as autism, than females. Skuse et al. also reported three Turner syndrome females, all with maternally inherited X chromosomes, who had been diagnosed with autism. Two more XO autistic individuals have recently been reported, one with a maternally derived X (102) and the other with an X of unknown origin (Wassink et al., submitted).

The gene for Rett syndrome was recently identified on Xq28 (1). Rett syndrome, considered to be a subtype of PDD, is a disorder occurring only in girls that is characterized by mental retardation, loss of speech, and stereotypic hand movements after 1 to 2 years of normal development. The gene for Rett syndrome, (*MECP2*), is widely expressed and codes for a DNA binding protein that regulates gene expression (1).

Linkage screens of the X chromosome in autism have generally been negative, excluding genes of even small effect (37,103), and have contributed to a reluctance to examine the sex chromosomes for autism disease genes. The evidence from sex chromosome abnormalities and from X-linked disorders with phenotypic similarities, however, suggests that such pessimism is premature, and that the X and Y chromosomes should continue to be a focus of attention in autism.

BROADER AUTISM PHENOTYPE

In addition to describing the hereditary basis of autism, family and twin studies have demonstrated, in nonautistic relatives of autistic probands, the presence of milder traits that are qualitatively similar to the defining features of autism. These collective traits, referred to as the "broader autism phenotype" (BAP), were first observed by Kanner in parents of autistic children. Bailey et al., replicating and extending findings from the original Folstein and Rutter (104) twin study, found a substantially higher concordance rate for the presence of mild social and communication deficits in MZ versus DZ twin pairs (92% versus 10%) (21). These results are supported by several family studies using the family history method of assessment (105,106). In the London Autism Family Study, Bolton et al. (105) reported that familial aggregation of the BAP was associated with proband verbal IQ. In the Iowa Autism Family Study (Piven and Palmer, submitted) familial aggregation of the BAP was higher in relatives from families with two autistic siblings (multiple-incidence families) than in families ascertained through a single autistic child.

A more detailed examination of the BAP has been accomplished through direct assessment of relatives. Relatives from multiple-incidence families, for example, were found to have (a) elevated rates of personality characteristics such as aloofness and rigidity, (b) diminished pragmatic language and speech abilities, (c) fewer quality friendships, and (d) decreased scores on a number of specific cognitive measures (107–109).

Investigation of the BAP may, by clarifying the range of phenotypic expression of the underlying genetic liability to autism, provide a complementary approach to traditional linkage that increases power to detect genes by identifying more affected individuals, thereby enabling extension of typically small autism pedigrees. Understanding the boundaries and nature of the BAP may also help our efforts to detect genes in autism by enabling focused investigation of specific BAP components (e.g., language deficits, ritualistic-repetitive behaviors, or cognitive deficits) that may map on to separate genes that together cause the full syndrome of autism. This approach to disaggregating complex phenotypes has proved successful in dyslexia, where separate linkages were found to single-word reading and phonemic awareness (110). Clearly, clarification of the genetically relevant aspects of both the autism and the broader autism phenotype is an important strategy to pursue in our search for genes in this disorder.

RELATED DISORDERS

Autism is characterized by dysfunction in three symptom domains: language; social interaction; and repetitive, stereotyped movements and behaviors. As autism is a heterogeneous, genetically complex disorder, it may be that each of these domains has unique, independent genetic determinants. Studying disorders that resemble these individual domains, therefore, may provide insight into their etiology in

autism. There are also related disorders, such as tuberous sclerosis, and domains of investigation, such as immunogenetics, that may provide insight into autism.

Disorders of Language

Specific language impairment (SLI) is a disorder characterized by isolated impairment of language skills, and may be characterized by grammatical impairment, word finding difficulties, or an underlying perceptual deficit (111). Though traditionally considered distinct disorders, SLI has been found to be common in autistic individuals and to occur at higher rates than expected in their nonautistic relatives (108). Conversely, an increased rate of autistic disorder has recently been found in siblings of children with SLI (112). Tying this in to chromosome 7, an association study found significant associations between two 7q31 genetic markers and a group of SLI trios (113). Also, a family has been identified with a severe speech and language disorder characterized by deficits in grammar, expressive language, articulation, and coordination of orofacial musculature (114). A genome-wide screen of this three-generation pedigree found a maximum LOD score of 6.62 at a marker in 7q31, with fine mapping narrowing the region to a 5.6-cM interval (SPCH1 locus). These findings, therefore, may represent localizations of heritable components of the autism phenotype and are of particular interest given the evidence for linkage of autism to this same region of chromosome 7.

Disorders of Repetitive and Stereotyped Behaviors

The phenomenologic overlap between autism, obsessive-compulsive disorder (OCD), and Tourette's syndrome has led some to wonder whether these disorders have etiologic mechanisms in common. For example, caudate volumes have been found to be abnormal in both Tourette's (115) and OCD (116). Therefore, we examined caudate volumes in an MRI study of autistic children and found enlargement of the caudate that was correlated to ritualistic, stereotyped behaviors but not to social or communication deficits (17). In an earlier family study, we reported higher familial aggregation of autism and the BAP in families ascertained through a Kanner proband (more severe ritualistic behavior) versus more broadly defined (DSM, third edition, revised) probands (117). Findings such as these suggest that traits such as stereotypies or ritualistic behavior may have unique genetic determinants that, when combined with genes that give rise to other traits such as language or communication deficits, could give rise to the syndrome of autism.

Disorders of Social Activity

Examples of disorders that involve significant social deficits include Turner syndrome and the fragile X syndrome, both of which have been discussed above (see Other Sex Chromo-some Abnormalities). Additional research related to social deficits that may have relevance to autism comes from studies of various neuropeptide systems. For example, nematode worms that lack receptors for neuropeptide Y become strikingly isolated in situations where they would normally congregate with other worms (118). Genetic variability in receptors for oxytocin/vasopressin in mice and other rodents is also associated with clear variability in social behavior (119). Thus, though there is significant evolutionary distance between worms, rodents, and humans, these transmitter systems may merit closer examination in individuals with autism.

Tuberous Sclerosis

Tuberous sclerosis complex (TSC) is a neurocutaneous disorder characterized by benign tumors affecting numerous organs, most commonly the brain, eyes, skin, kidneys, and heart (120), with a population prevalence estimated at 1/10,000 (121). The occurrence of autism and other behavioral and psychiatric disturbances in the context of TSC has long been recognized (122). Clinic-based and epidemiologic studies of autism in TSC suggest that up to 25% of individuals with TSC meet diagnostic criteria for autism and over 40% meet criteria for any PDD (24,123). Conversely, 1% to 3% of autistic individuals will have TSC (124), though this rate approaches 10% for autistic individuals with seizure disorders (24,123).

TSC is an autosomal-dominant disorder caused by mutation in one of either two genes, *TSC1* or *TSC2* (125). *TSC2* is located on chromosome 16p13 and codes for the protein tuberin, whereas *TSC1* is located on 9q34 and codes for the protein hamartin. Tuberin has numerous functions, acting as a tumor suppressor or a chaperone, and having an influence on cell cycle passage. Dysfunction of tuberin may result in constitutive activation of RAP1, a protein that regulates DNA synthesis and cellular transition, thereby producing excessive proliferation and impaired differentiation of a variety of cell types. Hamartin is one of the proteins for which tuberin acts as a cytosolic chaperone. Other than an ill-defined role in tumor suppression, the function of hamartin remains unknown (126). Approximately two-thirds of TSC cases are sporadic and one-third familial. Half of the familial cases and 75% to 80% of sporadic cases arise from mutations of *TSC2*, with the remainder attributed to *TSC1*. Two studies have shown that TSC due to *TSC2* mutations is more likely to be associated with either mental retardation or intellectual impairment than TSC due to *TSC1* mutations (127,128). Despite the strong association between TSC and autism, however, the mechanistic link between the two disorders remains unclear. Autism in the context of TSC may arise directly from the TSC mutations, from the tubers they produce, or from some other as yet undiscovered mechanism. One group, for example, has reported an association between the presence of temporal lobe tubers and autism (129), though this finding has not been replicated (24).

Immunogenetics

A number of investigators have suggested that some cases of autism may be attributable to interactions between infections, the immune system, and genetic factors (130). Subjects with autism have been shown to have deficits in the number and function of various immune cell subtypes (131–136). A series of investigations, therefore, performed primarily by one research group, has investigated specific components of the major histocompatibility complex (MHC) on chromosome 6p21 for association with autism (130,137–140) (Table 41.2). The samples in these studies were generally small and overlapping, and associations emerged primarily when probands were compared to a population control group as opposed to a parent-based test. Nonetheless, the authors report consistently significant findings that await replication by others.

FUTURE DIRECTIONS

Alternative Sampling Designs

As noted above, all of the genome-wide linkage screens performed to date have focused almost exclusively on affected sibling-pair (ASP) pedigrees. This is in part due to the difficulty of gathering extended autism pedigrees. Also, however, because the mode of inheritance for autism is unknown, many researchers appear to be more comfortable with the simple "model-free" linkage analysis methods available for ASP data than with the more complicated methods required for the analysis of general pedigree structures. Yet, limiting pedigrees solely to ASPs may be unnecessarily and detrimentally restrictive.

The optimal pedigree structure for the detection of linkage depends on the true, underlying genetic model for the trait, which in this case is unknown. Large, multiplex pedigrees, for example, are optimal when a trait is transmitted as a rare dominant but tend to be uninformative if the trait is a common recessive. Furthermore, an ASP data set contains only two primary pieces of information—the number of sibling pairs sharing 1 allele identical by descent and the number of pairs sharing 2 alleles—thereby limiting the complexity of any model that can be fit to ASP data. By limiting the families that are gathered to ASPs, the ability to detect linkage under all possible modes of transmission may be similarly limited. It could be argued, therefore, that precisely because the mode of inheritance for autism is unknown, the optimal strategy would be to ascertain all types of potentially informative pedigree structures. This could include large multigenerational extended pedigrees and moderate-sized pedigrees with more than just two affected individuals, in addition to ASPs.

The difficulty is that there are few larger pedigrees for autism, thus highlighting the potential benefit and utility of the broader autism phenotype. As noted above, classifying individuals with familial autism–related traits as "affected" may increase the prevalence of extended pedigrees and reveal patterns of segregation within those pedigrees beyond what is seen for autism itself. Segregation of such traits (and their underlying genetic diatheses), however, will only be detected if extended pedigrees are sought to begin with. A further benefit to using the BAP is that it enables us to diagnose parents, and possibly siblings, of ASPs as well. Thus even within the nuclear families already collected through existing genomic screens, the amount of linkage information may be greatly increased if indeed the BAP is genetically related to autism.

Another sampling design enabled by the BAP is discordant sibling pair (DSP) analysis. The BAP conceptualizes and measures the traits related to autism along continua. Those subjects who exceed severity thresholds on these measures but do not meet criteria for PDDs are considered to have some form of the BAP. These measures also indicate, however, family members who are very *unaffected*, and therefore discordant, with their autistic siblings. If the traits measured by the BAP truly reflect underlying genetic diatheses toward autism, one would expect reduced allele sharing between DSPs at susceptibility loci as opposed to the excess allele sharing expected in ASPs (141).

The major advantage of the DSP method is that, theoretically, it may require far fewer sibling pairs than the ASP method under some circumstances. Risch and Zhang (142) estimated that DSP analysis may provide the same power to detect disease genes as the ASP method with a sample 10- to 40-fold smaller. The primary disadvantage of the DSP method is that DSPs are difficult to find, and require an extensive screening effort to identify enough pairs.

Microarray Technology

DNA chip microarray technology, which enables the simultaneous analysis of tens of thousands of DNA or RNA sequences, may be of significant benefit to autism research in two primary domains. First, an important focus of the current effort to sequence the human genome is the identification of single nucleotide polymorphisms (SNPs) (143). SNPs are sites of single base pair substitutions, are much more common than di-, tri-, or tetranucleotide polymorphisms, and, in contrast to current methodologies, can be easily and rapidly genotyped using DNA chips (144). The hope, therefore, is that DNA SNP chips will be used to rapidly screen a dense marker map, thereby enabling the performance of genome-wide association studies (145). Implementation of DNA chips for this purpose awaits the development of such SNP maps as well as the statistical and computational tools with which to analyze and interpret the resultant data.

The second potential use of microarray technology is to examine gene expression patterns in relevant tissues. DNA chips can be created that recognize all possible mRNAs that a tissue is currently producing (146). Thus, the expression

of thousands of genes from the brains of autistic individuals and controls can be compared in order to detect etiologically meaningful differences. The primary limitations of this method are the collection of brain tissue, which must be done rapidly and according to exact protocols, and interpretation of the data generated from such experiments (147). Altered gene expression, for example, may be due to medication effects as opposed to an etiologically relevant mechanism. The most meaningful studies of gene expression at this time, therefore, may come from animals genetically designed to exhibit autistic behaviors, where the environment and timing of the tissue analysis can be controlled.

Refining the Phenotype Through Biological Correlates of Illness

The primary purpose for investigating the BAP is to enable identification of meaningful genetic subgroups based on the core symptom domains of autism. Similar strategies are being pursued with various biological correlates of the disorder, including serotonin metabolism, minor physical anomalies, head circumference, brain morphology and function, and others.

CONCLUSION

Numerous complementary strategies are currently being employed to attempt to locate autism disease genes. Linkage studies have identified a number of suggestive loci, most notably distal 7q. Interest in this region is supported by findings from language-disorder families and from a small number of 7q chromosomal anomalies in individuals with autism. Chromosomal anomalies also implicate 15q11-q13 as a region of interest, though linkage and association studies of the region have not been as impressive. These findings clearly demonstrate progress in the effort to find regions harboring genes in autism. As sample sizes continue to grow through collaborative efforts and molecular and statistical methods improve, and as complementary strategies such as the use of the BAP emerge, it seems plausible that in the near future more disease loci will be uncovered and specific autism disease genes may be identified.

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