Autism: recent molecular genetic advances

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Autism (MIM 209850) is a severe neuropsychiatric disorder of unknown aetiology with profound consequences for patients and their families. Strong evidence from twin and family studies indicates the importance of genetic factors in the development of idiopathic autism, although it is clear that these influences are complex. This review focuses on recent molecular investigations to identify susceptibility loci implicated in autistic disorder.

INTRODUCTION

Autism (MIM 209850) is a severe neuropsychiatric disorder characterized by social and communicative impairments and repetitive and stereotyped behaviours and interests. Developmental abnormalities are apparent in the first 3 years of life and the characteristic impairments persist into adulthood. Autism was first described by Kanner (1), and the clinical similarities between autism and Asperger’s syndrome (2) are now recognized; both disorders currently are classified under the diagnostic rubric of Pervasive Developmental Disorders (3,4). The general population prevalence of autism is 5 in 10 000, with a male to female sex ratio of ~3:1 (5,6). Only a minority of cases are associated with recognized medical disorders, estimates varying between 10 and 25% (7–10). The most common associations are with tuberous sclerosis (11) and fragile X (12,13).

From Kanner’s earliest description, particular personality traits were noted in some parents and these seemed to resemble the behavioural characteristics of the affected children. These observations initially were interpreted as evidence of environmental causation: it was suggested that such parental traits, by acting on child-rearing practices, might lead to the development of the disorder. The subsequent recognition that autism was associated with mental retardation in some 75% of cases (14) and with epilepsy in ~33% (15) led to a realization that autism in fact had an organic basis.

There is now strong evidence from twin and family studies for the importance of genetic factors in the development of idiopathic autism, although it is also clear that these influences are complex. Several epidemiological same-sex twin studies have clearly demonstrated significant differences in the mono-zygotic (MZ) and dizygotic (DZ) twin concordance rates (16–18). In the largest of these studies, Bailey et al. (17) found that 60% of MZ pairs were concordant for autism compared with none of the DZ pairs, suggesting a heritability to the liability to autism of >90% assuming a multifactorial threshold model (19). The rate of autism in singleton siblings is also 2–6% (20) which is some 50–100 times the general population prevalence, and together these findings suggest that autism is one of the most strongly genetic childhood-onset psychiatric disorders.

The findings from several twin studies (16,17) have suggested that the autism phenotype in fact extends beyond the traditional diagnostic boundaries. The combined 1995 study found that most of the non-autistic MZ co-twins exhibited milder related social and communicative abnormalities. Family studies similarly have shown a marked increase in the rate of these abnormalities amongst the relatives of autistic individuals compared with the relatives of controls (21,22). With regard to modes of inheritance, the marked difference in pairwise concordance between MZ and DZ twins and the rapid fall off in the rate of autism with decreasing genetic relatedness point to the action of several genes, probably acting epistatically. Latent class modelling of family and twin data also strongly suggests the probable involvement of multiple genetic loci acting epistatically, with three or four loci providing the best fit (23). The association of autism with tuberous sclerosis (11) and fragile X (12) indicates the presence of genetic heterogeneity, and it is likely that idiopathic autism is similarly heterogeneous, although clinical markers have been difficult to identify (20).

The past decade has seen a rapid growth of interest in the aetiology of autism, and many studies utilizing genetic, pharmacological, biochemical and neurobiological approaches are attempting to pinpoint its cause. This review aims to summarize the current state of knowledge and recent progress in autism genetics, which, for the purposes of this review, has been subdivided into three parts. The first reviews cytogenetic and chromosomal abnormalities reported in autism, with particular reference to findings on chromosome 15; the second considers candidate gene studies; whereas the third part reviews the results of recently published genetic linkage studies.

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CYTOGENETIC STUDIES AND CHROMOSOMAL ABNORMALITIES IN AUTISM

Studies of the location of chromosomal abnormalities and breakpoints can be extremely useful in the identification and mapping of genes predisposing to disease. To date, there have been a number of reports in the literature of chromosome aberrations in autism (comprehensively reviewed in ref. 24) covering a broad spectrum of anomalies, including terminal and interstitial deletions, balanced and unbalanced translocations and inversions. Furthermore, instances of marker chromosomes and autosomal or sex chromosome aneuploidies have been reported in autism. With the exception of chromosomes 14 and 20, abnormalities of all of the chromosomes have been associated with autistic behaviour, although no publications involving classically defined autism were identified for chromosomes 12 and 19 (24). Abnormalities of chromosomes 15 (see below) and structural and numerical abnormalities of the sex chromosomes have been the most frequently documented.

However, there have been very few reports of cytogenetic abnormalities associated with autistic disorder in multiplex families. Recently, Ashley-Koch et al. (25) identified an autistic disorder family in which the three siblings had inherited from their mother an identical paracentric inversion on the long arm of chromosome 7, with the breakpoints appearing to coincide with two chromosome 7 common fragile sites. In this family, the two male siblings have autistic disorder, whereas the female sibling has expressive language disorder. This family is of particular interest in relation to recent genetic linkage findings.

The majority of these chromosome abnormalities arise de novo, but the functional significance of these aberrations in autism remains to be established, as balanced translocations occur in otherwise phenotypically normal individuals. It is also interesting to note that several cases of autism have been reported in association with a number of common fragile sites other than those involved in fragile X syndrome (24). These fragile sites may be associated with unstable regions of DNA (26). However, fragile sites appear to be a part of normal chromosomal architecture, and many of these findings may prove to be incidental to autistic disorder. It remains to be determined to what extent submicroscopic chromosomal deletions occur in autism, and whether this disorder is associated with unusually high rates of small cytologically undetectable abnormalities.

Structural abnormalities of chromosome 15 in autistic disorder

Over the last decade or so, there have been numerous reports in the literature of abnormalities of chromosome 15 associated with autism, in particular in those cases associated with mental retardation and seizures (27–30). These abnormalities often take the form of a supernumerary isodicentric chromosome 15, or less frequently as a maternally derived interstitial duplication of the 15q11–q13 region (28,31–35). Recently, a submicroscopic genomic deletion has also been identified in this region (36) with a higher frequency in chromosomes in the autistic population than in unrelated control chromosomes ($P = 0.017$), suggesting that this may be a potential susceptibility marker in the autism population. However, the deletion did not show parent-specific inheritance and did not always segregate with autism in multiplex families. The predominant phenotype of supernumerary chromosome 15 is typified by features characteristic of autistic disorder, including developmental delay, mental retardation, neurological signs and behavioural disturbances. Speech is often absent, and, when present, parroted, echolalic or otherwise abnormal. In addition, individuals may be clumsy, seizures are common and abnormal behaviour may include aggression, hyperactivity, short attention span, frustration, ritualistic behaviour, stereotypic movements, self-mutilation and other autistic behaviours (34,37,38).

The 15q11–q13 region is also of particular interest as it is the critical region for Prader–Willi/Angelman syndromes. Angelman syndrome (AS) (for a review see ref. 39) is a neurological disorder some of whose clinical features resemble autistic behaviours (40). Considerable evidence suggests that the gene or genes implicated in AS are expressed normally only from the maternal copy of chromosome 15 (41). This syndrome results most frequently from a de novo maternal microdeletion of the 15q11–q13 region, but in a few cases is caused by paternal uniparental disomy, or from a putative imprinting defect. The majority of the remaining cases of the disease can be attributed to a mutation in the UBE3A gene, which encodes E6-AP ubiquitin–protein ligase. A more severe AS phenotype has been observed in deletion patients compared with an age-matched non-deletion group (42), and 15q11–q13 deletions were associated with more severe epilepsy than other causes of AS (43).

Chromosome 15 is one of the most complex regions of the genome so far identified in terms of genomic instability and imprinting. It shows a high frequency of deletion events, and accounts for ~50% of all supernumerary marker chromosomes observed in man (44,45). It has been proposed that the genomic instability in the 15q11–q13 region might be due to the involvement of large duplicated genomic segments (46) mediating the frequent rearrangements observed for this chromosome. However, the genotype–phenotype correlation between chromosome 15 structural abnormalities and autistic disorder remains unclear. The frequency of these abnormalities in autism is unknown and, whilst constituting only a small percentage of the total incidence of autism, still appears too high to be incidental. Further studies are required to characterize the relationship between chromosome 15 breakpoints and autism.

CANDIDATE GENE STUDIES IN AUTISM

Candidate gene studies of chromosome 15

The numerous reports of autistic individuals with cytogenetic abnormalities of chromosome 15 have indicated that this region may harbour a potential susceptibility gene or genes for autistic disorder. Several genes have been identified within this chromosomal region, which are potential candidate genes for autism.

One of the most interesting of these is the $\gamma$-aminobutyric acid (GABA$\_\gamma$) receptor gene cluster, containing genes coding for the $\alpha 5$, $\beta 3$ and $\gamma 2$ receptor subunits. GABA is the principal inhibitory neurotransmitter in the mammalian central nervous system, controlling excitability in the adult brain. The
GABAergic system has long been implicated in epilepsy, both in humans and in experimental animal models. GABA receptor expression has been demonstrated to be regulated both regionally and developmentally; therefore, deficits in the GABAergic system have the potential to lead to neuro-developmental abnormalities. The GABA$_A$-$\beta$3 subunit is of particular interest as it is expressed early in development, and loss of the single gabrb3 gene in mice is sufficient to produce electroencephalographic abnormalities, seizures and phenotypic traits resembling the clinical features of AS (47,48). Several studies in humans have tested for association between different forms of epilepsy and the gene encoding this subunit (49,50).

In order to determine whether this gene complex plays a role in autism susceptibility, several studies have screened markers in this region for allelic association in families with autistic disorder. Cook et al. (51) tested nine markers in 140 predominantly singleton families. They found evidence for association to a marker GABRB3 135CA-2 in GABA$_A$-$\beta$3 ($P$ = 0.0014), but not to the two closest flanking markers. No evidence for parent-of-origin effects on allelic transmission was found. A study of 94 multiplex families from the International Molecular Genetic Study of Autism Consortium (IMGSAC) sample (52) examined seven microsatellite markers spanning the 15q11-q13 region, and found no evidence for linkage or association in their families at any of the markers tested (53). In addition, a recent study by Veenstra-VanderWeele et al. (54) found no evidence for a functional mutation in the UBE3A gene in autistic disorder. Thus, at present, there is no strong evidence for association in this region in idiopathic cases. Further work on the cytogenetic rearrangements may narrow down the region of interest, but the possibility that autism cases associated with rearrangements represent some form of contiguous gene syndrome cannot be excluded.

Other candidate gene studies in autism

Numerous studies over the last 2–3 decades have reported increased platelet and/or urine serotonin (5-HT) levels in some autistic patients compared with controls (55,56). Results from Piven et al. (57) have also suggested that increased platelet 5-HT levels in autistic subjects may be associated with a genetic liability in their relatives. Furthermore, treatment with serotonin re-uptake inhibitors has been shown to ameliorate some autistic symptomatology in some individuals (58,59). These results have prompted interest in the role of the serotonergic system in autism, in particular because of its involvement in a wide variety of behavioural and physiological functions. Recently, the serotonin transporter gene (5-HTT) and various serotonin receptors have formed the focus of candidate gene investigations. A study by Cook et al. (60) in 86 singleton families revealed no evidence for linkage or association between autistic disorder and a polymorphism in the second intron of 5-HTT. However, preferential transmission of the short variant of an insertion/deletion polymorphism of the 5-HTT promoter (5-HTTLPR) was found in the same families ($P$ = 0.030). In contrast, a study by Klauke et al. (61) found transmission of the long variant of 5-HTTLPR in 65 autistic singleton families ($P$ = 0.032). Analysis of both polymorphisms of the 5-HTT gene in the IMGSAC families (53) showed no evidence for linkage or association to either marker. In addition, Zhong et al. (62) found no significant association of variants in autistic subjects. Further studies on the serotonin 5-HT2A receptor gene (41) and the 5-HT2A receptor gene (63) found no evidence of association in autistic disorder.

A number of other studies investigating potential candidate genes in autism have been reported, including studies of the neurofibromatosis type 1 gene (64), and the c-Harvey-Ras gene (65,66). The results of many of these candidate gene studies studies remain equivocal, and until positive findings are replicated in larger samples it is difficult to determine their significance. The number of loci tested for linkage disequilibrium in autism, the need to correct for multiple testing and the frequent lack of significant association with flanking markers suggests that some significant results may represent false positives. Transmission ratio distortion may also contribute to positive findings.

GENETIC LINKAGE STUDIES OF AUTISM

Since the neurobiological basis of autism is largely unknown, several groups recently have undertaken systematic screening studies of the whole human genome in multiplex families in order to identify autism susceptibility loci (52,67–70). The results of the four genome screens published to date are summarized in Table 1, showing the most significant linkage results for each study and overlapping regions between studies.

The first of these studies was carried out by the IMGSAC (52) who conducted a two-stage screen for autism susceptibility loci in 87 affected sib pairs plus 12 non-sib affected relative pairs, for a total of 99 pairs. In stage 1, 354 markers were genotyped in 39 families, with 60 additional families genotyped in stage 2 using a subset of 175 markers focusing on regions of interest. Regions on six chromosomes (chromosomes 4, 7, 10, 16, 19 and 22) were identified that overlapped those previously identified by IMGSAC. The most significant linkage was to a region on chromosome 7q between markers D7S530 and D7S684 with an MLS of 2.53, followed by a region on 16p between markers D16S407 and D16S3114 with an MLS of 1.51. The whole X chromosome was excluded for a $\lambda_c$ of 2.5, as was the HLA region on the short arm of chromosome 6. Subsequent follow-up of the initial chromosome 7 linkage findings was carried out by fine mapping of the 7q32–q35 region (71). Seventy-four markers were genotyped in 125 families, comprising the original 99 families and 26 families that were identified subsequently. These results provided further support for an autism susceptibility locus in this region generating a multipoint MLS of 3.63.

In a subsequent study by the Paris Autism Research International Sibpair Study (67), 264 microsatellite markers were genotyped in 51 multiplex families. Using two-point or multipoint affected sib pair analyses, 11 chromosomal regions were positively linked to autism, giving nominal $P$ values of 0.05 (chromosomes 2, 4, 5, 6, 7, 10, 15, 16, 18, 19 and X). Four of these regions on chromosomes 2q, 7q, 16p and 19p overlapped those previously identified by IMGSAC. The most significant multipoint linkage was on the long arm of...
chromosome 6, just distal to marker D6S283, with an MLS of 2.23.

The largest genome screen to date was carried out by Risch et al. (69) in an initial set of 90 multiplex families using 519 markers, with promising regions followed up using 149 of these markers in a second set of 49 families. The strongest evidence for linkage in this study in the combined family data was a multipoint MLS of 2.15 near marker D1S1675 on chromosome 1. Three additional regions on chromosomes 17p, 7p and 18q gave an MLS ≥ 1. The region on 18q overlapping that identified by Philippe et al. (67). Modestly positive LOD scores were also detected on chromosomes 7q and 13q in regions identified by IMGSAC (52) and the Collaborative Linkage Study of Autism (CLSA) (68). The observed increase in identity by descent (IBD) sharing in the affected sib pairs compared with the discordant sib pairs in this study was due to a modest increase in the entire distribution of IBD, rather than a small number of loci, leading the authors to conclude that these results are most compatible with a disease model specifying a large number of loci (perhaps ≥15).

The most recently published study was the first stage of a two-stage autosomal genomic screen carried out by the CLSA (68) using 416 markers in 75 multiplex families. Using model-based linkage analysis, their strongest multipoint results were on regions on chromosomes 13 and 7, with a maximum multipoint heterogeneity LOD (MMLS/het) score of 3.0 at D13S800 and the next highest peak an MMLS/het score of 2.3 between markers D13S217 and D13S1229, both under a recessive model. The third highest score of 2.2 was on chromosome 7q at marker D7S1813 under the recessive model.

In addition to the results of these four published studies, the first stage of a two-stage genome screen by Busbaum et al. (72) in ∼60 families found evidence consistent with linkage to chromosome 7q, although no evidence of linkage to chromosomes 6, 13 or 15 was observed.

The combination of results from these studies strongly indicates that a locus on chromosome 7q may be involved in the aetiology of autistic disorder. Based on these observations, and the cytogenetic finding in their own studied families, Ashley-Koch et al. (25) genotyped 76 multiplex families for nine markers in this region. In this study, two-point linkage analysis yielded a maximum heterogeneity LOD score of 1.47 and a maximum LOD score of 1.03 at marker D7S495. Multipoint MLS and non-parametric linkage (NPL) analyses resulted in peak scores of 1.77 at D7S522 and 2.01 at D7S640, respectively. They also found significant paternal, but not maternal, IBD sharing and linkage disequilibrium at markers D7S640 (P = 0.007) and D7S1824 (P = 0.02), respectively, again supporting evidence for the presence of an autism susceptibility locus on chromosome 7q. The regions supporting linkage to chromosome 7q from this study, and in the genome screens discussed previously, are summarized schematically in Figure 1.

The studies by IMGSAC (52) and Risch et al. (73) found no evidence for linkage to the 15q11–q13 region associated with cytogenetic abnormalities in autism. The CLSA (68) found a maximum MMLS/het score of 0.51 at marker D15S975. Philippe et al. (67) found positive linkage results in their families, with a maximum MLS of 1.10 at marker D15S118, ~20 cm distal to the GABRB3 subunit gene. In addition, a genetic linkage study undertaken by Bass et al. (74) in an independent set of 63 multiplex families found evidence in support of linkage using 14 markers in the 15q11–q13 region. The highest LOD score found was under a recessive model for marker D15S217, with a peak LOD of 1.37. This marker also generated the maximum LOD score under a dominant model,
and an NPL Z score of 1.78 using non-parametric multipoint analysis. The results of these genetic linkage studies on chromosome 15 are summarized in Table 2.

It is worth noting that the majority of studies reviewed here find no support for linkage to the X chromosome. These X chromosome findings are consistent with previously published results demonstrating that X-linked transmission does not account for the majority of cases of autism (75). The higher incidence of autism in males than in females and the association of autism with fragile X syndrome previously have suggested an involvement of the X chromosome in autism. However, results from the IMGSAC data, and from other family studies (E. Cook, personal communication), suggest that the incidence of fragile X may be lower than that previously suggested (13).

Similarly, a number of studies previously have suggested an involvement of autoimmune disorders and a role for the HLA complex in the genetic susceptibility to autism. However, the studies reviewed here show no evidence for linkage to this region. Furthermore, there is no evidence for linkage to the region of the serotonin transporter gene on chromosome 17p11.2 in the studies reviewed here.

**DISCUSSION**

Although a number of genome screens and genetic linkage studies for susceptibility loci for autistic disorder have now been reported, the comparison of linkage results from multiple studies and the possibility of meta-analysis across groups is complicated by several factors. Methodological limitations include the use of different genetic markers and marker maps, variations in statistical analysis and the varying power of different studies. In addition, reporting bias makes the identification of negative results more difficult. Differences between studies in diagnostic and inclusion/exclusion criteria for such a complex psychiatric disease mean that variance in ascertainment may give rise to fundamentally different samples. Comparison of diagnostic criteria used in the studies reviewed here suggests that variation in patient age and IQ may contribute to any inter-study differences. Different linkage findings between studies may be explained partially by weak genetic effects that apply to only a small subset of autistic patients. The strongest linkage results of the CLSA (68) were achieved assuming a model of genetic heterogeneity, therefore suggesting that only a subgroup of families are linked to the specific loci detected in their study. It should be possible in the future to use increasingly sophisticated diagnostic tools and knowledge of individual components of the phenotypic expression to tease apart the underlying genetic mechanisms using quantitative trait loci (QTL) analysis. Subdivision of the phenotype may prove helpful and/or necessary in order to increase power to detect underlying susceptibility genes. In order to achieve this, larger collections of families will be necessary.

The lack of strong evidence for linkage to chromosome 15 in the genome screens reviewed here is surprising given the relatively high incidence of chromosome abnormalities in this region. However, the majority of these studies report exclusion of patients with chromosome abnormalities. High rates of chromosome instability and the increased genetic recombination observed in this region of chromosome 15 (74) and on chromosome 7 (25) may act to confound linkage results. Furthermore, the majority of cases of autism associated with chromosome 15 anomalies reported in the literature are sporadic, and therefore may represent a genetically discrete subgroup from the multiplex families ascertained in the studies reviewed here, with different underlying genetic mechanisms. This may also pose a problem for replication of multiplex associations in singleton families, if findings in multiplex studies cannot be generalized to sporadic cases.

**Table 2. Summary of chromosome 15 linkage results**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Position (cM)</th>
<th>Result</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABRB3 155CA-2</td>
<td>9.8</td>
<td>Linkage disequilibrium (P = 0.0014)</td>
<td>Cook et al., 1998 (140)</td>
</tr>
<tr>
<td>D15S217</td>
<td>ND</td>
<td>NPL Z score 1.78 (P = 0.03)</td>
<td>Bass et al., 1999 (63)</td>
</tr>
<tr>
<td>D15S975</td>
<td>13.1</td>
<td>MMLS/het score 0.51</td>
<td>CLSA, 1999 (75)</td>
</tr>
<tr>
<td>D15S118</td>
<td>32.6</td>
<td>Multipoint MLS 1.10</td>
<td>Philippe et al., 1999 (51)</td>
</tr>
</tbody>
</table>

Positions are in centimorgans from pter from the Marshfield chromosome 15 sex-averaged linkage map. For each study, the number of families is indicated in parentheses. See text for details.
However, with these caveats, the majority of studies reviewed here support evidence for a susceptibility locus for autistic disorder on chromosome 7q. This leads us to question the extent to which these separate linkage findings may represent a single underlying genetic effect, and whether these studies constitute replication of the chromosome 7 linkage. Computer simulation models chosen to represent typical complex traits indicate a large degree of variation in location estimates (regions giving maximum evidence for linkage) (76). In this study, 95% confidence intervals for location estimates covered up to 25 cM for a typical complex trait with 200 affected sib pairs. Not surprisingly, the variation in location estimate was also found to be a function of the magnitude of the expected LOD score. A previous study by Suarez et al. (77) has also demonstrated that substantially larger numbers of families are required for replication of true linkages than for initial detection. A chromosomal region of the size indicated by Roberts et al. (76) is comparable to that covered by the chromosome 7 linkage findings reviewed here, although these studies all have a smaller family sample size. Nevertheless, these linkage findings are still more consistent than those reported in the literature for other psychiatric disorders.

CONCLUDING REMARKS

In summary, the identification of susceptibility genes in autistic disorder will depend on an interdisciplinary approach involving interaction between many groups using a combination of diagnostic, neurobiological, cytogenetic, affected sib pair and candidate gene approaches. Despite formidable challenges, the future of autism research looks increasingly optimistic, and the goal of mapping genes for autistic disorder may soon be attainable.

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REFERENCES


