Analysis of IMGSAC autism susceptibility loci: evidence for sex limited and parent of origin specific effects


Background and methods: Autism is a severe neurodevelopmental disorder, which has a complex genetic predisposition. The ratio of males to females affected by autism is approximately 4:1, suggesting that sex specific factors are involved in its development. We reported previously the results of a genomewide screen for autism susceptibility loci in 83 affected sibling pairs (ASP), and follow up analysis in 152 ASP. Here, we report analysis of an expanded sample of 219 ASP, using sex and parent of origin linkage modelling at loci on chromosomes 2, 7, 9, 15, and 16.

Results: The results suggest that linkage to chromosomes 7q and 16p is contributed largely by the male–male ASP (MLS = 2.55 v 0.12, and MLS = 2.48 v 0.00, for the 145 male–male and 74 male–female/ female–female ASP on chromosomes 7 and 16 respectively). Conversely linkage to chromosome 15q appears to be attributable to the male–female/female–female ASP (MLS = 2.62 v 0.00, for non-male and male–male ASP respectively). On chromosomes 2 and 9, all ASP contribute to linkage. These data, supported by permutation, suggest a possible sex limited effect of susceptibility loci on chromosomes 7, 15, and 16. Parent of origin linkage modelling indicates two distinct regions of paternal and maternal identity by descent sharing on chromosome 7 (paternal MLS = 1.46 at −112 cM, and maternal MLS = 1.83 at −135 cM; corresponding maternal and paternal MLS = 0.53 and 0.28 respectively), and maternal specific sharing on chromosome 9 (maternal MLS = 1.99 at −30 cM; paternal MLS = 0.02).

Conclusion: These data support the possibility of two discrete loci underlying linkage of autism to chromosome 7, and implicate possible parent of origin specific effects in the aetiology of autism.

Autism (OMIM #209850) is a severe neurodevelopmental disorder which affects approximately 200 in 100 000 individuals, although related disorders affect a further 400 in 100 000 people. Onset is before 3 years of age, and the core deficits, comprising qualitative impairments in reciprocal communication and social interaction, and repetitive and stereotyped behaviours and interests, persist throughout life. There is evidence for a complex genetic susceptibility to most cases of autism, and statistical modelling suggests the involvement of perhaps three or four loci, although up to 15 may be implicated. Many groups have undertaken genomewide screens for autism susceptibility loci using affected sibling pairs (ASP), followed by a positional functional candidate gene approach (see Lamb et al1 for review). Encouragingly, there is convergent evidence for linkage for several chromosome regions. However, the identified genomic regions are large and, in the absence of replicated association findings, researchers have tried to minimise clinical and genetic heterogeneity to improve localisation of the linkage signals. Three quarters of individuals affected by autism are male, suggesting the involvement of sex specific factors in autism susceptibility. However, there is little evidence from genetic studies for involvement of genes on the X chromosome in the majority of individuals with autism. Linkage that is dependent on the sex of the affected individual has been reported previously in studies of other complex diseases including inflammatory bowel disease, hypertensin, diabetes, and the personality trait neuroticism, therefore we reasoned that proband sex might be a useful index of heterogeneity in our sample. We have reported previously the results of a genomewide screen for autism susceptibility loci in 83 ASP, and follow up analysis in 152 ASP. Here, we report linkage analysis in an expanded sample of 219 ASP at the principal loci on chromosomes 2, 7, 9, 10, 16, and 17. In addition, loci on chromosomes 3, 13, and 15 have been investigated further because of reports of linkage of autism and specific language impairment to chromosomes 3 and 13, and linkage and an increased incidence of cytogenetic abnormalities of chromosome 15q11-q13 in autism (see Lamb et al1 for review). Analysis by proband sex and identity by descent (IBD) sharing partitioned into paternal and maternal components was carried out at the loci on chromosomes 2q, 7q, 9p, 15q, and 16p.

MATERIALS AND METHODS

Family collection and phenotypic assessment

The identification of families and assessment methods used by the IMGSAC has been described in detail previously. In families passing an initial screen, parents were given the Autism Diagnostic Interview Revised (ADI-R)20 and the Vineland Adaptive Behaviour Scales.21 Potential cases, all ≥4 years old, were also assessed using either the Autism Diagnostic Observation Schedule (ADOS)22 or ADOS Generic (ADOS-G).23 When possible, psychometric evaluation was also conducted using Raven’s progressive matrices24 or the Mullen Scales of Early Learning,25 and the British Picture Vocabulary Scale26 on the Peabody Picture Vocabulary Test.

Abbreviations: ADI-R, Autism Diagnostic Interview Revised; ADOS, Autism Diagnostic Observation Schedule; ADOS-G, Autism Diagnostic Observation Schedule Generic; ASP, affected sibling pairs; IBD, identity by descent
Families with autism were collected and genotyped in five successive stages. A total of 420 affected individuals and 219 non-independent affected sibling pairs from 207 families were studied. The male-to-female ratio of these affected individuals is 3.94:1. The 219 ASP comprised 145 male–male ASP, 59 male–female ASP, and 15 female–female ASP. The male–female and female–female ASP combined to give a group of 74 non-male ASP. There is no known overlap between these families and those ascertained by other research groups.

Physical examination was undertaken to exclude recognizable medical causes of autism. Blood samples were taken for DNA extraction when possible from all probands and available first-degree relatives, and lymphoblastoid cell lines generated. Buccal swabs were taken when a blood sample could not be obtained (3.2% of samples). Karyotypes were obtained on all affected individuals when possible, and Fragile X results obtained on one affected individual per family where possible. Karyotype abnormalities were excluded in at least one affected individual per family in 93% of families and in both affected individuals in 83% of families. Fragile X was excluded in at least one affected individual in 98% of families. The study has been reviewed by the relevant local ethics committees.

Genomic DNA extraction and genotyping

Genomic DNA extraction, amplification, and genotyping were performed as described previously according to standard protocols. Genetic marker positions were taken from the deCODE genetics sex averaged map, and additional loci, by taking into account the interdependency between the two linked loci. TwoLoc calculates a two locus MLS for affected sibling pairs by specifying the joint two locus allele sharing probabilities as a function of the variance components and disease prevalence. The joint IBD sharing probabilities are maximized with respect to the genetic model specified. Analyses in TwoLoc were performed under two genetic models: a general two locus model, under which the joint IBD can vary freely within the valid ranges, and a single locus model under which the IBD probabilities are restricted. To obtain the joint two locus IBD sharing probabilities, TwoLoc uses family likelihood calculation output from Vitesse, which allows for sex-specific recombination rates but is limited to eight markers. Therefore, we also used Merlin to calculate the joint two locus IBD probabilities using all markers on chromosome 7, but with sex averaged recombination rates only. Support for linkage to the second hypothetical disease locus in TwoLoc is calculated as the difference in MLS between a two locus and a single locus model.

RESULTS

Linkage analysis was carried out in 219 ASP at the principal loci on chromosomes 2, 7, 9, 10, 16, and 17 indicated by our previous results in 152 ASP. In addition, loci on chromosomes 3, 13, and 15 were investigated based on published research. The position, heterozygosity and effective sample size of the 396 markers used in these analyses are shown in the supplementary table online. Mean marker heterozygosity across the genome was 0.78.

Multipoint linkage analysis using ASPEX generated MLS > 2 on chromosomes 2q, 7q, and 9p (fig 1A), with MLS of 2.54 near D2S2314 on chromosome 2q, 2.31 near D7S530 on chromosome 7q, and 2.12 near D9S161 on chromosome 9p. The next highest MLS were 1.73 at D16S497 on chromosome 16p, and 1.67 on chromosome 9q towards the telomere in the interval D9S158-D9S905. These data were supported by the single point SPLINK results (data not shown). There was no strong evidence for linkage on chromosomes 3, 13, or 15 (MLS<1). The highest ASPEX multipoint MLS generated at the loci genotyped in the 219 ASP are presented in table 1.

Analysis according to the sex of the affected sibling pairs was carried out at the principal loci on chromosomes 2, 7, 9, and 16, and also on chromosome 15 because of the suggestion of genetic heterogeneity at this locus. The 219 ASP comprised 145 male, 59 male–female, and 15 female–female pairs. Because the subset of female pairs was considered too small to be analysed independently, male–female and female–female pairs were combined to give a group of 74 non-male pairs. Analysis of the 145 male pairs generated MLS of 2.55 and 2.48 on chromosomes 7 and 16 respectively (fig 1B; table 1). No linkage to these regions was observed in the 74 non-male ASP (MLS = 0.12 and 0.00 on chromosomes 7 and 16). Conversely, linkage to chromosome 15q was attributable to the non-male ASP (MLS = 2.62 v 0.00, for the non-male and male ASP respectively). On chromosomes 2 and 9, all ASP contributed to linkage. Empirical significance levels were determined by permutation (see methods), suggesting that this apparent sex limited effect on chromosomes 15 (p = 0.0011) and 16 (p = 0.026) is significant, whereas on chromosome 7 this effect failed to reach significance (p = 0.075).

Paternal and maternal contributions to the linkage on chromosomes 2, 7, 9, 15, and 16 were evaluated using ASPEX sex split (Fig 2). On chromosomes 2, 15, and 16, parent of origin linkage modelling showed IBD sharing from both parents at essentially the same position. On chromosome 7, two distinct regions of paternal and maternal IBD sharing were identified, with paternal MLS of 1.46 between markers D7S477 and D7S2453, and maternal MLS of 1.83 between
markers D7S530 and D7S640 ~20 cM distally. The corresponding maternal and paternal MLS at these loci were 0.53 and 0.28 respectively. This effect was more apparent in the subset of male ASP (data not shown). On chromosome 9p, IBD sharing appeared to be largely maternally derived; maternal MLS = 1.99 at ~30 cM (paternal MLS = 0.02).

In order to assess support for the presence of two autism susceptibility loci on chromosome 7, data was analysed in a maximum likelihood framework using TwoLoc. Analysis in Twoloc using all chromosome 7 markers and sex averaged recombination rates generated a peak MLS of 3.34 under the two locus general model, and MLS of 2.13 and 2.31 at locus 1 and locus 2 respectively under the single locus model.

This analysis was restricted to a subset of eight markers at the interval D7S477–D7S530 (133 cM). Linkage support for the presence of two autism susceptibility loci at 111 cM, independent of the locus at D7S477 (locus 1, 111 cM) and the interval D7S530–D7S640 (133 cM). Linkage support for the presence of two susceptibility loci on chromosome 7, data was analysed in a maximum likelihood framework using TwoLoc. Analysis in TwoLoc using all chromosome 7 markers and sex averaged recombination rates generated a peak MLS of 3.34 under the two locus general model, and MLS of 2.13 and 2.31 at locus 1 and locus 2 respectively under the single locus model.

DISCUSSION
The addition of further ASP and analysis of existing data continues to support the existence of loci influencing autism susceptibility in the IMGSAC sample, with the loci on chromosomes 2 and 7 showing suggestive evidence for linkage. Nevertheless, the addition of further families has reduced the multipoint MLS on chromosomes 2, 10, 16, and 17 compared with previously reported results. The MLS on chromosome 9 has increased, and the maximum multipoint MLS on chromosome 7 is essentially unchanged, although the peak of linkage has moved ~23 cM distally. Previous studies have demonstrated a large degree of variation in linkage location estimates in studies of complex disease with modest power.

The high ratio of males with autism indicates the possible involvement of sex specific factors in the development of the disorder, and a possible basis for genetic heterogeneity. A number of hypotheses have been posited to account for the higher incidence of autism in males, including X linked inheritance. However, this is not consistently supported by genetic linkage studies, and evidence suggests a higher incidence of the broader autistic phenotype in fathers than in mothers. There is little evidence for the involvement of the X chromosome in the majority of cases. Analysis according to the sex of ASP suggests that linkage to chromosome 16 is limited to male affected sibling pairs in the IMGSAC sample, a finding supported by empirical significance thresholds determined by simulation. The same trend is observed on chromosome 7, but narrowly fails to reach significance.

Evaluation of parent of origin contributions at these loci represents the same locus. It is not yet clear whether the cytogenetic abnormalities of chromosome 15, in particular maternally inherited duplications of 15q11-q13, the most commonly documented chromosomal abnormalities leading to an autistic phenotype, suggest a possible cause of genetic heterogeneity at this locus. However, the linkage reported here in the non-male ASP is to 15q21.3-q22.31, at least 25 Mb distal to 15q11-q13, so it is unlikely that this linkage represents the same locus. It is not yet clear whether the cytogenetic abnormalities of 15q11-q13 are at an increased frequency in autistic females. These results support the usefulness of linkage analysis by affected sibling pair sex, and suggest that this approach may be useful for other neurodevelopmental disorders in which a similar sex bias is observed.

The results of two locus analysis using TwoLoc suggest that the majority of evidence...

<table>
<thead>
<tr>
<th>Chrom. no</th>
<th>Marker Position</th>
<th>All (219)</th>
<th>Male (145)</th>
<th>Non-male (74)</th>
<th>Pat</th>
<th>Mat</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>D2S2314-D2S2310</td>
<td>177–181.4</td>
<td>2.54</td>
<td>1.25</td>
<td>1.39</td>
<td>1.35</td>
</tr>
<tr>
<td>3</td>
<td>D3S157</td>
<td>10</td>
<td>0.65</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>D7S477-D7S3435</td>
<td>113–118.1</td>
<td>2.55</td>
<td>0.075</td>
<td>0.12</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>D7S550-D7S640</td>
<td>133–141.2</td>
<td>2.31</td>
<td>0.03</td>
<td>0.29</td>
<td>1.83</td>
</tr>
<tr>
<td>9</td>
<td>D9S157</td>
<td>25.1–34.4</td>
<td>2.12</td>
<td>0.02</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D9S158-D9S161</td>
<td>40.4</td>
<td></td>
<td>1.92</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>D10S188</td>
<td>24</td>
<td>1.15</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>D13S189</td>
<td>24</td>
<td>1.15</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>D15S129-CYP19</td>
<td>46.6–53.8</td>
<td>1.07</td>
<td>0.90</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>D16S407-D16S497</td>
<td>24</td>
<td>1.15</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>16</td>
<td>D16S407-D16S497</td>
<td>32.8–35.9</td>
<td>2.48</td>
<td>0.00</td>
<td>2.62</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>D16S497</td>
<td>35.9</td>
<td>1.73</td>
<td>(p = 0.026)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D16S3102</td>
<td>37.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>D17S513</td>
<td>10</td>
<td>1.13</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
for linkage comes from locus 2 (D7S530-D7S640), and the additional support for linkage at locus 1 (D7S477), assuming that locus 2 is linked, is MLS = 0.83, indicating that these results should be interpreted with caution.

The involvement of parental sex in autism has previously been indicated by the maternally inherited duplications of 15q11-q13, and several developmental disorders have been associated with imprinting defects, including Angelman, Beckwith-Wiedemann, and Prader-Willi syndromes. Prader-Willi and Angelman syndromes result from loss of expression at oppositely imprinted neighbouring genomic loci in the 15q11-q13 region. This clustering of differentially imprinted genes has been reported for a number of other genomic loci. The association of aberrant imprinting with several human cancers is interesting, given the mapping of

Figure 1 Multipoint linkage results generated by ASPEX under an additive model with no dominance variance. The results are presented after removal of possible errors identified using Merlin. (A) Multipoint map across all chromosomes from pter to qter (red line). Loci typed in the whole sample of 219 ASP are indicated by triangles on the x axis. The results of analysis after stage 4 (152 ASP) are shown for comparison (black line). The results presented here differ slightly from those reported previously for stage 4 due to refinement of the genetic map, and differences in the method of error detection. (B) Linkage analysis of chromosomes 2, 7, 9, 15, and 16 according to ASP sex. Red, total sample of 219 ASP; blue, 145 male ASP; green, 74 non-male ASP; x axis, position from pter in cM using the deCODE sex averaged map. The positions of markers are represented by diamonds on the x axis with the effective sample size (ESS) in the total sample indicated by: blue, ESS<50; red, ESS 50–100; green, ESS>100.

Figure 2 Parent of origin linkage modelling on chromosomes 2, 7, 9, 15 and 16 generated using ASPEX sex_split: chromosomes 2, 7, and 9; multipoint linkage in total sample of 219 ASP; chromosome 15; multipoint linkage in 74 non-male ASP; and chromosome 16; multipoint linkage in 145 male ASP. Red, total sample of 219 ASP; blue, 145 male ASP; green, 74 non-male ASP; turquoise, paternal identity by descent (IBD) sharing; yellow, maternal IBD sharing. The black lines below the chromosome 7 figure show the one-lod support interval for regions of paternal and maternal IBD sharing; x axis, position from pter in cM using the deCODE sex averaged map. The positions of markers are represented by diamonds on the x axis.

Figure 3 Increase in the MLS for a two locus model, assuming a primary susceptibility locus at 134 cM, versus the single locus MLS results. Results were obtained across all markers on chromosome 7 using TwoLoc with sex averaged recombination rates from the deCODE sex averaged map in the total sample of 219 ASP. Thin line, single locus MLS; thick line, two locus MLS. The positions of markers are represented by diamonds on the x axis.
loci for acute myeloid leukaemia and acute lymphoblastic leukaemia to our regions of linkage on chromosomes 7 and 9 respectively.

The identification of possible sex limited and parent of origin specific effects on chromosomes 7, 9, 15, and 16 should facilitate a more focused approach to candidate gene screening. The human reelin gene maps to 7q22, within the region of maternal linkage, and has been suggested as a candidate for autism susceptibility (see Bonora et al8 for review). Interestingly, a progressive Purkinje cell loss has been reported in the cerebellum of male heterozygous reeler mice, while the females are spared.18 However, screening the coding sequence of this gene for autism susceptibility variants in the IMGSAC sample did not reveal any putative aetiological variants with a high enough frequency to explain the strength of the linkage findings.19 A number of imprinted genes have already been identified on chromosome 7, including several genes within the region of maternal linkage.19 21 Future studies will determine if other genes/ transcripts in this region are preferentially expressed from one parental allele.

These results suggest the usefulness of genomewide analysis by affected sibling pair sex and evaluation of parent of origin effects, although future studies are necessary to determine if these results can be replicated. The suggestion of narrower confidence intervals for the linkage signals on chromosomes 7, 9 demarcates a region suitable for high resolution association mapping. However, the possible involvement of epigenetic rather than genetic variation may be an explanation for the lack of causative variants so far identified in candidate genes in these regions.

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Competing interests: none declared

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