Whole genome linkage scan of recurrent depressive disorder from the depression network study

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Genome-wide linkage analysis was carried out in a sample of 497 sib pairs concordant for recurrent major depressive disorder (MDD). There was suggestive evidence for linkage on chromosome 1p36 where the LOD score for female–female pairs exceeded 3 (but reduced to 2.73 when corrected for multiple testing). The region includes a gene, MTHFR, that in previous studies has been associated with depressive symptoms. Two other regions, on chromosomes 12q23.3–q24.11 and 13q31.1–q31.3, showed evidence for linkage with a nominal P < 0.01. The 12q peak overlaps with a region previously implicated by linkage studies of unipolar and bipolar disorders and contains a gene, DAO, that has been associated with both bipolar disorder and schizophrenia. The 13q peak lies within a region previously linked strongly to panic disorder. A fourth modest peak with an LOD of greater than 1 on chromosome 15q lies within a region that showed genome-wide significant evidence of a recurrent depression locus in a previous sib-pair study. Both the 12q and the 15q findings remained significant at genome-wide level when the data from the present study and the previous reports were combined.

INTRODUCTION

Genetic risk factors are well established for major affective disorders, and a recent twin study has suggested that unipolar depression has a stronger genetic influence than previously thought. McGuffin et al. (1) have estimated that the heritability may be >70% in a clinically ascertained twin sample, whereas a population-based twin study resulted in a very similar estimate using a re-test method of assessing lifetime diagnosis (2). The majority of studies suggest a relative risk to siblings (lrs) of affective disorder in the region of 3 (3). However, a recent study comparing the siblings of unipolar depressives with the siblings of healthy controls using strict definitions of both depression and health found a substantially higher lrs of over 9 (4). Further, the inheritance of unipolar depression is complex and involves an interplay of genetic and environmental factors.
environmental factors, including certain types of severe and life-threatening events such as events associated with humiliation or loss (5,6).

Despite the excess of females to males of approximately two to one for unipolar depression, the heritability in a clinically ascertained sample was the same in men and women (1). Some population-based twin studies suggest at least some of the genes conferring liability differ between the sexes (7), whereas other studies do not (8). Although it has been suggested that early-onset depression is more clearly familial than later onset, this is not supported by a meta-analysis (9). The only characteristics of probands associated consistently with higher familiality or heritability are recurrence of episodes and severity of disorder (1,9).

Previous linkage studies of unipolar depression

Most previous linkage studies have been carried out in families identified by a bipolar proband and where unipolar and bipolar relatives are frequently grouped together into a broad definition of affective disorders. Most such studies have focussed on multiple affected extended pedigrees on the assumption that there may be a subset segregating a gene of major effect. This approach has been successful in complex disorders such as early-onset Alzheimer’s disease and breast cancer. However, consistent evidence of major gene effects in bipolar disorder has not been forthcoming (10). In addition, the unknown mode of inheritance creates inherent difficulties in classic linkage approaches, and consequently, sib-pair methods are attractive in the study of complex familial disorders. An affected sib-pair genome scan study of recurrent depression has now been published, suggesting that there is a depression susceptibility locus on chromosome 15q (11). Another genome scan focussing on multiple affected extended pedigrees found the strongest evidence for linkage on chromosome 12q (12). In addition, a genome scan of multiple affected families with alcoholism, and in whom, some individuals had depression or co-morbid alcoholism and depression, found evidence of a depression-linked locus on chromosome 1p (13). These results merit further scrutiny and attempts at replication.

RESULTS

Allele frequencies across sites

Nominal significance of allele frequency differences between centres were detected across both depression linked and unlinked regions, but none of these remained after Bonferroni correction. However, as a check against biased results from allele frequency mis-specification, we also investigated whether incorrectly specified marker allele frequencies could have resulted in false positive linkage signals using an approach based on Morton’s (14) ‘pre-divided samples’ heterogeneity test. Parametric linkage analysis of the most significant markers was carried out with the same allele frequencies across centres and with centre-specific allele frequencies. The difference in the sum of the maximum LOD score multiplied by 4.6 is approximately a$ \chi^2 $, where the degrees of freedom equal the number of different centres. The results provided no evidence of heterogeneity, suggesting that observed differences in the allele frequencies had not artificially inflated the LOD scores.

Linkage findings

The MOD score parametric analyses and the non-parametric analyses using MERLIN yielded closely similar results identifying the same regions, with little difference in signal size. For the ease of presentation, only the results from MERLIN are given here. The findings from MERLIN multipoint analyses, where LOD scores exceeded 1, are summarized in Table 1. Although the only LOD score greater than 3 is on chromosome 1p for female sib pairs, it is worth noting that there are also positive findings on chromosomes 1q, 13q and 15q, where there is overlap with the regions previously reported to be linked to depression or associated traits.

Chromosome 1. A peak with a maximum LOD score of $ \sim 2 $ overall and just over 3 in females, was observed on chromosome 1p36 between 13.8 and 21.8 cM and extending from 7.2 to 14.5 Mb on NCBI Build 35 (Fig. 1).

Chromosome 12. A narrow peak with a maximum LOD score of $ \sim 1.6 $ overall, $ \sim 1.85 $ in females, was observed between D12S1636 and D12S1583 at chromosome 12q23.3/24.11, 104.2–108.4 Mb (Fig. 2).

Chromosome 13. A peak with a maximum LOD score of $ \sim 1.5 $ was observed on chromosome 13q31.1–q31.3 between 75 and 87 cM and extending from 76.1 to 92.6 Mb (Fig. 3).

Chromosome 15. We observed a modest peak (two markers) with a maximum LOD of 1.14 on chromosome 15q25.2 from 88 to 91.7 cM and from 78.7 to 84 Mb for depression in the overall sample, but little other evidence. Although not striking on its own, this is of interest because it lies completely within a linkage region for depression previously reported by Holmans et al. (11) (Fig. 4).

Parametric linkage analysis of markers on chromosomes 1, 10, 12, 13, 15, 21 and X revealed LOD scores of greater than 1 under either or both models at or near the peaks identified with the non-parametric analysis. The result on chromosome 20 was the only non-parametric LOD of greater than 1, not confirmed by parametric analysis. However, this is a small signal and may well be a false positive.

Combined analysis with previously published results

The phenotypic definitions used in the two previous whole genome scans of depressive disorder are similar to those used here so that it is reasonable to undertake a combined analysis. For both chromosomes 12q and 15q, the results from the depression network (DeNt) data set were smallest and hence treated as the ‘secondary P-values’ (15). In the analysis of chromosome 15, the empirical P-value was used (rather than that derived from the test statistic). In the analysis of chromosome 12, we have used the genome wide P-value. This ensures appropriately conservative estimates.

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Table 1. LOD scores greater than 1 from DeNt linkage scan for depression

<table>
<thead>
<tr>
<th>Marker name</th>
<th>Chromosome</th>
<th>Position (cM)</th>
<th>LOD Score</th>
<th>Female LOD</th>
<th>Male LOD</th>
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<tr>
<td>D1S450</td>
<td>1</td>
<td>16.993</td>
<td>1.9</td>
<td>3.03</td>
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<td>D12S1613</td>
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<td>1.42</td>
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<td>13</td>
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<td>1.47</td>
<td>0.58</td>
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<td>20.363</td>
<td>1.44</td>
<td>2.54</td>
<td>−0.13</td>
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<tr>
<td>D15S08</td>
<td>1</td>
<td>13.895</td>
<td>1.38</td>
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<td>−0.06</td>
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<tr>
<td>D15S34</td>
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<td>1.96</td>
<td>−0.06</td>
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<tr>
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<td>1.08</td>
<td>0.7</td>
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<tr>
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<td>127.220</td>
<td>1.25</td>
<td>1.86</td>
<td>0.68</td>
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<tr>
<td>D12S1609</td>
<td>12</td>
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<td>1.18</td>
<td>0.38</td>
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<td>13</td>
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<td>1.17</td>
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<tr>
<td>D15S1047</td>
<td>15</td>
<td>88.194</td>
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<td>0.21</td>
<td>0</td>
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<tr>
<td>D20S171</td>
<td>20</td>
<td>100.839</td>
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<td>1.01</td>
<td>0.22</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*Including opposite sex pairs.

Chromosome 12. The analysis of the DeNt data revealed a P-value of 0.004 for marker D12S1613 at position 106.15 Mb on NCBI Build 35, this adjusts to 0.1008. Abkevich et al. (12) report a P-value of 0.00003 for marker D12S1706 (position 95.37 Mb). The overall P-value is therefore 0.00004.

Chromosome 15. The analysis of the DeNt data revealed a P-value of 0.011 for marker D15S1047 at position 78.92 Mb, this adjusts to 0.278898. Holmans et al. (11) report a P-value of 0.023 for marker D15S652 (position 90.32 Mb). The overall P-value is therefore 0.038803.

**DISCUSSION**

Our study provides support for the main positive findings from the only whole genome scans of depression published to date indicating loci on chromosomes 12q (12) and 15q (11). We also have a modest linkage peak on chromosome 13q in a region previously implicated in panic disorder and a variety of somatic symptoms (16). This is potentially noteworthy because there is evidence of overlap between the genes that contribute to anxiety and depressive symptoms (17,18). It is also worth noting that a region on chromosome 13q (15) has been previously implicated in both bipolar disorder and schizophrenia, and a region on 15q has been found to be linked with lithium responsive bipolar disorder (19). However, neither of these regions overlap with those reported in the present study.

Our most interesting new result was a peak with a maximum LOD score of ~2 overall and just over 3 in females, but with no contribution from male–male pairs, observed on chromosome 1p36 between 13.8 and 21.8 cM and extending from 7.2 to 14.5 Mb on Build 35. There have been no previous reports of linkage on chromosome 1 in studies of depression as such. Moreover, the region is separate from a chromosome 1 region, reported by Nurnberger et al. (13) to be linked with depression co-morbid with alcoholism. Nor does it overlap with a region linked to neuroticism, a personality trait associated with vulnerability to depression, reported from the London center of the DeNt consortium using a separate sib-pair sample (20). Both these are centromeric to the DeNt linkage region at ~60–70 cM. The findings concerning the 1p region need to be interpreted with caution. An LOD of 3.03 does not quite meet the guideline level for significance of 3.3 (or 3.6 for samples consisting only of sib pairs) suggested by Lander and Kruglyak (21) and reduces to 2.73 if we subtract log_{10}(2) for the correct number of independent tests introduced by dividing the non-parametric analysis by sex. Strictly speaking, the result is ‘suggestive’ rather than ‘significant’.

Nevertheless, it is intriguing that a gene associated with depression, MTHFR, encoding for the enzyme methylenetetrahydrofolate reductase (MTHFR) is in the middle of the DeNt chromosome 1 linkage region (at 11.78 Mb). MTHFR catalyses the conversion of 5,10-5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. Bjelland et al. (22) examined the association between folate, total homocysteine, vitamin B12 and an MTHFR 677C/T polymorphism and anxiety and depression, as measured by the Hospital Anxiety and Depression Scale, in approximately 6000 subjects. The T/T genotype (OR, 1.69) was associated with depression but not with anxiety and, in contrast to our finding of a linkage signal only in women, there was no evidence of sex-specific effects. In addition, studies by Hickie et al. (23) and Arinami et al. (24) have found this association. There has been one negative study (25), but it contained less than 100 depressed cases and probably lacked power to detect the effect observed by Bjelland et al. (22). Although most of the linkage signal comes from female–female pairs, the number of male–male pairs is small with low power on their own to detect an effect. Indeed, if we perform the test of Morton (14) for linkage heterogeneity between male and female pairs, the result is not significant (x^2 = 5.198, two degrees of freedom, P = 0.074). Furthermore, Bjelland et al. (26) in their association study found no differences between men and women, so that if our linkage finding largely or entirely reflects the presence of MTHFR within this region, it is unlikely that there are true sex differences (22).

A narrow peak with a maximum LOD of ~1.6 was observed between D12S1636 and D12S1583 at chromosome 12q23.3/24.11, extending from 104.2 to 108.4 Mb. Thus, it is just adjacent to the region reported as linked to major depression by Abkevich et al. (12) on chromosome 12q22–23.2. Combined analysis of these two data sets results in a P-value of 0.00004. It is also entirely within the bipolar linkages reported by Craddock et al. (27) and others. However, an important caveat is that the evidence for linkage in the study of Abkevich et al. (12) came only from male pairs in contrast to the present study and previously reported bipolar study findings (26).

It is also of interest that the gene encoding d-amino acid oxidase (DAO), that has been reported to be associated with schizophrenia (28), lies within the region at 107.7 Mb. DAO is expressed in the brain, where it oxidizes d-serine, a potent...
Figure 1. Graph showing LOD scores on chromosome 1 versus centiMorgan position.

Figure 2. Graph showing LOD scores on chromosome 12 versus centiMorgan position.
activator of \( N \)-methyl-\( d \)-aspartate-type (NMDA) glutamate receptors. Glutamate and aspartate are excitatory neurotransmitters that have been implicated in a number of pathological states of the nervous system. DAO has also been found to interact with G72 protein by which it becomes activated (27), and the \( G72 \) gene has also been implicated recently in the bipolar affective disorder (29).

A peak with a maximum LOD score of 1.5 was observed on chromosome 13q31.1–q31.3 between 75 and 87 cM and extending from 76.1 to 92.6 Mb. This region is relatively gene poor, with about half the normal density of genes and has relatively low GC content. However, this locus is within a region reported to be strongly linked to panic disorder. In a study of panic disorder, Weissman et al. (30) identified a
group of families with a syndrome that includes frequency of micturition, migraine and mitral valve prolapse. They suggested that this phenotype may represent a subtype of panic disorder. Their maximum LOD score was 3.6 at D13S779 on chromosome 13q (~99.2 Mb) with panic as the affected phenotype and a LOD of 4.2 was obtained when individuals with the broader syndrome were included as affected. The same group (16) later produced a replication of this finding with a different set of families.

Finally, we also observed a modest peak (two markers) with a maximum LOD of 1.14 on chromosome 15q25.2 from 88 to 91.7 cM and from 78.7 to 84 Mb. Although not striking on its own, this is of interest because it lies completely within a linkage region for depression. Holmans et al. (11) performed a genome scan of families multiple affected with recurrent, early-onset major depressive disorder (MDD), thereby selecting a strictly defined phenotype closely similar to that used by ourselves. The sample consisted of 297 informative families containing 415 independent affected sib pairs. Affected cases had recurrent MDD with onset before 31 years of age for probands or 41 years of age for other affected relatives; the mean age of onset was 18.5 years and the mean number of depressive episodes was 7.3. Genome-wide significant linkage with a maximum LOD score of 3.7 was observed on chromosome 15q25.3–q26.2. The linkage was not sex-specific. Combined analysis of our highest LOD score on chromosome 15 and Holman’s highest LOD score gave a significant result at a genome-wide level.

In conclusion, our findings provide further evidence of loci conferring susceptibility to depression on chromosomes 12q and 15q and lend support for the existence of a locus associated with a range of anxiety related and depressive symptoms on chromosome 13q. Our novel linkage peak on chromosome 1p suggests that there may be depression susceptibility locus within this region, and the region does contain a gene with a common variant that has been implicated in depression in three independent association studies.

MATERIALS AND METHODS

Subjects

Sibling pairs affected with recurrent unipolar depression were recruited from eight clinical sites: Aarhus, Denmark; Bonn, Germany; Dublin, Ireland; Lausanne, Switzerland; St Louis, USA and London, Cardiff and Birmingham, UK. In addition, where available, parents of the affected sibling pairs were also included in the study.

Subjects were identified from psychiatric clinics, hospitals and general medical practices and from volunteers responding to media advertisements. Caucasian subjects over the age of 18 were included if they had experienced two or more episodes of unipolar depression of at least moderate severity separated by at least 2 months of remission as defined by the Diagnostic and Statistical Manual 4th edition operational criteria (DSM-IV) (31) or the International Classification of Diseases 10th edition operational criteria (ICD-10), for unipolar depression (32). Probands were all white and of white European parentage. They were included in the study if they had at least one biological sibling, not a monozygotic twin, over the age of 18 years meeting the same diagnostic criteria. Subjects were excluded if either sibling had ever fulfilled criteria for mania, hypomania or schizophrenia.

Subjects were also excluded if they experienced psychotic symptoms that were mood incongruent or present when there was no evidence of a mood disturbance. Other exclusion criteria were intravenous drug use with a lifetime diagnosis of dependency; depression occurring solely in relation to alcohol or substance abuse or depression only secondary to medical illness or medication and a clear diagnosis of bipolar disorder, schizophrenia, schizo-affective disorder or acute or transient psychotic disorders in first- or second-degree relatives.

The sociodemographic characteristics of subjects, their recruitment and assessment have been described in detail by Farmer et al. (33). Data pertinent to the present report are summarized in Table 2.

Clinical assessment

All subjects were interviewed using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (34,35). Items of psychopathology in the SCAN interview were rated for presence and severity according to the worst and the second worst episodes of depression identified by the subjects. For the purposes of rating severity, subjects were asked to identify within each of these episodes of depression a 4–6 week period when their symptoms were at their worst (peak intensity). The majority of the SCAN items were coded as follows; 0—indicates the absence of the item, 1—the item was present but to a mild degree or intermittently throughout the peak intensity 4–6 weeks, 2—item moderately severe and present for >50% of the peak intensity period or severe but present for <50% of the peak intensity period, 3—item severe for >50% of the peak intensity period. The computerized version of the SCAN2.1 is built on top of the IShell system, which is a computer-aided personal interviewing tool produced by the World Health Organization (36) and provides diagnoses according to DSM-IV and ICD-10 operational definitions.

Interviewer training and reliability across sites

All interviewers from each site attended a 4-day SCAN training course in the UK. Each site also undertook further inter-rater reliability meetings regularly and annually; all interviewers from all sites took part in a joint inter-rater reliability exercise. Further details are provided by Farmer et al. (33).

Ethical approval

All sites obtained ethical approval for the DeNt study within their own countries and institutions. All study participants gave written informed consent for participation in the study.

Blood samples

At the time of the SCAN interview, interviewers obtained 25 ml of whole blood that was collected in 37.5 ml (EDTA containing) monovettes. In addition, drops of blood were placed on a Guthrie blood spot card. The blood samples
were labelled with a bar code, gently mixed and stored frozen upright in a −20°C freezer pending DNA extraction.

**Phenotypic data analysis**

All phenotypic information from interviews and questionnaires was coded by assigning a number to each subject and removing any personal identifying information. The same codes were used on the blood sample tubes, using a bar code system. The phenotypic information was first entered on an EXCEL spreadsheet after which a data file was created using the Statistical Package for the Social Sciences (SPSS) version 10 for Windows for the statistical analyses.

**Genotype checking**

Genotyping of microsatellite markers was carried out by DeCode, and the results were checked for mis-specified relationships by the programmes RELPAIR (37) and Graphical Representation of Relationships (GRR) (http://www.sph.umich.edu/csg/abecasis/GRR/index.html). RELPAIR compares the multipoint probability of the genotype data under different possible relationships, whereas GRR calculates the IBS mean and SD for each pair and plots these values representing each type of relative pair using a different colour. Decisions about each problematic pair were made on the basis of the results from both programmes; if there was discrepancy between the programmes, the GRR results were used because these are less sensitive to genotyping errors. Mendelian errors were investigated using PEDSTATS (38) and were dealt with on a case-by-case basis. As a further error-checking measure, MERLIN (37) was used to run analyses including and excluding unlikely genotypes (i.e. those implying double recombination in a small interval); both options gave similar results.

If parental genotypes are missing, MERLIN calculates identical by descent probabilities on the basis of population allele frequencies. Therefore, it is important to check whether there are significant differences between study sites. Allele frequency heterogeneity between the different centres was investigated using χ² tests.

These data cleaning processes resulted in 929 individuals from 417 families. Using an ‘N − 1’ method of counting affected sib pairs in sibships with more than two affecteds, we had a total of 497 sib pairs. They consisted of 266 same-sex female, 58 same-sex male and 173 opposite-sex pairs. They were genotyped at 963 autosomal markers and 44 X chromosome markers. Success rates for the autosomal markers were >86% for 90% of the markers, with a minimum success rate of 61%. For the X chromosome, the success rate was >66%. For individuals, the average genotyping success rate was 94% with a minimum of 73% for autosomal markers and 61% for the X chromosome.

**Genotypic data analysis**

**Linkage.** Non-parametric linkage analysis was performed using MERLIN and MINX (http://www.sph.umich.edu/csg/abecasis/merlin). MERLIN (37) is a multipoint engine for rapid likelihood inference and uses sparse inheritance trees for pedigree analysis. It performs rapid haplotyping, genotype error detection and affected pair linkage analyses and can handle more markers than other pedigree analysis packages. The analysis of dichotomous trait data implemented in MERLIN is essentially a model-free approach, where the Kong and Cox (39) LOD score type statistic is calculated on the basis of allele sharing. The Kong and Cox approach is robust and highly appropriate to the DeNt sib-pair design. However, as some DeNt sites (particularly St Louis) adopted an ascertainment strategy that derives affected sib pairs from extended multiplex families, there was a strong argument for also exploring the data using an approach that takes full advantage of larger as well as small pedigrees. In particular, it has been proposed (40) that analysing linkage data over a dominant model and a recessive model—in effect maximizing the LOD score over model as well as position—is more powerful than a model-free method and nearly as robust. This type of the so-called MOD score analysis was performed using the heterogeneity LOD score option available in GENEHUNTER (41). The following parameters, corresponding to a population frequency of recurrent depression of ~5%, were used to specify a dominant model $q = 0.05$, $f_1 = 0.05$, $f_2 = 0.5$ and $f_3 = 0.5$ and parameters $q = 0.33$, $f_1 = 0.05$, $f_2 = 0.05$ and $f_3 = 0.5$ were used to specify a recessive model. Parametric analysis was carried out on the eight chromosomes that had LOD scores greater than 1 in the non-parametric analysis. Owing to susceptibility of multipoint parametric analysis to mis-specified allele frequencies, single point analysis was also undertaken. Gender-specific analyses were also performed using only same-sex pairs.

**Combined data analysis.** Badner and Gershon’s (15) method was used to combine the results of chromosomes 12q and 15q with those from previous studies by Abkevich et al. (12) and Holmans et al. (11). This method involves correcting all but the P-value from the original study by an equation that takes into account the distance between the peak of the initial

<table>
<thead>
<tr>
<th>Site</th>
<th>Gender</th>
<th>Number of probands</th>
<th>Number of siblings</th>
<th>Mean age at illness onset (SEM)</th>
</tr>
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<td>17</td>
<td>21.39 (1.8)</td>
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<td>54</td>
<td>63</td>
<td>18.44 (0.9)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>21</td>
<td>33</td>
<td>17.58 (1.5)</td>
</tr>
</tbody>
</table>

Table 2. Probandes and affected siblings recruited from each site
and subsequent studies (42):

\[ \text{adjusted}_P = CP + 2 \Delta G[Z(P)] \ln [Z(P) \sqrt{4(4 \Delta)]}, \]

where \( C \) is the number of chromosomes, \( P \) the observed \( P \)-value, \( \Delta \) the rate of crossovers per Morgan, \( G \) the size of region in Morgans, \( Z(P) \) the standard normal inverse of \( P \) and \( \Phi(Z(P)) \) the standard normal density function. The final part of the equation is a correction for the fact that the test is made at discrete points, i.e. each marker. \( \Delta \) is the average marker spacing in Morgans, and when \( Z(P) \sqrt{4(4 \Delta)} \) is less than 2, as it was in all our analysis, \( v \) can be approximated as \( \exp (-0.583 \Delta) \). The \( P \)-value of the original study and the adjusted \( P \)-values of the subsequent studies are combined using the equation given by Fisher:

\[ \chi^2 = -2 \Sigma \ln (P_i), \]

where the degrees of freedom is twice the number of studies.

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