ORIGINAL ARTICLE

Genome wide significant linkage in schizophrenia conditioning on occurrence of depressive episodes

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Background: Schizophrenia shows substantial clinical heterogeneity. One common important clinical variable in presentation is the occurrence of episodes of major depression.

Methods: We undertook analyses in an attempt to detect loci that influence susceptibility to, or modify the clinical expression of, schizophrenia according to the occurrence of episodes of major depression. We used a logistic regression framework in which lifetime presence/absence of major depression was entered as a covariate in the linkage analysis of our UK schizophrenia affected sibling pair series (168 affected sibling pairs typed for a 10 cM map of microsatellite markers).

Results: Inclusion of presence/absence of depression as a covariate detected a genome wide significant linkage signal on chromosome 4q28.3 at 130.7 cM (LOD=4.59; $p=0.038$; increase in maximum LOD over univariate analysis (ILOD)=3.62). Inclusion of the depression covariate also showed suggestive evidence of linkage on 20q11.21 (LOD=4.10; expected to occur by chance 0.093 times per genome scan, ILOD=2.83).

Conclusions: Our findings identify loci that may harbour genes that play a role in susceptibility to, or modify the risk of, episodes of major depression in people with schizophrenia.

Schizophrenia is a common disorder with a lifetime morbid risk of 1%, and more if spectrum disorders are included.¹ A large number of family, twin, and adoption studies shows that individual differences in liability are very largely genetic, with heritability estimates of around 80%.² Several genes are probably involved in the transmission.² However, the number of susceptibility loci, the disease risk conferred by each locus, the extent of genetic heterogeneity, and the degree of interaction among loci all remain unknown. As for other common genetic disorders, the small amount of risk conferred by variation at any given locus makes the task of identifying susceptibility genes by positional genetics difficult. However, in recent years, as sample sizes and hence power have increased, so have replicated, positive linkages to a number of chromosomal regions accumulated.^{3–5} Moreover, systematic mapping studies of several of these regions have produced replicated evidence for association between schizophrenia and polymorphisms in specific genes.⁶

The great majority of linkage studies of schizophrenia have sought to locate susceptibility alleles using categorical definitions of affection status. However, schizophrenia shows substantial clinical variation, offering the potential to use subtypes or covariates to enhance the power of analyses to detect loci, as has been used successfully with some other complex genetic disorders, such as asthma. One important area of variation is the occurrence of major depression; this is the focus of the current report.

We chose to study major depression for several reasons. Firstly, episodes of major depression occur commonly in individuals diagnosed with schizophrenia.⁷ Secondly, major depression occurs at increased frequency in relatives of schizophrenic probands compared with relatives of controls.^{8,9} Thirdly and importantly, familial aggregation of

lifetime occurrence of major depression has been demonstrated within samples of sibling pairs diagnosed with schizophrenia.^{10,11} These latter observations indicate a possible genetic effect.

Given that the above evidence is consistent with the existence of loci that influence the risk of major depression in schizophrenia, we used major depression as a covariate in linkage analyses aimed at detecting loci conferring increased risk to depressive syndromes in a large sample of sibling pairs affected by schizophrenia.

MATERIALS AND METHODS

Sample

The sample consisted of 170 affected sibling pairs (ASPs) drawn from 137 families collected in the UK and the Republic of Ireland. The sample was referred to as the "UK sample" in our recent schizophrenia genome scan.¹² All individuals were white, had been born in the UK or Republic of Ireland, and gave written, informed consent as approved by the multi-centre research ethics committee and relevant local research ethics committee. The distribution of diagnoses following the criteria of the *Diagnostic and statistical manual of mental disorders*, 4th ed (DSM-IV) among sibling pairs was as follows: schizophrenia-schizophrenia, 140 pairs; schizophrenia-schizoaffective, depressed type, 13 pairs; schizophrenia-schizoaffective, bipolar type, 12 pairs; schizoaffective, depressed type-schizoaffective, bipolar type, 3 pairs; schizoaffective, depressed type-schizoaffective, depressed type, 1 pair; schizophrenia-delusional disorder, 1 pair. All family

Abbreviations: ASPs, affected sibling pairs; DSM-IV, *Diagnostic and statistical manual of mental disorders*, 4th ed; IBD, identity by descent; ILOD, increase in logarithm of the odds ratio; OPCRIT, Operational Criteria Checklist

relationships were confirmed by examination of the degree of allele sharing across the genome as described previously.¹²

Diagnosis

Diagnoses were made by trained raters based on all available clinical information including a semistructured interview, examination of case notes, and information from relatives and mental health professionals. Additional rating scales measuring illness severity and psychopathology were completed. Details are given in Williams *et al.*¹² Psychiatrists or psychologists conducted all interviews. Vignettes were prepared on background, interview, and case note data. Clinical information was collated using the Operational Criteria Checklist (OPCRIT).¹³ Two independent raters separately rated each case and a consensus diagnosis was reached. If there were any discrepancies, the case was brought to a full team meeting so consensus could be reached or the case was excluded. Forty cases were rated against consensus by each rater in order to measure reliability. Inter-rater reliability for diagnosis was excellent, with an average kappa score of 0.90 for all raters for the duration of the study.

Mood syndrome rating

The lifetime presence or absence of at least one major depressive episode was obtained from the mood item ratings on the OPCRIT for each patient by counting the symptom items recorded on the OPCRIT for the worst episode of depression, and determining whether the threshold for a diagnosis of DSM-IV major depressive episode was met. Use of OPCRIT has been shown to be a valid research diagnostic approach for mood disorder syndromes.¹⁴ Within the current study, reliability of the OPCRIT data was assessed on 30 cases with a range of functional psychoses. The OPCRIT defined depression episode had a reliability of 0.86.¹⁵

Genotyping

In total, 372 microsatellite markers were included in this study and were typed within the context of our previously reported genome scan of schizophrenia.¹² Most were selected from the ABI linkage mapping set (version 2; $n = 352$) with a further 20 markers being added from the Marshfield genetic map (<http://research.marshfieldclinic.org/genetics/default.htm>). This resulted in an average intermarker distance of 10.22 cM across the genome. The marker order and the distances between them were checked by referring to the high resolution genetic map determined by Kong *et al.*¹⁶ where possible and by using CriMap.¹⁷ To help interpret the results, physical distances were obtained from the UCSC Human Genome Browser (May 2004 assembly; <http://genome.ucsc.edu>). A detailed description of the DNA preparation and genotyping methods can be found in Williams *et al.*¹²

Statistical analysis

In total, 282 affected individuals were included in the analysis, of whom 76 (27.0%) were rated as having experienced a definite major depressive episode.

The depression data were dichotomous: + and – were used to indicate presence and absence, respectively, of the trait for each individual. For the analysis, a measure was required that described the phenotype of the affected sibling pair rather than the individual. The dichotomous covariate information was reduced to –/–, –/+, and +/+ pairs of affected individuals. The resulting distribution of sibling pairs was: –/–, 84 (50%); –/+, 73 (43%); and +/+, 11 (7%). Genes that do not influence overall risk of schizophrenia, but do influence the distribution of covariate values in schizophrenia cases (“modifying genes”) are expected to show increased sharing in –/– or +/+ pairs (or both), with –/+ pairs showing decreased sharing. Genes that increase risk for a form of

schizophrenia that is partly characterised by the phenotype used as a covariate would be expected to increase sharing in pairs concordant for that value, with the other pairs showing little deviation from the identity by descent (IBD) probabilities expected in the absence of linkage. This is because individuals without the covariate reflect other sources of risk for schizophrenia, and are thus uninformative for the risk genotype at this particular locus. Finally, a gene that influences both schizophrenia risk and covariate values may exhibit increased or decreased sharing in –/+ pairs (depending on allele frequency and penetrances). However, the IBD sharing in the –/+ pairs should not be greater than the sharing in both –/– and +/+ pairs. Given that expected IBD probabilities within each of these model categories can vary considerably depending on the parameters (gene frequencies and penetrances), it is difficult to infer the likely genetic model from IBD probability estimates alone.

An ASP could only be included if phenotype information was available for both siblings. Therefore, the total number of 168 ASPs is less than the full sample dataset of 170. Covariate multipoint linkage analysis was performed on the ASP linkage data using a logistic regression framework as first described by Rice *et al.*¹⁸ and programmed into the analysis (by PH). For ASPs, the probability of allele sharing ranges between 0 and 1 and is modelled using logistic regression. Under the null hypothesis of no covariate effect, the intercept parameter (unconstrained) is a measure of the divergence of IBD from the null in the sample as a whole. Additional parameters for the covariates are then incorporated into the model. To ensure the logistic regression model made sense biologically, a constraint was applied to the –/+ pairs, so that their estimated sharing could not exceed that observed in either of the –/– and +/+ pairs.

The statistical measure of linkage support used was the LOD score. For each chromosome, a maximum LOD score was obtained both with and without conditioning on the covariate of interest (with no requirement that maxima were in the same location). Note that for small genetic effects it is well known that linkage peaks from sibling pair analyses can be some distance from the disease locus.¹⁹ It is, therefore, to be expected that the covariate peak signal may be at a different location from the peak linkage signal without inclusion of covariates, and this is often the case in practice. When the covariate reflects a feature of the genetic model (for example, heterogeneity or a modifier effect) including it effectively increases the evidence for a genetic effect, so the covariate peak is likely to be closer to the trait locus. Essentially, we were interested in the genome wide significance of the maximum covariate LOD score for each chromosome—that is, the significance after adjusting for multiple testing of all chromosomes. To determine the level of significance, we simulated 1000 replicate genotype data sets under the null hypothesis of no linkage. These simulations maintained the same marker-allele frequencies, marker locations, family structures, and individuals typed at each locus as in the observed dataset. Each simulated dataset was analysed with the observed depression data and the maximum covariate LOD scores for each chromosome recorded. Regions of interest were defined using the Lander and Kruglyak²⁰ definitions of significant and suggestive linkage. A region was defined as genome wide significant if the maximum LOD score would have been expected to occur only once in every 20 genome scans in the absence of linkage. A genome wide suggestive region was defined as occurring on average less than once per genome scan in the absence of linkage.

A potential confounding influence on our results is variation in duration of time over which an individual has had the opportunity to develop depression. For example, an

individual early in the course of schizophrenia may not have developed a depressive episode because of insufficient time rather than lack of intrinsic susceptibility. This only affects those individuals who have not experienced an episode of depression. In the sample, the duration of illness in the individuals who had never experienced depression ranged from 0 to 57 years. To ensure the results of our analysis were not dependent on the duration of illness, the regions that reached criteria for genome wide significant (or suggestive) linkage were further tested for the effect of duration of illness. A single additional covariate was constructed to test this effect of duration. In the $-/+$ pairs it took the value of the duration of illness of the member of the pair who had not experienced depression, and in the $-/-$ pairs it took the mean duration of the pair, and in the $+/+$ pairs it took the value of zero. In a manner similar to that presented above, a covariate linkage analysis was then performed to test the effect of the duration covariate on the model already including the depression covariate.

RESULTS

Presence or absence of major depressive syndromes was tested within the logistic regression model to determine evidence for linkage on the chromosomes in our genome scan of schizophrenia affected sibling pairs. A region on chromosome 4 gave genome wide significant evidence for linkage. Furthermore, a region on chromosome 20 reached the criteria for genome wide suggestive evidence for linkage (table 1;

Figure 1 Multipoint covariate LOD score plots for chromosomes 4 (A) and 20 (B). Univariate and depression covariate multipoint LOD score plots. Overall evidence of univariate linkage can originate from decreased sharing of alleles between ASPs. The inclusion of the depression covariate increased the LOD score.

Table 1 Results of covariate linkage analysis

Chromosome	Nearest marker*	Physical location of marker (Mb)	Maximum LOD score	Expected no. of occurrences†
4q28.3	D4S1575	135.1	4.59	0.038
20q11.21	D20S195	31.3	4.10	0.093

*Marker nearest to peak covariate linkage signal; †of observed (or greater) maximum LOD scores expected per genome scan.

fig 1). Results in both regions were independent of the duration of illness (that is, including the duration of illness as a covariate did not diminish the evidence).

The two regions meeting criteria for genome wide significant or suggestive covariate linkage regions are presented. Physical location to the peak linkage signals are taken from the UCSC Human Genome Browser as above.

Chromosome 4

A maximum covariate LOD score of 4.59 at 130.7 cM (nearest marker to peak was D4S1575 at 135.1 Mb) reached genome wide significance. The maximum univariate LOD score (with no covariate) on this chromosome (LOD = 0.97) occurred at 54.7 cM (nearest marker to peak: D4S405 at 40.2 Mb). Addition of the depression covariate increased the maximum LOD score by 3.62. The estimated IBD sharing at the maximum in the $-/-$, $-/+$, and $+/+$ pairs were 0.52, 0.32, and 0.99, respectively.

Chromosome 20

A depression covariate LOD score of 4.10 at 51.8 cM (nearest marker to peak was D20S195 at 31.3 Mb) reached genome wide suggestive evidence for linkage. By chance, the number of maximum LOD scores in excess of 4.10, expected to occur in a genome scan, is 0.093. The univariate maximum LOD score of 1.27 at 56.8 cM (nearest marker to peak: D20S107 at 38.3 Mb) increased by 2.83. At the maximum, the estimated IBD sharing in the $-/-$, $-/+$, and $+/+$ pairs were 0.49, 0.59, and 0.90, respectively.

DISCUSSION

We have undertaken the first systematic genome scan of a set of schizophrenia pedigrees that has used lifetime occurrence of major depression as a covariate. We found that inclusion of lifetime presence/absence of depression as a covariate gave a maximum LOD score on chromosome 4q28.3 of 4.59 at 130.7 cM, which meets criteria for genome wide significance ($p = 0.038$). The maximum LOD -1 region spans 18cM between 124.7 and 142.7 cM. Both depression concordant and depression discordant siblings contributed to the linkage signal: siblings with schizophrenia who both experienced depression showed close to 100% estimated IBD sharing in this region, while siblings with schizophrenia who were discordant for depressive episodes showed sharing below the 50% chance level. This region of chromosome 4 has not emerged in meta-analyses of systematic genome scans for schizophrenia^{4,5} or bipolar disorder,^{5,21} although LOD scores in the range 2–3 have been reported under some analyses in individual scans of schizophrenia (LOD = 2 at D4S430; 126.2 cM)²² and bipolar disorder (LOD = 3.2 at D4S1625; 146.0 cM)²³ and LOD = 2 at D4S1629; 158.0 cM).²⁴

Assuming our finding is a true positive, there are three main possible interpretations. The first is that the locus may contain one or more susceptibility genes for major depression and have no influence on the expression of schizophrenia. Support for this possibility would need to come from linkage studies of large samples of families multiply affected by

major depression, unselected for occurrence of schizophrenia. Only three systematic genome scans of unipolar depression have been reported to date,^{25–27} but this region has not been implicated in any of these. Secondly, one or more susceptibility genes on chromosome 4 may predispose to a subtype of schizophrenia characterised by the presence of depressive episodes. This corresponds to a heterogeneity model. The third possibility is that one or more genetic variants in this region may modify the schizophrenia phenotype toward expression of depressive episodes without altering susceptibility to schizophrenia itself (one can think of this as a “disease modifying” model). Both the latter models are consistent with our linkage data. Identifying association at the pathogenically relevant locus will be helpful in resolving these possibilities. The very high estimate of IBD sharing (99%) in these pairs suggests the possibility of a major effect at this locus. However, there were only 11 sibling pairs who were concordant for depressive episodes, thus, although the increased sharing is genome wide significant (and there is also a substantial contribution to the signal from the discordant pairs), caution is required in interpreting the magnitude of the IBD estimate, and it is clearly important that large independent schizophrenia linkage samples are examined in an attempt to replicate this finding.

It is interesting to note that the other region of interest which reached genome wide suggestive linkage in our analysis of depressive syndrome, according to the criteria of Lander and Kruglyak²⁰ on chromosome 20q11.21 (LOD = 4.10, LOD-1 region = 44.8–58.8 cM) was identified as a region of interest in a recent meta-analysis of genome scans for schizophrenia.⁴ It was one of 12 regions of interest (each a “bin” of 30 cM in size) identified under both weighting models used in the analysis. Our findings suggest that this locus, which was not identified in our own conventional schizophrenia genome scan, may be most efficiently dissected genetically by careful consideration of the clinical phenotype to take account of the occurrence of depressive episodes.

We recently reported strong evidence for linkage to schizophrenia at chromosome 10 close to D10S217 at 162 cM and on chromosome 17 close to D17S1868 at 66 cM.¹² Our previous study included the UK families analysed in the present study and samples from Sweden and the US (it should be noted that we have only examined the UK sample within the current analysis because this was the only subset for which detailed mood episode data were available). The depression covariate investigated in this analysis was not found to significantly affect the UK sample linkage peaks in the chromosome 10 or 17 regions (fig 1). Thus, this covariate is unlikely to be helpful in refining these peaks.

The logistic regression approach that we have used makes efficient use of covariate data within the context of a sibling pair linkage design because data from all families contribute to the analysis; this is potentially important within the current sample because only 11 pairs were concordant for lifetime major depression. This contrasts with a frequently used method in which a primary analysis is undertaken with the whole dataset, followed by a secondary analysis on a subset selected according to the covariate. This approach overlooks a considerable number of data because the individuals are either excluded totally or they remain in the analysis with phenotype designated as “unknown”. Either way, a direct comparison between the results for the main and secondary analyses is not straightforward as the sample sizes differ. As an alternative to the logistic regression method employed here, Devlin *et al*²⁸ proposed mixture models for analysing ASP linkage data with covariates. Two methods were proposed, which aim to assign membership

probabilities of each ASP to either linked or non-linked groups. One method uses the results of a cluster analysis based on phenotypic information, the other also uses estimated IBD information at the region of interest. Given that most of the ASPs studied here do not have extended pedigrees from which to draw substantial IBD information, the latter method is not expected to be particularly powerful within a sample such as ours. Furthermore, the method does not allow for a group of families that have IBD sharing <50%, as expected under the disease modifying model. Another potential method for analysing covariates is to analyse ordered subsets of the data.²⁹ However, this approach is more easily applied to continuous rather than to dichotomous covariates, as considered here.

The major limitations of our study are inherent in all lifetime studies of mood symptoms in schizophrenia. Phenotypic information was collected retrospectively, and in the context of diagnostic systems in which mood features are “trumped” by schizophrenia features. One can speculate that as a result, when sufficient clinical features are present for a diagnosis of schizophrenia, there may be a temptation to downplay mood symptoms. We suspect therefore that the mood data collected on our schizophrenia sample almost certainly represent a lower boundary for the true lifetime prevalence of major depression. This limitation is conservative and would be expected to contribute to type II rather than type I error.

CONCLUSION

In summary, we have used a logistic regression framework in which lifetime presence/absence of major depression was entered as a covariate in the linkage analysis of our UK schizophrenia affected sibling pair sample. We detected a genome wide significant linkage signal on chromosome 4q28.3 and genome wide suggestive evidence of linkage with depression as a covariate on 20q11.21. Our findings identify chromosomal locations that may harbour genes that play a role in susceptibility to, or modify the clinical expression of, schizophrenia according to the occurrence of episodes of major depression.

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