Objective: Vulnerability to the triggering of bipolar episodes by childbirth aggregates in families and may define a genetically relevant subtype of bipolar disorder. The authors conducted a search by systematic whole genome linkage scan for loci influencing vulnerability to bipolar affective puerperal psychosis.

Method: The authors selected families with bipolar disorder from their previous bipolar disorder genome scan, in which there was at least one family member with a manic or psychotic episode with an onset within 6 weeks of delivery. Individuals were coded as affected if they had been diagnosed with bipolar I disorder, bipolar II disorder, or schizoaffective disorder, bipolar type, according to DSM-IV. A total of 36 pedigrees contributed 54 affected sibling pairs to the cohort. A genome scan with 494 microsatellite markers was analyzed using GENEHUNTER and MAPMAKER/SIBS.

Results: A genome-wide significant linkage signal was observed on chromosome 16p13, and a genome-wide suggestive linkage was observed on chromosome 8q24. No significant or suggestive linkage was observed in these regions in our original bipolar scan.

Conclusions: This study identifies chromosomal regions that are likely to harbor genes that predispose individuals to bipolar affective puerperal psychosis. The identification of susceptibility genes would enhance understanding of pathogenesis and offer the possibility of improvements in treatment and risk prediction.

There is substantial evidence from family, twin, and adoption studies concerning the importance of genes in influencing susceptibility to bipolar disorder (1, 2). Progress in identifying the genetic variants that confer risk has improved in recent years, with replicated findings emerging from molecular genetic studies (3–6). Despite this encouraging progress, however, findings have varied between studies, perhaps reflecting variation in the clinical and genetic characteristics of cohorts.

For complex genetic conditions such as bipolar disorder, there are marked benefits in focusing on a homogeneous clinical subtype, both to increase phenotypic and genetic homogeneity and to allow a specific subset of hypotheses to be tested (7). In the case of bipolar disorder, a number of clinical subtypes have been investigated in molecular genetic studies, including rapid cycling illness, early age at onset, and lithium responsiveness. The present study focused on a clinical subtype of bipolar disorder that is defined by a vulnerability to the triggering of severe bipolar episodes by childbirth: bipolar affective puerperal (postpartum) psychosis. We have previously reported evidence that a vulnerability to postpartum episodes clusters in families (8, 9) and episodes occurring in relationship to childbirth may be a marker for a more familial form of bipolar disorder (10). A focus on bipolar disorder with a vulnerability to postpartum triggering is therefore a promising approach in the search for bipolar disorder susceptibility genes, and we have undertaken a systematic genome scan for loci that influence vulnerability to this subtype.

Method

Subject Recruitment

All subjects were Caucasian and of British or Irish origin and recruited in the United Kingdom and the Republic of Ireland.
through mental health services, patient support groups, and articles in the national media. Ethical approval was obtained prior to data collection, and written informed consent was obtained after a complete description of the study was given to each subject.

Assessment

All subjects were interviewed by a trained psychologist or psychiatrist using a semistructured research interview (Schedule for Clinical Assessment in Neuropsychiatry) (11), and case note data were obtained. Consensus best estimate ratings of episode and lifetime diagnoses, according to DSM-IV criteria, were made by two independent investigators on the basis of all available clinical data. Any disagreements were rated by a third investigator and discussed in order to reach a consensus. Regular meetings were held between all interviewers and raters in order to maximize clinical consistency and reliability. Inter-rater reliability was assessed using clinical data from 20 cases (chosen to represent a typical cross section of subjects recruited within the study), which were rated by each investigator and compared against consensus to obtain individual kappa coefficients of reliability. Reliability was measured during the studies and shown to be excellent, with a mean kappa of 0.85 for DSM-IV diagnosis.

For female participants, information was obtained during interview and via case notes on the relationship of episodes of illness to childbirth. Consensus best estimate ratings were made for both the perinatal episode diagnosis and the timing of onset in relation to delivery by the methodology described previously with excellent reliability (mean kappa=0.90 and 0.93, respectively). This allowed us to identify the subset of female participants with a history of puerperal psychosis, defined in the present study as an episode of mania or psychosis with onset within 6 weeks of delivery. The 6-week onset was chosen as a compromise between very narrow (onset within 1 or 2 weeks) and very wide (onset within 6 months) definitions that have been used in previous studies on puerperal psychosis. The vast majority (95%) of women with an episode of puerperal psychosis in our cohort had an onset within 2 weeks of delivery. Additional details of the phenotypic assessment and diagnostic methods are provided elsewhere (12-14).

Families were selected for inclusion in the current analysis according to the following criteria: 1) At least one female relative had a lifetime diagnosis that met DSM-IV criteria for bipolar I; bipolar II; or schizoaffective disorder, bipolar type, and had suffered a history of puerperal psychosis, defined in the present study as an episode of mania or psychosis with onset within 6 weeks of delivery. 2) At least one additional family member had an illness that met DSM-IV criteria for bipolar I; bipolar II; or schizoaffective disorder, bipolar type, and 3) the family was informative for linkage analysis.

Genotyping

The genome scan involved a two-stage design based on simulation work that showed that it was possible to achieve a similar power and type I error rate while considerably reducing the amount of genotyping. The strategy involved screening the stage 1 cohort with a 10 cM grid and then following up areas of interest with a tighter grid of markers every 5 cM. Seventeen regions that achieved nominally significant linkage in the full bipolar I disorder stage screen were taken forward into the second stage. In this study, both the first- and second-stage data were utilized in the analysis of the subset of families.

Laboratory work was undertaken using consistent methodologies in the molecular psychiatry laboratories at the University of Birmingham (Dr. Craddock), Cardiff University (Dr. Craddock), and the Psychiatric Genetics laboratory at Trinity College Dublin, Ireland, (Dr. Gill). The consistency and reliability of cross-center genotyping strategies were validated via a joint pilot study using markers on chromosome 21 (15). All deoxyribonucleic acid (DNA) samples were either extracted from whole blood or from saline mouthwash samples using standard procedures. All polymerase chain reactions were performed on MJ Research Thermal Cycler. Postpolymerase chain reaction—products from individual and multiplex reaction—were pooled in empirically determined ratios into size-specific marker sets prior to gel electrophoresis. This permitted up to 20 discrete marker loci to be analyzed in a single gel lane, with allele peak fluorescence intensities remaining within optimal limits (typically ~200 to 4000 units). All markers were genotyped on either ABI377 XL DNA sequencers or ABI3100 sequencers using the software Genescan and Genotyper (Applied Biosystems, Foster City, Calif.). Further details of genotyping methodology are given in articles by Bennett et al. (13) and Lambert et al. (14).

Statistical Analysis

Genetic relationships between family members were confirmed using marker data from across the genome and a suite of software packages: RELATIVE (16), RELCHECK (17, 18), and PREST (19). In-house software and the program GRR (20) were employed to detect monozygotic twins and cohort duplications to ensure that no one individual was typed in two different families. The presence of non-Mendelian errors was detected using the software PedCheck (21).

 Autosomal multipoint analyses were performed using the GENEHUNTER software package (22, 23), which calculates the maximum likelihood logarithm of the odds ratio (LOD) at each point in the genome using the maximum likelihood sharing probabilities (identical by descent) for each sibling pair. Maximum likelihood LOD scores for the X chromosome were estimated using the MAPMAKER/SIBS software package (24). Marker allele frequencies were estimated using the program SPLINK (25) from our data set with maximum likelihood methods. Our method of analysis made no assumptions about the genetic model. The phenotypic model used was to define all individuals with a diagnosis of DSM-IV bipolar I; bipolar II; or schizoaffective disorder, bipolar type, as “affected.” All other individuals were considered as “phenotype unknown.”

We obtained empirical significance levels and the expected number of given LOD scores per genome screen by simulating 1,000 replicates of the entire data set under the null hypothesis of no linkage and then analyzing the data set with GENEHUNTER. These simulations maintained the same marker allele frequencies, marker locations, family structures, and individuals genotyped at each locus as in the observed data set. This accounted for the specific properties of the cohort studied and allowed for multiple testing.

Results

In the complete two-stage genome scan, 494 markers were genotyped in 232 bipolar families, with an average intermarker distance of 8.7 cM in stage 1 and 4.8 cM in the stage 2 regions (14).

In the current analysis, which was limited to families containing a woman who had suffered an episode of postpartum psychosis, a total of 54 affected sibling pairs were included from 36 families (of which, 44 pairs from 26 families were included in stage 1). Table 1 shows the location and magnitude of the maximum linkage signal for each chromosome.

Simulation studies demonstrated that a maximum LOD of 3.62 was required for genome-wide significance according to the criteria by Lander and Kruglyak (26). This equates to an LOD score of 3.62 or greater being obtained.
by chance (i.e., under the null hypothesis of no true linkage) only once in 20 genome scans. The maximum LOD of 4.07 observed on chromosome 16 was therefore genome-wide significant, with an empirical genome-wide significance p value of 0.02 (Figure 1). The closest genotyped marker to our peak was DS16S423, and the LOD-1 interval (roughly corresponding to a 95% confidence interval) spanned approximately 9 cM (from genetic distance 10.4 cM [i.e., D16S423 the most telomeric marker typed] to 19.4 cM). The estimated probability of allele sharing between affected siblings at the peak was 0.75, a substantial elevation above the null expectation of 0.50.

An LOD of 1.95 or higher was expected to occur by chance once per genome scan (i.e., genome-wide “suggestive” linkage according to the criteria by Lander and Kruglyak [26]). In addition to the genome-wide significant region on chromosome 16, we observed a genome-wide suggestive region on chromosome 8q24, with an LOD score of 2.03 close to marker D8S284 (Figure 2). The estimated probability of allele sharing between affected siblings at this peak was 0.69, which was again a substantial elevation above the null expectation of 0.50.

**Discussion**

Linkage studies in bipolar disorder have tended to focus on bipolar I disorder alone or have extended the phenotype to include related diagnoses such as bipolar II disorder, recurrent major depression, and schizoaffective disorder. It is highly likely, however, that the bipolar diagnostic label covers a heterogeneous collection of conditions with differing etiological factors. Increasing attention is now being given to clinical subtypes of the disorder that may represent more homogeneous forms of the illness.

In this study, we have focused on a clinical subtype defined by a vulnerability to severe episodes of illness being triggered by childbirth. We have previously reported 1) compelling evidence that a vulnerability to postpartum bipolar episodes clusters in families [8] and 2) data suggesting that a vulnerability to puerperal episodes is a marker for a more familial form of bipolar disorder [10]. Additionally, we have reported a number of candidate gene association studies in women with puerperal psychosis [27] and have shown suggestive, although preliminary, evidence for an association between puerperal psychosis and a variant at the serotonin transporter gene on chromosome 17 [28]. In the present study, we employed the complementary, positional approach of a genome-wide linkage study in sibling pairs, focusing on those families in which a female proband has suffered an episode of mania or psychosis within 6 weeks of delivery.

We found one chromosome region (16p13) with an LOD score of 4.07 meeting the Lander and Kruglyak criteria (26) for genome-wide significance (p=0.02). A finding of this magnitude would therefore be expected by chance only once in every 43 genome scans conducted with similar family pedigrees and markers. We also report an additional chromosomal region (8q24) that meets the Lander and Kruglyak (26) criteria for genome-wide suggestive linkage.

**TABLE 1. Location and Magnitude of the Maximum Linkage Signal for Each Chromosome**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Observed Maximum Logarithm of the Odds Ratio Score</th>
<th>Peak Location (cM)</th>
<th>Nearest marker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.60</td>
<td>150.2</td>
<td>D1S252</td>
</tr>
<tr>
<td>2</td>
<td>1.03</td>
<td>257.6</td>
<td>D2S336</td>
</tr>
<tr>
<td>3</td>
<td>0.66</td>
<td>20.3</td>
<td>D3S3706</td>
</tr>
<tr>
<td>4</td>
<td>1.50</td>
<td>131.7</td>
<td>D4S1615</td>
</tr>
<tr>
<td>5</td>
<td>0.77</td>
<td>147.7</td>
<td>D5S436</td>
</tr>
<tr>
<td>6</td>
<td>0.82</td>
<td>14.2</td>
<td>D6S309</td>
</tr>
<tr>
<td>7</td>
<td>0.83</td>
<td>160.3</td>
<td>D7S636</td>
</tr>
<tr>
<td>8</td>
<td>2.03</td>
<td>143.7</td>
<td>D8S284</td>
</tr>
<tr>
<td>9</td>
<td>0.42</td>
<td>148.8</td>
<td>D9S290</td>
</tr>
<tr>
<td>10</td>
<td>0.59</td>
<td>29.1</td>
<td>D10S547</td>
</tr>
<tr>
<td>11</td>
<td>0.23</td>
<td>66.8</td>
<td>D11S987</td>
</tr>
<tr>
<td>12</td>
<td>0.89</td>
<td>133.0</td>
<td>D12S1217</td>
</tr>
<tr>
<td>13</td>
<td>0.91</td>
<td>46.0</td>
<td>D13S153</td>
</tr>
<tr>
<td>14</td>
<td>0.28</td>
<td>109.5</td>
<td>D14S280</td>
</tr>
<tr>
<td>15</td>
<td>0.39</td>
<td>61.1</td>
<td>D15S128</td>
</tr>
<tr>
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<td>4.07</td>
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</tr>
<tr>
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<td>54.6</td>
<td>D17S7978</td>
</tr>
<tr>
<td>18</td>
<td>1.78</td>
<td>73.0</td>
<td>D18S1127</td>
</tr>
<tr>
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<td>39.0</td>
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</tr>
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<td>20.8</td>
<td>D20S115</td>
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<td>9.7</td>
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</tr>
<tr>
<td>22</td>
<td>0.03</td>
<td>39.1</td>
<td>D22S283</td>
</tr>
<tr>
<td>x</td>
<td>1.19</td>
<td>114.7</td>
<td>DXS1106</td>
</tr>
</tbody>
</table>

a Marker maps for linkage analysis were obtained from the Marshfield genetic map (http://www.marshfieldclinic.org/research/genetics/).

b “Suggestive” genome-wide linkage.

c “Significant” genome-wide linkage.
Previous Findings at Loci 16p13 and 8q24

Although, to our knowledge, this is the first linkage study to focus on families containing cases of bipolar affective puerperal psychosis, it is of interest to compare the location of these chromosomal regions with those previously reported in linkage studies of bipolar disorder families unselected for onset in the puerperium. Although the two loci did not emerge from our own genome scan of bipolar disorder (13, 14), there have been previous reports of linkage in these regions.

16p13. The 16p13 region has not been implicated in meta-analyses of bipolar linkage scans (3, 4, 29), but a number of individual studies have reported evidence of linkage in this chromosomal location. Ewald et al. (30) reported an LOD score of 2.5 in two Danish pedigrees under a recessive model; their peak was less than 3 cM from our own and lay within our LOD-1 region. Other studies have also reported linkage in this general region. McInnes et al. (31) reported modest evidence for linkage (LOD=1.46) in two Costa Rican pedigrees, with a peak 12 cM telomeric from ours. Finally, multiple waves of the National Institute of Mental Health (NIMH) Genetics Initiative on Bipolar Disorder reported evidence for linkage in this region, with LOD scores >2 across a 17 cM region beginning 10 cM centromeric from our peak (32–34). Most recently, the consortium has reported that the evidence for linkage in this region is substantially increased (LOD score=4.5) when employing mania at onset as a covariate in the analysis (35).

The location of the peak of our genome-wide significant linkage signal on chromosome 16p13, therefore, coincides with a region that has been associated with bipolar disorder in previous linkage studies. It is possible that the families in the previous studies were particularly susceptible to postpartum triggering of illness. In the NIMH study, for example, it has been reported that almost 50% of the female bipolar probands reported episodes of severe illness in relation to childbirth (36). However, it is also possible that the short arm of chromosome 16 contains two or more loci harboring vulnerability genes for various forms of bipolar disorder.

8q24. The 8q24 region has also been implicated in previous linkage studies. Our peak at 143 cM was flanked by two previously reported linkage signals. Cichon et al. (37) reported an LOD score of 3.62 in 75 families that peaked at 130 cM, and 65 pedigrees were reported by the Johns Hopkins group (38, 39), with a maximum LOD score of 3.32 that peaked at 148 cM, the latter falling within our LOD-1 region. Although two of the published meta-analyses of bipolar linkage scans (3, 4) did not implicate this region of chromosome 8, a recent pooled-data analysis proved to be more germane at this locus (29). This analysis included 11 genome scans covering 5,179 individuals from 1,067 families and benefited from combining the original genotype data rather than the linkage statistics or p values. Employing a “broad” affected model that included both bipolar I and II probands, the study found evidence for genome-wide significant linkage (LOD=3.40) in this region with a peak at 151 cM.

Limitations

Despite the encouraging findings reported in this study, they must be interpreted in the light of a number of limitations.

Cohort size. In the context of studies on complex disorders, our cohort size was relatively modest (54 sibling pairs). The fact that such a cohort could yield one signal that is genome-wide significant and another suggestive signal suggests that our study has indeed benefited from increasing genetic homogeneity and illustrates the point that carefully selected cohorts of modest size may assist in finding genes for bipolar disorder. With this cohort size, however, genes conferring a small or modest increase in vulnerability to illness are unlikely to be identified. Further, it is desirable that our findings are replicated in independent cohorts, although this may require a number of further scans before it will become clear whether the loci reported in the present study actually contain vulnerability genes for bipolar affective puerperal psychosis.

Biological model. It is worth considering the model of puerperal triggering of bipolar episodes that underlies the current analysis. We consider bipolar disorder to represent an etiologically heterogeneous collection of disorders, including a form in which women are vulnerable to the triggering of episodes by childbirth. In this model, the genetic variants influencing vulnerability to puerperal triggering are the same variants that influence vulnerability to bipolar disorder. By selecting families through an index case of bipolar affective puerperal psychosis, we have sought to identify a more homogeneous cohort, which despite being smaller in number will facilitate the search for genes influ-
enancing disease susceptibility. An alternative model is that the trigger genes are independent of the bipolar genes and may be considered additional course modifying factors. In order to identify such course modifying genes we would need to study families multiply affected with puerperal psychosis. In the present study, we have identified families with an index proband that has suffered from puerperal psychosis; however, the proband may be the only individual in the family to suffer with a postpartum episode. The number of families in which there were two or more women with puerperal psychosis was prohibitively small for meaningful analysis of puerperal psychosis sibling pairs alone. Further recruitment of families multiply affected with perinatal mood disorder would be beneficial and allow an exploration of the alternative, course modifying model of postpartum triggering.

**Specificity.** We selected families for inclusion in this study based upon the occurrence of an episode of postpartum psychosis. There was no evidence of systematic differences between illness in the nonpuerperal relatives in these families and other families in our sib pair cohort. However, it remains possible that there is one or more correlated characteristics that accounts for the linkage obtained. Future linkage studies examining a range of clinical variables as covariates may prove beneficial in refining more heterogeneous bipolar disorder phenotypes.

**Gene identification.** The regions of interest we have identified contain many hundreds of genes. More extensive investigation is needed to narrow the regions of interest and to examine the potential candidate genes they contain. There are, however, a number of potentially relevant genes in these regions, including ABAT and GRIN2A (chromosome 16) and ADCY8 (chromosome 8) that will benefit from further study.

In summary, we report the first systematic genome scan aimed at localizing genes that influence susceptibility to bipolar affective puerperal psychosis and provide further support for the hypothesis that this is a genetically meaningful subtype of bipolar disorder. We have identified regions of interest on chromosomes 16p13 (LOD=4.07, genome-wide significant) and 8q24 (LOD=2.03, genome-wide suggestive). Our linkage findings point to the benefits of focusing on the subset of bipolar disorder in which women have a vulnerability to the triggering of episodes by childbirth and provide strong evidence for a susceptibility locus on chromosome 16. Considerable investigation is required to identify the genetic variant or variants involved, but finding genes that influence susceptibility to the postpartum triggering of bipolar episodes will, it is hoped, lead to a better understanding of the etiology of these conditions and to the development of better treatment and prevention for women who suffer such devastating episodes during this time.

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