Genome Scan of a Second Wave of NIMH Genetics Initiative Bipolar Pedigrees: Chromosomes 2, 11, 13, 14, and X

As part of the ongoing NIMH Genetics Initiative on Bipolar Disorder, we have ascertained 153 multiplex bipolar pedigrees and genotyped them in two waves. We report here the genome scan results for chromosomes 2, 11, 13, 14, and X in the second wave of 56 families. A total of 354 individuals were genotyped and included in the current analyses, including 5 with schizoaffective/bipolar (SA/BP), 139 with bipolar I disorder (BPI), 41 with bipolar II disorder (BPII), and 43 with recurrent unipolar depression (RUP). Linkage analyses were carried out with multi-point parametric and non-parametric affected relative pair methods using three different definitions of the affected phenotype: (model 1) SA/BP and BPI; (model 2) SA/BP, BPI, and BPII; and (model 3) SA/BP, BPI, BPII, and RUP. The best findings were on 11p15.5 (NPL = 2.96, P = 0.002) and Xp11.3 (NPL = 2.19, P = 0.01). These findings did not reach conventional criteria for significance, but they were located near regions that have been identified in previous genetic studies of bipolar disorder. The relatively modest but consistent findings across studies may suggest that these loci harbor susceptibility genes of modest effect in a subset of families. Large samples such as that being collected by the NIMH Initiative will be necessary to examine the heterogeneity and identify these susceptibility genes.

KEY WORDS: bipolar disorder; linkage; genome scan; heterogeneity

INTRODUCTION

Bipolar disorder is a serious mental illness that affects nearly 1% of the population. The causes of bipolar disorder are unknown. However, several lines of evidence suggest that genetic factors play an important role. Given the pattern of segregation in families, it is likely that the inheritance of bipolar disorder is complex and probably involves multiple genes acting independently and interactively [Craddock and Jones, 1999]. A growing number of studies have tried to map bipolar susceptibility genes with limited success. Although several interesting loci have been identified across the genome [Nurnberger and Foroud, 2000; Baron, 2002], the findings remain inconclusive, and no genes have yet been cloned.

In 1988, the NIMH began an initiative to establish an archival resource for genetic studies of bipolar disorder [NIMH Genetics Initiative Bipolar Group, 1997]. The goal was to collect a large sample of families with multiple first-degree relatives affected with bipolar disorder and/or other affective illnesses ascertained using similar procedures. The four original sites collaborating in the initiative were Indiana University; Johns Hopkins University; Washington University, St. Louis; and the NIMH Intramural Research Program. In 1997, these sites collectively reported findings from...
a genome scan of a first wave of data collection on 97 bipolar families [Detera-Wadleigh et al., 1997; Edenberg et al., 1997; Rice et al., 1997; Stine et al., 1997].

A second wave of data collection on 56 new families has since been completed. As in the first wave, each of the collaborating sites was responsible for studying a portion of the genome. The JHU site studied chromosomes 2, 11, 13, 14, and X and we now report the findings from a scan of these chromosomes in the second wave of families. The results from scans carried out by the other sites on their respective chromosomes can be found elsewhere [Dick et al., 2002].

MATERIALS AND METHODS
Ascertainment and Assessment

The four sites used common ascertainment and clinical assessment procedures that have been described in detail elsewhere [Genomic survey of bipolar illness in the NIMH genetics initiative pedigrees: a preliminary report, 1997; Dick et al., 2002]. Families were ascertained either systematically through one of the participating treatment facilities or non-systematically through advertisements, advocacy groups, or other non-clinical sources. Systematically ascertained families were included if the proband had bipolar I disorder (BPI), and he/she had an available first-degree relative with either BPI or schizoaffective disorder bipolar sub-type (SA/BP). The family of origin of the proband could not be bilineal (both parents with BPI or SA/BP). Non-systematically ascertained families were also accepted into the study if they met more stringent criteria. These families had to have two relatives with BPI or SA/BP who were either first-degree relatives or second-degree relatives connected through another family member with at least bipolar II disorder (BPII), and the families had to have two additional relatives with BPI, SA/BP, BPII or recurrent unipolar depression (RUP). The study was approved by the local Institutional Review Boards at each collaborating site, and informed written consent was obtained from all family members who agreed to participate.

Participants were assessed by trained interviewers using the Diagnostic Instrument for Genetic Studies (DIGS) [Nurnberger et al., 1994] and the Family Interview for Genetic Studies (FIGS). At Johns Hopkins the interviewers were psychiatrists, while at the other sites the interviewers were specially trained nurses, social workers, or experienced research interviewers. Medical records were requested for all inpatient and outpatient psychiatric treatment. The interviewer and an editor separately provided working diagnoses based on the DIGS. Final diagnoses were assigned using a best estimate procedure. Two clinician reviewers made separate assessments using all available data. They then discussed the case and agreed on a diagnosis. If the two reviewers were unable to reach an agreement, then a third clinician was referred to for making a final decision. Diagnoses of BPI and SA/BP were made using Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R) criteria, while diagnoses of BPII and RUP were made using Research Diagnostic Criteria (RDC). BPII was diagnosed with RDC because DSM-III-R does not provide such a diagnosis. A diagnosis of BPII required RUP, therefore to be consistent across the two diagnostic categories RUP was diagnosed with RDC as well.

A total of 56 families with at least two available siblings diagnosed with one of the core illnesses were included into the second wave of the study. Affected relatives from these families were selected for genotyping. Parents, unaffected siblings, and other intervening biological relatives were also selected for genotyping in order to improve the ability to estimate allele sharing identical-by-descent. A total of 354 individuals were genotyped and included in the current analyses.

Genotyping

The fluorescently labeled tandem repeat microsatellite markers were amplified using polymerase chain reaction (PCR) following a modified standard protocol [Stine et al., 1995]. Electrophoresis and signal recording were performed on ABI PRISM 377 DNA Sequencers (Applied Biosystems, Foster City, CA). Genotype scoring was done automatically with ABI PRISM Genotyper software and then checked by a technician. The recorded genotypes were binned and examined for inheritance using the Genetic Analysis System (GAS v2.0) [Young, 1995] and UNKNOWN v5.2. Alleles that did not segregate were re-examined. In those cases where it was not possible to correct the errors, the data for that marker from that portion of the pedigree were deleted.

Marker Maps

We genotyped a total of 105 polymorphic markers; approximately 73% of the markers genotyped in the wave one families were genotyped here. There were 27 markers on chromosome 2, with an average spacing of 9.66 cM; 18 markers on chromosome 11, with an average spacing of 8.6 cM; 18 markers on chromosome 13, with an average spacing of 6.89 cM; 15 markers on chromosome 14, with an average spacing of 6.69 cM; and 27 markers on the X chromosome, with an average spacing of 3.02 cM. There were approximately 10 alleles per marker for an average heterozygosity of 0.76. The average information content across all the chromosomes as calculated from Genehunter-Plus was 0.60. The order of the markers was taken from Marshfield when available [Broman et al., 1998]. Those markers not included in the Marshfield map were placed using the BUILD option of CRIMAP [Lander and Green, 1987], and the final order was then tested using the FLIPS option. The distances (in Kosambi cM) between markers on the autosomes were estimated by CRIMAP using the FIXED option with the final marker order. The distances for the X-chromosome markers were taken from the Marshfield map.

Statistical Analyses

All genotype and phenotype data were imported into Discovery Manager (Visualize, Inc., Phoenix, AZ). This
software package was used to prepare all data and parameter files for subsequent analyses. It also provided estimates of allele frequencies based on the entire data set.

Multipoint non-parametric and parametric linkage analyses were carried out with Genehunter-Plus for the autosomes and X-Genehunter v1.3 for the X-chromosome. In the non-parametric analyses, non-parametric linkage (NPL) scores for affected relative pairs were calculated using the S(all) option. In the parametric analyses, LOD scores were calculated using only affected family members (all other family members were coded with a phenotype of unknown) under dominant and recessive models. The models were determined based on prevalence estimates for bipolar disorder and allowed for age-dependent phenotype rates of 0.014 (<30-years old), 0.016 (<30- and ≥40-years old), and 0.040 (>40-years old). The dominant model assumed a disease allele frequency of 0.02, while the recessive model assumed a frequency of 0.2. Both the non-parametric and parametric analyses were performed using three different definitions of the affected phenotype: (model 1) SA/BP and BPI; (model 2) SA/BP, BPI, and BPII; and (model 3) SA/BP, BPI, BPII, and RUP.

For the best finding on chromosome 11 (see below), an additional analysis was carried out using Genefinder [Liang et al., 2001]. Genefinder uses general estimating equation (GEE) methods to estimate the location of an unobserved susceptibility gene within a chromosomal region framed by multiple markers based on IBD sharing among affected sib pairs. The method has been shown to be robust in that no assumption about the genetic mechanism is required other than that the region contains no more than one susceptibility gene.

RESULTS

Table I describes the breakdown of the 56 pedigrees by the three diagnostic categories. A total of 144 individuals were diagnosed with BPI or SA/BP. These individuals comprised 101 affected relative pairs under model 1. An additional 41 individuals were diagnosed with BPII, providing 193 affected relative pairs under model 2. Another 43 individuals were diagnosed with RUP, yielding 206 affected relative pairs under model 3.

Only two loci on chromosomes 11 and X had NPL scores that exceeded 2.0 (Fig. 1). On chromosome 11, NPL scores greater than 2.0 spanned approximately 20 cM from markers D11S1984 to D11S2362 under model 2. The peak NPL score was 2.96 ($P = 0.002$) at marker D11S1923. Parametric analyses yielded similar results in this region with a peak HLOD of 2.00 near the same marker under model 2 and dominant inheritance, with an estimated 40% of the families ($z$) linked to the locus (Fig. 2). Further analysis with Genefinder suggested that the location of a bipolar disorder susceptibility gene on this chromosome was 15.5 cM from the p-terminal marker (and approximately 1 cM away from marker D11S1923) with a 95% confidence interval for the true location ranging from 6.82 to 23.48 cM. Addition of families from the first wave of the NIMH Bipolar Genetics Initiative and analysis of data on all wave one and two markers genotyped in the region of interest yielded somewhat diminished results. The peak NPL score dropped to 2.26 ($P = 0.012$) and the HLOD was 0.90 with an estimated 18% of the families linked.

On the X chromosome there was a broad region of approximately 60 cM showing excess sharing (Fig. 1), with a peak NPL score of 2.19 ($P = 0.01$) at marker GATA144D04 under model 1. Parametric analyses yielded similar but more focused results. The peak NPL score dropped to 2.26 ($P = 0.012$) and the HLOD was 0.90 with an estimated 18% of the families linked.

DISCUSSION

We studied chromosomes 2, 11, 13, 14, and X in 56 families collected as part of the NIMH Genetics Initiative on Bipolar Disorder. The best findings from both the parametric and non-parametric analyses were on chromosomes 11p15.5 and Xp11.3. These findings did not meet conventional criteria for significance [Lander and Kruglyak, 1995]. However, they are located near regions that have received considerable attention in previous genetic studies of bipolar disorder.

The first report of linkage on 11p15 came from a study of a large Old Order Amish family [Egeland et al., 1987]. This finding received a great deal of attention, but several subsequent studies of independent samples were unable to replicate it [Deterra-Wadleigh et al., 1987; Hodgkinson et al., 1987], and a follow-up of the original pedigree yielded reduced evidence for linkage [Kelsoe et al., 1989]. A systematic genome scan in the first wave of the Bipolar Genetics Initiative [Stine et al., 1997] failed to implicate 11p15, and addition of
these families to the current sample did not improve our findings in the region. However, only one of the six markers in the region of interest was genotyped in both sets of families. Moreover, several other studies of the region have found that there might be linkage only in a subset of families [Byerley et al., 1992; Lim et al., 1993; Smyth et al., 1996, 1997b; Malafosse et al., 1997], suggesting the possibility of genetic heterogeneity. These latter findings in particular could help explain the differences observed across samples.

Interestingly, in their study Smyth et al. [1997b] reported evidence that linkage to 11p15 was strongest in those families that were not linked to another locus on chromosome 21q22. We tested for such heterogeneity by stratifying the families based on linkage to 21q22. We found no evidence for increased linkage at 11p15 in those families that were not linked to 21q22 (results not shown), and therefore were not able to provide further support for the finding of Smyth et al. [1997b].

We estimated from our sample of families that the most likely location of a gene on 11p15 is approximately 1 cM telomeric from marker D11S1923. This marker is approximately 1.5 Mb from the tyrosine hydroxylase (TH) gene. Because TH is the rate-limiting enzyme in the synthesis of catecholamines, which are thought to play a role in the pathophysiology of bipolar disorder, it is a plausible candidate gene. Several studies reported that bipolar disorder was associated with polymorphisms at TH [Leboyer et al., 1990; Meloni et al., 1995], but a later meta-analysis was unable to substantiate these claims [Turecki et al., 1997].
Another plausible candidate gene in this region is the dopamine D4 receptor (DRD4) gene. DRD4 is a G-protein coupled receptor that inhibits adenyl cyclase and has a high affinity for the anti-psychotic clozapine [Van Tol et al., 1991]. Studies of an association between DRD4 and bipolar disorder have been mixed. Several smaller studies showed little evidence of such an association [Lim et al., 1995; Perez dC et al., 1994; Weiss et al., 1996; Oruc et al., 1997; Li et al., 1999; Serretti et al., 1999]. However, one relatively large study [Serretti et al., 2001] suggested that polymorphisms at DRD4 might be associated specifically with delusional symptoms in bipolar patients, while another more recent study [Muglia et al., 2002] reported evidence of a parent-of-origin effect at this locus with bipolar disorder. Given the difficulties in demonstrating an association between a gene of modest effect and a complex disease with a heterogeneous etiology such as bipolar disorder [Bellivier et al., 1998], inconsistent findings may not be unexpected. Thus, further study of the region, and TH or DRD4 specifically, may be warranted.

There have also been notable but inconsistent findings for bipolar disorder susceptibility genes on the X chromosome. Several earlier studies found that bipolar disorder co-segregated in some families with X-linked traits such as color blindness and glucose-6-phosphate dehydrogenase deficiency [Smyth et al., 1997a; Mendlewicz et al., 1979, 1980]. A later study reported that bipolar disorder was linked to DNA markers on Xq26-28 in three Israeli pedigrees [Baron et al., 1987]. However, a follow-up examination of the same pedigrees was unable to confirm the initial findings [Baron et al., 1993].

![Chromosome 11](image1.png)
![Chromosome X](image2.png)

Fig. 2. Results from parametric analyses of chromosomes 11 (phenotype model–2, genetic model–dominant) and X (phenotype model–1, genetic model–recessive).

Mixed results, with some providing evidence in support of linkage [Mendlewicz et al., 1987; Lucotte et al., 1992; Pekkarinen et al., 1995] and others not [Berrettini et al., 1990; Gejman et al., 1990; Smyth et al., 1997a; Vallada et al., 1998]. The previous scan of families collected in the first wave of the NIMH Bipolar Genetics Initiative reported evidence for linkage on Xq26-28 and Xp22. Only one other study has reported linkage to Xp22 [Mendlewicz and Fleiss, 1974].

In the current study, we found increased evidence for linkage in Xp11.3, which is approximately 15–20 cM away from Xp22 and 35–40 cM from Xq26-28. It has been shown through simulation studies that the localization of findings in replicated samples may vary across considerable distances [Roberts et al., 1999], therefore it difficult to assess whether our finding corresponds to one of those from the previous scan of NIMH Bipolar families. The addition of the two waves of families did not help resolve the finding, but the best marker in our current sample was not genotyped in both samples. Nevertheless, the finding on Xp11.3 is interesting and has received considerable attention because it harbors the monoamine oxidase A (MAOA) gene. MAOA catalyzes the oxidative degradation of biogenic amines, including neurotransmitters such as norepinephrine, epinephrine, dopamine, and serotonin. It is a plausible candidate gene for bipolar disorder since inhibitors of the enzyme can successfully treat depression, and a mutation in the gene has been associated with a syndrome of mental retardation that exhibits a behavioral phenotype similar to mania [Brunner et al., 1993]. Studies of the association between polymorphisms in MAOA and bipolar disorder have reported both positive [Lim et al., 1995; Rubinsztein et al., 1996; Ho et al., 2000; Lin et al., 2000; Preisig et al., 2000] and negative [Craddock et al., 1995; Parsian and Todd, 1997; Sasaki et al., 1998; Furlong et al., 1999; Kunugi et al., 1999; Turecki et al., 1999] findings, but two recent meta-analyses [Furlong et al., 1999; Preisig et al., 2000] provided overall support for such an association. Although we were unable to formally localize our linkage finding on the X chromosome as we were for chromosome 11, the peak NPL score occurred at a marker that is less than 1 Mb from MAOA. This finding is consistent with the hypothesis that MAOA, or a gene nearby, has a modest effect on bipolar disorder susceptibility.

Finally, the previous scan of families from the first wave of the NIMH Bipolar Genetics Initiative identified a locus of interest on chromosome 13q14-32. Two previous studies of bipolar disorder have reported similar findings at this locus [Detera-Wadleigh et al., 1999; Kelsoe et al., 2001]. Interestingly, two studies of schizophrenia also found significant evidence for linkage on 13q32 [Blouin et al., 1998; Brzustowicz et al., 1999], leading some to hypothesize that the locus harbors a susceptibility gene shared in common by the two disorders [Berrettini, 2000]. We were unable to provide further support for this hypothesis. We genotyped two of the markers that showed the greatest evidence of linkage in the wave one families, plus seven additional markers in between these, but could not replicate a finding of linkage in the region.
In summary, the current survey of chromosomes 2, 11, 13, 14, and X in the 56 families collected during the second wave of the NIMH Bipolar Genetics Initiative provided relatively modest evidence for linkage on 11p15.5 and Xp11.3. Intriguingly, these findings occurred in regions implicated in previous linkage and association studies. Because bipolar disorder is a complex disorder with a heterogeneous etiology, it may not be surprising that the previous findings are not entirely consistent.

The NIMH Bipolar Genetics Initiative has recently collected a third wave of families for study. Combining families from all waves of the initiative will lead to one of the largest samples ascertained under common criteria for study of the genetics of bipolar disorder. Although the addition of families from the first two waves did not help clarify the findings reported here, large samples like the one being collected by the NIMH Bipolar Genetics Initiative will be necessary to examine the genetic heterogeneity that underlies bipolar disorder. Only by taking this heterogeneity into consideration will we be able to more definitively map the regions on 11p15.5 and Xp11.3 identified in this report. Given the poor overlap in markers, the successful mapping of these regions will require the genotyping of additional markers at a greater density than currently exists across all families in order to maximally derive the information available from this growing and important sample.

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