

ORIGINAL RESEARCH ARTICLE

Linkage of a bipolar disorder susceptibility locus to human chromosome 13q32 in a new pedigree series

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Bipolar (BP) disorder or manic depressive illness is a major psychiatric disorder for which numerous family, twin and adoption studies support a substantial genetic contribution. Recently, we reported the results of a genome-wide search for BP disorder susceptibility loci in 20 pedigrees. Suggestive evidence for linkage was found in this study at three markers on 13q, representing possibly two peaks separated by 18 cM. We have now collected a second set of 32 pedigrees segregating BP disorder and have tested for evidence of linkage to markers on human chromosome 13q. In this sample, we have replicated the linkage result in 13q32 at D13S154 (lod = 2.29), the more proximal of the two original peaks. When all 52 pedigrees were combined, the multipoint maximum lod score peaked approximately 7 cM proximal to D13S154 (lod = 3.40), with a second peak occurring between D13S225 and D13S796 (lod = 2.58). There have been several other reports of significant linkage to both BP disorder and schizophrenia in this region of chromosome 13. These pedigrees provide additional evidence for at least one locus for BP disorder in 13q32, and are consistent with other reports of a possible genetic overlap between these disorders. *Molecular Psychiatry* (2003) 8, 558–564. doi:10.1038/sj.mp.4001267

Previously we have reported suggestive linkage results to chromosome 13q32 as part of a genome-wide scan for bipolar (BP) disorder^{1,2} susceptibility loci in a collection of 20 pedigrees.³ From this genome scan, lod scores greater than 1.0 were found at nine markers spanning an 18 cM interval. At the proximal end of this interval, a lod score of 2.40 at D13S154 was found using a broad diagnostic phenotype (BP plus recurrent major depression) and an autosomal recessive medium penetrance genetic model. In addition, markers approximately 6 and 18 cM distal to D13S154, respectively, were suggestive under different diagnostic and genetic models (D13S225, lod = 2.22, BP only, autosomal recessive medium penetrance model; D13S796, lod = 2.34 BP plus recurrent major depression, autosomal dominant high penetrance model). In order to replicate these find-

ings, we have collected a second set of 32 pedigrees and have genotyped 12 microsatellite markers spanning over 50 cM from 13q22 to 13q34 in this new pedigree set.^(4–6)

Prior to linkage analysis of the new pedigree set, simulation studies were conducted to determine the power to detect various lod score thresholds (1, 2, 3) in both the set of 32 replication pedigrees and the combined set of 52 pedigrees, and the expected lod scores at various values of θ . The program FASTLINK^{7,8} was used to simulate 1000 replicates of genetic marker data with four equi-frequent alleles simulating a marker with 75% heterozygosity. The simulated data were analyzed using a narrow diagnostic phenotype and two of the genetic models used in the linkage analysis, autosomal dominant high penetrance and autosomal recessive medium penetrance. In addition, the data were analyzed under both homogeneity and heterogeneity with 25% unlinked pedigrees. Expected lod scores were calculated at $\theta = 0.05$. Under homogeneity and the dominant and recessive models, respectively, the 32 pedigrees had an expected lod score of 3.65 and 3.35, and 79 and 68% power to detect a lod score greater than 3. Under 25% heterogeneity and dominant and recessive models, respectively, the 32 pedigrees had an expected lod score of 2.04 and 1.91, and 29 and 22% power to detect a lod score greater than 3. The original 20 pedigrees previously described³ were combined with the new set of 32 pedigrees for a combined sample size of 52 pedigrees. Under homogeneity and the dominant and recessive models, respectively, the 52 pedigrees had an expected lod score of 6.98 and 5.88, and 99.6 and 97.1% power to detect a lod score greater than 3. Under 25% heterogeneity and dominant and recessive models, respectively, the combined 52 pedigrees had expected lod scores of 3.98 and 3.39, and 74 and 61% power to detect a lod score greater than 3.

We, therefore, conducted two-point linkage analyses using both parametric (MLINK^{9,10}) and nonparametric (MERLIN¹¹) methods in the new 32-pedigree set using the same diagnostic and genetic models as previously described.³ Briefly, two diagnostic cate-

gories for 'affection' were used for the analyses: a narrow category that included BP I and II and schizoaffective disorder bipolar type (BP only) and a broad category that added recurrent major depression (BP + RD). Individuals who were determined to have no mental illness after interview were considered 'unaffected'. All other individuals were considered 'unknown'. For parametric analyses, the three genetic models included an autosomal dominant 85% penetrance model (AD85), a dominant 50% penetrance model (AD50), and a recessive 50% penetrance model (AR50) with seven age-dependent liability classes with a minimal risk below age 15 years and maximum penetrance at age 40 years. Analyses were conducted assuming homogeneity.

The results of the two-point analyses in the new set of 32 pedigrees are shown in Table 1. From the parametric linkage analysis, two markers separated by approximately 6 cM had lod scores greater than 1.5: D13S154 (lod = 2.29, BP only, AD85) and D13S225 (lod = 1.65, BP + RD, AD85). In order to assess empirically the significance of these two suggestive lod scores, 5000 unlinked genotypes were simulated in the 32 pedigrees. The data were analyzed using the same genetic models used to obtain the maximum lod scores. *P*-values were empirically calculated from the resulting lod scores of the simulated data using the program BINOM.¹² At D13S154, the lod score of 2.83 had a resulting *P*-value of 0.0004 (95% CI = 0.00001–0.002), and at D13S225 the lod score of 2.02 had a resulting *P*-value of 0.002 (95% CI = 0.00065–0.005). These empirical *P*-values do not take the multiple genetic models tested into account. Using nonparametric allele sharing tests in MERLIN, the same two markers demonstrated evidence for linkage (D13S154 lod score = 0.65, *P*-value = 0.04; D13S225 lod score = 1.36, *P*-value = 0.006). Therefore, both parametric and nonparametric linkage analysis in the second set of 32 pedigrees replicated the two markers, D13S154 and

D13S225, at the more proximal region with suggestive evidence for linkage in the first set of 20 pedigrees. However, little evidence was found to support the more distal region near the marker D13S796, which was suggestive in the first study.

The set of 32 replication pedigrees was also analyzed using multipoint linkage analysis using both parametric and nonparametric methods. For the parametric multipoint analysis, markers D13S800, D13S154, D13S128, D13S225, and D13S796 were used under all six diagnostic and genetic models. The markers used in the multipoint analyses included the two markers with peak lod scores under the two-point analysis and the most informative flanking markers. Marker D13S317 was not used in any of the multipoint analyses because of the discrepancy in map order between the genetic maps and the physical Mapview location. The resulting multipoint parametric linkage curve is shown in Figure 1a (BP-only phenotype) and b (BP + RD phenotype). The multipoint linkage curves were very similar between the BP only, autosomal dominant high and medium penetrance models, with peak lod scores of 3.39 and 3.30, respectively, occurring approximately 7 cM proximal to D13S154 between markers D13S800 and D13S154. For the nonparametric multipoint linkage analysis, the same five markers used in the parametric analysis were used under both diagnostic models. The resulting multipoint nonparametric linkage curve is shown in Figure 2. The peak lod score in the nonparametric analysis had a similar location to that of the parametric analysis, occurring approximately 11 cM proximal to D13S154 between markers D13S800 and D13S154 using the BP-only phenotype (lod = 1.83, *P*-value = 0.002). The addition of the recurrent major depression phenotype to the BP-only phenotype did not improve the results in either the parametric or nonparametric multipoint linkage results (Figures 1 and 2). Both nonparametric and parametric multi-

Table 1 Two-point linkage analysis in the new set of 32 pedigrees segregating BP disorder

Marker	Mapview loc. (kbp)	Map position (pter cM)	Parametric linkage analysis				Nonparametric linkage analysis			
			Dx	Model	Z_{max}	θ	Dx	Lod score	<i>P</i> -value	Information content
D13S800	73 827	55.31	BP only	AD 85	0.96	0.15	BP only	0.79	0.03	0.54
D13S317	61 134	65.07	BP+RD	AD 85	0.79	0.20	BP+RD	0.08	0.30	0.51
D13S154	95 125	73.45	BP only	AD 85	2.29	0.10	BP only	0.65	0.04	0.74
D13S793	96 704	75.23	BP	AD 85	0.72	0.15	BP+RD	0.25	0.14	0.62
D13S128	97 823	76.54	BP	AD 50	0.80	0.15	BP only	0.38	0.09	0.77
D13S770	98 601	78.00	BP+RD	AR	0.59	0.15	BP+RD	0.06	0.30	0.57
D13S1240		78.49	BP only	AD 85	1.12	0.20	BP+RD	0.43	0.08	0.58
D13S779	100 355	78.97	BP+RD	AD 85	0.56	0.15	BP+RD	0.23	0.20	0.46
D13S225	100 482	78.97	BP+RD	AD 85	1.65	0.15	BP+RD	1.36	0.006	0.66
D13S173	107 150	94.75	BP+RD	AR	1.28	0.15	BP+RD	0.50	0.06	0.53
D13S796	107 232	96.32	BP only	AD 50	0.45	0.15	BP only	0.06	0.30	0.50
D13S285	112 505	110.64	BP+RD	AR	0.03	0.30	BP only	0.07	0.30	0.69

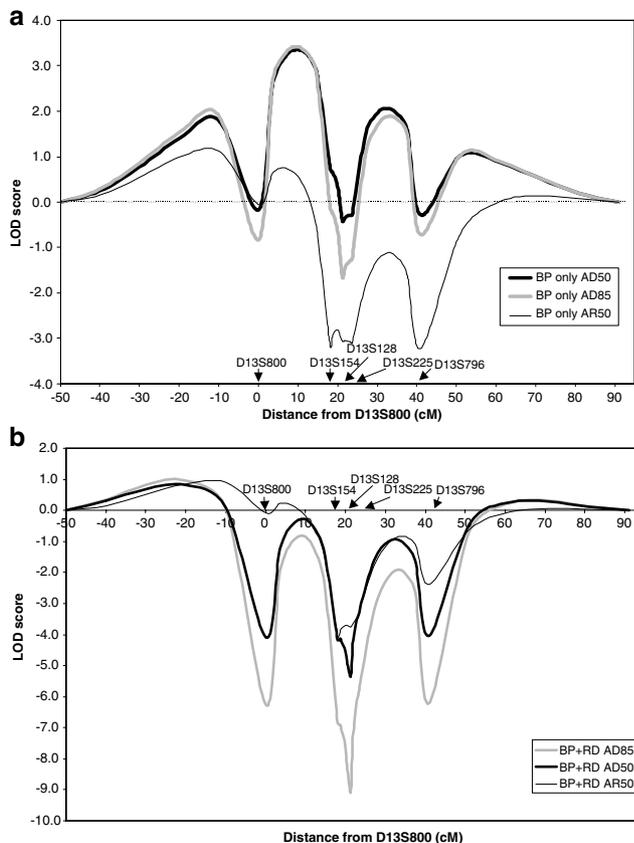


Figure 1 Parametric multipoint linkage analysis of the new set of 32 pedigrees using LINKMAP of FASTLINK. The multipoint lod scores are shown on the Y-axis, and distance in cM from D13S800 is shown below the X-axis. The placement of each marker in the multipoint analysis is shown above the X-axis. The multipoint analysis was conducted using (a) narrow (BP only), and (b) broad (BP plus recurrent major depression) diagnostic phenotypes. Both phenotypes were analyzed using three genetic models: autosomal dominant high penetrance (dark bold line), autosomal dominant medium penetrance (light bold line), and autosomal recessive medium penetrance (dark thin line).

point analyses demonstrated evidence for linkage in the region of D13S154 but did not provide much evidence for linkage in the more distal region near D13S796.

In order to maximize power of the linkage analysis and to better define the linkage interval, the initial set of 20 pedigrees plus the second set of 32 pedigrees were combined for additional analyses. The results of the two-point analysis using both MLINK and MERLIN are listed in Table 2. Using two-point parametric linkage analysis in the combined 52 pedigrees, the highest lod score of 2.83 occurred at D13S154 under a BP-only, autosomal dominant medium penetrance model. In addition, marker D13S225, approximately 6cM distal to D13S154, had a lod score of 2.02 under the same diagnostic and genetic model. The more distal marker, D13S796, did not show as much evidence for linkage as the

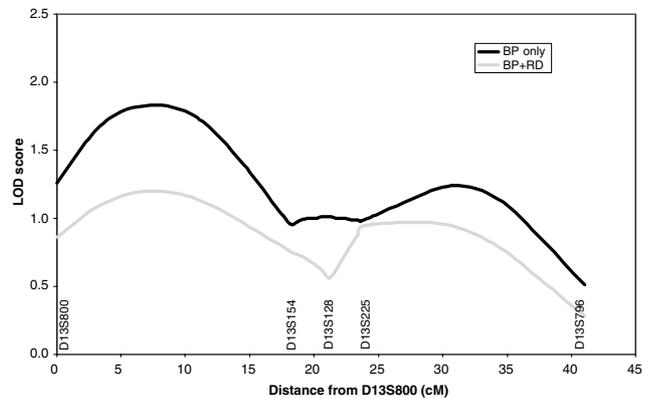


Figure 2 Non-parametric multipoint linkage analysis of the new set of 32 pedigrees using MERLIN. Lod score is shown on the Y-axis, and distance in cM from D13S800 is shown below the X-axis. The placement of each marker in the multipoint analysis is shown above the X-axis. The dark line indicates the linkage curve using the narrow (BP only) diagnostic phenotype. The light line indicates the linkage curve using the broad (BP plus recurrent major depression) diagnostic phenotype.

markers in the more proximal region: however, the lod score did improve in the combined set of pedigrees (lod = 1.51, BP only, AD85). The significance of the two suggestive lod scores was empirically assessed using the same methods as in the 32-pedigree cohort. At D13S154, the lod score of 2.83 had a resulting P -value of 0.0002 (95% CI = 0.000005–0.001), and at D13S225 the lod score of 2.02 had a resulting P -value of 0.0008 (95% CI = 0.0002–0.002). Markers D13S154 and D13S225 were also tested for linkage under heterogeneity. Both loci had peak lod scores at $\alpha = 1$ (all families linked), and therefore the lod scores did not differ from the analysis under homogeneity.

Using nonparametric linkage analysis, markers D13S154 (lod = 1.09, P -value = 0.01), D13S128 (lod = 1.15, P -value = 0.01) and D13S225 (lod = 1.47, P -value = 0.005) yielded evidence for linkage using the BP-only diagnostic phenotype. Under the BP + RD diagnostic phenotype, the most proximal marker, D13S800, had evidence for linkage with a lod score of 1.1 (P -value = 0.01).

Finally, multipoint linkage analyses were done using both parametric and nonparametric linkage analysis methods in the combined set of 52 pedigrees. For the parametric multipoint analysis, markers D13S800, D13S154, D13S128, D13S225, and D13S796 were used under a BP-only, autosomal dominant medium penetrance model. This model had the best results in the two-point parametric analysis for the combined set of 52 pedigrees for the markers chosen for the multipoint analysis. The markers chosen for the multipoint analysis are the same as those chosen for the set of 32 pedigrees and included the two markers with peak lod scores > 2 under the two-point analysis and the most informa-

Table 2 Two-point linkage analysis in the combined set of 52 pedigrees segregating BP disorder

Marker	Mapview loc. (kbp)	Map position (pter cM)	Parametric linkage analysis				Nonparametric linkage analysis			
			Dx	Model	Z _{max}	θ	Dx	lod score	P-value	Information content
D13S800	73 827	55.31	BP+RD	AD50	1.28	0.2	BP+RD	1.1	0.01	0.55
D13S317	61 134	65.07	BP only	AR	0.38	0.25	BP only	0.06	0.3	0.57
D13S154	95 125	73.45	BP only	AD50	2.83	0.1	BP only	1.09	0.01	0.72
D13S793	96 704	75.23	BP+RD	AR	1.02	0.2	BP only	0.6	0.05	0.64
D13S128	97 823	76.54	BP only	AD50	1.3	0.15	BP only	1.15	0.01	0.79
D13S770	98 601	78	BP+RD	AR	0.78	0.2	BP only	0.04	0.3	0.62
D13S1240		78.49	BP+RD	AR	0.9	0.2	BP only	0.45	0.08	0.57
D13S779	100 355	78.97	BP only	AD50	1.54	0.1	BP only	0.64	0.04	0.49
D13S225	100 482	78.97	BP only	AD50	2.02	0.1	BP only	1.47	0.005	0.67
D13S173	107 150	94.75	BP+RD	AR	1.71	0.15	BP only	0.36	0.1	0.52
D13S796	107 232	96.32	BP only	AD85	1.51	0.15	BP only	0.43	0.08	0.54
D13S285	112 505	110.64	BP+RD	AR	0.22	0.3	BP only	0.06	0.3	0.67

tive flanking markers. The resulting parametric multipoint linkage curve is shown in Figure 3. The highest peak lod score of 3.40 occurred approximately 7 cM proximal to D13S154. A second peak lod score of 2.58 occurred 23.2 cM distal to the first peak, between markers D13S225 and D13S796. These results were, therefore, consistent with the original study in suggesting the possibility of two distinct linkage peaks.

The resulting multipoint nonparametric linkage curve using a BP-only phenotype and the same markers as in the parametric analysis is shown in Figure 4. The nonparametric linkage curve was broad over the entire region, with three main peak areas. The first occurred between markers D13S800 and D13S154, approximately 7 cM proximal to D13S154 (lod = 1.86, $P = 0.002$). The second peak occurred at marker D13S228 (lod = 1.97, $P = 0.001$), and the third peak occurred between markers D13S225 and D13S796 (lod = 1.93, $P = 0.001$).

Overall, the nonparametric MERLIN results were less significant than the parametric linkage results. Model-free linkage analysis can be a favorable method of analysis for complex disease traits since there are no assumptions about mode of inheritance, disease allele frequencies, and penetrance. However, even without specifying models correctly, parametric analyses can be a more powerful test given the type of pedigree collection. In this study, many pedigrees included multiple generations and were, therefore, more extended than simple affected sib-pair families. It is not surprising that the parametric linkage analysis was more powerful given the pedigree structures in this study. In addition, one pedigree belonging to the original 20 pedigrees was too large to be analyzed intact due to MERLIN's pedigree size limitation and therefore had to be pared down (17 genotyped individuals were discarded), although all affected individuals remained for the analyses. Using parametric linkage analysis in the combined 52

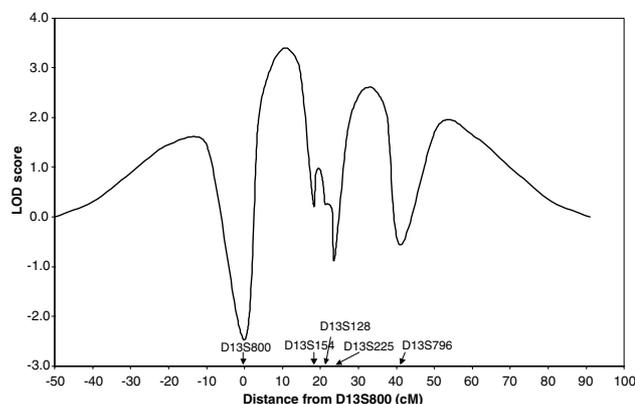


Figure 3 Parametric multipoint linkage analysis of the combined set of 52 pedigrees using LINKMAP of FASTLINK. The multipoint lod scores are shown on the Y-axis, and distance in cM from D13S800 is shown below the X-axis. The placement of each marker in the multipoint analysis is shown above the X-axis. The multipoint analysis was conducted using the narrow (BP only) diagnostic phenotype and autosomal dominant medium penetrance model.

pedigrees, this pedigree had the largest contribution of all pedigrees to the overall linkage results (lod = 0.71 and 0.64 at D13S154 and D13S225, respectively). Therefore, this loss of information most likely contributed to the less significant results with the combined pedigree analysis.

Lander and Kruglyak¹³ have suggested guidelines for reporting 'suggestive' and 'significant' linkage findings for complex disease traits. For parametric lod score analysis, they suggested a lod score threshold of 3.3 as evidence for significant linkage as part of a genome scan, and for replication of a significant linkage result they suggested a P -value < 0.01 . Our results presented here in the combined sample set exceed the genome-wide threshold for significant

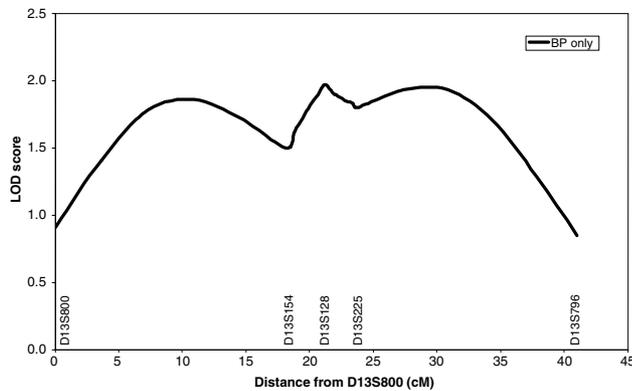


Figure 4 Nonparametric multipoint linkage analysis of the combined set of 52 pedigrees using MERLIN. Lod score is shown on the Y-axis, and distance in cM from D13S800 is shown below the X-axis. The placement of each marker in the multipoint analysis is shown above the X-axis. The multipoint analysis was conducted using the narrow (BP-only) diagnostic phenotype.

linkage in the multipoint parametric linkage analysis for markers in 13q32. We have also presented suggestive evidence for linkage in 13q32 in our new pedigree set alone that surpasses the threshold of replication of a significant linkage result. Since evidence for significant linkage was not obtained in the first family set, these data do not represent replication in these two family samples. However, both these independent family sets do provide replication for other recently reported results in BP disorder.

In addition to our study presented here, there have been several reports of both significant and suggestive linkage to 13q32 in both BP disorder and schizophrenia. Detera-Wadleigh *et al*⁴ recently reported a significant linkage result in 13q32 in a set of 22 multiplex BP pedigrees. This study found a peak nonparametric lod score of 3.5 ($P = 2.8 \times 10^{-5}$) using a broad phenotypic model (BP I and II, schizoaffective disorder, and recurrent unipolar depression) between markers D13S1252 and D13S1271 (Marshfield cumulative map position = 77.5 and 79.5 cM, respectively). These markers are in the interval between our two suggestive proximal markers, D13S154 and D13S225 (Marshfield cumulative map position = 75.2 and 83.6 cM, respectively). Also, Stine *et al*¹⁴ reported positive linkage findings in 13q32 using sib-pair analysis as part of the NIMH Genetics Initiative genome screen for BP disorder susceptibility genes. In the 97-pedigree cohort, they reported evidence for linkage at markers D13S800 and D13S793 (P -value = 0.04 and 0.02, respectively; approximate Marshfield cumulative map position = 55 and 76 cM, respectively) using a narrow diagnostic phenotype (BP I and schizoaffective disorder BP type). Although our first study suggested the possibility of two distinct linkage peaks, the evidence for linkage from our second set of families, as well as that from these other studies, clusters around the more proximal peak

near the D13S154–D13S225 interval, and provides limited support for the more distal peak near D13S796 seen in the first family set.

To date, there have been several other genome scans for BP disorder that have not found evidence for linkage to chromosome 13. Foroud *et al*¹⁵ conducted a separate analysis using GENEHUNTER of the same genome scan dataset reported previously by Stine *et al*¹⁴ and failed to find support for 13q. Cichon *et al*¹⁶ conducted a genome screen of 75 BP pedigrees of German, Israeli, and Italian ancestry. Markers on chromosome 18q24 were significant for linkage. 13q11 was one of nine other genomic regions suggestive of linkage in their study. The region in 13q11 is far from the 13q32 region reported here. Coon *et al*¹⁷ conducted a genome scan on eight BP pedigrees and found evidence for linkage to chromosomes 5 and 11. No markers on chromosome 13 had lod scores >1.0. Friddle *et al*¹⁸ conducted a genome scan of 50 BP pedigrees and did not find evidence for linkage to any genomic region. They concluded that there were no major genes with larger effects present in their pedigree collection and that their pedigree series was most likely limited in power to detect genes of relatively small effect. Bennett *et al*¹⁹ investigated 151 BP pedigrees from the UK and Ireland and did not find any genomic regions that were significant or suggestive of linkage. They did identify several genomic regions to follow-up in a second pedigree cohort; however, no markers on chromosome 13 surpassed their lod score threshold for follow-up studies. All the genome scans that did not find evidence for linkage to chromosome 13q32 underscore the complex nature of the genetic contribution to BP disorder. Several genes and other environmental and epistatic factors most likely contribute to the onset of disease. However, the replication of the chromosome 13q32 BP linkage result in several studies with sufficient power indicates that a locus on chromosome 13 may be emerging as one of the loci contributing to BP disorder in some families.

Interestingly, there have also been several reports of linkage of a schizophrenia susceptibility locus to 13q32. This overlap, also observed on 22q11–13, 18p, and 10p, has stimulated a discussion of the possibility of genetic overlap between BP disorder and schizophrenia.^{4,20–22} Blouin *et al*⁵ reported significant linkage to 13q32 in a collection of 54 pedigrees segregating schizophrenia. Using nonparametric linkage methods, a peak NPL score of 4.18 (P -value = 2×10^{-5}) was observed at D13S174 (cumulative Marshfield map position = 85 cM). In addition, Brzustowicz *et al*⁶ reported significant linkage to 13q32 in a collection of 21 extended pedigrees using a broad diagnostic phenotype (schizophrenia plus schizotypal personality disorder, paranoid personality disorder, and nonaffective psychotic disorder). This study reported a maximum lod score under a heterogeneity of 4.42 at D13S793. There have also been reports of suggestive linkage results of schizo-

phrenia to 13q. Lin *et al*²³ reported suggestive evidence of linkage to D13S122/D13S128 (cumulative Marshfield map positions = 73 and 76 cM, respectively) using multipoint nonparametric linkage methods. Shaw *et al*²⁴ reported evidence for linkage as part of a genome-wide scan for schizophrenia to D13S170 ($P=0.03$; cumulative Marshfield map position = 63.9 cM) using nonparametric linkage methods in a cohort of 70 pedigrees. Although there is evidence of genetic heterogeneity in both BP disorder and schizophrenia, all the reports of significant and suggestive linkage in both disorders to 13q32 demonstrate consistent, robust linkage results that warrant a next step of evaluating candidate genes in this region using fine-mapping methods.

Materials and methods

Family collection

Families were ascertained from the general North American population through both a systematic survey of clinical facilities and through advertising and patient support groups from three sites: San Diego, Vancouver and Cincinnati. Families were ascertained through a proband with BP I or II disorder, and included if at least two additional members had either BP disorder or recurrent major depression. The family sample included 32 families and 194 subjects. A total of 43 subjects had a diagnosis of BP I or schizoaffective bipolar type, 26 had BP II, and 49 had recurrent major depression. There was an average of 6 subjects per family, and 2 and 3.7 affected members per family under the narrow and broad diagnostic category, respectively.

Following informed consent procedures approved by the local IRBs of the collaborative sites, family members were interviewed directly using the Structured Clinical Interview for DSM-3-R (SCID).²⁵ Diagnoses were made using DSM-3-R criteria. The definition of hypomania was modified to require only a 2-day minimum duration. Information was obtained from other family informants and medical records and reviewed along with the interview by a panel of experienced psychiatric clinicians. Using this process, a consensus, best-estimate diagnosis was obtained. All interviewers underwent a standardized training course for the SCID and were regularly tested for diagnostic reliability.

Genotyping

Blood was drawn on all subjects for the establishment of lymphoblastoid cell lines, and DNA prepared by phenol/chloroform extraction. Genotyping was conducted as follows: 50 ng of DNA was amplified in a 20 μ l reaction using an MJ Research PTC-200 thermal cycler (MJ Research, Waltham, MA, USA). Reactions contained 1.5 mM MgCl₂, 50 mM KCl, 200–500 nM of each primer, 10 mM Tris-HCl (pH 8.3), 0.001% gelatin and 1 unit of either AmpliTaq or AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). The forward primer was fluorescently labeled. Reactions

were first incubated for 10 min at 95°C (AmpliTaq Gold only), then the PCR reaction was conducted using the touchdown protocol²⁶ in which the annealing temperature was decreased by 1°C every two cycles from 65 to 55°C, followed by a final 10 cycles at 55°C. PCR products were loaded onto a gel and electrophoretically separated using an ABI 377 (Applied Biosystems, Foster City, CA, USA). Genescan and Genotyper software (Applied Biosystems, Foster City, CA, USA) were used by two independent readers to call alleles. Markers with different molecular weights and labeled with different fluorors were pooled for efficiency. Standardized molecular weights were obtained by running a CEPH grandparent DNA in multiple lanes in all gels. The resulting genotype data were checked using the program PedCheck²⁷, detecting an overall error rate of approximately 0.5%. In addition, there was approximately 1% missing genotype data.

Statistical analysis

For all analyses, allele frequencies were derived from the total family data. For suggestive lod scores, analyses were rerun using allele frequencies derived from founders only and there were no significant differences in results. Genetic maps were constructed from the 52-pedigree genotype data using the program MULTIMAPv2.0.²⁸ CHROMPIC from CRI-MAP²⁹ was run after establishing map order to identify double recombinants. Four double recombinants were identified and discarded from subsequent analyses. Map distances between each marker were compared to the Marshfield map (see Broman *et al*,³⁰ <http://research-marshfieldclinic.org/genetics/>) and were very similar, both maps spanning a total of 55 cM. Map order was also compared to physical map position based on sequence from NCBI (<http://www.ncbi.nlm.nih.gov/>). Marker orders were identical for all markers except D13S317. Since there was a discrepancy in map order for this marker, it was removed from all multipoint analyses.

For parametric linkage analysis, genetic data were analyzed using the program MLINK of FASTLINK.^{9,10} Empirical P -values were calculated for two-point lod scores >2 from the parametric linkage analysis of the combined sample of 52 pedigrees. A total of 2500 replicates of unlinked genotype data were generated using SIMULATE³¹ keeping the pedigree structure and diagnoses intact and simulating genetic marker data independently from diagnostic information. The data were analyzed using the same models as in the linkage analysis. P -values and respective 95% CI for lod scores were generated using the program BINOM¹² from the resulting lod scores of unlinked simulated data. Suggestive lod scores were also calculated under heterogeneity using the program HOMOG.³² Multipoint parametric analyses were conducted using LINKMAP of FASTLINK.

Two-point and multipoint nonparametric allele sharing tests among affected relatives were done using MERLIN.¹¹ Lod scores and corresponding P -

values were converted from nonparametric scores using the Kong and Cox function³³ and were computed for each marker for two-point analyses. For the multipoint analyses, Lod scores and *P*-values were computed at each marker position and four equally spaced positions between each marker. In addition, the information content was calculated for each marker from pedigree data in both two-point and multipoint analyses using the program GENEHUNTER³⁴. Map distances were derived from pedigree data as described above.

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