

Initial Genome Screen for Bipolar Disorder in the NIMH Genetics Initiative Pedigrees: Chromosomes 2, 11, 13, 14, and X

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We report on an initial genome screen of 540 individuals from 97 families collected as part of the NIMH Genetics Initiative Bipolar Group. Among the individuals studied, 232 were diagnosed with bipolar (BP) I, 72 with BP II, 88 with major depressive disorder-recurrent type (UPR), and 32 with schizoaffective disorder, bipolar type (SA/BP). A total of 53 markers on chromosomes 2, 11, 13, 14, and X (average spacing: 11.5 cM) were studied at Johns Hopkins University. Tests for linkage were performed using nonparametric affected sib-pair and whole pedigree methods with three definitions of affected status. Three regions of interest were identified (13q14–32, Xp22, and Xq26–28). On chromosomes 2, 11, and 14, a disease locus with relative risk $\lambda_1 = 1.5$ could be excluded in <10% of the genetic distance studied, while a locus conferring $\lambda_1 = 3$ or greater could be excluded across at least 96%. The autosomal region that could not be excluded even with $\lambda_1 = 5$ was near 13q14–32. In this region, two-point affected sib-pair analyses revealed a pair of consecutive loci with ex-

cess sharing ($P < 0.05$) and a multipoint affected sib-pair LOD score of 1.12. On the X chromosome, nonparametric multipoint affected sib-pair analyses revealed peak total LOD scores of 0.94 on Xp22 and 1.34 on Xq26–28. A locus linked to the markers in Xp22 would have $\lambda_1 = 3.6$ in affected brother-brother pairs, while a locus linked to the markers in Xq26–28 would have $\lambda_1 \geq 1.9$ in affected sister-sister pairs. The results on 13q14–32, Xp22, and Xq26–28 suggest areas of interest for further studies. *Am. J. Med. Genet.* 74:263–269, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: linkage; occlusion mapping; affective disorder

INTRODUCTION

There is good evidence for a genetic contribution to the risk of bipolar (BP) affective disorder, as discussed in the companion article [NIMH Genetics Initiative Bipolar Group, 1997]. Although many linkages have been reported, few have been confirmed by subsequent studies [Risch and Botstein, 1996]. These studies are complicated because the limits of the phenotype, the mode of inheritance, and the number of loci involved are all unknown.

In an attempt to further elucidate the genetics of BP affective disorder, 97 families ascertained and clinically assessed by the NIMH Diagnostic Centers for Linkage Analysis, Bipolar Disorder, were studied using highly polymorphic genetic markers and subjected to linkage analysis. A genome-wide screen was initiated by the four collaborating centers. The center at Johns

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Hopkins University has screened chromosomes 2, 11, 13, 14, and X. The results of the first set of markers on these chromosomes are reported here.

Many previous linkage analyses have surveyed part or all of the genome for loci predisposing to BP affective disorder. No studies have reported evidence for linkage on chromosomes 2 and 14. The reported significant linkage on chromosome 11 [Egeland et al., 1987] was not replicated in subsequent studies [Hodgkinson et al., 1987; Detera-Wadleigh et al., 1987] or in the same pedigree [Kelsoe et al., 1989]. More recent studies have suggested linkage, if heterogeneity is considered [Gurling et al., 1996; Smyth et al., 1996]. On chromosome 13, a single study suggests some evidence of linkage [Ginns et al., 1996]. On the X chromosome, linkages have been reported variously to Xq27–q28 [Winokur et al., 1969; Baron et al., 1987; Mendlewicz et al., 1972], but have not withstood further molecular studies [Bertolini et al., 1990; Gejman et al., 1990; Del Zompo et al., 1991; Baron et al., 1993]. Recently, Pekkarinen et al. [1995] reported a maximum LOD of 3.54 at DXS994 in Xq26 in one large Finnish pedigree. There is a single report of possible linkage to Xp22 [Mendlewicz and Fleiss, 1974].

Our genetic analyses reveal regions of interest which require further study near 13q14–32, Xp22, and Xq26–28. These results exclude a gene with relative risk (λ_i) >3 across 96% of chromosomes 2, 11, and 14. Our results should be read in the context of the companion papers in this issue [NIMH Genetics Initiative Bipolar Group, 1997; Detera-Wadleigh et al., 1997; Edenberg et al., 1997; Rice et al., 1997].

PATIENTS AND METHODS

The common ascertainment and clinical assessment protocol for the four sites is described elsewhere in this volume [NIMH Genetics Initiative Bipolar Group, 1997]. All subjects at the Johns Hopkins site were interviewed by a psychiatrist trained in the use of the Diagnostic Interview for Genetic Studies (DIGS) [Nurnberger et al., 1994]. Best-estimate diagnoses were assigned by 2 noninterviewing, experienced psychiatrist reviewers [see Simpson et al., 1992; Stine et al., 1995].

A total of 97 families was included in the genetic analyses. There were 623 individuals with best-estimate diagnoses. DNA was available from 540 individuals for genotyping. Of these, 232 were diagnosed with bipolar disorder (BP) I, 72 with BP II, 88 with major depressive disorder-recurrent (UPR), and 32 with schizoaffective disorder-bipolar type (SA/BP) disorder; 71 had other diagnoses and 45 were never mentally ill. Affected family members and their parents, when available, were chosen to maximize the informativeness for linkage [see NIMH Genetics Initiative Bipolar Group, 1997].

We analyzed a total of 53 marker loci for chromosomes 2, 11, 13, 14, and X, with an average spacing of 11.5 cM. The fluorescently labeled markers used for genotyping at Johns Hopkins University were selected from the CHLC/Weber version 6 set (Research Genetics). PCR reactions were performed following a stan-

dard protocol [Stine et al., 1995]. ABI 373 or 377 fluorescent sequencing machines were used with Genescan and Genotyper software (ABI). Initial scoring was done automatically, but then was checked by a technician. The recorded genotypes were binned and tested for inheritance using the Genetic Analysis System [Young, 1995]. Alleles that had sizes outside the fixed bin sizes or that did not segregate were rechecked. After rechecking, in those cases where a discrepancy remained, the data for that marker from that portion of the pedigree were deleted. All genotyping was performed by individuals blind to the clinical data. The BUILD option of CRIMAP [Lander and Green, 1987] was used to order the markers at 1,000-to-1 odds on each chromosome. In cases where the ratio was <1,000-to-1, a stepwise procedure was used to order the markers, including the FLIP option. Based on the order and genetic distances from CRIMAP, the genetic information content derived from the genotypes generated across each autosome was estimated using GENEHUNTER [Kruglyak et al., 1996]. Genetic information content on the X-chromosome was estimated using MAPMAKER/SIBS [Kruglyak and Lander, 1995].

Because some cases of BP II and UPR ascertained through BPI probands may have distinct etiologies [Endicott et al., 1985; Blacker and Tsuang, 1993; McMahon et al., 1994], analyses were performed using three definitions of the affected phenotype: 1) SA/BP, BPI; 2) SA/BP, BPI, BP II; and 3) SA/BP, BPI, BP II, UPR (referred to as affection status models 1, 2, and 3, respectively).

The mean proportion of alleles shared identical-by-descent (IBD) by affected sib pairs was calculated using SIBPAL from the S.A.G.E. [1994] software package. The degrees of freedom were calculated using $(n - 1)$ sibs per family to adjust for nonindependence [Suarez and Van Eerdewegh, 1984].

Multipoint nonparametric sib-pair sharing IBD and exclusion LOD scores were calculated using MAPMAKER/SIBS [Kruglyak and Lander, 1995]. For X-chromosome analyses, only the maternal X-chromosome was considered. For autosomes, multipoint nonparametric analysis using all affected relative pairs was performed using GENEHUNTER [Kruglyak et al., 1996].

RESULTS

Autosomes: 2, 11, 13, and 14

The observed order of markers on each autosome was consistent with the published order (Table I). There were 13 markers on chromosome 2, with an average spacing between markers of 14.1 cM; 11 markers on chromosome 11, with an average spacing of 13.6 cM; 7 markers on chromosome 13, with an average spacing of 13.4 cM; and 11 markers on chromosome 14, with an average spacing of 11 cM. The average information content calculated using GENEHUNTER on chromosome 2 was 66%; on chromosome 11, 66%; on chromosome 13, 66%; and on chromosome 14, 65%.

Exclusion of possible linked loci depends on the postulated relative risk (λ_i) of the putative disease locus (Fig. 1) [Risch, 1987]. For a locus with $\lambda_i = 1.5$, <10% of the genetic distance on these autosomes can be ex-

TABLE I. Results From Two-Point Affected Sib-Pair Analyses*

	Calculated cumulative distance	Model 1			Model 2			Model 3		
		Pair	IBD	<i>P</i>	Pair	IBD	<i>P</i>	Pair	IBD	<i>P</i>
D2S405	0.0	108	0.52		168	0.5		239	0.49	
D2S1356	15.3	81	0.46		133	0.44		181	0.46	
D2S441	45.9	90	0.49		151	0.51		213	0.49	
D2S1394	50.3	85	0.5		138	0.51		188	0.49	
D2S436	77.5	74	0.53		115	0.52		165	0.51	
D2S1328	88.3	99	0.49		160	0.48		232	0.48	
D2S1326	102.8	97	0.47		158	0.46		220	0.46	
D2S1353	118.1	93	0.49		155	0.49		213	0.5	
D2S1776	127.3	86	0.53		137	0.49		190	0.5	
D2S1391	142.7	98	0.51		163	0.49		223	0.5	
D2S434	163.8	95	0.47		154	0.49		216	0.49	
D2S1363	173.3	75	0.46		112	0.47		153	0.49	
D2S427	182.8	81	0.56	0.01	115	0.54		157	0.54	0.02
TH01	0.0	95	0.48		151	0.5		203	0.51	
D11S2362	7.0	80	0.53		133	0.52		180	0.52	
D11S1981	28.4	88	0.45		144	0.49		210	0.5	
ATA34E08	43.9	101	0.52		163	0.52		231	0.52	
D11S1392	56.9	90	0.49		136	0.5		185	0.5	
D11S1985	66.4	84	0.45		130	0.48		178	0.47	
D11S2002	99.5	87	0.5		149	0.49		214	0.49	
D11S2000	125.6	90	0.46		151	0.49		224	0.5	
D11S1986	130.0	93	0.47		153	0.5		212	0.49	
D11S1998	140.9	97	0.49		159	0.51		223	0.51	
GATA64D03	150.2	93	0.49		149	0.51		210	0.53	0.04
GGAA29H03	0.0	99	0.53		162	0.53		224	0.53	
D13S894	4.8	95	0.49		153	0.5		220	0.49	
D13S788	19.0	90	0.52		150	0.5		186	0.51	
D13S800	32.5	71	0.56	0.04	112	0.54		155	0.54	
D13S793	57.9	89	0.56	0.02	137	0.52		189	0.52	
D13S173	81.4	93	0.52		143	0.51		200	0.52	
D13S285	94.1	55	0.49		90	0.49		120	0.49	
D14S742	0.0	45	0.5		63	0.5		93	0.5	
GATA31B09	11.3	99	0.53		161	0.5		235	0.5	
D14S297	22.3	90	0.48		137	0.48		191	0.49	
D14S587	37.7	85	0.54		143	0.52		202	0.51	
D14S592	53.1	59	0.48		93	0.48		127	0.49	
D14S588	63.6	50	0.57	0.05	90	0.55		133	0.51	
D14S53	83.5	105	0.51		172	0.5		238	0.51	
D14S606	92.3	64	0.51		108	0.5		150	0.5	
D14S617	108.4	73	0.52		121	0.49		173	0.51	
D14S749	112.3	98	0.49		153	0.48		206	0.5	
D14S611	120.9	101	0.45		166	0.46		225	0.48	

*Only *P* values ≤ 0.05 are reported.

cluded under any affection model. In contrast, for a locus with $\lambda_i = 3$, nearly all the distance on these four autosomes can be excluded, 86% assuming model 1, 96% for model 2, and 98% for model 3. A single region around D13S800 and D13S793 cannot be excluded, even if λ_i is increased to 5.

Positive evidence for linkage on 13q14–32 was suggested by two methods of analysis. Excess allele sharing by affected sib pairs was observed at consecutive markers D13S800 and D13S793. The mean proportion of sibs sharing alleles IBD was 56% ($P = 0.04$ and $P = 0.02$, respectively) (Table I). Maximum likelihood LOD scores of 1.12 at D13S793, and 0.5 and 0.65 at the flanking markers D13S800 and D13S173, respectively (Fig. 2), were produced by nonparametric multipoint analyses.

Little evidence was observed for linkage on the autosomes 2, 11, and 14 by any analytic method. No significant ($P \leq 0.05$) excess allele sharing was seen at any consecutive pair of markers. Excess sharing at the

$P = 0.01$ level was seen at the single telomeric marker D2S427 under affection status model 1. The mean proportion of sibs sharing alleles IBD ranged from 0.44–0.56 (Table I). Nonparametric multipoint analyses produced no LOD score > 0.4 for any affection status model.

X-Chromosome Analyses

The observed order of markers was consistent with the published order (Table II). The observed total distance (96 cM) was somewhat shorter than expected (140 cM). Three intervals (DXS987 to DXS989, DXS6800 to DXS6804, and DXS1001 to GATA 31E08) were more than 12 cM shorter than expected, accounting for most of the discrepancy. The average spacing between markers was 8.7 cM. The genetic information content, derived from the genotypes generated across the X chromosome, ranged from 28–76% with an average of 48% (MAPMAKER/SIBS).

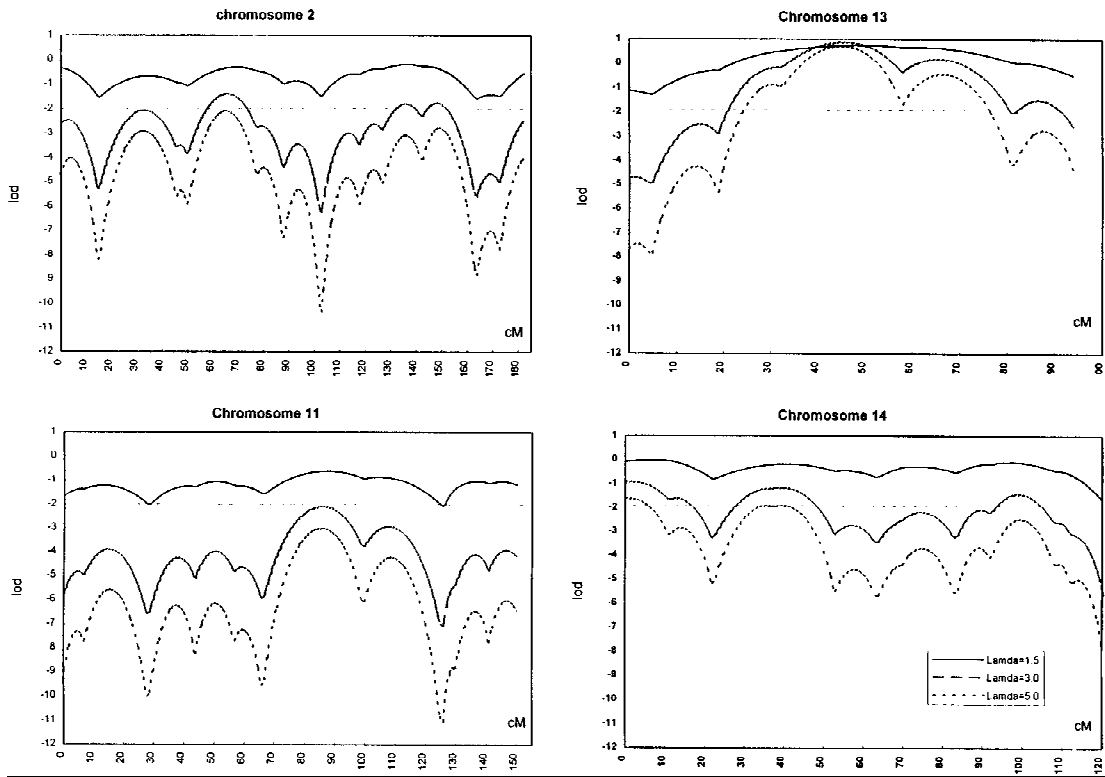


Fig. 1. Exclusion curves for $\lambda_i = 1.5$ (thick lines), $\lambda_i = 3.0$ (thin lines), and $\lambda_i = 5.0$ (dotted lines), for autosomes 2 (upper left), 11 (upper right), 13 (lower left), and 14 (lower right). Each curve is plotted assuming affection status model 1.

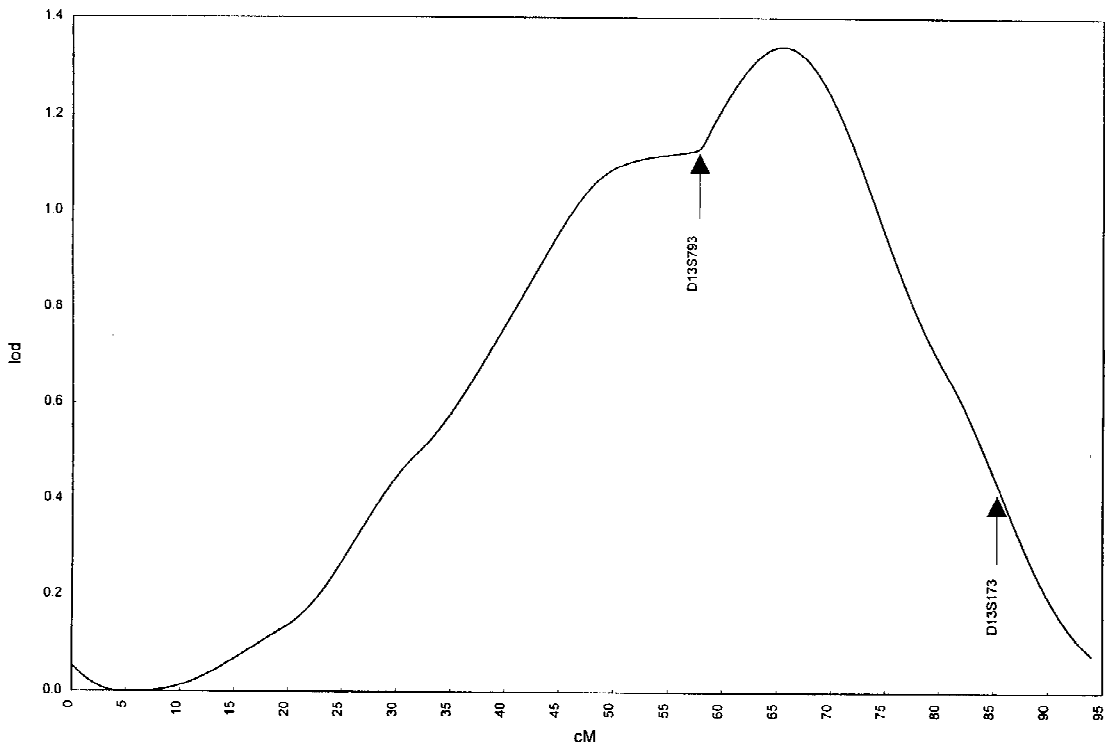


Fig. 2. Multipoint nonparametric maximum likelihood LOD assuming affection status model 1 for chromosome 11.

TABLE II. Multipoint Affected Sib-Pair Results for the X Chromosome*

Calculated cumulative distance (cM)	Model 1				Model 2				Model 3				
	Total	B-B	B-S	S-S	Total	B-B	B-S	S-S	Total	B-B	B-S	S-S	
	LOD	IBD (N = 30)	IBD (N = 75)	IBD (N = 40)	LOD	IBD (N = 37)	IBD (N = 124)	IBD (N = 84)	LOD	IBD (N = 43)	IBD (N = 237)	IBD (N = 152)	
DXS987	0.0	0.79	0.75	0.5	0.57	0.79	0.69	0.5	0.63	0.15	0.59	0.5	0.54
DXS989	2.7	0.92	0.79	0.5	0.55	0.74	0.71	0.5	0.59	0.16	0.6	0.5	0.52
DXS1068	21.7	0.16	0.55	0.54	0.58	0.07	0.5	0.5	0.55	0.0	0.5	0.5	0.5
DXS6810	29.2	0.03	0.5	0.5	0.54	0.0	0.5	0.5	0.5	0.0	0.5	0.5	0.5
DXS1003	34.9	0.07	0.5	0.5	0.5	0.0	0.5	0.5	0.5	0.0	0.5	0.5	0.5
DXS7132	48.4	0.16	0.5	0.58	0.5	0.0	0.5	0.51	0.5	0.0	0.5	0.5	0.5
DXS6800	50.2	0.24	0.5	0.6	0.5	0.0	0.5	0.51	0.5	0.0	0.5	0.5	0.5
DXS6804	69.0	0.03	0.5	0.54	0.5	0.02	0.5	0.5	0.53	0.07	0.5	0.55	0.5
DXS1001	86.2	0.0	0.5	0.5	0.5	0.0	0.5	0.5	0.5	0.07	0.5	0.54	0.5
DXS1047	92.5	0.41	0.5	0.5	0.68	1.23	0.5	0.5	0.72	0.3	0.5	0.5	0.59
GATA31E08	96.2	0.26	0.5	0.5	0.63	1.27	0.5	0.5	0.71	0.5	0.5	0.5	0.61

*B-B, brother-brother; B-S, brother-sister; S-S, sister-sister. IBD scores are constrained by Holman's triangle. Thus, all scores ≤ 0.5 are reported as 0.5.

The total multipoint nonparametric LOD scores at each marker position for each affection status model are presented in Table II. Two peaks occur in the LOD score statistic, one on Xp and the other on Xq. Assuming model 1, the highest observed LOD score is 0.92 at DXS989 in Xp22. The calculated maximum LOD score, 6 cM centromeric, is 0.94. Assuming model 2, the highest observed LOD scores are 1.23 and 1.27 at DXS1047 and GATA31E08, respectively, in Xq26–28. The calculated maximum LOD score 1.34 occurs between these markers.

DISCUSSION

This initial survey of chromosomes 2, 11, 13, 14, and X revealed regions of interest on 13q14–32, Xp22, and Xq26–28. A susceptibility gene for bipolar affective disorder, conferring a relative risk of 3 or greater in this sample, could be excluded across 96% of the distance on chromosomes 2, 11, and 14. On the X chromosome, two regions of interest were observed, Xp22 and Xq26–28. These regions of interest require additional studies, employing more markers and additional families.

The region of interest on chromosome 13q was suggested by two analyses. First, two-point affected sib-pair analyses revealed that D13S793 and D13S800 were the only two consecutive markers in this set with $P < 0.05$. In a simulated data set, Goldin and Chase [1996] showed that consecutive markers with low P values are a good indicator of loci associated with complex disorders. These P values need be interpreted cautiously, since they are observed only in model 1 of the three affected status models tested. However, model 1 is the narrowest model and may be expected to have the fewest phenocopies. Second, the only multipoint maximum likelihood score (MLS) LOD score >1 occurred at D13S793, using the observed allele frequencies from our sample. As a test for sensitivity to changes in allele frequency, the analyses were repeated using equal allele frequencies. The results were consistently positive, and the maximum MLS LOD at D13S793 was 1.6. Thus, our reported LOD scores reflect a conservative interpretation of the data. A disease locus in this region is predicted to have a λ_i of 1.1.

Chromosome 13 has been previously implicated as the site of a possible susceptibility gene for BP affective

disorder [Ginns et al., 1996]. The implicated regions, 13q13 [Ginns et al., 1996] and 13q14–32 (this study) are very broad and are separated by 10 cM based on the Location Data Base (LDB) map [Collins, 1997]. At loci where the proportion of sibs sharing alleles IBD is <0.6 , it may be expected that the gene will lie outside the range of maximal sharing [Kruglyak and Lander, 1996]. Therefore, it is possible that these two studies may implicate the same region.

The X chromosome appears to show two regions of interest: Xp22 and Xq26–28. The peak multipoint nonparametric LOD scores, 0.94 and 1.34, occurred in Xp22 and Xq26–28, respectively. When the data are divided into gender-specific pairs, the LOD score peaks appear to result from sharing by affected brother-brother pairs on Xp22, and affected sister-sister pairs on Xq26–28 (Table II). Affected brother-brother pairs showed excess sharing near DXS989. For example, the maximum proportion of brother-brother pairs sharing the X-chromosome IBD in this region under model 1 was 0.86 (the highest proportion of sharing actually observed at a marker was 0.79 at DXS989). Among sister-sister pairs, excess sharing was observed near DXS1047 and GATA31E08 in Xq26–28. For example, the proportion of sister-sister pairs sharing the maternal X chromosome IBD in this region under model 2 was 0.74.

The calculated λ_i for a disease allele depends on the disease model. For a disease allele on Xp22, λ_i equals 3.6 for affection status model 1. For a disease allele on Xq26, where there is sister-sister sharing, the calculated λ_i equals 1.6, 1.9, and 1.3 for models 1, 2, and 3, respectively, and is the lower bound of relative risk [Cordell et al., 1995]. Sib-pair sharing for brother-brother, brother-sister, and sister-sister pairs may be considered separately because the distinct types of pairs share different proportions and components of the genetic variance [Cordell et al., 1995] and may have distinct relative risks (λ_{xs}) [Hallmayer et al., 1996].

It is of interest that more affected sister-sister pairs were seen in our sample. Our sample had more affected sister-sister pairs compared to brother-sister and brother-brother pairs than expected by random segregation ($\chi^2 = 17.1$, $P < 0.0002$) for model 2, and ($\chi^2 = 45.2$, $P < 2 \times 10^{-10}$) for model 3. This finding is consistent with previous family studies of bipolar disorder

that showed that sisters of female probands had a higher morbid risk than brothers of female probands [Reich et al., 1969; Cadoret et al., 1970; Mendlewicz and Rainer, 1974; Smeraldi et al., 1981]. In the context of same-sex allele sharing, this may reflect gender-specific genetic vulnerability factors for BP affective disorder in these families. This hypothesis should be tested prospectively in future studies.

In our study, the genetic length of the X chromosome was shorter than the published length. Xp22 and Xq26–28 showed reductions in length of 50% or more. These two regions also showed excess sharing between affected sib pairs. The apparent reduction in length of these segments may be the result of an ascertainment bias because we analyzed only affected sibs. This occurs when affected sibs share a disease allele and the segment of the chromosome surrounding that allele. Thus, in this shared interval, there will be a decrease in the number of recombination events and hence an apparent reduction in genetic length when only affected sibs are analyzed.

Previous linkage studies have suggested possible loci for bipolar affective disorder on Xp22 and Xq26–28. An Xp22 location has been suggested by Mendlewicz and Fleiss [1974]. We are not aware of any other data testing for the presence of a gene important in BP affective disorder on Xp22. On Xq24–27, Pekkarinen et al. [1995] found a maximum LOD score of 3.54 near DXS994 in a single large Finnish kindred ascertained through a proband with BPI. The observed location overlaps with our result. Several other earlier studies using fewer and less informative markers also found evidence for linkage to this same general region [Winokur et al., 1969; Baron et al., 1987; Mendlewicz et al., 1972, 1987; Craddock and Owen, 1992; Gill et al., 1992; Lucotte et al., 1992; Jeffries et al., 1993; Stine et al., 1997]. Other studies in this region using pedigrees consistent with X-linkage (i.e., no father-to-son transmission) have produced conflicting results [Berrettini et al., 1990; Gejman et al., 1990; Del Zompo et al., 1991; Baron et al., 1993].

The lack of evidence for linkage to loci on chromosomes 2, 11, and 14 is consistent with most previous reports. A locus with $\lambda_1 = 3.0$ could be excluded in 96% or more of the genetic distance on these autosomes, while a gene with $\lambda_1 = 1.5$ or less could be excluded from <10%. It is possible that genes with small effects or with a large effect but only in a few pedigrees may be present on these autosomes. Since only two thirds of the potential genetic information has been obtained, it is possible that additional information from markers spaced at narrower intervals will point to additional linked loci on these chromosomes.

Our initial survey of chromosomes 2, 11, 13, 14, and X suggests regions of interest for bipolar affective disorder near Xp22, Xq26–28, and 13q14–32. These results should be interpreted in the context of the companion papers in this issue [NIMH Genetics Initiative Bipolar Group, 1997; Detera-Wadleigh et al., 1997; Edenberg et al., 1997; Rice et al., 1997] and are consistent with some previous studies.

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