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Evidence for Linkage of Bipolar Disorder to Chromosome 18 with a Parent-of-Origin Effect

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Summary

A susceptibility gene on chromosome 18 and a parent-of-origin effect have been suggested for bipolar affective disorder (BPAD). We have studied 28 nuclear families selected for apparent unilineal transmission of the BPAD phenotype, by using 31 polymorphic markers spanning chromosome 18. Evidence for linkage was tested with affected-sib-pair and LOD score methods under two definitions of the affected phenotype. The affected-sib-pair analyses indicated excess allele sharing for markers on 18p within the region reported previously. The greatest sharing was at D18S37: 64% in bipolar and recurrent unipolar (RUP) sib pairs ($P = .0006$). In addition, excess sharing of the paternally, but not maternally, transmitted alleles was observed at three markers on 18q: at D18S41, 51 bipolar and RUP sib pairs were concordant for paternally transmitted alleles, and 21 pairs were discordant ($P = .0004$). The evidence for linkage to loci on both 18p and 18q was strongest in the 11 paternal pedigrees, i.e., those in which the father or one of the father's sibs is affected. In these pedigrees, the greatest allele sharing (81%; $P = .00002$) and the highest LOD score (3.51; $\theta = 0.0$) were observed at D18S41. Our results provide further support for linkage of BPAD to chromosome 18 and the first molecular evidence for a parent-of-origin effect operating in this disorder. The number of loci involved, and their precise location, require further study.

Introduction

Bipolar affective disorder (BPAD) is a severe psychiatric condition characterized by episodes of depression and mania. This common disorder has a prevalence of 1%–2% (Robins et al. 1984). While the etiology is unknown, family, twin, and adoption studies have consistently supported a genetic basis (reviewed by Tsuang et al. 1990). The mode of inheritance remains unclear. Segregation analyses have yielded inconsistent results (Crowe and Smouse 1977; Goldin et al. 1983; O'Rourke et al. 1983; Rice et al. 1987; Spence et al. 1993). The relative risk for siblings of affected probands varies from 5 to 20 (Mendlewicz and Rainer 1974; Rice et al. 1987). Thus, BPAD does not appear to be a simple Mendelian trait and may be genetically heterogeneous.

Recently, Berrettini et al. (1994) reported evidence of a susceptibility locus for BPAD on chromosome 18. Attempts to replicate this finding are important, since several earlier genetic linkage studies of BPAD were not confirmed. Initial reports of linkage to Xq28 (Reich et al. 1969; Mendlewicz et al. 1972; Baron 1977) were not consistently replicated in other pedigrees (Gershon et al. 1979) or in some of the original pedigrees when highly polymorphic DNA markers were utilized (Baron et al. 1993). An initial report of linkage to chromosome 11p in a large pedigree (Egeland et al. 1987) was not supported by further analyses and extensions of this same pedigree (Kelsoe et al. 1989).

We have proposed a parent-of-origin effect in BPAD, on the basis of analysis of clinical data collected for our genetic linkage study (McMahon et al. 1995). Our study is distinguished by (1) relatively small, densely affected families with apparent unilineal transmission of the affected phenotype and (2) direct clinical evaluation of family members by psychiatrists. A parent-of-origin effect was suggested by the observations that transmitting mothers are more common than transmitting fathers and have a higher proportion of affected relatives. Thus, families with transmitting mothers and families with transmitting fathers show different susceptibility to BPAD.

In this paper, we report affected-sib-pair and LOD score analyses on 28 families by using a set of markers spanning chromosome 18. We have tested for evidence

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of linkage to these markers and for parent-of-origin effects. Our results provide further support for linkage of BPAD to chromosome 18 and the first molecular evidence for a parent-of-origin effect operating in this disorder. The number of loci involved, and their precise location, require further study.

Methods

Family Ascertainment

Clinics and inpatient units in Baltimore, Maryland, and Iowa City, Iowa, were screened for treated bipolar I probands with two or more affected siblings or one affected sibling and one (but only one) affected parent. For ascertainment purposes, subjects with a bipolar or recurrent unipolar disorder (RUP) based on family history were considered affected. In addition, families were solicited through the media and local clinicians and studied if they met the ascertainment criteria. The ascertainment plan sought families exhibiting unilineal transmission and required that the putative unaffected parent undergo direct clinical assessment. Families with an affected phenotype among the parents, aunts, uncles, or grandparents in both parental lineages were excluded (Simpson et al. 1992).

Clinical Evaluation

After the procedure was explained and informed consent was obtained, all available family members were interviewed by one of four psychiatrists (S.G.S., J.R.D., F.J.M., or M.G.M.) trained in the use of the Schedule for Affective Disorders and Schizophrenia-Lifetime version (SADS-L; Endicott and Spitzer 1978). The psychiatrists have established good interrater reliability (test-retest κ = 0.72 for RUP, 0.74 for bipolar I, and 0.61 for bipolar II). Best estimate diagnoses were assigned by two psychiatrists (not including the interviewer) who reviewed the SADS-L, family informant data, and any available medical records. Diagnoses were based on the Research Diagnostic Criteria (Spitzer et al. 1975), except that both hypomania and recurrent major depression were required for a diagnosis of bipolar II. There was excellent interrater reliability for the best estimate procedure (κ = 0.96). The clinical personnel were blind to genotype data.

A "phenotype uncertain" category was established to encompass threshold cases of affective disorder, subjects whose disease status was unclear after best estimate review, and subjects with "affective spectrum" conditions that have been associated with BPAD in family studies. The most common diagnosis of individuals in the uncertain category was a single episode of major depression. Unaffected subjects included those with no psychiatric diagnosis and those with diagnoses considered outside the affective spectrum. The uncertain and unaffected categories are detailed in the study by Simpson et al. (1992).

All interviewed subjects assigned an uncertain or unaffected diagnosis received an annual follow-up contact. Subjects reporting new symptoms were reinterviewed whenever possible. No diagnoses were changed on the basis of follow-up interviews in these 28 families.

The Sample

The sample consisted of 243 interviewed and genotyped individuals from 28 pedigrees. On average, there were 4.5 affected individuals per pedigree. There were 59 bipolar I, 42 bipolar II, 24 RUP, 54 phenotype uncertain, and 64 unaffected individuals. The mean age at onset was 21.2 ± 9.8 years for individuals with a diagnosis of bipolar I or II and 28.9 ± 11.9 years for individuals with recurrent unipolar disorder. These mean ages at onset are similar to those seen in other family studies (Coryell et al. 1985; Endicott et al. 1985).

There were 11 "paternal" pedigrees, in which the father of the proband or at least one of the father's sibs was affected (in one family, the affected father was the proband), and 16 "maternal" pedigrees, in which the mother or at least one of her sibs was affected. Only one family could not be classified because there were no affected relatives in either parental lineage. In the 11 paternal pedigrees, there were 103 interviewed and genotyped individuals: 27 bipolar I, 21 bipolar II, 5 RUP, 23 phenotype uncertain, and 27 unaffected. In the 16 maternal pedigrees, there were 134 interviewed and genotyped individuals: 31 bipolar I, 20 bipolar II, 18 RUP, 29 phenotype uncertain, and 36 unaffected.

Cell Lines and DNA Isolation

Leukocytes were isolated and transformed using Epstein-Barr virus in order to establish permanent cell lines. DNA was isolated using a Puregene kit, following the instructions of the manufacturer (Gentra).

Markers

Markers were genotyped at both Johns Hopkins and Stanford Universities. We studied 31 highly polymorphic markers on chromosome 18. For 16 of these markers, the observed heterozygosity was >0.8 (table 1).

The oligonucleotides used to initiate the PCR for all marker loci were purchased from Research Genetics or Généthon. The annealing temperature was adjusted for some pairs of oligonucleotides. PCR was carried out in a 10-μl volume containing 50–100 ng DNA, 15 nM primers, 200 μM dNTPs, 1.5 mM $MgCl_2$, and 10 mM Tris pH 8.3. The resulting amplified products were separated on 5% or 6% denaturing polyacrylamide gels. Fragments were detected by one of two methods. At Johns Hopkins, one primer was end-labeled prior to PCR amplification with γ-^{32}P ATP by using T-4 poly-

Table 1

Results of Affected-Sib-Pair Analyses

Marker	Observed Heterozygosity	Cumulative Distance (cM)	Bipolar and Recurrent Unipolar			Bipolar Only		
			Pairs	IBD[a]	P[b]	Pairs	IBD[a]	P[b]
D18S59	.87	.0	109	.50	...	88	.49	...
D18S54	.79	13.9	61	.50	...	57	.51	...
D18S62	.71	21.2	86	.50	...	71	.50	...
D18S843	.78	35.9	91	.51	...	67	.50	...
D18S464	.48	38.7	68	.55	.05	54	.59	.005
D18S53	.82	41.8	112	.56	.02	90	.56	.04
D18S71	.82	46.3	96	.49	...	84	.49	...
D18S37	.56	49.0	46	.64	.0003	39	.60	.005
D18S482	.48	50.9	56	.53	.09	44	.55	.04
D18S40	.88	54.3	84	.57	.02	70	.59	.01
D18S45	.76	54.3	45	.52	...	34	.54	...
D18S56	.77	62.3	89	.51	...	78	.53	...
D18S57	.89	65.8	84	.48	...	69	.51	...
D18S36	.76	67.4	81	.44	...	64	.44	...
D18S34	.80	73.9	26	.58	.06	21	.59	.07
D18S46	.82	83.3	56	.46	...	46	.50	...
D18S363	.82	83.3	48	.48	...	41	.49	...
D18S39	.84	87.3	102	.53	...	81	.54	.09
D18S69	.84	87.9	93	.50	...	77	.51	...
D18S35	.70	89.2	56	.50	...	50	.51	...
D18S381	.84	91.5	74	.50	...	61	.50	...
D18S41	.70	91.5	96	.55	.04	74	.57	.02
D18S64	.74	95.0	97	.55	.04	78	.57	.03
D18S38	.81	97.8	39	.57	.06	35	.57	.07
D18S42	.80	100.6	90	.49	...	74	.48	...
D18S60	.59	100.6	91	.51	...	71	.52	...
D18S55	.80	103.0	90	.52	...	76	.53	...
D18S61	.90	109.3	98	.53	...	79	.54	...
D18S58	.79	119.6	79	.51	...	64	.50	...
D18S880	.42	124.6	111	.54	.01	86	.53	.05
D18S70	.88	141.4	101	.50	...	81	.51	...

[a] Proportion of sibs sharing alleles identical by descent.
[b] All nominal *P*-values <.1 are reported.

nucleotide kinase and buffer supplied by the manufacturer (New England Biolabs). Allele sizes were determined by using a phosphoimager (Molecular Dynamics) and CEPH controls. At Stanford, the separated PCR fragments were transferred to nylon membranes, hybridized with a digoxigen-labeled primer, immunoreacted with a conjugated antibody, and detected by alkaline phosphatase–mediated cleavage of the chemiluminescent substrate Tropix (Clark and Gschwend 1994). The label was detected by exposure to x-ray film. Allele sizes were determined by using the Whole Band Analyzer (BioImage).

Genotypes

Genotypes were read by two independent raters blind to diagnosis. Allele sizes (in bp) were determined for 13 markers (D18S59, D18S464, D18S53, D18S71, D18S40, D18S56, D18S39, D18S69, D18S41, D18S64, D18S38, D18S60, and D18S70) by comparison with a CEPH individual with known allele sizes loaded on the same gel. The other markers were scored with relative allele sizes within each family. Each marker in every family was tested for proper segregation.

CRIMAP (Lander and Green 1987) was used to establish the order of the markers on our map (table 1). The map order of our markers is similar to that of published maps (Gyapay et al. 1994; Straub et al. 1994; CHLC 1995). The map order was used to identify double recombinants. Double recombinants occurring within 30 cM were reassayed in the laboratory. To avoid bias in repeat genotyping, a sample of families without double recombinants was also included for repeat assay.

Eleven markers were assayed independently in each laboratory to test the discrepancy rate and confirm posi-

tive results. The discrepancy rate between the two laboratories was <2%. When a discrepancy occurred, readers at both institutions rechecked the original data to check for mistakes in the assignment of the alleles. If the discrepancy was not resolved, the sample was reassayed or the genotype was considered unknown in the analyses. All analyses were based on the corrected data set.

Genetic Analyses

Because some cases of RUP disorder ascertained through bipolar I probands may have distinct etiologies (Blacker and Tsuang 1993; McMahon et al. 1994), all analyses were performed using two definitions of the affected phenotype: (1) bipolar I and II and RUP and (2) bipolar I and II only. Hereafter, all subjects with either bipolar I or II are referred to as "bipolar."

When paternal and maternal pedigrees were analyzed separately, the affected sibs of the mother or father were considered phenotype unknown, since the phenotypes of their parents (in general, the proband's grandparents) are in most cases unknown.

The proportion of affected sibs sharing alleles identical by descent was calculated using SIBPAL from the SAGE (1994) software package. The degrees of freedom were calculated by using $(n - 1)$ sibs per family to adjust for nonindependence (Suarez and Van Eerdewegh 1984). The Genetic Analysis System (GAS; Young 1995) program was used to detect differences in sharing of paternally versus maternally transmitted alleles identical by descent. Unless otherwise specified, nominal *P*-values are reported in text and tables; empirical *P*-values are presented below.

LOD scores were calculated using LINKAGE (Lathrop et al. 1984) and ANALYZE (Terwilliger 1995). Initial analyses were conducted by using an "affecteds-only" approach under both definitions of affected status. For these analyses, phenotype data from unaffected and uncertain family members were not considered, but their genotype data were used in determining phase. Because previous segregation and linkage analyses have been unable to determine the mode of inheritance, both dominant and recessive models of inheritance were analyzed. The dominant model assumed a phenocopy rate of 1%–5%, depending on the age at onset, and a .02 frequency of the disease allele. The recessive model assumed the same phenocopy rate but a .2 frequency of the disease allele. A maximum age-dependent penetrance of 85% was assumed. These values are consistent with previous linkage studies of BPAD (Egeland et al. 1987; Baron et al. 1993; Berrettini et al. 1994). For markers with preliminary evidence for linkage, analyses were repeated with the inclusion of phenotype data from unaffected family members.

Multipoint likelihoods were calculated using LINKMAP (Lathrop et al. 1984) for several sets of three to four loci, to determine the most likely location of a disease locus. Multipoint-polylocus LOD scores were calculated using ANALYZE (Terwilliger 1995); if a family was uninformative at a given marker, the genotypic data from flanking markers were used with allowance for the recombination distance between the markers (Terwilliger and Ott 1993).

Homogeneity tests were performed using the admixture test (HOMOG; Ott 1991) to test for between-family heterogeneity and to test for the presence of a second disease locus on chromosome 18. M-tests were performed to test for homogeneity of the LOD scores between the maternal and paternal pedigrees (Morton 1956).

Simulations

Empirical *P*-values were determined by performing computer simulations (SIMULATE; Ott and Terwilliger 1992) using the actual pedigree structures of the families in this study. The observed allele frequencies at D18S41 (the marker with the highest two-point LOD scores) were used. Simulated data were generated under the assumption of no linkage; 2,080 replicates were performed. Simulated data were analyzed in exactly the same manner as the actual data.

For sib-pair analyses, the simulated data were analyzed under both definitions of the affected phenotype by using SAGE (1994). The summed results revealed that a *P*-value of <.01 occurred 3.6% of the time and a *P*-value of <.001 occurred 0.6% of the time. When the parent of origin of the shared allele was determined under both definitions by using GAS (Young 1995), the summed results revealed that a *P*-value of <.001 was observed <0.3% of the time. In simulations using only the paternal pedigrees and summing over all models, a *P*-value of <.0001 was seen <0.3% of the time.

Eight LOD score analyses were performed on each simulated data set. We tested the entire set of pedigrees and the subset of paternal pedigrees by using the two definitions of the affected phenotype and assuming both dominant and recessive models. In the simulations, LOD scores >3.0 in any of the 8 analyses were observed at a rate of 0.002; LOD scores >2.0 were observed at a rate of 0.02.

Results

Evidence for Linkage to Chromosome 18

Affected-sib-pair analyses, using both definitions of affected status, provided evidence for linkage to chromosome 18. In bipolar and RUP-affected sib pairs, there was an excess of allele sharing ($P < .05$) for seven markers (D18S464, D18S53, D18S37, D18S40, D18S41, D18S64, and D18S880) (table 1). The greatest sharing

Table 2

Results of LOD Score Analyses for Selected Markers

	Dominant Model—All Pedigrees			
	Bipolar and Recurrent Unipolar		Bipolar Only	
Marker	LOD	θ_{max}	LOD	θ_{max}
D18S62	.04	.38	.00	...
D18S464	.00	...	.00	.40
D18S53	.10	.30	.00	...
D18S71	.32	.30	.14	.30
D18S37	1.45	.10	.72	.14
D18S482	.03	.18	.02	.14
D18S40	.08	.34	.00	...
D18S45	.27	.22	.23	.16
D18S56	.00	...	.00	...
D18S39	.00	...	.06	.26
D18S41	.76	.20	1.45	.10
D18S64	.34	.24	.88	.16
D18S38	.26	.22	.36	.20
D18S880	.84	.10	.93	.06

NOTE.—θ_{max} is reported when <.5. The region identified by Berrettini et al. (1994) extends from D18S62 to D18S56.

was at D18S37, 64% (P = .0003); when RUP sibs were excluded, the sharing was 60% (P = .005). Excess allele sharing was observed at four flanking markers (D18S464, D18S53, D18S482, and D18S40), which map within an 18-cM region on 18p. The other markers with excess allele sharing, D18S41, D18S64, and D18S880, map to 18q.

Affected-only LOD score analyses, using both definitions of affection status and a dominant mode of transmission, provided suggestive evidence of linkage to chromosome 18 (table 2). LOD scores of 1.45 (θ_{max} = .1) were observed for D18S37 (on 18p), defining bipolar and RUP as affected, and for D18S41 (on 18q), defining only bipolar disorder as affected.

The results of the two-point LOD score analyses were supported by multipoint-polylocus LOD scores. LOD scores for a dominant model were 1.69 at D18S38 (on 18q) (bipolar and RUP) and 1.04 at D18S37 (bipolar only).

Less evidence for linkage was found by using a recessive model. Defining bipolar disorder and RUP as affected, 3 of 31 markers had LOD scores >.5 (θ_{max} ranged from .04 to .14); the largest were 1.12 (θ_{max} = .06) at D18S880, and 0.96 (θ_{max} = .04) at D18S37. Defining bipolar disorder only as affected, 4 of 31 markers had LOD scores >.5 (θ_{max} ranged from .06 to .20); the largest was 0.84 (θ_{max} = .06) at D18S880.

Multipoint LOD scores, using two sets of markers, provided weak but consistent evidence for a susceptibility locus on 18q. A dominant model, defining bipolar and RUP as affected, was used. For the marker set D18S53, D18S56, and D18S41, the maximum LOD score was 1.41, 10 cM toward the q telomere from D18S41. The LOD scores for loci on 18p were uniformly negative. For D18S59, D18S56, and D18S880, the maximum LOD score was 1.32, 17 cM toward the q telomere from D18S56. Again, the LOD scores for 18p loci were uniformly negative. The admixture test provided no evidence for a second disease locus on chromosome 18 (HOMOG2R; Ott 1991).

Parent-of-Origin Analyses

Excess sharing of paternally transmitted alleles identical by descent was observed among bipolar and RUP sibs at three neighboring markers within an 8-cM region on 18q (table 3). The numbers of sib pairs concordant or discordant for paternally transmitted alleles were, respectively, 51 and 21 (P = .0004) for D18S41, 45 and 20 (P = .002) for D18S64, and 20 and 7 (P = .01) for D18S38. For bipolar sibs, similar results were observed, with excess sharing of paternally transmitted alleles. In contrast, maternally transmitted alleles assorted randomly among affected sib pairs at these three markers.

Further evidence for a parent-of-origin effect on 18q was found when the 11 paternal and 16 maternal pedigrees were analyzed separately (table 4). If sharing of alleles from either parent was examined, excess allele sharing at loci on both 18p and 18q was observed in the paternal but not in the maternal pedigrees. If only paternally transmitted alleles were examined, excess allele sharing was again seen in paternal but not maternal pedigrees, and only at markers on 18q (D18S39, D18S41, D18S64, and D18S38). The greatest sharing was seen at D18S41, 81% (P = .00002). Maternally transmitted alleles exhibited random segregation at these 18q loci. In the maternal pedigrees, there was random segregation of both paternal and maternal alleles. Similar results were observed when RUP sibs were included or excluded.

Two-point LOD scores for paternal pedigrees are presented in table 5. The highest LOD scores were observed on 18q at D18S41: 3.51 (θ_{max} = .00; bipolar and RUP); and 3.16, (θ_{max} = .02; bipolar only). LOD scores over 2.6 were also seen at D18S64. When unaffected family members were included, the LOD score at D18S41 was 3.07 (θ_{max} = .001). In the maternal pedigrees, no positive LOD scores were observed at D18S41, D18S64, or D18S38. There was suggestive evidence for heterogeneity between paternal and maternal pedigrees at D18S41 (M-test: χ^2 = 11.60; df = 1; P = .0007) and at several flanking markers (table 5).

The results of the two-point LOD score analyses were supported by multipoint-polylocus LOD scores. In these analyses, the highest LOD scores in the paternal pedi-

Table 3

Number of Affected Sib Pairs Sharing Alleles Identical by Descent, by Parent of Origin, for Selected Markers (28 Pedigrees)

	Sex-Specific Cumulative Distance (cM)		Bipolar and Recurrent Unipolar						Bipolar Only					
			Paternal Allele Sharing			Maternal Allele Sharing			Paternal Allele Sharing			Maternal Allele Sharing		
Markers	Male	Female	1	0	*P*	1	0	*P*	1	0	*P*	1	0	*P*
D18S62	39.8	4.8	5	8	...	17	16	...	5	5	...	15	15	...
D18S843	54.3	21.9	27	33	...	37	30	...	22	24	...	29	26	...
D18S464	54.3	27.8	18	18	...	21	8	.02	17	14	...	19	5	.004
D18S53	54.3	34.4	36	42	...	52	35	.07	34	33	...	42	32	...
D18S71	57.9	39.9	24	26	...	32	36	...	19	22	...	31	31	...
D18S37	61.4	41.6	9	3	...	9	3	.08	6	3	...	6	3	...
D18S482	61.9	45.9	14	11	...	4	5	...	13	9	...	4	4	...
D18S40	65.9	46.0	40	34	...	38	25	.10	35	28	...	34	21	.08
D18S45	67.7	46.0	9	14	...	3	5	...	8	10	...	1	2	...
D18S56	67.7	64.0	32	24	...	24	26	...	30	17	.06	22	22	...
D18S39	80.9	103.9	37	25	...	37	37	...	35	19	.03	30	34	...
D18S41	86.8	106.6	51	21	.0004	28	37	...	41	15	.0005	25	28	...
D18S64	89.1	111.0	45	20	.002	34	39	...	40	17	.002	31	32	...
D18S38	92.8	114.9	20	7	.01	11	16	...	19	6	.009	10	14	...
D18S880	112.5	150.4	14	13	...	15	8	...	12	10	...	5	6	...

Note.—The region identified by Berrettini et al. (1994) extends from D18S62 to D18S56. All nominal *P*-values <.1 are reported.

grees were 3.11 (bipolar only) and 2.86 (bipolar and RUP) at D18S64 (fig. 1). In the maternal pedigrees, no LOD scores >0.52 were observed.

Discussion

The results of this study provide further support for linkage of BPAD to chromosome 18 and the first molecular evidence of a parent-of-origin effect in BPAD. Evidence for linkage in complex disorders is subject to variable interpretations, depending on the criteria for significance. By convention, a LOD score of 3, or a *P*-value of <0.001 for nonparametric analyses, is required because of the prior odds against linkage, when a single model is assumed. When multiple models are tested, the LOD scores and *P*-values can be adjusted by correcting the LOD scores for multiple models (Weeks et al. 1990) or using more stringent *P*-values. Use of the LOD score correction in our data set would require a score >3.9 for statistical significance. However, these corrections assume no prior hypothesis of linkage. This assumption is not valid for marker loci in the region implicated by Berrettini et al. (1994) (see fig. 2). Thus, the conventional level of significance for LOD scores may be too conservative in this case.

An alternative approach, recommended by Lander and Schork (1994), is to calculate empirical *P*-values by computer simulation. We generated simulated data based on observed allele frequencies for D18S41 and analyzed the simulated data in precisely the same manner as our actual data. The results of these simulations indicate that our evidence for linkage to chromosome 18 is unlikely to represent a false positive. The *P*-value of .0003 observed in the affected-sib-pair analysis at D18S37 would be expected to be a false positive <6/1,000 times. Our evidence for a parent-of-origin effect is also unlikely to be a false-positive result. The *P*-value of .0004 observed in the affected-sib-pair analysis of paternal alleles would be expected to be observed by chance <8/1,000 times. Finally, the LOD score of 3.51 at D18S41 in paternal pedigrees would be expected to be a chance observation <2/1,000 times.

Our results from affected-sib-pair analyses are compared to those of Berrettini et al. (1994) in figure 2. Our analyses indicated excess allele sharing for markers on 18p within the region reported by Berrettini et al. (1994). These results could be considered a replication of those of Berrettini et al. (1994). However, our study found evidence for a parent-of-origin effect and found the strongest evidence of linkage on 18q, 30 cM (sex averaged) away from D18S56 on the map of Berrettini et al. (1994) (fig. 1). Initial evidence for linkage can occur at substantial distances from a disease locus because of both genetic heterogeneity and model misspecification. Indeed, we calculated a 95% confidence interval (Kruglyak and Lander 1995) by using the data from the paternal pedigrees; the interval includes both arms of chromosome 18. Similarly, BRCA1 ultimately

Table 4

Affected Sib-Pair Results (Bipolar and RUP), Broken Down by Parent of Origin of Shared Alleles, in Paternal and Maternal Pedigrees for Selected Markers

	Paternal Pedigrees—Shared Alleles									Maternal Pedigrees—Shared Alleles								
	Paternal			Maternal			Either			Paternal			Maternal			Either		
Marker	Pairs	IBD[a]	*P*	Pairs	IBD[a]	*P*	Pairs	IBD[a]	*P*	Pairs	IBD[a]	*P*	Pairs	IBD[a]	*P*	Pairs	IBD[a]	*P*
D18S62	3	.33	...	34	.54	...	27	.52	...	5	.50	...	9	.45	...	17	.47	...
D18S843	32	.56	...	38	.61	...	70	.58	...	15	.33	...	10	.48	...	36	.42	...
D18S464	28	.57	...	25	.80	.004	53	.68	.01	3	.33	...	1	.00	...	4	.25	...
D18S53	43	.51	...	49	.63	...	92	.58	...	27	.44	...	28	.46	...	53	.43	...
D18S71	21	.61	...	36	.44	...	57	.51	...	27	.37	...	30	.53	...	57	.46	...
D18S37	7	1.00	...	6	1.00	...	13	1.00	.0002	5	.40	...	6	.50	...	11	.45	...
D18S482	18	.66	...	5	.60	...	23	.65	...	6	.33	...	4	.25	...	10	.30	...
D18S40	46	.61	...	37	.70	.02	83	.65	.008	22	.45	...	20	.45	...	42	.44	...
D18S45	16	.37	...	1	.00	...	17	.35	...	2	1.00	...	2	.50	...	4	.75	...
D18S56	37	.67	...	23	.52	...	60	.62	...	14	.43	...	22	.45	...	37	.44	...
D18S39	40	.72	.006	44	.50	...	84	.61	.06	17	.35	...	25	.36	...	40	.38	...
D18S41	48	.81	.00002	47	.47	...	95	.64	.007	13	.54	...	12	.42	...	25	.48	...
D18S64	45	.77	.0003	41	.49	...	101	.63	.009	17	.53	...	14	.29	...	31	.42	...
D18S38	16	1.00	.00003	21	.48	...	37	.70	.02	9	.33	...	4	.00	...	13	.23	...
D18S880	18	.38	...	10	.50	...	28	42	...	6	1.00	...	4	.50	...	10	.80	...

NOTE.—The region identified by Berrettini et al. (1994) extends from D18S62 to D18S56. Nominal *P*-values <.1 are reported.

[a] Proportion of sibs sharing alleles identical by descent.

Table 5

Results of LOD Score Analyses and M-Tests for Selected Markers in Paternal and Maternal Pedigrees

	Bipolar and Recurrent Unipolar						Bipolar Only					
	Paternal		Maternal		M-Test		Paternal		Maternal		M-Test	
Marker	LOD	θ_{max}	LOD	θ_{max}	χ^2	*P*	LOD	θ_{max}	LOD	θ_{max}	χ^2	*P*
D18S62	...	...	...	...	...	...	...	...	.00	.46	...	...
D18S843	.01	.32	...	...	...	...	.23	.10	...	...	...	...
D18S464	.00	.46	...	...	...	...	.37	.10	...	...	...	...
D18S53	...	...	.18	.28	...	...	.80	.00	...	...	...	...
D18S71	.24	.24	.00	.38	...	...	1.14	.06	...	...	3.59	.06
D18S37	1.50	.00	.45	.14	...	...	1.29	.00	.07	.24	...	...
D18S482	.37	.00	...	...	...	...	.35	.00	...	...	...	...
D18S40	.19	.12	...	...	...	...	1.57	.00	...	...	7.23	.007
D18S56	.09	.18	...	...	...	...	1.45	.00	...	...	5.48	.02
D18S39	.66	.10	...	...	...	...	1.15	.04	...	...	5.24	.02
D18S41	3.51	.00	...	...	11.60	.0007	3.16	.02	...	...	5.30	.02
D18S64	2.62	.00	...	...	7.83	.005	2.86	.00	...	...	8.15	.004
D18S38	1.23	.00	...	...	4.93	.003	1.23	.00	...	...	4.79	.03
D18S880	.04	.00	1.11	.04	...	...	...	...	.80	.00	...	...

NOTE.—Only positive LOD scores are reported. All *P*-values refer to the M-test for heterogeneity; those <.1 are reported. The region identified by Berrettini et al. (1994) extends from D18S62 to D18S56.

mapped 20 cM away from the first linked marker to be identified (Hall et al. 1990; Easton et al. 1993). Thus, although our results differ from those of Berrettini et al. (1994) by most likely map location and by the presence of a parent-of-origin effect, both studies support the existence of a gene for bipolar affective disorder (*BPAD1*) on chromosome 18.

The question arises whether our observations may be the result of two loci on chromosome 18 linked to

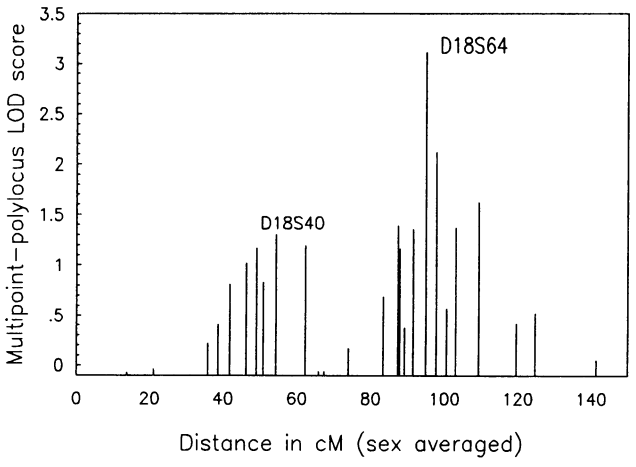

Figure 1 Multipoint-polylocus LOD scores for markers on chromosome 18, in paternal pedigrees. For these analyses, only bipolar subjects were defined as affected. Each marker is indicated on the x-axis by a bar proportional to its LOD score.

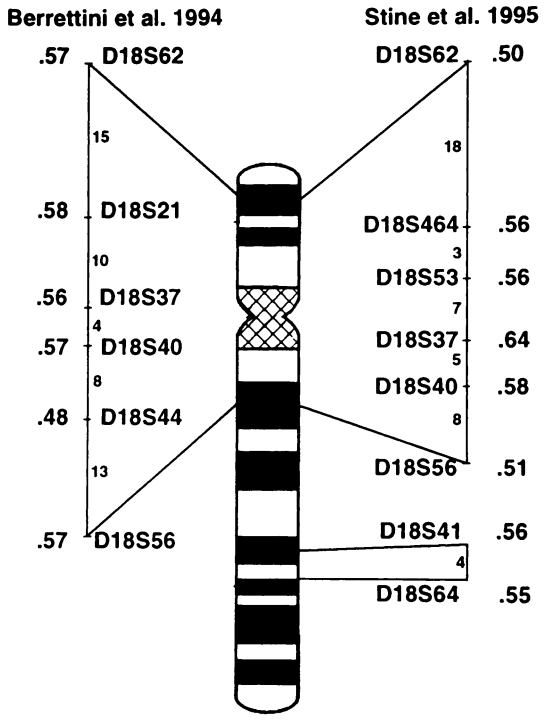

Figure 2 Affected-sib-pair results for selected markers on chromosome 18, compared to results of Berrettini et al. 1994. The proportion of affected sib-pairs sharing alleles identical by descent is indicated next to the marker names. Distances (in cM) are indicated between named markers.

BPAD. Two marker loci, D18S37 and D18S41, ~40 cM apart, produced the strongest evidence for linkage in affected-sib-pair analyses. However, the statistical test for the presence of two loci on the same chromosome was not significant (HOMOG2R; Ott 1991). This test does not allow for interaction between loci or for heterogeneity within families. Multipoint LOD score analyses were consistent with a single dominant disease gene on 18q. However, the purpose of multipoint analyses is to determine the most likely location of a single disease gene, not to test for the presence of two loci. Thus, we can neither establish nor exclude the presence of a second disease locus on chromosome 18.

The parent-of-origin effect, suggested by our clinical findings (McMahon et al. 1995), has not been described elsewhere in linkage analyses of BPAD and therefore requires replication. Our claim of a parent-of-origin effect is based on two observations. Our evidence for linkage on 18p is strongest in paternal pedigrees, i.e., those in which the father or one of the father's sibs is affected. However, the shared alleles appeared to be transmitted by either the mother or the father. In contrast, the evidence for linkage on 18q was derived solely from the paternally transmitted alleles. Thus, while there is a clear parent-of-origin effect, the underlying genetic mechanism appears complex. It may involve genetic heterogeneity, imprinting, and/or mitochondrial inheritance (McMahon et al. 1995).

An alternative explanation of the parent-of-origin effect is that it results from the observed differences between male and female recombination rates on chromosome 18 (Straub et al. 1993). On our map, the ratio of the sex-specific recombination rates in the region from D18S46 to D18S61 is ~1.6:1. The genetic distances in the sex-specific and sex-averaged maps are not significantly different ($\chi^2 = 10.68$; df = 11; P is not significant), on the assumption that, under the null hypothesis, the differences follow a χ^2 distribution. Thus, it is unlikely that our results are simply due to a difference in recombination rates.

Our data do not clarify whether RUP should be considered part of the BPAD phenotype. While previous studies have suggested that RUP may in some cases be etiologically distinct from the bipolar subtypes of BPAD (Blacker and Tsuang 1993; McMahon et al. 1994), we observed little variation in the affected-sib-pair and LOD score results when RUP was included as an affected phenotype. Similarly, there is little change in the evidence for linkage when unaffected subjects are included in the analyses.

There are several known candidate loci on chromosome 18. These include an ACTH receptor (Gantz et al. 1993), G_{olf} (Jones and Reed 1989), N-cadherin (Walsh et al. 1990), and RED-1 (Schalling et al. 1993). The latter two candidates contain triplet repeats. Triplet repeats have been associated in other diseases, e.g., Huntington disease, (Huntington's Disease Collaborative Research Group 1993) with clinical anticipation similar to that observed in some of the pedigrees described in our study (McInnis et al. 1993).

In summary, our affected-sib-pair and likelihood analyses provide further support for linkage of BPAD to markers on chromosome 18 and the first molecular evidence for a parent-of-origin effect operating in this disorder. The number of loci involved, and their precise location, require further study.

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