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No Evidence for Significant Linkage between Bipolar Affective Disorder and Chromosome 18 Pericentromeric Markers in a Large Series of Multiplex Extended Pedigrees

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Summary

Bipolar affective disorder (BP) is a major neuropsychiatric disorder with high heritability and complex inheritance. Previously reported linkage between BP and DNA markers in the pericentromeric region of chromosome 18, with a parent-of-origin effect (linkage was present in pedigrees with paternal transmission and absent in pedigrees with exclusive maternal inheritance), has been a focus of interest in human genetics. We reexamined the evidence in one of the largest samples reported to date (1,013 genotyped individuals in 53 unilineal multiplex pedigrees), using 10 highly polymorphic markers and a range of parametric and nonparametric analyses. There was no evidence for significant linkage between BP and chromosome 18 pericentromeric markers in the sample as a whole, nor was there evidence for significant parent-of-origin effect (pedigrees with paternal transmission were not differentially linked to the implicated chromosomal region). Two-point LOD scores and single-locus sib-pair results gave some support for suggestive linkage, but this was not substantiated by multilocus analysis, and the results were further tempered by multiple test effects. We conclude that there is no compelling evidence for linkage between BP and chromosome 18 pericentromeric markers in this sample.

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Introduction

Bipolar affective disorder (BP), a severe and common illness characterized by manic and depressive episodes, is a major public health problem. Although family, twin, and adoption studies strongly support genetic factors in the etiology of BP, the search for susceptibility genes has been fraught with uncertainty attributable in part to the complex inheritance of the disorder (Turecki et al. 1996*a*; Risch and Botstein 1996; Baron 1997). Early linkage reports have not been substantiated by subsequent studies. But recent advances—in particular, statistical techniques for the analysis of complex traits and detailed genomic maps saturated with marker loci—have bolstered the prospects of linkage studies in this area. Indeed, a string of recent reports points to possible disease genes on several chromosomal regions (for reviews, see Risch and Botstein [1996] and Baron [1997]).

Much attention has been focused on one of these findings because of potential replications, the "gold standard" of verifiable results. Berrettini et al. (1994) reported a putative disease locus, near the centromere of chromosome 18, in a series of 22 multiplex pedigrees comprising 368 individuals; a slightly enlarged sample (8 additional individuals in 2 pedigrees) gave similar results (Berrettini et al. 1997). Stine et al. (1995) observed excess allele sharing, between affected siblings, for chromosome 18 pericentromeric markers (as well as for more distal markers on 18q) in 28 families comprising 243 individuals. The evidence for linkage was strongest in paternally transmitted pedigrees; pedigrees with exclusive maternal transmission showed no evidence of linkage. Similarly, a reanalysis of the Berrettini et al. (1994) data for parent-of-origin effect also showed that linkage was restricted to pedigrees with paternal transmission (Gershon et al. 1996). Freimer et al. (1996) reported linkage to chromosome 18q markers in two large pedigrees obtained from a population isolate. In evaluating the evidence, Berrettini et al. (1997) con-

cluded that the published data on BP and chromosome 18 appear to meet the significance and replication criteria for linkage results. However, as discussed (Risch and Botstein 1996; Pauls et al. 1996; Baron 1997), the interpretation of these findings is far from clear.

First, there are apparent map inconsistencies between the various chromosome 18 findings. Although there was some overlap in linkage results in the studies of Berrettini et al. (1994) and Stine et al. (1995), the strongest evidence of linkage obtained by Berrettini et al. (1994) was at D18S21, >60 cM away from D18S41, the 18q locus with the most pronounced linkage in the Stine et al. (1995) report. The locus reported by Freimer et al. (1996), in distal 18q, does not overlap with either of these regions. Short of invoking separate susceptibility loci (which would be inconsistent with the claim of replication for any particular locus), the substantial map distances, which span both arms of the chromosome, cannot be readily reconciled even when allowance is made for uncertainties in pinpointing the map location of complex disease genes.

Second, the decision to classify pedigrees according to type of transmission (paternal vs. maternal) was crucial for producing the strongest linkage results (Stine et al. 1995; Gershon et al. 1996). This decision was based on excess maternal transmission of the disease in the family data, a pattern suggestive of a distinct genetic mechanism, such as mitochondrial inheritance, in a subset of the pedigrees. However, there was no prior statistical support for linkage heterogeneity in the sample as a whole. The reported parent-of-origin effect may simply be an artifact resulting from the small sample size, for example, differential reporting of illness in maternal versus paternal pedigree branches. Indeed, Kato et al. (1996) did not find a parent-of-origin effect in a series of bipolar families 10 times as large as either the Berrettini et al. (1994) or the Stine et al. (1995) samples.

Third, attempts at replication have generally resulted in negative or ambiguous results (Maier et al. 1995; Pauls et al. 1995; Coon et al. 1996; Debruyn et al. 1996; Labuda et al. 1996; Detera-Wadleigh et al. 1997; Ewald et al. 1997; Kalsi et al. 1997; Mynett-Johnson et al. 1997). However, since the samples required to replicate linkage to a modest-effect gene are generally larger than the sample needed for the initial detection of linkage (Suarez et al. 1994), it might be argued that the outcome of these studies—which, with one exception (Detera-Wadleigh et al. 1997), were not based on large samples and took no account of parent-of-origin effect—need not be construed as failed replication.

To address some of these issues, we have attempted to replicate the chromosome 18 pericentromeric finding in a sample substantially larger than the samples studied by Berrettini et al. (1994, 1997) and Stine et al. (1995): 1,013 genotyped individuals in 53 multiplex extended pedigrees classified according to the presence or absence of paternal transmission. This effort is part of our ongoing genomewide search for susceptibility genes in BP (Baron et al. 1994; Straub et al. 1994).

Methods

Sample

A full description of the pedigree set, including ascertainment, diagnostic procedures, affection status models, and simulation studies, has been published elsewhere (Baron et al. 1994). Our overall sample consists of 1,508 individuals ($\geqslant$16 years of age) in 57 extended pedigrees with a high density of BP. The size of the sample affords substantial statistical power to detect linkage, under various genetic models, and considerable heterogeneity. For example, statistical power to detect linkage with LOD score analysis, if we assume autosomal dominant transmission and a tightly linked marker of 70% heterozygosity, is nearly 100% for α (proportion of linked families) of 30% and 75% for α of 20% (Baron et al. 1994). The pedigrees (all Caucasian) were obtained in the United States and Israel under an identical research protocol. The panel of bipolar pedigrees included in this linkage study is somewhat smaller: 53 families (39 American, 14 Israeli) comprising 1,013 genotyped individuals. It consists of all pedigrees that are completed by way of diagnosis and cell lines and have no evidence of bilineality or non-Mendelization. The study was approved by the Columbia University–New York State Psychiatric Institute Review Board. Informed consent was obtained from all participants.

The clinical evaluation was based on personal interviews using the Lifetime Version of the Schedule for Affective Disorders and Schizophrenia (Endicott and Spitzer 1978) modified for BP and supplemented by family history information (Andreasen et al. 1977) and medical records. Best-estimate consensus diagnoses were made by independent diagnosticians using all available sources of information. Diagnostic determinations were based on the Research Diagnostic Criteria (Spitzer et al. 1978) and were made blind to familial relationships, clinical status of other relatives, and genetic marker information.

The "affected" category is broadened progressively from narrow to broader phenotypic boundaries under three diagnostic models: model I: manic syndrome, mostly bipolar I disorder (mania and major depression); model II: same as model I plus bipolar II disorder (hypomania and major depression); and model III: same as model II plus recurrent major depression. In all models, the "unaffected" category consists mainly of "never mentally ill"; mental disorders that do not aggregate in families of bipolar probands (single-episode minor de-

pression and "other psychiatric disorder") are included as well. The "uncertain" category includes all affective disorders that are not considered "affected" under a particular model plus some nonaffective conditions that show increased rates in the families of patients with some forms of affective illness but whose familial relation to BP is uncertain (acute and subacute schizophrenic disorder and unspecified psychosis) and patients whose phenotypic status is unknown because of insufficient diagnostic information.Our classification system is described in greater detail elsewhere (Baron et al. 1994). Among the 1,013 genotyped individuals, the numbers of affecteds under models I, II, and III are 145, 245, and 365, respectively. The corresponding numbers of affected sib pairs are 80, 157, and 326.

As proposed (Stine et al. 1995; Gershon et al. 1996), we classified our pedigrees as "maternal," "paternal," or "paternal/maternal" (both paternal and maternal) according to parental source of disease transmission. The three categories contained 19, 4, and 30 pedigrees, respectively. We combined the latter two categories in a single "paternal/mixed" group for the following reasons: (1) The mere presence of paternal transmission (with or without a maternal component) was the key to revealing linkage in previous studies (Stine et al. 1995; Gershon et al. 1996); (2) The number of pedigrees with exclusive paternal transmission was too small for a meaningful comparison; the rarity of "pure" paternal transmission in extended pedigrees was also observed by others (Gershon et al. 1996).

Genotypes

DNA was extracted from previously established lymphoblastoid cell lines. Identity of DNA samples was confirmed by means of autosomal and sex chromosome microsatellites. Primer pairs were either obtained from Research Genetics or synthesized locally. One primer per pair was labeled with γ^{32}P-ATP by use of the T4 polynucleotide kinase exchange reaction. PCR amplification and gel analysis of microsatellite markers were performed as described elsewhere (Knowles et al. 1998). Autoradiographs were prepared with Kodak XAR film and were read independently by two readers who were blind to the clinical phenotype; disagreements were resolved by a third reader, and the genotypes were redone, when required, for accuracy. Data were entered into the LABMAN software package for linkage and genetic studies (Adams 1996).

We studied 10 highly polymorphic microsatellite markers (heterozygosity >0.8) at the following chromosome 18 loci: the Golf gene, D18S62, D18S37, D18S53, D18S453, D18S45, D18S44, D18S66, D18S56, and D18S47. Spanning ~50 cM on 18p and 18q, including the centromere, these markers cover the region implicated by Berrettini et al. (1994).

Linkage Analysis

Three main approaches were used in analyzing our data. In approach A, we employed models and analytical schemes that are routinely used in our ongoing genome scan effort. In approach B, we subdivided our pedigrees into "maternal" and "paternal/mixed" (see Sample) and analyzed the two groups separately, using the same models and analyses used in approach A. In approach C, we attempted to replicate the Berrettini et al. (1994) study using their own disease models and analytical approaches.

In all three approaches, the FASTLINK 3.0 (Cottingham et al. 1993; Schaffer et al. 1994) version of MLINK (Lathrop et al. 1984) was used to compute two-point LOD scores. The HOMOG program (version 3.35; Ott 1991) was used to test homogeneity, and the ANALYZE package (Terwilliger 1994, 1995) was used for automation of the MLINK and HOMOG analyses. In addition, ANALYZE was used to perform haplotype relative risk (HRR) and transmission-disequilibrium testing (TDT), as well as linkage and heterogeneity analyses of the extended pedigrees subdivided into nuclear families. (Because it is likely that BP is caused by the interaction of several genes, more of these genes will be present in large pedigrees than in any of the individual nuclear families. Therefore, analyzing the nuclear families separately reduces heterogeneity and increases the power to detect linkage.) Also common to the three approaches were SimIBD (affected pedigree member [APM]) (Davis et al. 1996) and SIBPAIR (Terwilliger; described in Kuokkanen et al. 1996) for APM and affected sib-pair (ASP) analyses, respectively. For SimIBD (APM), the IBD statistic was computed at each locus, and 100 replicates were simulated to determine the empirical P values. We reanalyzed loci with $P < .05$, using 1,000 simulated replications, to confirm the P values. SimIBD is less susceptible to type I errors that are due to misspecified allele frequencies than is the previous, identity-by-state (IBS) APM method (Terwedow et al. 1996). SIBPAIR eliminates the increased type I error associated with treating all possible sib pairs as if they were independent by a pedigree-based likelihood method (Kuokkanen et al. 1996; Satsangi et al. 1996).

Approaches A and B.—Two-point LOD scores were computed for diagnostic models I and III, each under a dominant and recessive transmission model using affected-only analysis. For all models, the penetrance for the susceptible genotype was set to .80, with a penetrance ratio of 500:1 (genetic vs. nongenetic cases). The disease-allele frequencies were adjusted to allow for an observed population prevalence of .005 for diagnostic

Table 1

Maximum LOD Scores under Dominant Genetic Model at Any Recombination Fraction: Approaches A and B

	All Pedigrees		Maternal		Paternal/ Mixed	
LOCUS	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}
Diagnostic model I:						
D18S62	.00	.00	.02	.00	.00	.00
D18S53	.00	1.83	.00	.00	.00	2.12
Golf	.01	.11	.35	.07	.00	.05
D18S37	.08	.39	.02	.00	.06	.51
D18S453	.00	.00	.33	.00	.00	.08
D18S45	.23	2.67	.10	.55	.24	2.11
D18S44	.00	.15	.01	.00	.00	.38
D18S66	.49	.18	.04	.00	.70	.72
D18S56	1.05	.42	.50	.00	.69	.78
D18S47	.93	1.68	.03	.33	.96	1.34
Diagnostic model III:						
D18S62	.00	.00	.00	.00	.00	.00
D18S53	.08	.43	.28	.01	.00	.46
Golf	.00	.15	.06	.00	.00	.21
D18S37	.32	.00	1.11	.22	.01	.00
D18S453	.00	.00	.00	.00	.00	.14
D18S45	.96	.57	.28	.05	.68	.57
D18S44	.00	.00	.00	.00	.04	.01
D18S66	.18	.01	.02	.02	.21	.00
D18S56	.00	.19	.00	.01	.00	.19
D18S47	.71	.78	.01	.46	.90	.47

NOTE.—N-Z_{max} = Maximum LOD score under heterogeneity when extended pedigrees are broken into nuclear families.

model I and .06 for diagnostic model III. Marker allele frequencies were calculated from the founders of each pedigree by LABMAN (Knowles et al. 1992; Adams 1996). Alleles that were present in the pedigrees but not in the founders were given a frequency of .001, and the most frequent allele was decremented appropriately. Allele frequencies from the American and Israeli pedigree sets did not differ significantly, so frequencies calculated from the total pedigree set were used. Parametric multipoint LOD scores were computed by GENEHUNTER (Kruglyak et al. 1996). As proposed by Risch and Giuffra (1992), we used high disease-allele frequencies (.05 for the dominant model 1) to circumvent the problem of spuriously reduced LOD scores in parametric multipoint analysis of genetically complex traits. The average information content of our markers was 75%. MAPMAKER/SIBS (Kruglyak and Lander 1995) was used for multipoint sib-pair analysis. The map was constructed by the program MULTIMAP (Matise et al. 1994) using both publicly available data from CEPH pedigrees and data generated in our laboratory (Straub et al. 1993). The map order (Kosambi cM, sex averaged) was D18S62–24.7 cM–D18S53–2.9 cM–D18S453–3.8 cM–D18S45–2.6 cM–D18S44–3.8 cM–D18S66–3.6 cM–D18S56. Two additional markers—D18S37 and D18S47—were not included in this map because the former was completely linked to D18S453 and the latter was tightly linked to D18S66 and D18S56. Therefore, the relative positive positions of these markers could not be determined on this map. We did not include D18S62 in the multipoint analyses because of its considerable distance from the other markers.

Approach C.—Two-point LOD scores were computed for diagnostic models II and III, each under a dominant and recessive transmission model, using both all subjects (with age-dependent penetrances) and affecteds only. Penetrance values and disease-allele frequencies for the various models were the same as those used by Berrettini et al. (1994). Our diagnostic models II and III correspond to the Berrettini et al. (1994) affection status models I and II. SIBPAL (Tran et al. 1991) and Multipoint APM (Weeks and Lange 1992) were used for sib-pair and multilocus APM analyses, respectively, in addition to SIBPAIR and SimIBD (APM). The map distances used for multipoint APM analyses are the same as in the multipoint sib-pair analysis (see Approaches A and B).

Results

The maximum two-point heterogeneity LOD scores under approaches A, B, and C are shown in tables 1, 2, and 3. Moderately positive LOD scores ($1 < Z_{max} < 3$) were found at D18S53 (tables 1, 2, and 3), D18S37 (table 1), D18S45 (tables 2 and 3), D18S66 (table 2), D18S56 (tables 1 and 2), and D18S47 (tables 1 and 3). However, adjacent markers, which were equally typed and informative, showed no indication of linkage. Indeed, most of the multipoint LOD scores were substantially negative; the highest LOD score was 0.28 at D18S66. The multipoint results for models that gave the highest two-point LOD scores are shown in figure 1. The homogeneity LOD scores were generally smaller (data not shown). There was no appreciable difference in LOD scores between American and Israeli pedigrees (data not shown).

The single-locus APM results under approaches A and B are given in table 4. Marginal *P* values (.03–.05) were noted for D18S45, D18S66, and D18S47. Similar results were obtained under approach C (data not shown). The multilocus-multipoint APM results were not significant, for all three weighting functions of allele frequencies (data not shown).

Results of the single-locus ASP analyses under approaches A and B are presented in table 4. Increased allele-sharing IBD ($.0003 < P < .05$) was noted for D18S53, D18S45, D18S66, and D18S47. Similar results were obtained for D18S53, D18S45, and D18S47 under approach C (data not shown). However, the multilocus ASP results showed no indication of linkage. Most of the multilocus LOD scores were in the 0–0.1 range; the highest LOD score was 1.26 at D18S56. Some of the

Table 2

Maximum LOD Scores under Recessive Genetic Model at Any Recombination Fraction: Approaches A and B

	Diagnostic Model I						Diagnostic Model III					
	All Pedigrees		Maternal		Paternal/Mixed		All Pedigrees		Maternal		Paternal/ Mixed	
Locus	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}
D18S62	.05	.00	.20	.00	.03	.00	.02	.00	.00	.00	.05	.00
D18S53	.45	1.25	.00	.00	.73	1.62	.06	.33	.00	.00	.07	.44
Golf	.14	.00	.13	.00	.09	.01	.00	.00	.10	.00	.00	.00
D18S37	.03	.14	.00	.00	.07	.19	.04	.00	.14	.05	.00	.00
D18S453	.10	.00	.11	.00	.06	.00	.00	.05	.10	.00	.00	.10
D18S45	1.33	1.80	.97	.92	.66	1.14	.32	.12	.35	.21	.16	.02
D18S44	.01	.10	.15	.15	.00	.03	.00	.00	.06	.00	.00	.00
D18S66	.79	.45	.00	.00	1.96	1.44	.00	.00	.00	.00	.00	.00
D18S56	.80	.35	.03	.00	1.35	.85	.03	.07	.00	.00	.22	.13
D18S47	.72	1.00	.01	.54	.88	.62	.44	.85	.00	.47	.60	.59

Note.— N-Z_{max} = Maximum LOD score under heterogeneity when extended pedigrees are broken into nuclear families.

multilocus results for models that gave the most pronounced single-locus *P* values are presented in figure 2. The TDT and HRR analyses (including the Golf locus) under approaches A and B did not yield *P* values <.05; similar results were obtained with approach C (data not shown).

As noted in the preceding paragraphs, we did not observe LOD scores >3.0 in either maternal or paternal/mixed pedigrees. Moderately positive two-point LOD scores ($1 < Z_{max} < 3$) were observed in both groups of pedigrees, with some preponderance in paternal/mixed pedigrees under diagnostic model I (tables 1 and 2). However, the multilocus LOD scores were negative in both groups of pedigrees under most analytic schemes, reaching a maximum of 0.28 under diagnostic model I in paternal/mixed pedigrees (fig. 1). The single-locus APM analysis showed marginal *P* values (.03 and .04) for some loci in the paternal/mixed pedigrees under diagnostic model I (table 4), but the multipoint results did not reach statistical significance in either group of ped-

Table 3

Maximum LOD Scores under Dominant and Recessive Genetic Models: Approach C

	Dominant Model				Recessive Model			
	All Subjects		Affecteds Only		All Subjects		Affecteds Only	
Locus	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}	Z_{max}	N-Z_{max}
Diagnostic model II:								
D18S62	.00	.00	.00	.00	.08	.00	.02	.00
D18S53	.09	.45	.03	.20	1.80	1.49	.67	.84
Golf	.06	.04	.07	.00	.25	.00	.09	.00
D18S37	.01	.00	.03	.00	.10	.03	.02	.00
D18S453	.04	.00	.00	.00	.09	.00	.08	.00
D18S45	1.43	1.17	1.64	.84	2.01	1.54	1.88	1.26
D18S44	.00	.00	.00	.00	.02	.00	.00	.00
D18S66	.31	.10	.55	.11	.42	.25	.51	.24
D18S56	.36	.22	.15	.04	.08	.06	.00	.02
D18S47	1.07	.65	.73	.26	.64	.24	.25	.38
Diagnostic model III:								
D18S62	.00	.00	.00	.00	.04	.00	.02	.00
D18S53	.13	.64	.10	.47	1.23	.84	.10	.38
Golf	.00	.13	.00	.15	.12	.03	.00	.00
D18S37	.33	.05	.32	.00	.51	.20	.03	.00
D18S453	.02	.01	.00	.00	.00	.02	.00	.06
D18S45	1.28	.75	1.08	.65	.39	.24	.32	.16
D18S44	.00	.00	.00	.00	.02	.05	.00	.00
D18S66	.12	.01	.21	.02	.02	.00	.00	.00
D18S56	.00	.38	.00	.20	.00	.01	.04	.09
D18S47	1.26	1.23	.80	.84	.84	.76	.49	.95

Note.—N-Z_{max} = Maximum LOD score under heterogeneity when extended pedigrees are broken into nuclear families.

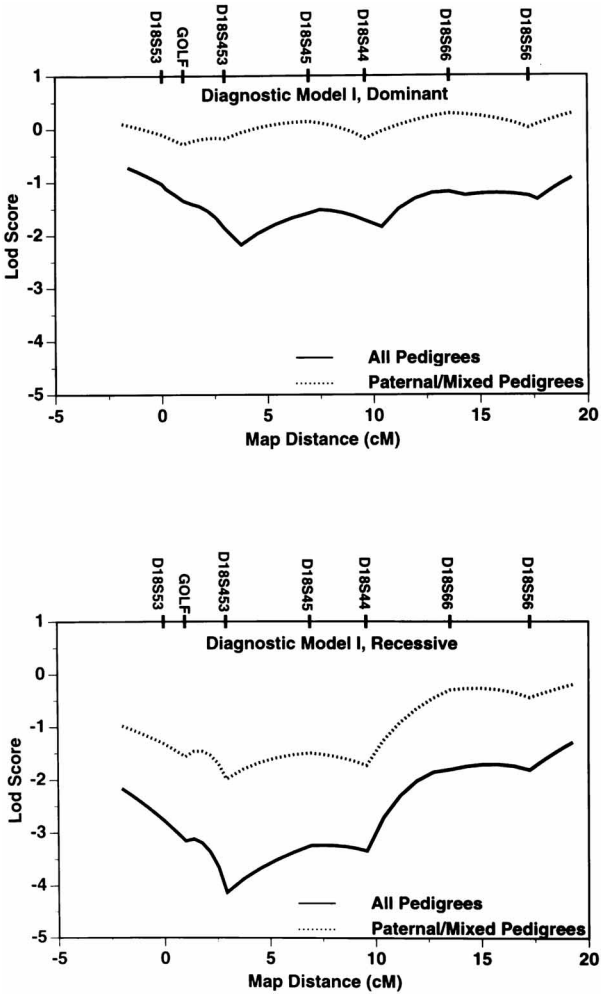

Figure 1 Parametric multipoint LOD scores plots showing parametric multipoint LOD scores obtained by GENEHUNTER.

igrees. Similarly, the single-locus ASP analysis yielded *P* values <.05 (range .03–.0005) for several loci in paternal/mixed pedigrees under diagnostic model I and for one locus ($P = .02$) in maternal pedigrees (table 4), but the multilocus LOD scores were all under 1.0, save for the D18S66–D18S56 interval in paternal/mixed pedigrees, which yielded a LOD score of 1.26 under diagnostic model I (fig. 2). The TDT and HRR test results failed to reach significance in either group of pedigrees ($P > .05$).

Discussion

To weed out false claims of linkage in complex genetic traits, such as BP, Lander and Kruglyak (1995) proposed guidelines for the interpretation of linkage results. They distinguished between significant linkage and suggestive linkage. In LOD score analysis, the two categories would correspond to LOD scores of 3.3 and 1.9, respectively; the corresponding pointwise significance levels in sib-pair analysis would be .000022 (LOD = 3.6) and .00074 (LOD = 2.2); for studies involving a mixture of relative types, as in APM analysis, a range can be used: .00005–.00001 and .005–.0001. Once a significant linkage is obtained in an initial study, a *P* value of .01 should suffice to declare credible replication. We interpret our findings in accordance with these guidelines.

Following up on prior reports (Berrettini et al. 1994, 1997; Stine et al. 1995) and using a range of diagnostic models and analytical schemes, we examined the pericentromeric region of chromosome 18 in our large series of multiplex bipolar pedigrees. There was no evidence for significant linkage. Some of our two-point LOD scores and single-locus ASP results were consistent with suggestive linkage, but this was not substantiated by multilocus analysis. Golf, the locus proposed by Berrettini et al. (1994) as a potential candidate gene for BP,

Table 4

Single-Locus APM and ASP Results: Approaches A and B

	Diagnostic Model I						Diagnostic Model III					
	All Pedigrees		Maternal		Paternal/ Mixed		All Pedigrees		Maternal		Paternal/ Mixed	
Locus	APM	ASP	APM	ASP	APM	ASP	APM	ASP	APM	ASP	APM	ASP
D18S62	.66	.50	.43	.50	.85	.50	.70	.49	.46	.50	.75	.47
D18S53	.18	.001	.81	.50	.14	.0005	.35	.02	.24	.34	.46	.02
Golf	.62	.39	.34	.46	.80	.39	.91	.50	.74	.50	.88	.39
D18S37	.07	.08	.46	.50	.16	.05	.32	.50	.13	.12	.31	.50
D18S453	.65	.50	.73	.50	.63	.38	.26	.44	.62	.50	.31	.13
D18S45	.05	.0003	.24	.02	.03	.002	.49	.02	.56	.19	.35	.13
D18S44	.49	.24	.72	.50	.40	.18	.73	.50	.90	.50	.67	.50
D18S66	.11	.16	.63	.50	.03	.008	.28	.50	.67	.50	.10	.45
D18S56	.13	.06	.49	.50	.11	.02	.40	.25	.82	.50	.15	.16
D18S47	.03	.03	.17	.05	.04	.07	.24	.07	.11	.08	.35	.12

Note.—Values in table are *P* values. APM analysis was performed with SimIBD (APM). ASP results were obtained with SIBPAIR.

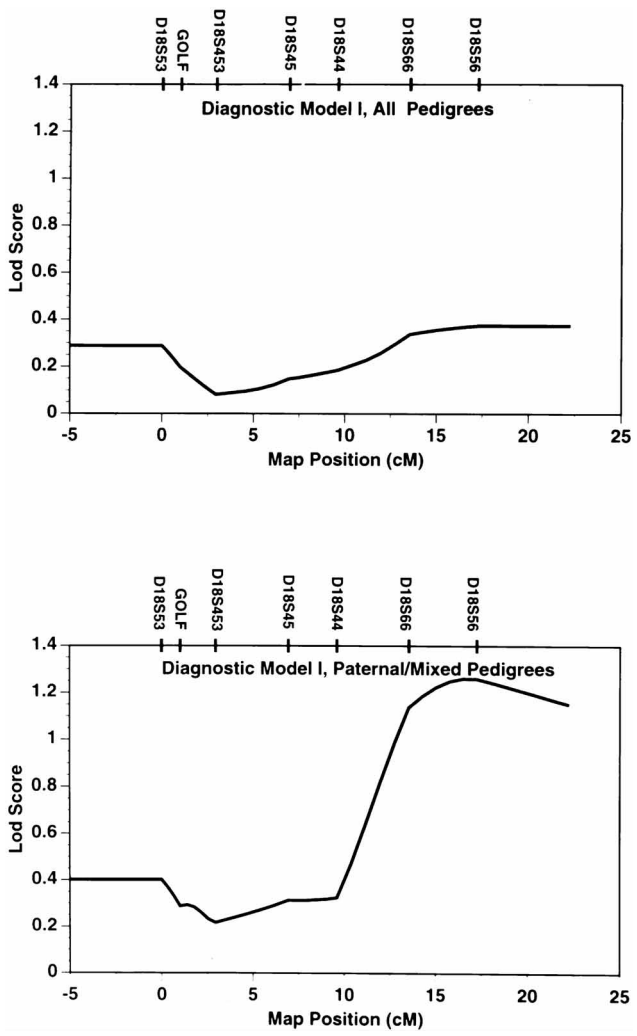

Figure 2 Multilocus sib-pair analysis plots showing multilocus sib-pair LOD scores obtained by MAPMAKER/SIBS. Results are based on families with at least one pair of affected siblings: 32 families in the all-pedigrees category and 18 families in the paternal/mixed category.

showed no evidence of linkage to the disease; similar results were reported by other investigators (Tsiouris et al. 1996; Turecki et al. 1996*b*).

We did not observe the hallmark of the putative chromosome 18 finding, namely, the pronounced linkage in pedigrees with paternal transmission and its virtual absence in maternal pedigrees (Stine et al. 1995; Gershon et al. 1996). There was some preponderance of moderately positive two-point LOD scores and single-locus ASP results, in paternal/mixed pedigrees, under diagnostic model I but not under diagnostic model III, the model that produced positive results in the samples reported by Stine et al. (1995) and Gershon et al. (1996); however, these results were not supported by multipoint analysis. Previous work has given the impression that paternal transmission can be readily distinguished from maternal transmission. In fact, such a distinction can best be made in nuclear families in which a parent and at least one offspring are affected. (When neither parent is affected, illness in a close relative—the parent's sibling, for example—can signify parental type of inheritance.) In extended pedigrees, the classification according to parental source of transmission is more complex because the disease may be transmitted from either parental source in different branches of the pedigree. For example, the rarity of extended pedigrees with exclusive paternal transmission limits the utility of comparing "pure" paternal transmission with maternal inheritance. The preferred solution, therefore, is to compare pedigrees with "mixed" paternal and maternal inheritance to pedigrees with pure maternal transmission (Gershon et al. 1996; present study). We noted, above, the uncertainties in characterizing pedigrees according to parent-of-origin effect (see Introduction and Methods).

Because the statistical criteria for replication, given prior evidence of linkage, are less restrictive than those advocated for the initial detection (Lander and Kruglyak 1995), and since multipoint analysis of complex traits, which gave no indication of linkage in our data, can result in reduced statistical power because of overestimation of recombination fractions (LOD score analysis), it might be argued that some of our positive single-locus results point to a bona fide gene effect. There are counterarguments, however: (1) For replication to be considered, the initial study must demonstrate significant linkage by Lander and Kruglyak's (1995) criteria. As discussed elsewhere (Baron 1997), it is far from clear whether the initial linkage results for the pericentromeric region (Berrettini et al. 1994; Stine et al. 1995; Gershon et al. 1996) surpassed the threshold for significant linkage. (2) As proposed elsewhere (Risch and Giuffra 1992), we used high disease-allele frequencies in our parametric multipoint analysis, to circumvent spuriously negative LOD scores. (3) Our use of multiple genetic and diagnostic models, while augmenting our chances of detecting true linkage, also increased the chance of spurious positive findings. (Numerous permutations were studied in the present analyses: dominant vs. recessive models; heterogeneity vs. homogeneity; extended pedigrees vs. pedigrees broken into nuclear families; maternal vs. paternal/mixed vs. all pedigrees; affected-only vs. all-subjects analyses; several nonparametric analyses; single-locus vs. multilocus analyses; and three diagnostic models.) Because some of these tests are not truly independent, there are no hard and fast rules for how to correct for multiple test effects. There is little doubt, however, that the *P* values are inflated and that, con-

sequently, even the "suggestive" significance levels in some of our analyses should be viewed with caution.

The considerably larger sample used in our study, compared with those used in the studies of Berrettini et al. (1994, 1997) and Stine et al. (1995), may have given us greater power to detect and assess linkage heterogeneity (see Methods for power estimates). If the putative chromosome 18 locus segregates in 25% of cases, as proposed elsewhere (Berrettini 1996), then our sizable sample should have revealed much stronger evidence of linkage. It is worth noting that the only other sample of comparable magnitude—the National Institute of Mental Health (NIMH) Genetics Initiative Bipolar Pedigrees, with 540 genotyped individuals in 97 pedigrees (including 424 subjects affected under diagnostic model III)—also failed to replicate the chromosome 18 finding, both in the sample as a whole and in paternal/mixed pedigrees (Detera-Wadleigh et al. 1997).

Finally, it might be argued that setting statistical thresholds for complex traits oversimplifies the difficulties in analyzing these disorders. For example, if Lander and Kruglyak's (1995) criteria are too stringent, some of the positive results reported to date, notwithstanding the aforementioned methodological uncertainties, might reflect a true positive linkage. On the other hand, results with significance levels in this range are not at all rare in genomewide searches; more often than not, they prove to be false positives. Even significant linkage by these criteria is associated with a 5% false-positive rate, and this rate likely increases as the criteria are relaxed to reflect suggestive linkage or potential replication.

In conclusion, coupled with uncertainties in interpreting the earlier positive findings, our study does not furnish compelling evidence of a vulnerability locus for BP in the pericentromeric region of chromosome 18. The results published elsewhere may be false positives, possibly because of chance statistical fluctuations or uncertainties in research procedures. However, given the reduced power of linkage analysis for genetically complex disorders and for possible heterogeneity across samples, a modest gene effect in some families cannot be excluded at present. Also, we did not examine, in this study, marker loci on distal 18q, which appeared linked to BP in some data sets (Stine et al. 1995; Freimer et al. 1996). Further study will be needed to cast light on this issue.

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