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A Comprehensive Linkage Analysis of Chromosome 21q22 Supports Prior Evidence for a Putative Bipolar Affective Disorder Locus

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Summary

Previously, we demonstrated evidence of linkage to bipolar affective disorder (BP) in a single large, multigenerational family with a LOD score of 3.41 at the PFKL locus on chromosome 21q22.3. Additional families showed little support for linkage to PFKL under homogeneity or heterogeneity, in that study. We have expanded on that analysis, with 31 microsatellite markers at an average marker spacing of ≤2 cM, in the largest multigenerational BP pedigree series reported to date. A two-point heterogeneity ($\alpha = 0.5$) LOD score of 3.35 ($P < .000156$) was found at the D21S1260 locus, 5 cM proximal to PFKL. Polylocus analysis with a cluster of three neighboring markers was consistent with these results (PL-HetLOD = 3.25). In the design of this study, 373 individuals from 40 families (from a total set of 1,508 individuals in 57 families) were chosen, as a cost-effective approach to genotyping this large sample set. Linkage analyses were performed with an "affecteds-only" method. As such, our results are based solely on genetic information from affected individuals, without assumptions about the disease-locus genotypes of the unaffecteds. Furthermore, for ease of comparison, this study was performed with the same approach as a 10-cM genome scan for BP loci, the results of which will be reported elsewhere.

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Introduction

Bipolar affective disorder (BP), or manic-depressive illness, is a severe and common psychiatric disorder with a lifetime prevalence of 0.5%–1.5%. An estimated 20%–25% of patients commit suicide, giving BP a mortality rate higher than that of many types of heart disease and cancer (Goodwin and Jamison 1990).

Family, twin, and adoption studies strongly implicate a discernable role for genetics in the etiology of BP, but no genes have been identified conclusively. One chromosomal region identified as possibly harboring a locus for susceptibility to BP is 21q22. Prompted by an initial report from our group (Straub et al. 1994), several laboratories have investigated linkage of BP to 21q22. Detera-Wadleigh et al. (1996) observed an excess of allele sharing among affected individuals at nine 21q loci spanning distal 21q. More recently, support for linkage to 21q was reported in the National Institute of Mental Health (NIMH) Genetics Initiative Bipolar Study (Detera-Wadleigh et al. 1997), with multilocus affected sib-pair analyses indicating excess allele sharing in the D21S1265–D21S1255 region. Smyth et al. (1997) reported a "suggestive" multipoint LOD score of 1.29, using the markers PFKL and D21S171; this score increased to 3.87 under a two-locus admixture model of PFKL/D21S171 and the tyrosine hydroxylase locus (on 11p15). A detailed review of studies in this area has been published (Baron 1997), and a synopsis of current linkage studies on chromosome 21 has been compiled as part of the Fifth World Congress of Psychiatric Genetics (Gurling 1998).

We previously reported evidence for linkage to BP on 21q22 based primarily on a single large, multigenerational pedigree (family 01001) with a two-point LOD score of 3.41 at the PFKL locus (Straub et al. 1994). At the time, 46 additional families were tested with two markers, PFKL and the more distal marker, D21S171, without significant evidence for linkage. However, we found modest evidence ($P < .0003$) at PFKL, using the affected-pedigree-member method. Here we report evi-

dence for the presence of a putative BP locus on 21q22, based on an analysis of 31 highly polymorphic microsatellite markers, including a concentration of 27 markers spanning the most distal 30 cM of the chromosome. These markers were tested on 373 individuals, 307 of whom are affected, who provide the bulk of the power to detect linkage in this sample set.

Material and Methods

Pedigrees, Clinical Classification, and Selection of Samples for Genotyping

A full description of the pedigree set, including ascertainment, diagnostic procedures, and power calculations, has been published (Baron et al. 1994). The overall sample consists of 1,508 individuals, from 57 extended pedigrees with a high density of BP, from the United States (39 pedigrees) and Israel (18 pedigrees). The study was approved by the Columbia University–New York State Psychiatric Institute Review Board. Informed consent was obtained from all participants.

Affected individuals were placed into one of three diagnostic models, which were devised on the basis of the research diagnostic criteria classification of affective disorders (Spitzer et al. 1978). All field interviews followed the Lifetime Version of the Schedule for Affective Disorders and Schizophrenia modified for BP (Endicott and Spitzer 1978) and were 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 and were blinded to familial relationships, clinical status of other relatives, and genetic data. The diagnostic models are as follows: model I: bipolar I disorder, unipolar manic disorder, bipolar and manic forms of schizoaffective disorder; model II: model I plus bipolar II disorder with major depression; and model III: model II plus recurrent unipolar major depressive disorder and recurrent unipolar schizoaffective disorder (depression only).

For the sake of efficiency, a subset of this sample was chosen for genotyping. The subset consisted of 373 individuals from the 40 most informative families (an average of 7 or 8 affected individuals per pedigree), providing the bulk of the power to detect linkage in the entire pedigree sample. They were the same individuals used in a systematic genomewide screen for bipolar loci, described elsewhere (Liu et al. 1997; J. Liu, V. M. Aita, J. Terwilliger, T. C. Gilliam, and M. Baron, unpublished data), as well as other candidate regions for BP (Aita et al. 1997). They were selected on the basis of a computerized algorithm described by J. Liu, V. M. Aita, J. Terwilliger, T. C. Gilliam, and M. Baron (unpublished data), and they contribute most of the genetic information available in the sample set (for dominantly inherited alleles). The algorithm selected affected individuals and connecting lineal pedigree members while excluding married-in spouses. It has been shown previously (Holmans and Craddock 1997) that genotyping can be made more efficient by excluding founders or married-in spouses.

Genotyping

DNA was extracted from blood and lymphoblastoid cell lines by established standard methods (Sambrook et al. 1989). A semiautomated high-throughput genotyping approach using fluorescence-labeled microsatellite markers was performed. All DNA samples and PCR reagents were pipetted with a TECAN Genesis RSP 150 robotic workstation. DNA was amplified by PCR with PTC 225 thermocyclers (MJ Research). For all markers, the last nucleotide of the reverse, nonfluorescent primer was modified to a guanine (G) residue to promote the nontemplated addition of adenine by *Taq* DNA polymerase onto the complementary, fluorescence-labeled strand. This method has been described to consistently shift the allelic profile of PCR products toward the allele-plus-A, which allows for more-accurate allele calling (Magnuson et al. 1996). PCR products were electrophoresed on 377 DNA sequencers (PE Applied Biosystems).

Generation of genotypes followed a strategy for analysis of large-scale, fluorescence-labeled genotyping data described elsewhere (Ghosh et al. 1997). In brief, raw data were collected by PRISM 377XL data collection software (PE Applied Biosystems) and sized by GENESCAN version 2.1; alleles were initially called by GENOTYPER v.1.1.1. The computer-generated genotypes were then checked by two independent researchers; any conflicts were resolved by a third. These manually inspected genotypes were then entered into a database, alleles were binned and labeled, Mendelization checks were performed, and linkage output files were generated by LABMAN (Adams 1994). Marker heterozygosity and polymorphism information content (PIC) were estimated by DOWNFREQ (Terwilliger 1995). Genotypes were determined with the researchers blinded to disease phenotypes.

Linkage Analysis

A pseudomarker strategy that approximates "model-free" affected relative pair analysis was used (Trembath et al. 1997; J. D. Terwilliger and H. H. Goring, unpublished data). This analytical method also maintains an important property of LOD score analysis—namely, that pedigree correlations between all relatives are considered jointly, and the pedigree is not broken into a set of all possible relative pairs, which are not in reality indepen-

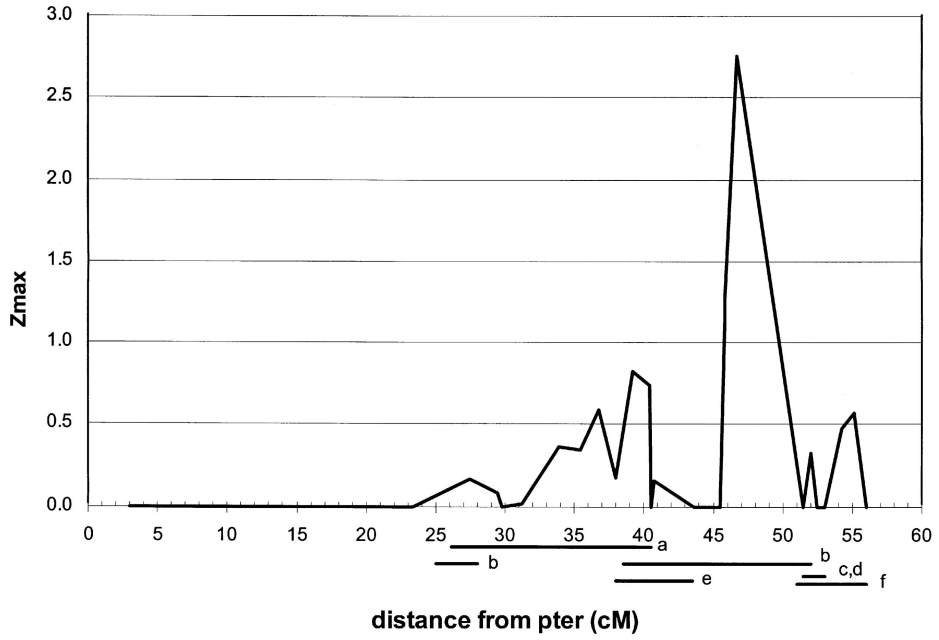

Figure 1 Two-point LOD scores in diagnostic model III, dominant inheritance. Regions shown are (a) implicated by Detera-Wadleigh et al. (1997); (b) implicated by Detera-Wadleigh et al. (1996); (c) implicated by Straub et al. (1994); (d) implicated by Smyth et al. (1997); (e) Down syndrome critical region from Osoegawa et al. (1996); and (f) mapped by Lapenta et al. (1998).

dent of each other. If there is a disease susceptibility allele that is shared identical by descent (IBD) by all affected individuals in a given pedigree, then it must have been transmitted along a single pathway within the pedigree—the individuals along this pathway are exactly the subset of individuals genotyped in this study, as described above. A LOD score analysis can be done between a "pseudomarker" locus (representing the maximal possible IBD sharing among affecteds) and the marker loci genotyped. As has been shown elsewhere (Trembath et al. 1997; J. D. Terwilliger and H. H. Goring, unpublished data), this strategy is a robust and powerful analogue of "model-free" linkage analysis in which all meioses are considered to be independent and identically distributed with respect to the segregation of the trait locus.

Two-point LOD scores were computed by means of the MLINK program from the LINKAGE package (Lathrop et al. 1984). Polylocus LOD scores were computed by POLYLOCUS from the ANALYZE package (Terwilliger 1994). Marker allele frequencies for linkage analyses were estimated on the basis of this data set by LABMAN (Adams 1994). Tests for heterogeneity (Smith 1961) were performed by the HOMOG program (Ott 1991). Affected sibpair analysis was performed by SIBPAIR from the ANALYZE package (Kuokkanen et al. 1996; Satsangi et al. 1996). For the sake of analytical completeness and comparison with other bipolar linkage studies, analyses were performed with dominant and recessive modes of inheritance, and whole families were broken into nuclear families. In addition, all analyses were performed with all three diagnostic models.

Results

We analyzed chromosome 21 with 31 markers, focusing 27 markers on the most distal 30 cM of 21q. The LOD scores from two-point pseudomarker analysis (Trembath et al. 1997; J. D. Terwilliger and H. H. Goring, unpublished data) under diagnostic model III and a dominant mode of inheritance are graphed in figure 1. Also shown are regions significant to the study of BP on chromosome 21 (fig. 1). In this study, the maximum two-point LOD score (Z_{max}) is found at D21S1260, with $Z_{max} = 2.76$, assuming locus homogeneity, and $Z_{max} = 3.35$, assuming locus heterogeneity ($\alpha = 0.5$) (table 1). As with any other LOD score reported under the assumption of heterogeneity, the extra degree of freedom in the analysis results in a less significant P value associated with that LOD score. As a result, the two LOD scores for D21S1260 have virtually equivalent P values ($P < .000183$, homogeneity, vs. $P < .000156$, heterogeneity). Map distances listed correspond to the genetic distance from the p-terminus of chromosome 21 and are taken or derived from the Marshfield Genetics map.

In lieu of multipoint linkage analyses, which are highly

Table 1

Two-Point LOD Score Analysis

		LOD Score	
cM	Marker	Homogeneity	Heterogeneity
2.99	D21S1432	.00	.44
13.05	D21S1437	.00	.01
17.67	D21S1409	.00	.00
23.34	D21S1435	.00	.00
27.4	D21S1270	.16	.16
29.4	D21S262	.08	.08
29.78	D21S1413	.00	.00
31.26	D21S1254	.01	.01
33.84	D21S1895	.36	.36
35.45	D21S1252	.34	.34
36.77	D21S1440	.59	.59
	D21S1290	.18	.28
39.22	D21S1255	.83	.92
40.45	D21S168	.74	.85
40.47	D21S1412	.00	.00
40.49	D21S268	.00	.19
40.49	D21S2055	.16	.17
43.67	D21S1893	.00	.00
45.55	D21S231	.00	.39
45.87	D21S1906	1.14	1.14
45.87	D21S1887	1.30	1.30
45.87	D21S266	1.27	1.39
46.71	D21S1260	2.76	3.35
51.49	D21S1411	.00	.11
	PFKL	.33	.34
52.5	D21S1890	.00	.00
52.5	D21S1885	.00	.09
53.05	D21S1912	.00	.01
54.22	D21S1897	.47	.47
	D21S1809	.57	.60
57.77	D21S1446	.00	.01

dependent on accurate estimates of intermarker recombination fractions, marker allele frequencies, and precise marker ordering, polylocus linkage analysis was performed. Table 2 shows the results of polylocus analysis, with four markers calculated in the context of each individual marker location. Polylocus analysis under the assumption of genetic heterogeneity had little effect on the LOD scores, with a $Z_{max} = 3.25$ at D21S1260 (table 2).

In this study, we genotyped 12 of the 45 individuals available from family 01001, consistent with the selection procedure described in Material and Methods. We included 8 of the 9 affecteds under diagnostic model III (the ninth individual was accidentally omitted because of a clerical error during the selection of samples for genotyping). In a two-point pseudomarker analysis of this family, D21S1260 gave a LOD score of 0.41. Analysis of the pedigree set after the removal of family 01001 gave a two-point heterogeneity LOD score of 3.07 at D21S1260 (table 3).

We compared the analytical models used here with those of Straub et al. (1994), using data from the PFKL locus (the only marker shared between the two studies). In the present study, family 01001 gave a LOD score of 0.67. Examination of the same data using the analytical methods of Straub et al. (1994) gave a LOD score of 0.70. Inclusion of the ninth affected individual, who was inadvertently excluded from this pedigree (see above), would have resulted in LOD scores of 0.95 and 0.94, respectively.

Two-point pseudomarker analysis of other diagnostic models, under both dominant and recessive inheritance, produced no significant results (data not shown). In addition, after all pedigrees were divided into their component nuclear families, affected sib pair analysis and pseudomarker analysis were performed, with no significant findings (data not shown). Table 4 provides information on the heterozygosity and PIC of the markers we used, as estimated from the individuals in this study.

Discussion

Our recent genome scan for linkage to BP (J. Liu, V. M. Aita, J. Terwilliger, T. C. Gilliam, and M. Baron, unpublished data) provides a frame of reference for the interpretation of this report. The subset of samples genotyped and the linkage methods are identical in both studies; this allows for a direct comparison of LOD scores. This comparison reveals that the highest homogeneity LOD score in this study ($Z_{max} = 2.76$ at D21S1260; table 1) ranks as the highest (whole pedigree) homogeneity LOD score found across all diagnostic models tested in our genome scan to date. This observation, coupled with the findings noted earlier (see Introduction and Results), is consistent with the hypothesis that 21q22 harbors a vulnerability locus for BP.

This report expands on our previous study (Straub et al. 1994) in that it is based on a denser marker coverage of 21q22 in the entire pedigree series selected for genotyping, without deference to any one particular family. Although family 01001—the family that provided the strongest support for linkage in our previous report—was included in this sample set, only 12 of the 45 individuals available for genotyping were studied. Our results suggest that the linkage evidence in the current

Table 2

Polylocus Linkage Analysis

Marker	PL-Hom	PL-Het
D21S1906	1.23	1.23
D21S1887	1.41	1.41
D21S266	1.20	1.33
D21S1260	2.61	3.25

Note.—PL-Hom = polylocus linkage under the assumption of homogeneity; PL-Het = polylocus linkage under the assumption of heterogeneity.

Table 3

Two-Point LOD Scores (Family 01001 Excluded)

	LOD Score	
Marker	Homogeneity	Heterogeneity
D21S1906	.97	.97
D21S1887	1.24	1.24
D21S266	1.45	1.53
D21S1260	2.24	3.07
D21S1411	.01	.15
PFKL	.24	.25

study comes from the entire pedigree series rather than just this previously identified family. Specifically, at our most "significant" marker, D21S1260, the removal of family 01001 still results in a two-point heterogeneity LOD score of 3.07 (table 3).

As shown above, when we recomputed LOD scores for family 01001 from the Straub et al. (1994) article, using only the 12 people chosen for genotyping in the current study, the results for PFKL (the only marker used in both studies) were nearly identical: 0.70 with the methods of Straub et al. (1994) versus 0.67 in the current study. This suggests that the reduced LOD scores in this family—0.67 (or 0.95, if the missing individual is added; see Results) versus 3.41 (Straub et al. 1994)—are likely the result of fewer persons genotyped (12 vs. 45 in Straub et al. 1994) rather than different analytical models in the two studies.

Of interest, the results from our genome scan (J. Liu, V. M. Aita, J. Terwilliger, T. C. Gilliam, and M. Baron, unpublished data) do not corroborate the evidence for linkage to 21q22. However, the marker spacing over the entire genome averaged 10 cM (compared with <2 cM in the present study), and the marker nearest to D21S1260 (the locus with the peak LOD score in the present study) was ~8 cM away. This serves as an important caveat to the interpretation of results from genome scans—in particular, overconfidence in the marker coverage reported for linkage to complex diseases. Random fluctuations in marker informativeness in any given family across a pedigree series could result in errors in linkage detection.

In designing this study, we used an "affecteds-only" approach. This ensured that all results were derived under the assumption that affecteds have similar genotypes at the disease locus, whereas no assumptions are made about disease-locus genotypes of the unaffecteds. The approach, therefore, does not require the interpretation that unaffected individuals do not carry alleles involved in predisposition to BP. Given the complexities of clinical diagnosis of this phenotype, this approach seemed prudent. In addition, choosing only affected individuals and critical connecting persons for genotyping is a cost-effective way of investigating a sample set as large as this one. Elimination of founders and married-in spouses maximizes the power per genotype in an analysis but could lead to a higher type I error rate (Holmans and Craddock 1997). In addition, genotyping errors could be less detectable without the genotyping of married-in spouses, and this could lead to additional errors. In the current study, the genotyping data were checked for Mendelization errors, and intermarker recombination rates were consistent with published genetic maps.

In an attempt to extract the maximal linkage information from a given region containing multiple markers, researchers often supplement two-point linkage methods with multipoint analyses. At extremely close marker spacing, however, multipoint analyses often become more difficult to perform because currently reported genetic map distances <2 cM are only tentatively resolved and cannot be estimated more accurately without thousands of informative meioses. Alternatively, polylocus linkage analysis combines data from multiple linked loci into one "superlocus," which increases the power of the analysis by increasing the number of in-

Table 4

Marker Heterozygosity and PIC

Marker	Heterozygosity	Polymorphism Information Content
D21S1432	.70	.65
D21S1437	.77	.74
D21S1409	.73	.69
D21S1435	.74	.71
D21S1270	.76	.74
D21S262	.76	.73
D21S1413	.72	.67
D21S1254	.57	.50
D21S1895	.86	.85
D21S1252	.85	.83
D21S1440	.71	.66
D21S1290	.78	.76
D21S1255	.83	.80
D21S168	.76	.73
D21S1412	.85	.84
D21S268	.88	.87
D21S2055	.89	.88
D21S1893	.76	.72
D21S231	.61	.57
D21S1906	.52	.46
D21S1887	.74	.70
D21S266	.60	.58
D21S1260	.80	.78
D21S1411	.88	.87
PFKL	.64	.60
D21S1890	.89	.88
D21S1885	.65	.62
D21S1912	.80	.78
D21S1897	.67	.63
D21S1809	.67	.62
D21S1446	.79	.77

formative meioses in a given pedigree series (Terwilliger and Ott 1993). As noted above, our polylocus results are consistent with the results of the two-point pseudomarker analysis (table 2).

Although our use of several analytical models enhanced the prospects of detecting true linkage, it also increased the chances of a false-positive finding. However, the prior evidence of linkage to 21q22 and the fact that some of these tests are correlated—for example, there is some overlap in disease definitions (diagnostic model I is nested in model II, and model II is nested in model III), and recessive inheritance models mimic results from affected sibpair analysis—serve to offset the inflationary impact of multiple testing on our finding. In addition, the highest LOD scores reported here were obtained with the same phenotypic model (diagnostic model III) and mode of inheritance (dominant) reported in our previous study (Straub et al. 1994). This provides a measure of confidence in the current results. Although the exact mode of inheritance for BP is unknown, some studies advocate a role for dominant transmission (Rice et al. 1987; Spence et al. 1995). Our pedigrees show primarily a "dominant-like" pattern—our ascertainment plan sought families with unilineal transmission (Baron et al. 1994)—and our genotyping strategies were optimized accordingly (see Material and Methods).

Identification of possible candidate genes for the BP locus implicated on chromosome 21q22 is greatly facilitated by the fortuitous colocalization of this region with the Down syndrome critical region (DCR) (Peterson et al. 1994; Osoegawa et al. 1996; Ohira et al. 1997). Our linkage analyses maximize at a marker within 3–5 cM of the DCR. Given the difficulty with pinpointing specific intervals implicated in linkage studies of complex traits and the further proximal linkage finding of Detera-Wadleigh (1996, 1997), consideration of the DCR is warranted. Figure 1 provides a graphical representation of LOD scores in this report, as well as relative map positions of other reported BP findings on 21q and the DCR. Several authors have even suggested that the implication of this region in both disorders may be biologically relevant and that some of the same genes may be involved in both disorders (reviewed by Craddock and Owen 1994). Extensive physical mapping of the DCR using YACs, phage artificial chromosomes, and cosmids has been described (Dufresne-Zacharia et al. 1994; Osoegawa et al. 1996), and sequencing of the region is currently underway as part of the Human Genome Project. Detailed transcript maps of the region have been generated by a variety of methods, including cDNA selection (Dahmane et al. 1998), exon trapping (Ohira et al. 1997), and *alu*-splice PCR (Fuentes et al. 1997).

To investigate the role of specific genes in this region, Smith et al. (1997) generated a 2-Mb in vivo library of large-insert transgenic mice from human chromosome 21q22. They further used this library to identify one specific human gene (*DYRK*), the human homologue of the *Drosophila* minibrain gene, that is responsible for learning defects in mice and possibly the learning defects associated with Down syndrome (reviewed by Smith and Rubin 1997). Other animal models, including both gain- and loss-of-function transgenic mice, have been generated for a number of genes on distal chromosome 21, although none are thought to model exactly any of the disorders associated with this chromosome (reviewed by Kola and Hertzog 1997).

In addition to the DCR, an integrated physical and transcript map of 2.5 Mb of 21q22.3 has been described (Lapenta et al. 1998). That report precisely positions 13 genes and 4 expressed sequence tags, including PFKL (fig. 1*f*). Collectively, to date, extensive physical and transcript map data have been reported for at least 6–7 Mb of 21q22.

In summary, chromosome 21q22 is one of the most extensively studied regions in the human genome. This should greatly enhance the search for candidate disease genes, including the putative BP locus, in this region. Our results, together with previous data supporting linkage of BP to 21q22, may justify such an effort.

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Electronic-Database Information

URLs for data in this article are as follows:

Human Genome Project, http://www.ncbi.nlm.nih.gov/HUGO
Marshfield Genetics, http://www.marshmed.org/genetics (for genetic map distances)

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