Loci on Chromosomes 6q and 6p Interact to Increase Susceptibility to Bipolar Affective Disorder in the National Institute of Mental Health Genetics Initiative Pedigrees

Thomas G. Schulze, Silvia Buervenich, Judith A. Badner, C.J.M. Steele, Sevilla D. Detera-Wadleigh, Danielle Dick, Tatiana Foroud, Nancy J. Cox, Dean F. MacKinnon, James B. Potash, Wade H. Berrettini, William Byerley, William Coryell, J. Raymond DePaulo, Jr., Elliot S. Gershon, John R. Kelsoe, Melvin G. McInnis, Dennis L. Murphy, Theodore Reich†, William Scheftner, John I. Nurnberger, Jr., and Francis J. McMahon

Background: *We have reported genetic linkage between bipolar disorder and markers on chromosome 6q16.3–22.1 in the National Institute of Mental Health Genetics Initiative wave 3 pedigrees. Here we test for: 1) robustness of the linkage to differing analysis methods, genotyping error, and gender-specific maps; 2) parent-of-origin effects; and 3) interaction with markers within the schizophrenia linkage region on chromosome 6p.*

Methods: *Members of 245 families ascertained through a sibling pair affected with bipolar I or schizoaffective-bipolar disorder were genotyped with 18 markers spanning chromosome 6. Nonparametric linkage analysis was performed.*

Results: *Linkage to 6q is robust to analysis method, gender-specific map differences, and genotyping error. The locus confers a 1.4-fold increased risk. Affected siblings share the maternal more often than the paternal chromosome ($p = .006$), which could reflect a maternal parent-of-origin effect. There is a positive correlation between family-specific linkage scores on 6q and those on 6p22.2 ($r = .26$; $p < .0001$). Linkage analysis for each locus conditioned on evidence of linkage to the other increases the evidence for linkage at both loci ($p < .0005$). Logarithm of the odds (LOD) scores increased from 2.26 to 5.42 on 6q and from .35 to 2.26 on 6p22.2.*

Conclusions: *These results support linkage of bipolar disorder to 6q, uncover a maternal parent-of-origin effect, and demonstrate an interaction of this locus with one on chromosome 6p22.2, previously linked only to schizophrenia.*

Key Words: Epistasis, GRIK2, LOD score, manic-depressive illness, nonparametric linkage analysis, prolyl endopeptidase

The largest genome-wide linkage analysis of bipolar affective disorder (BPAD) to date was recently published by the National Institute of Mental Health (NIMH) Genetics Initiative group (Dick et al 2003). That genome scan (wave 3), based on a set of 1163 subjects in 245 families, revealed suggestive evidence of linkage, as defined by the criteria of Lander and Kruglyak (1995), for the chromosomal regions 17q25.3 (marker D17S928; maximum LOD score of 2.4) and 6q16.3–22.1 (adjacent markers D6S1021 and D6S474: maximum LOD score of 2.2).

Loci on chromosome 6 have previously been implicated in the etiology of other psychiatric disorders such as autism (Philippe et al 1999) and schizophrenia. In schizophrenia, linkage has been reported to both 6q (Cao et al 1997) and 6p (Straub et al 1995; Schwab et al 1995). The purpose of this study is to characterize the genetic properties of the putative BPAD susceptibility locus on 6q. We assessed the robustness of the linkage finding by using two distinct, model-free analysis methods. Additional analyses were carried out to assess potential parent-of-origin effects and the increased risk of disease conferred by the 6q locus in this sample. Finally, we tested for genetic interaction between the 6q and the 6p loci in our sample. The results support the previously reported linkage to 6q and uncover a previously unsuspected maternal parent-of-origin effect at this locus. The most intriguing finding, however, was a significant interaction between the 6q locus and the region on chromosome 6p that was previously reported to be linked only to schizophrenia. This finding suggests that some genes that contribute to schizophrenia interact with genes that contribute to BPAD.

Methods and Materials

Sample

Families were ascertained from ten study sites across the United States. Each site obtained prior approval from its Institutional Review Board and written informed consent from each participant. Data were rendered anonymous before being made available to the authors. All families contained at least one sibling affected with bipolar I disorder (BPI) and a second sibling

†Dr. Reich is deceased.

From the Division of Genetic Epidemiology in Psychiatry (TGS), Central Institute of Mental Health, Mannheim, Germany; Genetic Basis of Mood and Anxiety Disorders (TGS, SB, CJMS, SDD-W, FJM), Mood and Anxiety Program, and Laboratory of Clinical Science (DLM), National Institute of Mental Health, Bethesda; Department of Psychiatry (DFM, JBP, JRD, MGM), The Johns Hopkins University, Baltimore, Maryland; Departments of Psychiatry (JAB, ESG, FJM), and Human Genetics (NJC, ESG), University of Chicago; Rush University Department of Psychiatry (WS), Chicago, Illinois; Departments of Medical and Molecular Genetics (DD, TF, JIN) and Psychiatry (TF, JIN), Indiana University School of Medicine, Indianapolis, Indiana; University of Pennsylvania (WHB), Philadelphia, Pennsylvania; University of California (WB), Irvine, California; University of Iowa (WC), Iowa City, Iowa; University of California (JRK), San Diego, California; Department of Psychiatry (TR), Washington University, St. Louis, Missouri.

Address reprint requests to Thomas G. Schulze, M.D., Central Institute of Mental Health, Division of Genetic Epidemiology in Psychiatry, J5, 68159 Mannheim, Germany; E-mail: schulze@zi-mannheim.de.

Received December 5, 2003; revised March 23, 2004; revised April 13, 2004; accepted April 16, 2004.

0006-3223/04/$30.00
doi:10.1016/j.biopsych.2004.04.004

affected with BPI or the closely related phenotype of schizoaffective disorder, bipolar-type (SABP). Parents and additional ill relatives were included when possible. Families in which both parents suffered from BPI or SABP were excluded. Subjects with another disorder that made the mood disorder diagnosis uncertain were excluded. Detailed ascertainment information has been published elsewhere ("Genomic survey of bipolar illness," 1997).

Subjects underwent a semistructured interview by a trained professional (Nurnberger et al 1994). Subsequently, the interview, available medical records, and information from family informants were reviewed independently by two clinicians, who assigned a final diagnosis. When the first two clinicians disagreed, a third clinician reviewed all materials and assigned the final diagnosis. This procedure has been shown to produce diagnoses that are highly reliable (Leckman et al 1982).

The sample consisted of 1163 diagnosed individuals in 245 families. Of these, 50.21% had BPI or SABP, 4.60% had bipolar II disorder (BPII), 8.86% had recurrent major depressive disorder (RUP), and 36.29% were unaffected with a major mood disorder. On average, there were 3 individuals per family who were affected with a major mood disorder (range 2–9). BPI, SABP, and RUP were defined by standard diagnostic criteria (American Psychiatric Association 1994). In keeping with our earlier studies, the diagnosis of BPII was defined by Research Diagnostic Criteria (Feighner et al 1972), with the additional requirement of recurrent episodes of major depression (Rice et al 1992).

Pedigree familial relationships were confirmed with the computer program PREST (Lei Sun, Chicago, Illinois, http://galton.uchicago.edu/~mcpeek/software/prest/). Twelve monozygotic twin pairs included in the original sample (Dick et al 2003) were excluded from the present sample.

Genotyping

DNA samples were extracted from whole blood or lymphoblastoid cell cultures at the Rutgers University Cell and DNA Repository (www.rucdr.org). DNA was available for 1118 subjects. Genotyping was performed at the Center for Inherited Disease Research (CIDR). See www.cidr.jhmi.edu for details of markers, maps, and genotyping procedures. Briefly, CIDR used their 2001 marker panel, derived from the Marshfield version 8 screening set (Research Genetics, Huntington, AL), consisting of 392 markers (mean heterozygosity 76%) that span the genome at a mean spacing of 8.9 centimorgans (cM), with no gaps greater than 18 cM. Samples were amplified by polymerase chain reaction and loaded on automated capillary sequencers. Marker alleles were assigned using standard, semiautomated procedures blind to phenotype and binned across families.

The results presented herein are based on the 18 chromosome-6 markers in the CIDR marker panel. A total of 19,854 genotypes were generated for chromosome 6. Of these, 14 genotypes (.07%) represented an allele that could not have been transmitted from parents and were removed. This low rate of Mendelian inconsistencies is similar to that observed in the genome-wide data. Genotypes from 17,707 blind duplicates indicate an error rate of .05%.

Linkage Analysis

Model-free linkage analysis was performed with GenehunterPlus (GHP), version 1.2 (Bioinformatics Applications, Cambridge, England) with the correction of Kong and Cox (1997) implemented in their program Allele Sharing Modeling (ASM; Center for Parallel Computers, Stockholm, Sweden; www.pdc.kth.se/doc/genehunter/asm). GHP-ASM is a modification of the Genehunter software package (Kruglyak et al 1996) and assesses multipoint marker allele sharing in pairs of affected relatives (ARP) within small or moderately extended pedigrees, typical of those in the present data set. GHP-ASM calculates both nonparametric linkage (NPL) and multipoint logarithm of the odds (LOD) scores We used the default (equal) weighting of families, the pairs scoring function, the Kosambi map function, the observed allele frequencies, and the exponential allele-sharing model. Marker order and gender-averaged genetic distances were based on the CIDR map.

Positive results in the GHP-ASM analysis were confirmed using ASPEX version 2.2 (Hinds and Risch 1996). ASPEX compares observed versus expected allele-sharing in affected sibling pairs (ASP). ASPEX returns both gender-specific and gender-combined multipoint LOD scores. Genotyping errors presenting only as double recombinants can influence linkage results (Douglas et al 2000), so we used the option *sib_clean* in ASPEX to identify and remove these kinds of errors. ASPEX also was used to generate gender-specific marker maps, which are important when female and male genetic maps differ substantially (as they do on chromosome 6), since misspecification of intermarker distances can falsely inflate linkage scores (Paterson et al 1999). Finally, ASPEX provided an estimate of the proportion of affected siblings who share zero alleles identical-by-descent at a locus (z_0), which is useful in estimating the disease risk attributable to that locus in the sample, λ_{si} (Risch 1987). Since most families in this sample consisted of a single affected sibling pair, data were analyzed using all possible pairs (n = 453).

In the genome-wide analysis, a broad case definition that included subjects with SABP, BPI, BPII, and RUP performed best, so we used the same case definition for our additional analyses. Subjects without a major mood disorder were conservatively considered "phenotype unknown" in these analyses. Overall, the GHP-ASM analysis considered 103 avuncular and 47 first-cousin pairs not considered by the ASPEX analysis.

The statistical significance of differences in paternal and maternal multipoint LOD scores was assessed by simulation. Parental genders were randomly permuted, while pedigree structures, phenotypes, and genotypes remained constant. The number of times, out of 1000 replicates, that the difference in paternal and maternal multipoint LOD scores was at least as large as that observed in the actual data was taken as the empirical p-value.

The assessment of a potential interaction between the loci on 6q and 6p followed the approach of Cox et al (1999). Family-specific NPL scores at both locations were identified, and the Pearson correlation between family-specific NPL scores at the peaks on 6q (113.4 cM; between marker D6S1021 and D6S474) and 6p (42.0 cM; marker D6S2439) was calculated. Two conditional GHP-ASM analyses were performed on the chromosome 6 data (6q conditional on 6p and vice versa. Since the Pearson correlation was positive, families yielding NPL scores larger than 0 at the correlated locus were assigned a weight of 1, while families with scores equal to or smaller than 0 were assigned a weight of 0. If the correlation had been negative, the weight assignment would have been reversed.

Given that any correlation between two loci on the same chromosome could be in part due to undetected linkage between the respective peaks, we designed the following simulation procedure to evaluate the empirical significance of the observed interaction: Using the same pedigree structures and affection status designations as in the original data, 3 loci were simulated using SLINK (Ott 1989; Weeks et al 1990; Cottingham

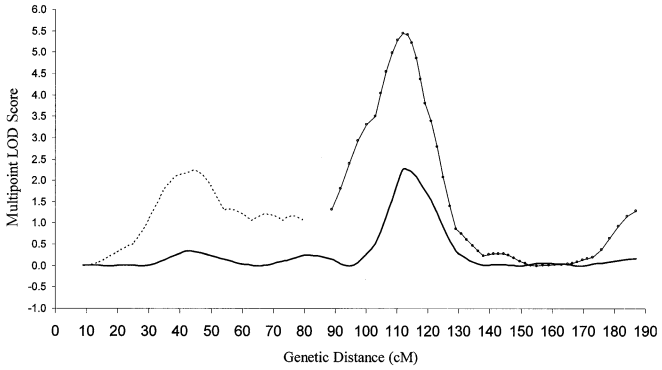

Figure 1. Affected relative-pair (ARP) linkage analysis of 18 polymorphic microsatellite markers on chromosome 6. Both the analysis on the full set of families (continuous line) yielding multipoint logarithm of the odds (LOD) scores of 2.26 (6q) and .35 (6p) and the conditional analyses yielding multipoint LOD scores of 5.42 for 6q (line with data points) and 2.18 for 6p (dashed line) are shown. Since the graphs for the conditional peaks are derived from two separate analyses, they are not joined. Intermarker distances are shown in gender-averaged centimorgans (cM), and correspond to the Center for Inherited Disease Research (CIDR) map (www.cidr.jhmi.edu).

et al 1993): a susceptibility locus with $\lambda_s = 1.6$, a marker with 4 equifrequent alleles that is tightly linked to the susceptibility locus, and a second multiallellic marker ~70 cM away from the first marker (i.e., distance between the 6q and 6p peaks in our sample). Each replicate was analyzed with GHP-ASM.

Annotation

The RefSeq track (Maglott et al 2000) in the Draft Genome Browser (UCSC Genome Bioinformatics 2002) was used to estimate the gene content of the region between markers D6S1021 and D6S474.

Results

Linkage Analysis

Suggestive evidence of linkage (Lander and Kruglyak 1995) was detected to markers on chromosome 6q16.3–22.1. In ARP, the strongest linkage signal occurred at position 113.4 cM between the adjacent markers D6S1021 and D6S474 (Figure 1). The peak multipoint LOD score was 2.26. Secondary analyses of ASP, using gender-specific maps and data cleaned of 5 apparent genotyping errors, confirmed these findings. The peak multipoint LOD score was 3.05 in the same marker interval that produced the strongest linkage evidence in the ARP analysis (Table 1).

The linked markers D6S1021 and D6S474 are 6.4 cM apart on the gender-averaged map. The region between the linked markers spans approximately 8.20 mega base pairs (Mb) of finished sequence (Draft Genome Browser, 11/02 build), including at least 39 genes.

Little evidence of linkage was detected on 6p in single-locus analyses. The peak multipoint LOD score was .35 in the GHP-ASM and 1.04 in the ASPEX analysis at the marker D6S2439, in 6p22.2 (Figure 1, Table 1).

Locus-Specific Relative Risk

We did not study a population-based sample of families, which limits our ability to draw general conclusions about the properties of the chromosome 6q locus; however, some values can be estimated from this sample. About half the families contribute positive NPL scores to the total (Figure 2). The minimum proportion of affected sib pairs sharing no parental alleles (Z_0) is 18%, indicating that this locus confers a 1.4-fold

Table 1. Affected Sibling-Pair Linkage Analysis of Chromosome 6 Markers. Results from the ASPEX Analysis Using all Possible Pairs.

Marker	Physical Position (kb)[a]	Cytogenetic Location	Sex-Average cM[b]	Female cM[c]	Male cM[c]	% Sharing[d]	Z_0[e]	Paternal LOD	Maternal LOD	Gender-Combined LOD
F13A1	6,089	6p25.1	9	.0	.0	50.3	.25	−.03	.04	.00
D6S2434	13,954	6p23	25	23.3	15.8	50.0	.25	.00	.00	.00
D6S2439	24,315	6p22.2	42	54.2	26.4	54.2	.21	.00	1.16	1.04
D6S2427	39,422	6p21.2	54	71.4	33.7	52.4	.23	.09	.32	.37
D6S1017	41,624	6p21.1	63	77.7	37.3	51.5	.24	.01	.14	.13
D6S2410	50,590	6p12.3	73	98.0	43.4	50.0	.25	.00	.00	.00
D6S1053	64,487	6q12	80	112.2	43.4	50.0	.25	.00	.00	.00
D6S1031	77,357	6q14.1	89	124.0	49.6	50.0	.25	.00	.00	.00
D6S1056	93,993	6q16.1	103	147.7	57.0	53.7	.21	.11	.86	.86
D6S1021	104,619	6q16.3	112	158.8	63.5	57.3	.18	.57[f]	2.86[f]	3.05[f]
D6S474	112,825	6q21	119	187.7	70.9	56.1	.19	.33	1.92	1.98
D6S1040	130,866	6q23.1	129	215.9	80.1	52.6	.23	.14	.28	.37
D6S1009	137,183	6q23.3	138	227.3	84.5	50.9	.24	.07	−.01	.05
C6s1848	147,848	6q24.3	146	245.8	94.9	52.3	.23	.29	.05	.30
D6S2436	154,067	6q25.2	155	262.7	101.5	50.0	.25	.00	.00	.00
D6S1035	159,836	6q25.3	165	274.6	109.5	50.0	.25	.00	.00	.00
D6S1277	164,107	6q26	173	286.2	115.5	50.0	.25	.00	.00	.00

cM, centimorgens; kb, kilo base pairs; LOD, logarithm of the odds.
[a]Positions are taken from the UCSC Genome Browser (UCSC Genome Bioinformatics 2003).
[b]Sex-average map in cM (Center for Inherited Disease Research; www.cidr.jhmi.edu).
[c]Sex-specific map in cM estimated from the sample with ASPEX sib_map.
[d]Percentage of marker alleles shared by affected siblings.
[e]Fraction of affected sibling pairs sharing 0 alleles.
[f]When only independent sib pairs are considered (count_once option in ASPEX), the LOD scores are .41, 2.28, and 2.37, respectively.

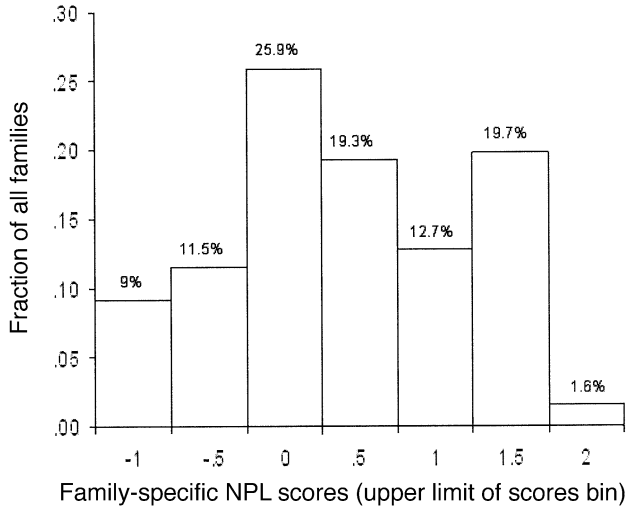

Figure 2. Distribution of family-specific nonparametric linkage (NPL) scores at the linkage peak. In 53.3% of families, an NPL score >0 was observed. LOD, logarithm of the odds.

increased risk of BPAD in this sample (Risch 1987). Since first-degree relatives of probands with BPAD have a five- to tenfold increased risk of BPAD (Gershon et al 1982), other familial factors appear to influence risk in this sample.

Evaluation of Potential Parent-of-Origin Effects

ASPEX calculates separate multipoint LOD scores based on marker alleles transmitted from the mother (maternal) and the father (paternal). Since the peak maternal score (2.86) was larger than the paternal score (.57; Table 1), we tested the statistical significance of this difference by simulation, randomly permuting parental gender in the actual data. The observed difference in maternal and paternal scores was met or exceeded 6 times out of 1000 replicates, yielding an empirical p-value of .006.

Two-Locus Interaction Between 6q and 6p

We observed a strong correlation between family-specific NPL scores at the 6q and 6p loci (Pearson $r = .264$, $p < .0001$ or $p = .004$ by simulation, i.e., observed by random 20 times out of 4989 replicates). Based on this result, linkage evidence on chromosome 6q was estimated conditional on the evidence of linkage to 6p and vice versa.

Conditional analysis resulted in an increase in the evidence for linkage to 6q that was significant at the $p = .00046$ level, by simulation. The multipoint LOD score on 6q increased from 2.26 to 5.42 at 113.4 cM, between D6S474 and D6S1021. Conditional analysis also led to a significant increase in the multipoint LOD score in the 6p region ($p = .00039$ by simulation), but the conditional score on 6p remained a modest 2.18.

Discussion

Linkage findings in complex diseases like BPAD can be difficult to replicate. These findings may also be so, but are based. The linkage results are based on a large sample and have been obtained without resort to assumptions about the genetic features of BPAD or subdivision of the sample, all techniques that increase the degrees of freedom in the linkage test. We have demonstrated that the results are not due to genotyping error. The linkage signals are detected reliably by two methods of analysis, and both methods point to the same locus. About half the families contribute positive scores to the total score on 6q (Figure 2), suggesting that the linkage signal is not driven by a few large families. The locus increases risk modestly and cannot fully account for the increased risk of disease among the first-degree relatives of probands with BPAD, but this is not unexpected in BPAD, where several loci (along with environmental factors) are thought to contribute to risk for disease. The magnitude of the risk conferred by the 6q locus is modest (relative risk < 1.4), so very large samples will be needed for replication.

The region on 6q between the linked markers D6S1021 and D6S474 contains several interesting candidate genes. Prolyl oligopeptidase, also known as prolyl endopeptidase, has been implicated in mood disorders by some clinical studies (Maes et al 1994, 1995) and may be a common target for mood-stabilizing drugs such as lithium that are effective treatments for BPAD (Detera-Wadleigh 2001; Williams et al 2002). Other genes encode RTN4IP1 (also known as NOGO-interacting mitochondrial protein), potentially involved in neuronal development and plasticity (Hu et al 2002), and the nuclear orphan receptor NR2E1, thought to be important for forebrain and limbic system development (Monaghan et al 1997). Neuronal development and plasticity, particularly in forebrain and limbic structures, may be abnormal in BPAD (Manji et al 2000). Another interesting gene in this region is GRIK2 (Paschen et al 1994), which encodes the glutamate receptor 6. Glutamate is the principal excitatory neurotransmitter in the brain and is directly involved in cognitive functions such as memory and learning. Recently, GRIK2 has been found to be associated with autism (Jamain et al 2002). Further studies are needed to fully delineate the linkage region and identify the genetic variation that accounts for the linkage findings.

Chromosome 6q has not been widely implicated in BPAD to date; however, at least two previous samples, including the earlier families collected by the NIMH Genetics Initiative, produced suggestive evidence of linkage to markers within 20 cM of the peak observed in the present sample (Rice et al 1997; Cichon et al 2001). Very recently, a genome scan on 25 multiplex families from the Azorean Islands also identified linkage between bipolar disorder and markers within the 6q linkage peak in our sample (Middleton et al 2004).

Linkage signals at markers in the 6q16.3–22.1 region were first detected in two independent samples of families with schizophrenia (Cao et al 1997). This finding was also supported by a meta-analysis of several data sets (Levinson et al 2000). A locus that accounts for the linkage evidence in this region has not yet been identified. Linkage to schizophrenia has also been detected on 6p23 (Straub et al 1995; Schwab et al 1995) and systematic fine mapping has shown that markers in the gene *dysbindin* are associated with the disease (Straub et al 2002). This finding has been supported by independent samples (Schwab et al 2003; Van Den Bogaert et al 2003). In light of the findings in schizophrenia and the growing evidence for shared genetic factors in the etiology of schizophrenia and BPAD (reviewed in Berrettini 2000), we hypothesized that the 6p and 6q loci may both be involved in BPAD, even though we initially detected little evidence of linkage to 6p markers in this sample. Indeed, we observed a strong correlation between the linkage evidence at the 6q and 6p loci, suggesting interaction, and the conditional linkage analysis led to a significant increase in the LOD score at both loci; however, it appears unlikely that dysbindin accounts

for the linkage evidence we detect on 6p22.2. The 6p marker that shows the strongest evidence of linkage on the conditional analysis (D6S2439) is located 8.9 Mb proximal to dysbindin (Table 1), while the marker that is closest to dysbindin (D6S2434, 1.4 Mb distal), does not show any linkage to BPAD in our sample and was thus not considered in the interaction analysis.

One might argue that the strong correlation we see could be in part due to undetected linkage between the 6q and 6p markers in those families that contribute to the linkage finding. This is unlikely, since the two loci are ~70 cM apart on a gender-averaged map. Furthermore, there is a distinct minimum point in the LOD score curve (Figure 1) between the two loci that argues against linkage between the markers inflating the linkage evidence at 6p. Nevertheless, since there are no clear precedents on how to best perform conditional linkage analysis for loci on the same chromosome, we assessed the potential impact of linkage between markers at the two loci by simulation. We simulated a linkage peak at one marker and correlated this with the linkage evidence at a marker 70 cM away, using the same data structure as in our sample. This simulation produced a correlation coefficient as large as that observed in our real data only 4 times out of 1000 replicates. This shows that the correlation we observe is unlikely to result from linkage between the markers at the 6p and 6q loci, but rather from a true interaction between the loci to increase susceptibility to BPAD in our sample.

To the best of our knowledge, this is the first study of linkage in a complex disorder that investigates interaction of two loci on the same chromosome. We outline an approach that combines correlation analysis, conditional linkage analysis, and a simulation procedure to assess significance empirically, taking into account any linkage between the two loci that may exist. While conditional linkage analysis can detect important interactions, in affected sib-pair data the method does not readily distinguish between two-locus heterogeneity and true epistasis (Vieland and Huang 2003).

We also detect evidence of a maternal parent-of-origin effect acting at the 6q locus in this sample. The ASP analysis with ASPEX, using gender-specific maps, yielded a significantly higher maternal than paternal LOD score. This demonstrates that the maternally transmitted marker alleles account for more of the linkage evidence on 6q in this sample. The first evidence of a parent-of-origin effect in BPAD was detected in a linkage region on chromosome 18q (Stine et al 1995), replicated in two independent samples (Gershon et al 1996; McMahon et al 1997) and supported by additional analyses with a dense set of markers (McMahon et al 2001; Schulze et al 2003). Subsequently, both maternal and paternal parent-of-origin effects were described for linkage signals on chromosomes 1q and 13q (McInnis et al 2003). On chromosome 6q, a maternal parent-of-origin effect has been detected in autism (Jamain et al 2002) and, more modestly, in schizophrenia (T. Bourgeron, personal communication October 6, 2003) in the same chromosomal region implicated by our findings. Moreover, 6q22–24 has been identified as a region with imprinted genes (Temple et al 1995; Temple and Shield 2002), suggesting a possible mechanism for the observed parent-of-origin effect. Nevertheless, our finding should be interpreted with caution, since the sample we studied contains more missing fathers than mothers, decreasing the certainty of identity-by-descent inferences for paternal chromosomes.

These results support the previously reported linkage to 6q, uncover a maternal parent-of-origin effect, and demonstrate that this locus interacts with a locus on chromosome 6p previously reported to be linked only to schizophrenia. Further research is needed to identify the actual susceptibility locus, explain the parent-of-origin effect, and elucidate the nature of the two-locus interaction we observe on chromosome 6.

We are sorry to report that TR passed away before the submission of the final version of this paper.

We thank Eric Meyer and John Rice for assistance with data management; the clinical coordinators, research interviewers, and diagnosticians for their clinical work; Alison Goate, Robert Desimone, and Dennis Charney for their critique of the manuscript; and the family volunteers for making this research possible.

This research was supported by grants from the National Institute of Mental Health (NIMH); the Edward F. Mallinckrodt, Jr. Foundation (FJM); the Chicago Brain Research Institute (FJM); and the National Alliance for Research in Schizophrenia and Depression (TGS, FJM). This work was also supported by the NIMH Intramural Research Program (TGS, SB, CJMT, SDW, FJM).

Genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract N01-HG-65403. We thank Jay Tischfield, Ph.D. (Rutgers University Cell and DNA Repository) for transforming cell lines and providing DNA samples.

American Psychiatric Association (1994): *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition. Washington DC: American Psychiatrc Association Press.

Berrettini WH (2000): Are schizophrenic and bipolar disorders related? A review of family and molecular studies. *Biol Psychiatry* 48:531–538.

Cao Q, Martinez M, Zhang J, Sanders AR, Badner JA, Cravchik A, et al (1997): Suggestive evidence for a schizophrenia susceptibility locus on chromosome 6q and a confirmation in an independent series of pedigrees. *Genomics* 43:1–8.

Cichon S, Schumacher J, Müller DJ, Hürter M, Windemuth C, Strauch K, et al (2001): A genome screen for genes predisposing to bipolar affective disorder detects a new susceptibility locus on 8q. *Hum Mol Genet* 10:2933–2944.

Cottingham RW, Idury RM, Schaffer AA (1993): Faster sequential genetic linkage computations. *Am J Hum Genet* 53:252–263.

Cox NJ, Frigge M, Nicolae DL, Concannon P, Hanis CL, Bell GI, et al (1999): Loci on chromosomes 2 (NIDDM1) and 15 interact to increase susceptibility to diabetes in Mexican Americans. *Nat Genet* 21:213–215.

Detera-Wadleigh SD (2001): Lithium-related genetics of bipolar disorder. *Ann Med* 33:272–285.

Dick DM, Foroud T, Flury L, Bowman ES, Miller MJ, Rau NL, et al (2003): Genomewide linkage analyses of bipolar disorder: A new sample of 250 pedigrees from the National Institute of Mental Health Genetics Initiative. *Am J Hum Genet* 73:107–114.

Douglas JA, Boehnke M, Lange K (2000): A multipoint method for detecting genotyping errors and mutations in sibling-pair linkage data. *Am J Hum Genet* 66:1287–1297.

Feighner JP, Robins E, Guze SB, Woodruff RA Jr, Winokur G, Munoz R (1972): Diagnostic criteria for use in psychiatric research. *Arch Gen Psychiatry* 26:57–63.

Genomic survey of bipolar illness in the NIMH genetics initiative pedigrees: A preliminary report (1997): *Am J Med Genet* 74:227–237.

Gershon ES, Badner JA, Detera-Wadleigh S, Ferraro TN, Berrettini WH (1996): Maternal Inheritance and Chromosome 18 Allele Sharing in Unilineal Bipolar Illness Pedigrees. *Am J Med Genet* 67:202–207.

Gershon ES, Hamovit J, Guroff JJ, Nurnberger JI, Goldin LR, Bunney WE (1982): A Family Study of Schizoaffective, Bipolar I, Bipolar II, Unipolar and Normal Control Probands. *Arch Gen Psychiatry* 39:1157–1167.

Hinds D, Risch N (1996): The ASPEX package: Sib-pair exclusion mapping [computer software]. Available online at http://aspex.sourceforge.net/. Accessed May 27, 2004.

Hu WH, Hausmann ON, Yan MS, Walters WM, Wong PK, Bethea JR (2002): Identification and characterization of a novel Nogo-interacting mitochondrial protein (NIMP). *J Neurochem* 81:36–45.

Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B, et al (2002): Linkage and association of the glutamate receptor 6 gene with autism. *Mol Psychiatry* 7:302–310.

Kong A, Cox NJ (1997): Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet* 61:1179–1188.

Kruglyak L, Daly MJ, Reeve-Daly M, Lander ES (1996): Parametric and nonparametric linkage analysis: A unified multipoint approach. *Am J Hum Genet* 58:1347–1363.

Lander E, Kruglyak L (1995): Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nat Genet* 11:241–247.

Leckman JF, Sholomskas D, Thompson WD, Belanger A, Weissman MM (1982): Best estimate of lifetime psychiatric diagnosis: A methodological study. *Arch Gen Psychiatry* 39:879–883.

Levinson DF, Holmans P, Straub RE, Owen MJ, Wildenauer DB, Gejman PV, et al (2000): Multicenter linkage study of schizophrenia candidate regions on chromosomes 5q, 6q, 10p, and 13q: Schizophrenia linkage collaborative group III. *Am J Hum Genet* 67:652–663.

Maes M, Goossens F, Scharpe S, Calabrese J, Desnyder R, Meltzer HY (1995): Alterations in plasma prolyl endopeptidase activity in depression, mania, and schizophrenia: Effects of antidepressants, mood stabilizers, and antipsychotic drugs. *Psychiatry Res* 58:217–225.

Maes M, Goossens F, Scharpe S, Meltzer HY, D'Hondt P, Cosyns P (1994): Lower serum prolyl endopeptidase enzyme activity in major depression: Further evidence that peptidases play a role in the pathophysiology of depression. *Biol Psychiatry* 35:545–552.

Maglott DR, Katz KS, Sicotte H, Pruitt KD (2000): NCBI's LocusLink and RefSeq. *Nucleic Acids Res* 28:126–128.

Manji HK, Moore GJ, Rajkowska G, Chen G (2000): Neuroplasticity and cellular resilience in mood disorders. *Mol Psychiatry* 5:578–593.

McInnis MG, Lan TH, Willour VL, McMahon FJ, Simpson SG, Addington AM, et al (2003): Genome-wide scan of bipolar disorder in 65 pedigrees: Supportive evidence for linkage at 8q24, 18q22,4q32, 2p12, and 13q12. *Mol Psychiatry* 8:288–298.

McMahon FJ, Hopkins PJ, Xu J, McInnis MG, Shaw S, Cardon L, et al (1997): Linkage of bipolar affective disorder to chromosome 18 markers in a new pedigree series. *Am J Hum Genet* 61:1397–1404.

McMahon FJ, Simpson SG, McInnis MG, Badner JA, MacKinnon DF, DePaulo JR (2001): Linkage of bipolar disorder to chromosome 18q and the validity of bipolar II disorder. *Arch Gen Psychiatry* 58:1025–1031.

Middleton FA, Pato MT, Gentile KL, Morley CP, Zhao X, Eisener AF, et al (2004): Genomewide linkage analysis of bipolar disorder by use of a high-density single-nucleotide polymorphism (SNP) genotyping assay: a comparison with microsatellite marker assays and finding of significant linkage to chromosome 6q22. *Am J Hum Genet* 74:886–897.

Monaghan AP, Bock D, Gass P, Schwager A, Wolfer DP, Lipp HP, et al (1997): Defective limbic system in mice lacking the tailless gene. *Nature* 390:515–517.

Nurnberger JI, Blehar MC, Kaufmann CA, Cooler CY, Simpson S, Friedman JH, et al (1994): Diagnostic Interview for Genetic Studies. *Arch Gen Psychiatry* 51(11):849–59.

Ott J (1989): Computer-simulation methods in human linkage analysis. *Proc Natl Acad Sci U S A* 86:4175–4178.

Philippe A, Martinez M, Guilloud-Bataille M, Gillberg C, Rastam M, Sponheim E, et al (1999): Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sibpair Study. *Hum Mol Genet* 8:805–812.

Paschen W, Blackstone CD, Huganir RL, Ross CA (1994): Human GluR6 kainate receptor (GRIK2): Molecular cloning, expression, polymorphism, and chromosomal assignment. *Genomics* 20:435–440.

Paterson AD, Naimark DM, Petronis A (1999): The analysis of parental origin of alleles may detect susceptibility loci for complex disorders. *Hum Hered* 49:197–204.

Rice JP, Goate A, Williams JT, Bierut L, Dorr D, Wu W, et al (1997): Initial genome scan of the NIMH genetics initiative bipolar pedigrees: Chromosomes 1, 6, 8, 10, and 12. *Am J Med Genet* 74:247–253.

Rice JP, Rochberg N, Endicott J, Lavori P, Miller C (1992): Stability of psychiatric diagnoses: An application to the affective disorders. *Arch Gen Psychiatry* 49:824–830.

Risch N (1987): Assessing the role of HLA-linked and unlinked determinants of disease. *Am J Hum Genet* 40:1–14.

Schulze TG, Chen Y-S, Badner JA, McInnis MG, DePaulo JR, McMahon FJ (2003): Additional, physically ordered markers increase linkage signal for bipolar disorder on chromosome 18q22. *Biol Psychiatry* 53:239–243.

Schwab SG, Albus M, Hallmayer J, Honig S, Borrmann M, Lichtermann D, et al (1995): Evaluation of a susceptibility gene for schizophrenia on chromosome 6p by multipoint affected sib-pair linkage analysis. *Nat Genet* 11:325–327.

Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M, et al (2003): Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. *Am J Hum Genet* 72:185–190.

Stine OC, Xu J, Koskela R, McMahon FJ, Gschwend M, Friddle C, et al (1995): Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect. *Am J Hum Genet* 57:1384–1394.

Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, et al (2002): Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am J Hum Genet* 71:337–348.

Straub RE, MacLean CJ, O'Neill FA, Burke J, Murphy B, Duke F, et al (1995): A potential vulnerability locus for schizophrenia on chromosome 6p24–22: Evidence for genetic heterogeneity. *Nat Genet* 11:287–293.

Temple IK, James RS, Crolla JA, Sitch FL, Jacobs PA, Howell WM, et al (1995): An imprinted gene(s) for diabetes? *Nat Genet* 9:110–112.

Temple IK, Shield JP (2002): Transient neonatal diabetes, a disorder of imprinting. *J Med Genet* 39:872–875.

UCSC Genome Bioinformatics (2002): Genome browser. Available online at http://genome.ucsc.edu. Accessed January 2, 2003.

UCSC Genome Bioinformatics (2003): Human Genome Browser Gateway. Available online at http://genome.ucsc.edu/cgi-bin/hgGateway. Accessed April 1, 2004.

Van Den Bogaert A, Schumacher J, Schulze TG, Otte AC, Ohlraun S, Kovalenko S, et al (2003): The DTNBP1 (dysbindin) gene contributes to schizophrenia, depending on family history of the disease. *Am J Hum Genet* 73:1438–1443.

Vieland VJ, Huang J (2003): Two-locus heterogeneity cannot be distinguished from two-locus epistasis on the basis of affected-sib-pair data. *Am J Hum Genet* 73:223–232.

Weeks DE, Ott J, Lathrop GM (1990): SLINK: A general simulation program for linkage analysis. Available online at http://linkage.rockefeller.edu/ott/SLINK.htm. Accessed May 27, 2004.

Williams RS, Cheng L, Mudge AW, Harwood AJ (2002): A common mechanism of action for three mood-stabilizing drugs. *Nature* 417:292–295.