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


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 between 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

Loci on chromosome 6 have previously been implicated in the etiology of other psychiatric disorders such as autism (Philipp 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 (BDI) and a second sibling...

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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 semi-structured 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. 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 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 (0.7%) 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 0.5%.

Linkage Analysis

Model-free linkage analysis was performed with Genehunter-Plus (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 sb_clean in ASPEX to identify and remove these kinds of errors. ASPEX was also 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 mis specification 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 (Z0), which is useful in estimating the disease risk attributable to that locus in the sample, Zlocus (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 (133.4 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
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 multiallelic 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 increase in risk.

![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).](#)

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

<table>
<thead>
<tr>
<th>Marker</th>
<th>Physical Location</th>
<th>Cytogenetic Location</th>
<th>Sex-Average cM</th>
<th>Female cM</th>
<th>Male cM</th>
<th>% Sharing</th>
<th>$Z_0^a$</th>
<th>Paternal LOD</th>
<th>Maternal LOD</th>
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cm, centimorgans; kb, kilo base pairs; LOD, logarithm of the odds.

*Positions are taken from the UCSC Genome Browser (UCSC Genome Bioinformatics 2003).

*Sex-average map in cm (Center for Inherited Disease Research; www.cidr.jhmi.edu).

*Sex-specific map in cm estimated from the sample with ASPEX sib_map.

*Percentage of marker alleles shared by affected siblings.

*Fraction of affected sibling pairs sharing 0 alleles.

*When only independent sib pairs are considered (count_once option in ASPEX), the LOD scores are .41, 2.28, and 2.37, respectively.

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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 RTN4P1 (also known as NOGO-interacting mitochondrial protein), potentially involved in neuronal development and plasticity (Hu et al 2002), and the nuclear orphan receptor NR2F1, 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 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.

**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.
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 in those families that contribute to the linkage finding. This is and was thus not considered in the interaction analysis.

1.4 Mb distal), does not show any linkage to BPAD in our sample (Table 1), while the marker that is closest to dysbindin (D6S2434, shows the strongest evidence of linkage on the 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 (McNnis 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.

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