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BRIEF REPORT

A linkage disequilibrium study of bipolar disorder and microsatellite markers on 22q13

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Bipolar disorder is a major psychiatric disorder characterized by extreme mood states that alternate between mania and depression. Family, twin, and adoption studies indicate a genetic component to the disease, but the etiology is suspected to be complex, with multiple genes contributing to an increased susceptibility to the disorder. We have previously reported a genome scan in which a genome-wide maximum LOD score indicated evidence of linkage at the marker D22S278 at 22q13. This area is of particular interest since it is also implicated in schizophrenia, and thus may harbor a susceptibility gene common to both disorders. In our further efforts to fine map this region, we examined 10 microsatellite markers spanning an interval of 2.3 MB in a set of 142 parent–proband triads. Linkage disequilibrium to illness was tested using the Transmission Disequilibrium Test. Haplotypes were determined and marker-to-marker linkage disequilibrium across the region was examined. D22S281 and D22S685 yielded suggestive evidence of linkage disequilibrium to bipolar disorder (empirical *P* values of 0.023 and 0.036, respectively), but a marker-to-marker analysis indicates that a higher density screen is needed to adequately analyze this region. Psychiatr Genet 12:231–235 © 2002 Lippincott Williams & Wilkins.

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INTRODUCTION

Bipolar disorder is a severe psychiatric disorder characterized by alternation between extreme mood states. It occurs in approximately 1% of the population, and is associated with psychosis and a substantial risk of suicide (Goodwin and Jamison, 1990). Twin and family studies indicate a genetic component to the disorder, but the non-Mendelian inheritance pattern of the disorder suggests the disease is complex with multiple susceptibility loci (Tsuang and Faraone, 1990). Numerous loci have been implicated by linkage studies but, to date, no specific gene has been definitively identified within these linked regions.

We have recently conducted a genome scan of 20 families with bipolar disorder from the general North American population. These data yielded a genome-wide maximum LOD score of 3.8 at D22S278 (Kelsoe *et al.*, 2001). Surprisingly, this was near the same region implicated in studies of schizophrenia. Subsequently, this overlap of linkage findings in bipolar disorder and schizophrenia has been reported in several other chromosomal regions and has led to the speculation that these disorders may share some common susceptibility genes (Kelsoe, 1999; Berrettini, 2000).

Based on these results, we have conducted linkage disequilibrium studies to examine the region near D22S278 in greater detail. By fine-mapping the region, we hope to better define the region implicated in bipolar disorder. We now report the results of linkage disequilibrium analysis in 142 triads using the Transmission Disequilibrium Test (TDT) with 10 microsatellite markers in the vicinity of D22S278.

METHODS

Subjects

Families for this study came from two different clinical collections. Thirty-six multiplex families were ascertained through a proband with bipolar I or bipolar II collected at one of three sites (San Diego, Vancouver, and Cincinnati) as part of a collaborative

linkage study of bipolar disorder. All subjects were interviewed using the Structured Clinical Interview for DSM-III-R (Spitzer *et al.*, 1992) for Diagnostic and Statistical Manual of the American Psychiatric Association DSM-III-R or DSM-IV. Diagnoses were determined by consensus review of interview and medical record materials by a panel of experienced clinicians as described previously (Kelsoe *et al.*, 2001). Nuclear families were selected from this sample for linkage disequilibrium studies using the TDT. Thirty-one of these families included a proband and both parents, two included an affected sibpair and both parents, and three families included three affected sibs and both parents.

DNA samples for an additional 106 nuclear families were obtained for subjects selected from multiplex families collected as part of the NIMH Genetics Initiative for Bipolar Disorder. These subjects were ascertained and interviewed using the Diagnostic Interview for Genetic Studies at one of four participating sites (Berrettini *et al.*, 1997; NIMH Genetics Initiative Bipolar Group, 1997). Thirty-eight of these families included a proband and both parents, while 68 included an affected sibpair and both parents.

Genotyping

After obtaining informed consent, blood was drawn on all subjects and immortalized lymphoblastoid cell lines were established. DNA was prepared from cultured cells by phenol/chloroform extraction. Microsatellite markers were selected from the Genome Database (http://www.gdb.org). Fluorescently labeled primers were used to amplify markers by polymerase chain reaction. Allele sizes were determined by electrophoresis on an ABI 377 as described elsewhere (Kelsoe *et al.*, 2001).

Statistical analyses

Linkage studies in complex diseases by nature implicate a large chromosomal region (Terwilliger *et al.*, 1997). However, linkage disequilibrium is subject to association between alleles due to population stratification and admixture rather than from an ancestral association. Family-based tests of linkage disequilibrium such as the TDT are robust to population admixture (Spielman *et al.*, 1993; Ewens and Spielman, 1995).

The extended TDT (ETDT) is a program that performs a TDT analysis for markers with multiple alleles (Sham and Curtis, 1995a). The ETDT employs two alternative approaches to calculate the TDT statistic. The allele-wise analysis examines transmission of alleles across all genotypes. The genotype-wise analysis considers every heterozygous parental genotype separately, and examines whether each allele of the genotype is transmitted to affected offspring. To evaluate the significance of the TDT statistic obtained for each marker, a Monte Carlo approach was employed using 1000 simulations with the MCETDT program (Sham and Curtis, 1995b). Haplotypes were generated using Simwalk2 (Sobel and Lange, 1996), and linkage disequilibrium statistics were calculated with GOLD (Abecasis and Cookson, 2000). Haplotypes could not be definitively determined for two parents.

RESULTS

Using the ETDT program, we analyzed 10 microsatellite markers spanning a 2.3 MB region encompassing

TABLE 1. Extended Transmission Disequilibrium Test results

Marker name	Chromosomal[a] location	Distance (base pairs)[b]	Allele wise	Allele-wise empirical	Genotype wise	Genotype-wise empirical
D22S1162	31106990–31107144	0	0.293	0.354	0.105	0.299
D22S281	31132757–31132897	25 613	0.582	0.634	0.007	0.023
D22S685	31391651–31391837	258 754	0.064	0.078	0.016	0.036
D22S691	31671712–31671955	279 875	0.24	0.324	0.212	0.337
D22S1152	31935156–31935426	263 201	0.9	0.909	0.052	0.074
D22S304	32166729–32166839	231 303	0.227	0.361	0.216	0.41
D22S277	33014905–33015070	848 066	0.84	0.91	0.018	0.129
D22S1142	33017284–33017473	2214	0.943	0.987	0.236	0.509
D22S278	33149958–33150195	132 485	0.19	0.254	0.321	0.53
D22S1173	33379930–33380191	229 735	0.746	0.874	0.903	0.952

[a]Chromosomal location is based on actual sequence position as reported in the public database at the time of writing, and it is interpolated using downloaded sequence data when actual position is not available (D22S281 and D22S685).
[b]Distance is calculated using distance between primers rather than actual distance between repeated elements.

TABLE 2. Marker-to-marker linkage disequilibrium

	Standardized disequilibrium coeffecient (D')									
	D22S1162	D22S281	D22S685	D22S691	D22S1152	D22S304	D22S277	D22S1142	D22S278	D22S1173
D22S1162		0.509	0.116	0.133	0.099	0.118	0.148	0.091	0.105	0.089
D22S281	<0.0001		0.142	0.115	0.119	0.088	0.096	0.078	0.057	0.121
D22S685	0.3028	0.0506		0.158	0.14	0.145	0.118	0.101	0.095	0.129
D22S691	0.05	0.6442	0.0016		0.258	0.104	0.142	0.109	0.085	0.103
D22S1152	0.8101	0.4559	0.0651	<0.0001		0.216	0.093	0.069	0.062	0.07
D22S304	0.5038	0.4557	0.0646	0.2525	<0.0001		0.074	0.121	0.076	0.099
D22S277	0.2202	0.6648	0.1334	0.0203	0.8014	0.9245		0.148	0.149	0.125
D22S1142	0.6759	0.8966	0.4471	0.1983	0.7555	0.2864	0.0055		0.278	0.066
D22S278	0.7537	0.9985	0.2535	0.8634	0.5529	0.7817	0.0053	<0.0001		0.111
D22S1173	0.9687	0.3693	0.7928	0.3294	0.3509	0.5826	0.0885	0.8066	0.7489	
	Chi-square P value									

Values above the diagonal are the standardized disequilibrium coefficient (D'). Values below the diagonal are the chi-square P values.

D22S278. As shown in Table 1, suggestive evidence of linkage disequilibrium to bipolar disorder was obtained at D22S281, 2 MB from D22S278 (genotype-wise empirical $P = 0.023$). D22S685, which is located 200 kb telomeric to D22S281, also yielded suggestive evidence of linkage disequilibrium (genotype-wise empirical $P = 0.036$). No other marker examined gave any evidence of linkage disequilibrium.

To interpret these results and to estimate the required marker density for a higher resolution study, we examined marker-to-marker linkage disequilibrium. Table 2 illustrates the standardized disequilibrium coeffecient (D') values and the χ^2 values from this analysis. Linkage disequilibrium was not strongly conserved in the 2.3 MB region examined as most of the D' values fall below 0.15. The D' values do not always correspond to P values since a single occurrence of rare alleles for each of the two loci on the same haplotype can produce a significant P value. D' corrects for allele frequencies, thereby preventing an inflation of the D' value resulting from such rare events. Several such instances of the lack of correlation between these measures are apparent in Table 2. As most of our markers were separated by more than 200 kb, these results are not unexpected. However, some close marker pairs did exhibit linkage disequilibrium, although this was not always consistent. In the case of markers D22S1162 and D22S281, significant linkage disequilibrium ($D' = 0.509$) was detected, and these markers are separated by 25 kb. D22S1142 and D22S277 are only separated by 2.2 kb; however, they show much less linkage disequilibrium ($D' = 0.148$).

DISCUSSION

We examined 142 families for linkage disequilibrium in 10 microsatellite markers. Approximately 2 MB centromeric to D22S278, we detected modest evidence of linkage disequilibrium at D22S281 and D22S685. No linkage disequilibrium was detected at marker D22S278. We also examined marker-to-marker linkage disequilibrium in the region, and only modest linkage disequilibrium was found overall. This suggests that a much higher marker density will be required to adequately examine this region for linkage disequilibrium with bipolar disorder.

Many statistical and genetic factors play into examining linkage disequilibrium, and thus these should be considered when interpreting our results. Since the allele-wise test was not significant at any of the markers tested, our results may be more indicative of linkage rather than linkage disequilibrium. Another major limitation to our study is the limited density of markers. Our analysis of marker-to-marker linkage disequilibrium argues that linkage disequilibrium is, in general, not preserved over large distances in this genomic region. Therefore, a much higher marker density than can be provided by microsatellite markers will be required to adequately cover this chromosomal segment and to interpret our linkage disequilibrium results in this region. Furthermore, as microsatellites have a higher mutation rate, it has been argued that linkage disequilibrium may be less likely to be preserved and, therefore, that they are less effective for linkage disequilibrium mapping. This factor, however, must be balanced against their greater informativeness. Finally, allelic heterogeneity

could substantially reduce our power to detect linkage disequilibrium.

Our data show suggestive evidence of linkage disequilibrium at markers D22S281 and D22S685, and although modest, in the context of existing linkage and linkage disequilibrium studies of this region, they provide some support for other reports of a susceptibility locus for bipolar disorder in this region. Detera-Wadleigh *et al.* (1999) reported a maximum LOD score of 2.5 between markers D22S685 and D22S689 in a linkage study of 21 families with bipolar disorder. Mujaheed *et al.* (2000) looked for evidence of linkage disequilibrium between bipolar disorder and D22S278 in an Arab-Palestinian population, and they found modest evidence of linkage disequilibrium ($P = 0.031$). Evidence for linkage with schizophrenia has been found at D22S278 and at nearby markers (Coon *et al.*, 1994; Pulver *et al.*, 1994; Vallada *et al.*, 1995; Gill *et al.*, 1996). Furthermore, D22S278 has also been implicated in linkage disequilibrium studies of schizophrenia (Moises *et al.*, 1995; Schizophrenia Collaborative Linkage Group for Chromosome 22, 1998).

Since this region is implicated in both schizophrenia and bipolar disorder, it may contain a susceptibility gene for both disorders. Only the identification of a disease susceptibility gene and functional variants will enable the definitive testing of this hypothesis. Such a discovery could provide new insight into fundamental pathophysiology and the relationship of the underlying neurobiology of these two disorders.

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