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Evidence for Linkage Disequilibrium Between the Dopamine Transporter and Bipolar Disorder

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A role for the dopamine transporter (DAT) in bipolar disorder is implicated by several lines of pharmacological evidence, as well as suggestive evidence of linkage at this locus, which we have reported previously. In an attempt to identify functional mutations within DAT contributing a susceptibility to bipolar disorder, we have screened the entire coding region, as well as significant portions of the adjacent non-coding sequence. Though we have not found a definitive functional mutation, we have identified a number of single nucleotide polymorphisms (SNPs) that span the gene from the distal promoter through exon 15. Of the 39 SNPs that are suitable for linkage disequilibrium (LD) studies, 14 have been analyzed by allele-specific PCR in a sample of 50 parent-proband triads with bipolar disorder. A haplotyped marker comprised of five SNPs, spanning the region between exon 9 and exon 15, was constructed for each individual, and transmission/disequilibrium test (TDT) analysis revealed this haplotype to be in linkage disequilibrium with bipolar disorder (allele-wise TDT $p = 0.001$, genotype-wise TDT $p = 0.0004$). These data replicate our previous finding of linkage to markers within and near DAT in a largely different family set, and provide further evidence for a role of DAT in bipolar disorder. Published 2001 Wiley-Liss, Inc.†

KEY WORDS: dopamine transporter; bipolar disorder; single nucleotide polymorphism; linkage disequilibrium; transmission/disequilibrium test

INTRODUCTION

The dopamine transporter (DAT) has been implicated as a candidate gene in several disorders, including bipolar disorder and attention-deficit hyperactivity disorder (ADHD) [Kelsoe et al., 1996; Cook et al., 1995; Waldman et al., 1998]. DAT plays a critical role in the regulation of dopaminergic transmission by mediating the active reuptake of dopamine from the synapse into the presynaptic terminal [Giros and Caron, 1993]. Several lines of pharmacological evidence suggest a possible role for dopamine in bipolar disorder, particularly in mania. Cocaine and amphetamine have been shown to increase synaptic dopamine concentrations by acting at DAT to inhibit dopamine reuptake [Giros et al., 1992]. The acute effects of such psychostimulants closely resemble mania, and chronic administration can provoke a manic episode in bipolar patients and trigger psychosis in nonbipolar patients [Angrist, 1994]. In addition, L-dihydroxyphenylalanine (L-DOPA), which also increases synaptic dopamine, has been observed to precipitate mania, and antipsychotic drugs, which block dopamine receptors, are an effective treatment for mania [Murphy et al., 1971; Goodwin and Jameson, 1990]. Finally, the antidepressant bupropion acts at least in part at DAT and has been reported to be of particular efficacy in bipolar depression [Sachs et al., 1994].

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We have reported previously on suggestive evidence of linkage between the DAT locus on chromosome 5p15.3 and bipolar disorder using several markers within and near the DAT gene [Kelsoe et al., 1996]. Although statistically significant results of 3.0 were not attained, the data suggested that DAT may play a causal role in a minority of families with bipolar disorder and justify further examination of the DAT gene. Our current efforts have thus focused on the identification of possible functional mutations within the DAT gene in coding and regulatory sequence. In the process of screening the introns and exons of DAT for possible mutations, we have identified a number of single nucleotide polymorphisms (SNPs) that span the gene from the distal promoter through exon 15. Although of no obvious functional significance, many of these SNPs are appropriate for linkage disequilibrium (LD) studies of bipolar disorder. We have therefore focused on the evaluation of LD, through transmission/disequilibrium test (TDT) analyses, between the DAT gene and bipolar disorder, as well as the detection of a possible shared haplotype among bipolar patients.

MATERIALS AND METHODS

Subjects

The genomic DNA from three individuals was used in screening DAT for functional mutations: a control subject with no history of psychiatric illness, the bipolar I proband (GM5927) of Old Order Amish pedigree 110, and a member of Family 16 (0244) from the UCSD family collection with schizoaffective disorder, bipolar type. These two affected subjects were chosen because suggestive evidence of linkage between DAT and bipolar disorder was found previously in these two families [Kelsoe et al., 1996]. All three subjects chosen for sequencing studies were caucasians of European ancestry.

A sample of 50 families, each consisting of either one or two children with bipolar disorder (bipolar I or bipolar II) and both parents, was chosen from the UCSD/UBC/UC family collection and Old Order Amish pedigree 110 for association studies with the 14 SNPs. Each of these triad or sib pair families was a subset of a more extended family that was ascertained for linkage studies. The family sample for this study exhibits some degree of overlap with the sample previously reported for our linkage study of the dopamine transporter, as 10 of the 50 triad or sib pair families included in this sample derived from 10 of the original 20 families from our previous linkage study [Kelsoe et al., 1996]. The UCSD/UBC/UC families were ascertained as part of a multi-site linkage study, which included collaborative sites at the University of California at San Diego, the University of British Columbia, and the University of Cincinnati. Families for this linkage study were selected for the availability of at least one member with bipolar disorder (I or II) and two other family members who were affected under a broad model (bipolar I, bipolar II, schizoaffective, bipolar type, or recurrent major depression). All subjects provided informed consent per local IRB approved procedures prior to participation. Blood was obtained from all subjects for the immortalization of lymphoblastoid cell lines, and DNA was prepared by phenol/chloroform extraction from cultured cells.

Diagnosis

Each family member was interviewed directly using the Structured Clinical Interview for DSM-III-R (SCID) [Spitzer et al., 1987]. However, the Schedule of Affective Disorders and Schizophrenia (SADS-L) was used to interview one family (Family 16) [Endicott and Spitzer, 1978]. Information from the interview, medical records, and at least one other family informant was reviewed by a committee of clinicians in order to make consensus, best-estimate diagnoses. All interviewers underwent the same extensive training, and diagnostic reliability, which was tested biannually by review of videotaped interviews, was consistently high.

Mutation Screening

DAT sequence for use as a reference was compiled from several sources (GenBank accession numbers U12320–U12327, U17990–U17992, U92262, D88556–D88570) [Vandenbergh et al., 1992; Donovan et al., 1995; Kouzmenko et al., 1997; Kawarai et al., 1997]. Primer sequences for the promoter, intron 1, and exon 15 were obtained from published sequence, and these regions were amplified in overlapping segments of approximately 500 bp each. Exons were amplified with up to 500 bp of flanking intronic sequence on either side. Primer sequences for the screening of exons 1, 3, 9, 11, and 12 and flanking intronic regions could be obtained from published sequence. A BAC containing DAT (BACH-278o18) was obtained from Genome Systems (St. Louis, MO) to generate sequence for the screening of exons 2, 4, 5, 6, 7, 8, 10, 13, and 14 in this manner. Following amplification, PCR products were purified and served as templates for dye-termination cycle-sequencing reactions to prepare fluorescent-labeled products for analysis on an Applied Biosystems 377 automated sequencer. Resultant sequences were aligned and manually screened for mutations.

Allele Detection and Haplotype Determination

Genotyping of the triads for the 14 SNPs was accomplished by allele-specific PCR (AS-PCR), in which primers were designed to specifically amplify either the baseline allele or its alternative in separate PCR reactions. The specific primers and reaction conditions for each SNP or pair of SNPs are listed in Table I. When possible, SNPs were combined in double-ended AS-PCR reactions to generate haplotyped markers. This was the case for the following SNPs: I1+1036/I1+1736, I1+1860/I1+1861/I2+28, and E9+59/I9+102. The reactions were assembled as follows: 170ng of genomic DNA, 0.2μM of each primer, 200μM dNTPs, 4% DMSO, 1.5mM or 2.5mM $MgCl_2$, GeneAmp 10X PCR Gold Buffer (Perkin-Elmer Applied Biosystems, Foster City, CA), and 1.0 unit of AmpliTaq Gold (Perkin-Elmer Applied Biosys-

TABLE I. Primers and PCR Conditions For AS-PCR of Selected SNPs*

SNP(s)	Genotype	Forward primer	Reverse primer	[Mg^{+2}]	TD temp	Cycle #	Product size
P+214	A	5′-GAGGAATCGTCTTTGTCTTGA-3′	5′-GGAGAATAGCTTGAAGCTGG-3′	2.5mM	60→50	40	329bp
	G	5′-GAGGAATCGTCTTTGTCTTGG-3′	5′-CCTCGAAGCTGTAGTGTGTCA-3′	2.5mM	63→53	40	737bp
I1+1036/I1+1736	CG	5′-CTGCTGGATCCAAATGC-3′	5′-CACACTTCCAGAGAGCAATC-3′	2.5mM	65→55	35	737bp
	CA	5′-CTGCTGGATCCAAATGC-3′	5′-CACACTTCCAGAGAGCAATT-3′	2.5mM	65→55	35	737bp
	AG	5′-GCTGCTGGATCCAAATGA-3′	5′-CACACTTCCAGAGAGCAATC-3′	2.5mM	67→57	35	737bp
	AA	5′-GCTGCTGGATCCAAATGA-3′	5′-CACACTTCCAGAGAGCAATT-3′	2.5mM	67→57	35	737bp
I1+1860/I1+1861/I2+48	AGG	5′-GGTGCAGGGGAAGGAGAG-3′	5′-CCCCGGCTGCACCTAC-3′	2.5mM	70→60	40	653bp
	AGA	5′-GGTGCAGGGGAAGGAGAG-3′	5′-CCCCGGCTGCACCTAC-3′	1.5mM	70→60	35	653bp
	GAG	5′-GTGCAGGGGAAGGAGGA-3′	5′-CCCCGGCTGCACCTAC-3′	2.5mM	70→60	40	653bp
	GAA	5′-GTGCAGGGGAAGGAGGA-3′	5′-CCCCGGCTGCACCTAT-3′	2.5mM	70→60	40	653bp
	AAG	5′-GGTGCAGGGGAAGGAGAA-3′	5′-CCCCGGCTGCACCTAC-3′	1.5mM	70→60	35	653bp
	AAA	5′-GGTGCAGGGGAAGGAGAA-3′	5′-CCCCGGCTGCACCTAT-3′	2.5mM	70→60	35	653bp
	GGG	5′-GTGCAGGGGAAGGAGGG-3′	5′-CCCCGGCTGCACCTAC-3′	1.5mM	70→60	35	653bp
	GGA	5′-GTGCAGGGGAAGGAGGG-3′	5′-CCCCGGCTGCACCTAT-3′	1.5mM	70→60	35	653bp
I6+96	G	5′-ACGGCATCAGAGCATACC-3′	5′-AACCCTGGTGTCTGCAAC-3′	2.5mM	70→60	35	166bp
	C	5′-CACAGCCACCATGCCATA-3′	5′-GAACCCTGGTGTCTGCAAG-3′	2.5mM	70→60	35	237bp
E9+59/I9+102	AG	5′-CACGCTCCCTCTGTCCTCA-3′	5′-AGGTCTGGGGGCCGTC-3′	1.5mM	70→60	35	190bp
	GG	5′-ACGCTCCCTCTGTCCTCG-3′	5′-AGGTCTGGGGGCCGTC-3′	2.5mM	70→60	35	190bp
	AT	5′-CACGCTCCCTCTGTCCTCA-3′	5′-GAGGTCTGGGGGCCGTA-3′	1.5mM	70→60	35	190bp
	GT	5′-ACGCTCCCTCTGTCCTCG-3′	5′-GAGGTCTGGGGGCCGTA-3′	1.5mM	70→60	35	190bp
I9-21	G	5′-CCGTGGATACAGTGGTC-3′	5′-ACACAGAGCACAGGGTC-3′	1.5mM	65→55	40	227bp
	A	5′-CTCACTACCAGGCATCC-3′	5′-ACACAGAGCACAGGGTT-3′	1.5mM	60→50	40	156bp
I10+117	G	5′-ACTTCCAGGGAGCTGCG-3′	5′-AACCGAATGCGGAACTTG-3′	2.5mM	70→60	35	261bp
	A	5′-ACTTCCAGGGAGCTGCA-3′	5′-TCCGATGGCTTCGATG-3′	2.5mM	67→57	35	578bp
E15+274	G	5′-GAGGTCCACCCCGTTG-3′	5′-GATTCTCAGCAGGTGCGT-3′	2.5mM	67→57	35	169bp
	C	5′-GAGGTCCACCCCGTTC-3′	5′-GATTCTCAGCAGGTGCGT-3′	2.5mM	67→57	35	169bp
E15+352	G	5′-CCTGCTCCCTGCTCCCG-3′	5′-CCATCCTCCAATGCCTCTGA-3′	2.5mM	73→63	40	304bp
	A	5′-CCTGCTCCCTGCTCCCA-3′	5′-CCATCCTCCAATGCCTCTGA-3′	2.5mM	73→63	40	304bp
E15+1812	T	5′-CCGTCAGCCTGTGAACT-3′	5′-CTGACTGCAGCAGCCA-3′	2.5mM	65→55	40	198bp
	C	5′-GAATGCAGTATCCGCGAG-3′	5′-CTGACTGCAGCAGCCG-3′	1.5mM	67→57	35	476bp

*Shown are the sequences of the primers used to detect the corresponding SNP genotype, the final millimolar (mM) magnesium concentrations ([Mg^{+2}]), touchdown (TD) PCR annealing temperatures, number of cycles at the final annealing temperature, and expected product size.

tems) in a total volume of 30μl. Thermocycling was performed using a PTC-200 thermocycler from MJ Research, Waltham, MA and a modified touchdown protocol with an initial denaturation at 94°C for 10 min to activate the AmpliTaq Gold, denaturation at 94°C for 30 sec, primer annealing for 30 sec (temperatures specified for each primer pair), and primer extension at 72°C for 1 min 30 sec [Don et al., 1991]. The annealing temperature was decreased by 0.5°C per cycle for a total of 20 cycles, with 15 or 20 additional cycles at the final annealing temperature. Amplified products were then analyzed by agarose gel electrophoresis and ethidium bromide staining.

Following genotyping, complete haplotypes comprised of all 14 SNPs were constructed for each individual using family relationships. These haplotypes could be conclusively determined for 47 of the 50 families. In three of the families, however, phase could not be unequivocally inferred for one SNP (I6+96). Therefore, haplotypes were determined for the remaining 13 SNPs, and the linkage phase of the I6+96 SNP was estimated based on the haplotype frequencies observed in the 47 families in which linkage phase could be unequivocally determined.

In addition to the SNP markers, the triads were genotyped for the 40 bp VNTR in the 3′ untranslated region of exon 15 [Vandenbergh et al., 1992]. Amplification of this repeat was accomplished using the same protocol as previously described with the following specifications: 2.5mM final $MgCl_2$ concentration, primers 5′-TGTGGTGTAGGGAACGGCCTGAG-3′ (forward) and 5′-CTTCCTGGAGGTCACGGCTCAAGG-3′(reverse), and a touchdown from 75 to 65°C. Products were then run on a 2% agarose gel at 70V for three hr to ensure proper allele separation.

Linkage Disequilibrium Analyses

The transmission/disequilibrium test strategy was chosen for LD studies because of its robustness against artifacts induced by population stratification [Spielman et al., 1993; Spielman and Ewens, 1996]. The standard TDT considers one allele versus all other alleles at a particular marker and tests for an excess of transmission to affected offspring, generating a composite χ^2 statistic and corresponding p value based on the total number of alleles. However, this approach may not detect association if more than one allele is transmitted in excess for a given marker. Therefore, we chose to use an extended version of the transmission/disequilibrium test (ETDT) for multi-allele marker loci, which provides for the analysis of parent-offspring data under two different models, allele-wise and genotype-wise [Sham and Curtis, 1995a]. The allele-wise model attempts to establish a pattern of preferential transmission of certain alleles across all parental genotypes, testing the hypothesis that different alleles may vary in the extent to which they are preferentially transmitted to affected offspring. The genotype-wise model considers every heterozygous parental genotype separately and tests each allele of a particular genotype for an excess of transmission. The empirical significance of the TDT statistics were evaluated using the Monte Carlo approach (MCETDT) and 1,000–10,000 replicates [Sham and Curtis, 1995b].

RESULTS

Mutation Screening

In the process of screening DAT for functional mutations, the entire coding sequence and the immediately adjacent non-coding sequence, including both 5′ and 3′ UTRs, 9.5 kb of flanking intronic sequence, and 2.5 kb of promoter sequence was screened. Intron 1 was screened in its entirety due to the presence of negative regulatory elements within this region [Kouzmenko et al., 1997]. Complete sequence for introns 2, 5, 7, and 9, as well as substantial sequence for introns 6, 11, 12, and 13, was obtained in the process of generating flanking sequence for the screening of the relevant exons (GenBank accession numbers AF306558 through AF306564).

This mutation screen led to the identification of 92 deviations from the reference sequence, which include 17 insertions, eight deletions, and 67 base substitutions. The one coding sequence variant identified, an A to G substitution in exon 9, was found to be synonymous, consistent with previous studies of ADHD [Vandenbergh et al., 2000]. Although no variants of obvious significance to transcriptional or splicing regulation were found, 39 of the SNPs were polymorphic among the three genomic samples sequenced and are thus suitable for linkage disequilibrium studies.

Linkage Disequilibrium and Association Studies

Since none of the SNPs were of obvious significance to the function of DAT, those for analysis were chosen on the basis of location and feasibility of unequivocal allele detection by AS-PCR. The 14 SNPs thus chosen for a further examination of LD span the gene from the distal promoter through exon 15. The specific positions of these SNPs within the DAT gene, as well as their observed frequencies and heterozygosities among the parental genotypes, are indicated in Table II.

The use of double-ended AS-PCR to generate haplotyped markers where possible resulted in 10 markers for the initial analysis: three multi-allelic, PCR-haplotyped markers consisting of two or three SNPs and seven bi-allelic, single-SNP markers. The results of the ETDT analyses of the 10 markers are shown in Table III. No evidence for association between bipolar disorder and any of these SNPs was found under the allele-wise model. Under the genotype-wise model, only the PCR-haplotyped marker, ME9I9, was shown to be in linkage disequilibrium with bipolar disorder, with an empirical p value of 0.044. A slight trend toward LD was seen for the 40 bp VNTR in exon 15 (E15VNTR), although statistically significant results were not obtained.

Following these initial analyses, family relationships were used to construct haplotypes comprised of all 14

TABLE II. Selection of SNPs Observed in DAT

SNP	Location	Variant	Heterozygosity[a]	Relative frequency[b]
P+214	231bp 5′ of exon 1	A→G	0.537	0.572
I1+1036	1036bp 3′ of exon 1	C→A	0.509	0.587
I1+1736	1736bp 3′ of exon 1	G→A	0.509	0.582
I1+1860	1860bp 3′ of exon 1	A→G	0.519	0.577
I1+1861	1861bp 3′ of exon 1	G→A	0.519	0.577
I2+48	48bp 3′ of exon 2	G→A	0.343	0.721
I6+96	96bp 3′ of exon 6	G→C	0.528	0.582
E9+59	59bp within exon 9	A→G	0.407	0.736
I9+102	102bp 3′ of exon 9	G→T	0.389	0.269
I9-21	21bp 5′ of exon 10	G→A	0.315	0.817
I10+117	117bp 3′ of exon 10	G→A	0.120	0.933
E15+274	274bp within exon 15	G→C	0.287	0.798
E15+352	352bp within exon 15	G→A	0.278	0.803
E15+1812	1812bp within exon 15	T→C	0.324	0.788

[a]Heterozygosity among parental genotypes.
[b]Relative frequency among parental genotypes of baseline allele as determined from reference sequence.

SNPs. Pairwise LD analyses revealed the presence of almost complete disequilibrium ($p<0.00001$) between the seven SNPs in the 5′ region of DAT (promoter through intron 6), as well as a high degree of LD between the seven SNPs in the 3′ region (exon 9 through exon 15). Strikingly, there was a complete lack of LD between these two regions, indicating a complex structure that is likely the result of both mutation and recombination (data to be published in detail separately). This segmental pattern of LD was used to construct a limited number of haplotyped markers within the 5′ and 3′ regions separately. Haplo1 includes all seven SNPs from the 5′ region of DAT (P+214 through I6+96), whereas Haplo2 is comprised of six SNPs from the 3′ region (intron 9 through exon 15). Since the PCR-haplotyped marker ME9I9 produced marginally significant results, Haplo2 was separated into two smaller haplotyped markers in an attempt to further refine the area of interest within the 3′ region. The resultant haplotyped markers, Haplo3 and Haplo4, were comprised of SNPs from the intron 9-intron 10 region and exon 15, respectively. As the 3′ region displayed a great deal more diversity than the 5′ region, with some pairs of SNPs in almost complete LD, and others exhibiting only marginal LD, a final haplotyped marker, HaploLD, was constructed from the five SNPs in the 3′ region that displayed the greatest degree of LD to each other.

Haplo1, which consists solely of SNPs from the 5′ region, was not found to be in linkage disequilibrium with bipolar disorder. However, all of the haplotyped markers comprised of SNPs from the 3′ region of DAT were found to be in LD with bipolar disorder under the genotype-wise model. HaploLD was most striking of all, with a highly significant empirical p value of 0.0004 ($\chi^2 = 40.5$) under the genotype-wise model, as well as a significant empirical p value of 0.001 ($\chi^2 = 26.4$) under the allele-wise model. These p values remain significant even when corrected for multiple comparisons based on the number of TDT tests conducted using the conservative Bonferroni method, $p = 0.006$ and 0.016, respectively.

TABLE III. TDT Analyses*

Marker	SNP positions	Allele-wise	Empirical	Genotype-wise	Empirical
MP214	P+214	0.209	0.267	0.209	0.267
MI1	I1+1036 and I1+1736	0.686	0.745	0.686	0.745
MI1I2	I1+1860, I1+1861, and I2+48	0.403	0.472	0.246	0.355
MI6	I6+96	0.910	1.000	0.910	1.000
ME9I9	E9+59 and I9+102	0.321	0.324	**0.023**	**0.044**
MI9	I9-21	0.881	1.000	0.881	1.000
MI10	I10+117	0.370	0.475	0.370	0.475
M274	E15+274	0.763	0.892	0.763	0.777
M352	E15+352	0.068	0.094	0.068	0.070
E15VNTR		0.161	0.128	0.159	0.164
M1812	E15+1812	0.206	0.271	0.206	0.271
Haplo1	P+214, I1+1036, I1+1736, I1+1860-1, I12+48, I6+96	0.290	0.455	0.095	0.309
Haplo2	I9+102, I9-21, I10+117, E15+274, E15+352, E15+1812	-nan	0.000	**0.006**	**0.023**
Haplo3	I9+102, I9-21, I10+117	0.073	0.108	**0.010**	**0.024**
Haplo4	E15+274, E15+352, E15+1812	0.094	0.090	**0.031**	**0.036**
HaploLD	E9+59, I9+102, I9-21, E15+274, E15+1812	**0.0009**	**0.001**	**0.0002**	**0.0004**[a]

*Allele-wise and genotype-wise p values generated by the ETDT program. Empirical p values generated as an evaluation of ETDT statistics using the Monte Carlo approach. No corrections were made for multiple comparisons.
[a]10,000 replications were used to generate a p value, rather than the usual 1,000.
Nominally, significant p values are indicated in bold.

DISCUSSION

The TDT results presented here are consistent with the findings of suggestive evidence of linkage and linkage disequilibrium to the 3′ end of DAT that we have reported previously [Kelsoe et al., 1996]. It is noteworthy that some of the strongest evidence of linkage in our previous study came from markers in the 3′ end of DAT. Our previous results consistent with linkage of bipolar disorder to the highly polymorphic VNTR within intron 8 of DAT are replicated by the current findings of significant linkage disequilibrium for all haplotyped markers containing SNPs from the exon 9-intron 9 region, which is in close proximity to the intron 8 VNTR. Although our previously reported analysis of the VNTR within exon 15 also gave results consistent with linkage and linkage disequilibrium to bipolar disorder, the current ETDT analysis of this VNTR failed to reach statistical significance. However, analysis of a haplotyped marker consisting of 3 SNPs within exon 15 (Haplo4) yielded modest evidence of linkage disequilibrium (p=0.036). These SNPs not only flank the VNTR but are in complete linkage disequilibrium with it according to pairwise LD analyses. Furthermore, the 10 repeat allele, which previously has been reported to be associated with ADHD, segregates with the 3′ haplotypes that were found to be associated with illness in this study. It is therefore possible that the failure of this marker to reach statistical significance is due to the small sample size and resulting low statistical power. These results for the 40 bp VNTR are thus consistent with those that we and others have reported previously.

Our current results must be qualified as not being entirely independent of our original study, as 10 of the families in the current sample of 50 originated from 10 of the original 20 families reported previously. However, our current results derive from a largely different clinical sample and a different set of markers was used for analysis. It is also noteworthy that HaploLD remained significant under the genotype-wise model with a p value of 0.001 when these 10 original families were removed from the current sample, generating a sample entirely independent of that in our previous study (data not shown).

There are a great many ways one could conceivably construct haplotypes based on two or more of the 14 SNPs. For example, one could use a sliding window method, in which individual SNPs are progressively combined into haplotypes spanning the entire gene to assess the incremental benefit of adding each SNP to the haplotype. However, our pairwise LD data, which will be published in detail separately, indicated nearly complete LD between SNPs in the 5′ region of DAT and a very high degree of LD between SNPs in the 3′ region. Since the lack of LD between these two regions of DAT may be an indication that recombination has effectively unlinked them, we opted not to use a sliding window method, in which the haplotypes would ultimately span the gap between these two regions. Although the use of separate haplotyped markers for the 5′ and 3′ region has allowed us to narrow down the area of interest to the 3′ region of DAT, we were unsuccessful at more precisely defining the region possibly containing a functional polymorphism. Thus, more sequencing of this region is required to identify the functional elements within this gene that may be etiologically relevant.

DAT has been implicated in other disorders, such as ADHD, psychosis in cocaine abusers, and alcoholism [Cook et al., 1995; Waldman et al., 1998; Gelernter et al., 1994; Muramatsu and Higuchi, 1995]. It is striking that the 3′ end of the gene is implicated in each of these other disorders. The studies of ADHD are particularly intriguing in light of family epidemiological data that suggest a possible genetic relationship between ADHD and bipolar disorder [Bierderman et al., 1991a, b]. Furthermore, a recent study revealed an association between DAT protein availability and genotype of the VNTR in exon 15 [Heinz et al., 2000]. Together these data raise the possibility that a functional mutation within the 3′ region of the DAT gene may play a role in several behavioral syndromes.

The use of high density SNP maps and haplotypes in LD studies has been the focus of much recent attention. These results illustrate several points regarding these methods. First, haplotypes are much more informative than individual SNPs and, therefore, have much greater power to detect LD. SNPs may also be superior markers for the detection of LD because of their lower mutation rate as compared to microsatellites. However, LD relationships between SNPs may be quite complex, and their power for disease mapping may only be fully maximized when these relationships are taken into account.

In summary, the data presented here provide evidence for an association between DAT and bipolar disorder. They also implicate the 3′ end of DAT, exon 9 through exon 15, as the region of interest and are thereby consistent with previous studies of the 3′ VNTR. These data suggest the presence of a functional variant of a 3′ non-coding sequence element that is involved in the susceptibility to bipolar disorder. Thus, further investigation of this region is needed to define any regulatory elements that may be present, and to evaluate their relevance with regards to bipolar disorder.

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