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Possible Locus for Bipolar Disorder Near the Dopamine Transporter on Chromosome 5

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The dopamine transporter (DAT) plays a key role in the regulation of dopaminergic neurotransmission by mediating the active reuptake of synaptic dopamine. It is an important candidate gene for bipolar disorder because of data implicating dopamine abnormalities in mania, and because it is the site of action of amphetamine, which has activating and psychotogenic properties. DAT has recently been cloned by its homology to a family of transporters, and mapped to chromosome 5p15.3. We tested DAT for linkage to bipolar disorder in a collection of 21 families from the general North American population (University of California, San Diego/University of British Columbia [UCSD/UBC] families), three Icelandic pedigrees, and Old Order Amish pedigree 110. We examined three markers at DAT, including a 5′ *Taq*I RFLP (HDAT-*Taq*I), a highly polymorphic variable number of tandem repeats marker (VNTR) (HDAT-VNTR1), and a 3′ 40-bp repeat marker (HDAT-PCR1), as well as two nearby microsatellite markers, D5S392 and D5S406. A maximum lod score of 2.38 was obtained at D5S392 in one of the UCSD/UBC families under an autosomal-dominant model. A lod score of 1.09 was also obtained under the same dominant model in the Amish at HDAT-PCR1. In the combined set of families, a maximum lod score of 1.76 was obtained under an autosomal-recessive model at HDAT-*Taq*I. Positive results were also obtained at several markers, using three nonparametric methods in the UCSD/UBC family set: the affected pedigree member method (P = 0.001), an affected sib pair method (ESPA, P = 0.0008), and the transmission disequilibrium test (P = 0.024). These results suggest the presence of a susceptibility locus for bipolar disorder near the DAT locus on chromosome 5.

KEY WORDS: bipolar disorder, dopamine transporter, genetic linkage, chromosome 5

INTRODUCTION

A variety of data implicate the dopamine transporter (DAT) as a candidate gene for bipolar disorder. DAT mediates the active reuptake of dopamine from the synapse into the presynaptic nerve terminal, and thereby plays a critical role in the termination of dopamine synaptic signals, and in the regulation of dopaminergic neurotransmission in general [Giros and Caron, 1993]. The possible role of dopamine in bipolar disorder, and mania in particular, has been suggested by several lines of evidence. Amphetamines, and other psychostimulants, act at DAT to increase synaptic dopamine [Kuczenski and Segal, 1994]. The euphoria and activation of acute stimulant intoxication can bear a striking resemblance to mania, such that they can be confused clinically. Chronic administration of stimulants can trigger mania in bipolar patients, and psychosis in nonbipolar patients [Angrist, 1994]. Similarly, L-dihydroxyphenylalanine (L-DOPA), which also increases synaptic dopamine, has been observed to precipitate mania [Murphy et al., 1971]. Furthermore, antipsychotic drugs, which block dopamine receptors, are an effective treatment for mania [Goodwin and Jameson, 1990]. The role of dopamine in mania has also been suggested by reports of elevated dopamine metabolites in the CSF of manic patients [Jimerson, 1987]. Lastly, the antidepressant drug bupropion, which acts at least partly at DAT, has been reported to

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be of particular efficacy in bipolar depression [Sachs et al., 1994].

DAT has been recently cloned and found to be a member of a highly conserved family of transporters including those for gamma amino butyric acid (GABA), norepinephrine, and serotonin [Kilty et al., 1991; Shimada et al., 1991; Giros et al., 1992; Vandenbergh et al., 1992a]. These transporters all share a characteristic structure, which includes 12 membrane-spanning domains. DAT has been cytogenetically localized to chromosome 5p15.3 by both in situ hybridization and somatic cell hybrids [Giros et al., 1992; Vandenbergh et al., 1992b]. Several polymorphisms have also been identified, including a *Taq*I RFLP in the 5′ end of the gene, a 40-bp tandem repeat in the 3′ untranslated tail and a highly informative variable number of tandem repeats (VNTR) polymorphism [Vandenberg et al., 1992a,b; Byerley et al., 1993]. As part of an ongoing survey of the genome for loci linked to bipolar disorder, we examined this region in our set of 25 families. Our results suggest the presence of a locus for bipolar disorder near DAT on chromosome 5p15.3.

MATERIALS AND METHODS

Family Ascertainment and Diagnosis

Twenty-five families from three populations were examined. The size and diagnostic characteristics of these families are summarized in Table I. The Old Order Amish family and the Icelandic families represent moderate to large families from relative population isolates, and the UCSD/UBC family set is a larger collection of smaller families from the general North American population.

The UCSD/UBC families derive from a collaborative effort between the University of California at San Diego and the University of British Columbia. At UCSD, families are identified through the UCSD Mental Health Clinical Research Center, as well as through a variety of UCSD and community hospitals and clinics. At UBC, families are identified through systematic screening of the UBC Mood Disorders Clinic, which is a tertiary referral center for all of British Columbia. At each site, families are identified through a proband with bipolar I, bipolar II, or schizoaffective disorder, and selected for study if at least two other members with bipolar, schizoaffective, or recurrent major depression are available for study. Each family member was interviewed directly, using the Structured Clinical Interview for DSM-III-R [Spitzer et al., 1987], and blood was obtained for immortalization of lymphoblastoid cell lines. One family (family 16) was interviewed using the SADS-L [Endicott and Spitzer, 1978] as modified for DSM-III-R diagnoses. Information from the interview, from medical records, and from at least one other family informant was reviewed by a committee of clinicians, blind to genotypic or family membership information, in order to make consensus "best-estimate" DSM-III-R diagnoses. Common training procedures and regular reliability testing were employed to maintain high diagnostic reliability both within and between sites.

TABLE I. Families for Linkage Analysis

	UCSD/ UBC	Icelandic	Amish	Total
Total subjects	172	54	118	344
No. of families	21	3	1	25
Average members/ family	8.2	18	118	13.8
Bipolar I	33	11	22	66
Bipolar II	15	0	0	15
Major depression, recurrent	28	9	7	44
Major depression, single episode	14	2	3	19
Other psychiatric disorder	25	3	8	36
No psychiatric disorder	49	28	77	154
Total affected members	76	20	29	125
Average affected/ family	3.6	6.7	29	5

The Old Order Amish are a conservative religious sect who live in relative isolation in Southeastern Pennsylvania. The epidemiology of affective disorders in the Amish, their advantages for genetic studies, and the diagnostic methods employed have been previously described [Egeland and Hostetter, 1983; Egeland et al., 1990]. Briefly, each subject was interviewed directly using the SADS-L, and additional information was obtained from other family informants and medical records. These data were reviewed by a panel of psychiatric clinicians, who were blind to genotypic data and family relationships, in order to obtain a consensus diagnosis using the Research Diagnostic Criteria (RDC) [Spitzer et al., 1978]. Pedigree 110 was originally ascertained through a systematic epidemiological survey of the Lancaster County Old Order Amish. As used in this study, it is identical in membership to a previously published pedigree [Kelsoe et al., 1989], except for several subsequent new onsets of illness [Kelsoe et al., 1993].

The Icelandic population is also a relative genetic isolate, and has similar advantages to the Old Order Amish for genetic studies. The epidemiology of affective disorders in Iceland has been studied extensively by Helgason [1979]. Three families with bipolar disorder were ascertained by one of us (H.K.) from the National University Hospital in Reykjavik. These pedigrees and the diagnostic methods employed have been reported previously [Kelsoe, et al., 1993]. Briefly, information was obtained from direct interview using an Icelandic version of the SADS-L. This interview, along with medical records and information from at least one family informant, was used by a panel of psychiatrists, blind to genotypic data, to make RDC consensus diagnoses.

Genotyping

Three polymorphisms were used at the DAT locus: a 40-bp tandem repeat in the 3′ untranslated tail of the gene, a *Taq*I RFLP in the 5′ end of the gene, and a highly polymorphic large-allele VNTR [Vandenbergh et al., 1992a,b; Byerley et al., 1993]. The 40-bp tandem repeat (HDAT-PCR1) was detected by PCR amplification using primers selected from the published sequence [Vandenbergh et al., 1992a]. The left primer

extended from position 2527–2546 (GCGTTCAGTTGACACATTGC), and the right primer from position 3305–3286 (CGCGGATACTGCATTCTTGA). Three hundred ng of DNA were amplified in a 25-μl reaction including 0.3–1.0 μM of each primer, 200 μM dNTPs, 4.5 mM $MgCl_2$, 2.5 units Taq polymerase, and buffer provided by the manufacturer (Perkin Elmer, Norwalk, CT). Thermal cycling was conducted for a total of 40 cycles according to the "touchdown" protocol, with annealing temperatures decreasing from 75°C to 65°C over the first 20 cycles [Don et al., 1991]. PCR products were separated by electrophoresis in 2% agarose gels and visualized with ethidium bromide. Three alleles of sizes 700, 740, and 780 were observed.

The two-allele *Taq*I RFLP (HDAT-*Taq*I) was detected using a 558-bp fragment obtained by digestion of the cDNA clone (pHDAT, kindly provided by Dr. Kim Neve, Oregon Health Sciences University) with *Sst*II. This fragment spanned bases 253–810 in the 5′ end of the gene, and included the Taq492 probe (bases 301–793) described by Vandenbergh et al. [1992a]. The large-allele VNTR (HDAT-VNTR1) described by Byerley et al. [1993] was detected using a 462-bp *Rsa*I fragment as probe (bases 748–1209), which spans transmembrane domains 4–7. Five μg of DNA were digested with *Taq*I, and Southern blotting and RFLP detection using each of these probes were conducted as described previously [Kelsoe et al., 1993]. Two alleles were observed for HDAT-*Taq*I of 7 and 5.6 kb, and 20 alleles were observed for HDAT-VNTR1, ranging from 4.2–13 kb. Genotypes were not generated for the Icelandic samples at these two RFLPs because of a limited supply of DNA.

In addition to these polymorphisms at the HDAT locus, two microsatellite markers, D5S392 and D5S406, were also examined. These markers were chosen from the Généthon map of 1992 [Weissenbach et al., 1992] because of their proximity to the region to which DAT had been cytogenetically mapped. They were PCR-amplified and detected as described previously [Mirow et al., 1994]. Nineteen and 14 alleles were observed for D5S392 and D5S406, respectively.

Linkage Analysis

Parametric linkage analyses were conducted using the FASTLINK version of the LINKAGE 5.1 program package [Cottingham et al., 1993; Schaffer et al., 1994; Lathrop et al., 1984]. Two definitions of the affected phenotype were examined. Diagnostic model 1 included as affected those with bipolar I, bipolar II, or schizoaffective diagnoses; model 2 included these diagnoses plus recurrent major depression. Subjects with other psychiatric diagnoses were considered of unknown phenotype. Three genetic models of transmission, two dominant and one recessive, were employed, as detailed in Table II. Alleles were recoded in some of the parametric analyses in order to increase computational speed [Braverman, 1985]; otherwise, allele frequencies were estimated from the families themselves [Boehnke, 1991]. The statistical power of these families and models was examined by simulation using the SIMLINK package, version 4.1 [Ploughman and Boehnke, 1989]. Analyses under heterogeneity were conducted using HOMOG [Ott, 1991].

TABLE II. Models for Linkage Analysis*

Model	q	Mode	f1	f2	f3
1	0.021	Dominant	0.85	0.85	0.001
2	0.024	Dominant	0.5	0.5	0.001
3	0.218	Recessive	0.5	0.001	0.001

*q, frequency of disease gene; f1, penetrance of AA, where A is the disease allele; f2, penetrance of Aa; f3, penetrance of aa.

These data were also examined using three nonparametric methods. The affected pedigree member (APM) method was employed using the programs APM and APMMULT; empirical *P* values were estimated by simulation, using the programs SIM and SIMMULT [Weeks and Lange, 1988]. A maximum likelihood-based extension of the affected sib pair method was also employed using the ESPA program [Sandkuyl, 1989]. The transmission disequilibrium test (TDT) [Spielman et al., 1993] was used to examine the data for possible linkage disequilibrium, using a maximum likelihood method implemented in the program TDTLIKE [Terwilliger, 1995].

RESULTS

Prior to analyzing the DAT marker data, power analyses were conducted in order to determine the probability of detecting linkage in this family set. Under genetic model 1 (Dom. 85), diagnostic model 2 (bipolar + recurrent major depression), and an assumption of 50% heterogeneity, the combined family set has a 76% probability of a lod score >1, a 52% probability of a lod score >2, and a 33% probability of a lod score >3 for a tightly linked four-allele marker.

Lod scores for two-point analyses using each of the five markers are summarized by population in Table III. Results are presented for both diagnostic models and for two of the genetic models (models 1 and 3). Though no statistically significant lod scores were obtained, positive lod scores suggestive of linkage were found at each of the markers in both the UCSD/UBC and the Amish populations. The maximum lod score for each marker is indicated in boldface in Table III. The maximum lod score for any of the markers in this table is 1.76, which is the total of both the UCSD/UBC and Amish populations at HDAT-*Taq*I under the recessive model and diagnostic model 2. At HDAT-PCR1, a lod score of 1.09 was obtained in the Amish population under a dominant model. The highly informative marker HDAT-VNTR1 yielded a maximum lod score of 1.16 under a recessive model for all families. Similarly, a lod score of 1.02 was obtained for the UCSD/UBC family set under the dominant model.

The highest two-point lod scores observed were obtained in individual families, as illustrated in Table IV. Family 16 in the UCSD/UBC family set yielded maximum lod scores of 2.38 and 2.08 at D5S392 and HDAT-VNTR1, respectively, under genetic model 1 and diagnostic model 1. A lod score of 1.49 was also obtained at D5S406. The other two DAT markers were uninforma-

TABLE III. Two-Point LOD Scores by Population*

Diagnostic model	Bipolar only, θ							Bipolar + recurrent depression, θ						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40	0.00	0.01	0.05	0.10	0.20	0.30	0.40
Marker/Genetic Model														
HDAT-PCR1 Dom 0.85														
UCSD/UBC	−0.97	−0.88	−0.65	−0.44	−0.19	−0.06	−0.02	−1.28	−1.22	−1.04	−0.82	−0.45	−0.17	−0.06
Icelandic	−2.60	−1.90	−1.09	−0.68	−0.30	−0.12	−0.03	−3.73	−2.86	−1.58	−0.95	−0.39	−0.16	−0.04
Amish	0.67	0.68	0.68	0.67	0.59	0.42	0.19	0.64	0.75	0.99	**1.09**	1.00	0.68	0.27
Total	−2.90	−2.10	−1.06	−0.45	0.10	0.24	0.14	−4.37	−3.33	−1.63	−0.68	0.16	0.35	0.17
HDAT-PCR1 Rec 0.50														
UCSD/UBC	−0.17	−0.01	0.32	0.48	0.43	0.26	0.09	0.42	0.42	0.49	0.49	0.36	0.18	0.06
Icelandic	−1.05	−0.97	−0.75	−0.55	−0.28	−0.12	−0.03	−2.39	−2.12	−1.54	−1.10	−0.56	−0.24	−0.07
Amish	0.29	0.30	0.30	0.29	0.23	0.13	0.05	0.31	0.33	0.36	0.36	0.27	0.14	0.05
Total	−0.93	−0.68	−0.13	0.22	0.38	0.27	0.11	−1.66	−1.37	−0.69	−0.25	0.07	0.08	0.04
HDAT-*Taq*I Dom 0.85														
UCSD/UBC	−1.01	−0.65	−0.20	0.03	0.19	0.19	0.11	−1.88	−1.39	−0.55	−0.12	0.19	0.21	0.12
Icelandic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Amish	−4.26	−2.93	−1.67	−1.07	−0.49	−0.20	−0.06	−3.35	−2.54	−1.48	−0.92	−0.37	−0.11	−0.01
Total	−5.27	−3.58	−1.87	−1.04	−0.30	−0.01	0.05	−5.23	−3.93	−2.03	−1.04	−0.18	0.10	0.11
HDAT-*Taq*I Rec 0.50														
UCSD/UBC	0.09	0.15	0.32	0.41	0.36	0.23	0.08	0.91	0.88	0.77	0.66	0.44	0.23	0.10
Icelandic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Amish	0.60	0.58	0.49	0.40	0.22	0.09	0.02	0.85	0.82	0.72	0.60	0.36	0.17	0.04
Total	0.69	0.73	0.81	0.81	0.58	0.32	0.10	**1.76**	1.70	1.49	1.26	0.80	0.40	0.14
HDAT-VNTR1 Dom 0.85														
UCSD/UBC	−8.38	−5.46	−2.14	−0.65	0.42	0.55	0.26	−9.53	−7.30	−3.68	−1.59	0.17	0.56	0.32
Icelandic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Amish	−7.64	−4.82	−2.43	−1.20	0.00	0.34	0.18	−12.65	−8.67	−4.40	−2.19	−0.28	0.20	0.09
Total	−16.02	−10.28	−4.57	−1.85	0.42	0.89	0.44	−22.18	−15.97	−8.08	−3.78	−0.11	0.76	0.41
HDAT-VNTR1 Rec 0.50														
UCSD/UBC	−1.04	−0.68	0.14	0.62	0.78	0.53	0.20	−1.33	−1.01	−0.15	0.39	0.64	0.47	0.14
Icelandic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Amish	−2.21	−1.77	−0.73	−0.09	0.38	0.39	0.22	−2.48	−2.08	−1.03	−0.32	0.25	0.32	0.18
Total	−3.25	−2.45	−0.59	0.53	**1.16**	0.92	0.42	−3.81	−3.09	−1.18	0.07	0.89	0.79	0.32
D5S392 Dom 0.85														
UCSD/UBC	−4.53	−2.48	−0.29	0.61	**1.02**	0.80	0.30	−6.19	−4.75	−2.27	−0.78	0.45	0.66	0.33
Icelandic	−4.47	−3.34	−2.08	−1.40	−0.69	−0.28	−0.07	−5.04	−4.13	−2.68	−1.81	−0.89	−0.38	−0.10
Amish	−11.56	−7.48	−3.63	−1.73	−0.17	0.20	0.06	−14.08	−10.10	−5.84	−3.47	−1.26	−0.56	−0.37
Total	−20.56	−13.30	−6.00	−2.52	0.16	0.72	0.29	−25.31	−18.98	−10.79	−6.06	−1.70	−0.28	−0.14
D5S392 Rec 0.50														
UCSD/UBC	−0.91	−0.59	0.22	0.64	0.75	0.48	0.18	−1.09	−0.56	0.28	0.71	0.79	0.51	0.18
Icelandic	−2.12	−1.95	−1.44	−1.01	−0.48	−0.19	−0.05	−1.42	−1.35	−1.09	−0.82	−0.43	−0.19	−0.04
Amish	−5.13	−4.48	−2.83	−1.69	−0.54	−0.08	0.04	−5.10	−4.48	−2.86	−1.72	−0.59	−0.15	−0.01
Total	−8.16	−7.02	−4.05	−2.06	−0.27	0.21	0.17	−7.61	−6.39	−3.67	−1.83	−0.23	0.17	0.13
D5S406 Dom 0.85														
UCSD/UBC	−7.19	−5.21	−2.86	−1.69	−0.66	−0.21	−0.05	−5.47	−4.25	−2.16	−0.93	0.10	0.34	0.19
Icelandic	−3.81	−2.70	−1.56	−1.02	−0.46	−0.18	−0.04	−3.76	−3.44	−2.36	−1.53	−0.69	−0.25	−0.06
Amish	−2.16	−1.21	−0.18	0.34	**0.58**	0.38	0.11	−4.62	−3.62	−1.78	−0.76	0.00	0.10	0.02
Total	−13.16	−9.12	−4.60	−2.37	−0.54	−0.01	0.02	−13.85	−11.31	−6.30	−3.22	−0.59	0.19	0.15
D5S406 Rec 0.50														
UCSD/UBC	−3.12	−2.80	−1.87	−1.18	−0.48	−0.18	−0.04	−3.29	−2.96	−2.05	−1.32	−0.52	−0.19	−0.04
Icelandic	−1.13	−1.03	−0.71	−0.47	−0.21	−0.08	−0.01	−1.13	−1.05	−0.76	−0.51	−0.21	−0.05	0.00
Amish	−1.88	−1.48	−0.63	−0.19	0.09	0.08	0.03	−2.38	−2.06	−1.23	−0.68	−0.19	−0.04	−0.01
Total	−6.13	−5.31	−3.21	−1.84	−0.60	−0.18	−0.02	−6.80	−6.07	−4.04	−2.51	−0.92	−0.28	−0.05

*Lod scores on left-hand side were generated using the "bipolar only" diagnostic model (model 1), while those at right used the "bipolar + recurrent major depression" model (model 2). Lod scores are indicated by population for each of the five markers under two of the genetic models: autosomal-dominant with a maximum penetrance of 0.85 (Dom 0.85), and autosomal-recessive with a maximum penetrance of 0.50 (Rec 0.85). The maximum lod score for each marker is in bold face.

tive in this pedigree. Examination of the pedigree indicates that all 4 bipolar subjects and 2 of the 4 recurrent unipolars shared the same haplotype for the three DAT markers and D5S392. Positive lod scores were also obtained at all three of the DAT markers in the Amish pedigree. The maximum lod score in the Amish was 1.09 at HDAT-PCR1, as described above. Family 16 is particularly notable because, although the family is not Amish, they live in Southeastern Pennsylvania approximately 30 miles from the Old Order Amish. They also share the same German/Swiss ancestry as the Old Order Amish and most of the "Pennsylvania Dutch"

TABLE IV. Two-Point Lod Scores in Individual Families

Locus	Genetic Model	Diagnostic definition	Family	Lod score
D5S392	Dom .85	BP	16	2.38
HDAT-VNTR1	Dom .85	BP	16	2.08
D5S406	Dom .5	BP + RD	16	1.49
HDAT-PCR1	Dom .85	BP + RD	Amish	1.09
HDAT-*Taq*I	Dom .85	BP + RD	Amish	0.85
HDAT-VNTR1	Dom .5	BP + RD	Amish	0.53
HDAT-PCR1	Rec .5	BP	24	0.45
HDAT-PCR1	Rec .5	BP	2106	0.42
HDAT-*Taq*I	Dom .85	BP + RD	10	0.45
D5S392	Dom .85	BP + RD	25	0.47
D5S392	Rec .5	BP	10	0.48
HDAT-VNTR1	Dom .85	BP + RD	25	0.54
HDAT-VNTR1	Rec .5	BP	10	0.48
HDAT-VNTR1	Rec .5	BP	2107	0.48

Dom .85 = model 1; Dom .5 = model 2; Rec .5 = model 3

population who live in this region. It is therefore appealing to hypothesize that these two families share a common mutation in the DAT gene that predisposes to bipolar disorder. Though the highest lod scores were obtained in these two families, other families in the UCSD/UBC family set also yielded positive lod scores at several of these markers, as shown in Table IV. In fact, as family 16 was uninformative at HDAT-*Taq*I and HDAT-PCR1, the positive lod scores at these markers for the UCSD/UBC population described above resulted entirely from families other than family 16.

Application of the admixture test using the HOMOG program failed to reveal any significant evidence for genetic heterogeneity. Similarly, lod scores calculated under an assumption of heterogeneity did not differ appreciably from those calculated under an assumption of homogeneity (data not shown). Lod scores were also calculated allowing for sex-specific recombination (data not shown). No maxima were found which significantly exceed those calculated under $\theta_m = \theta_f$. Multipoint analyses were also conducted for the UCSD/UBC families using the following map, which is derived from our data and which will be reported in detail elsewhere: pter–D5S392–(0.02)–HDAT-VNTR1–(0.08)–D5S406. These analyses excluded bipolar disorder from the interval examined under genetic model 1 and either diagnostic model. Assuming that bipolar disorder is heterogeneous and transmitted in a non-Mendelian fashion, these results are not surprising, given the work of Risch and Giuffra [1990], who demonstrated that multipoint analyses are less robust to violations of the assumed model than two-point analyses.

The results of nonparametric analysis using the APM method are shown in Table V. Using the f(p) = 1/sqrt(p) weighting function, evidence consistent with linkage was seen in the UCSD/UBC population. The marker HDAT-PCR1 yielded an APM statistic of 2.877, with an empirical *P* value of 0.001. Suggestive evidence was also seen at D5S392 and D5S406, with empirical *P* values of 0.025 and 0.021, respectively. In a multipoint analysis incorporating four of the markers, further evidence suggestive of linkage was obtained with an APM statistic of 3.11 and an empirical *P* value of 0.001. Results using the two alternative weighting functions are provided for comparison purposes. These functions provide either no weighting (f(p) = 1) or a strong weighting (f(p) = 1/p) for allele frequency. The function f(p) = 1/sqrt(p) provides an intermediate weighting, and is recommended by the developers of the test because of its greater power. The Icelandic and Amish samples provided no support for linkage using the APM method at any marker.

Table VI summarizes the results of the affected sib pair analysis using the ESPA program in the UCSD/UBC family set at the marker HDAT-PCR1. In these analyses, results consistent with linkage were obtained for both diagnostic models. These results were

TABLE V. APM Analysis*

Population	Locus	Weighting function	Statistic	Empirical *P*
UCSD/UBC	HDAT-PCR1	f(p) = 1/sqrt(p)	2.877	0.001
	HDAT-*Taq*I	f(p) = 1/sqrt(p)	0.821	0.213
	HDAT-VNTR1	f(p) = 1/sqrt(p)	0.034	0.452
	D5S392	f(p) = 1/sqrt(p)	2.154	0.025
	D5S406	f(p) = 1/sqrt(p)	2.441	0.021
	Multipoint[a]	f(p) = 1/sqrt(p)	3.116	0.001
	HDAT-PCR1	f(p) = 1	1.109	0.133
	HDAT-*Taq*I	f(p) = 1	0.069	0.472
	HDAT-VNTR1	f(p) = 1	−0.630	0.735
	D5S392	f(p) = 1	1.757	0.039
	D5S406	f(p) = 1	2.229	0.012
	Multipoint[a]	f(p) = 1	2.386	0.016
	HDAT-PCR1	f(p) = 1/p	2.918	0.002
	HDAT-*Taq*I	f(p) = 1/p	1.213	0.112
	HDAT-VNTR1	f(p) = 1/p	0.438	0.331
	D5S392	f(p) = 1/p	1.405	0.080
	D5S406	f(p) = 1/p	0.689	0.245
	Multipoint[a]	f(p) = 1/p	2.258	0.029
Amish	Not significant at any locus			
Icelandic	Not significant at any locus			

*Diagnostic model 2 (bipolar + recurrent depression) was used for these analyses.
[a]The following map was used for multipoint analysis: D5S392–(.02)–HDAT-PCR1–(.001)–HDAT-VNTR1–(.079)–D5S406.

TABLE VI. Extended Sib Pair Analysis (ESPA) for HDAT-PCR1 in UCSD/UBC Families

Diagnostic model		Not shared	Shared	χ^2	P
BP only	Completely known	0.0	8.0	8.00	0.0024
	ESPA	4.0	10.72	3.07	0.039
BP + RD	Completely known	0.0	10.0	10.00	0.0008
	ESPA	11.0	18.35	1.84	0.087

particularly strong when the analysis was restricted to alleles which were completely known. The results were reduced though still suggestive when other alleles were estimated using a maximum likelihood method (ESPA results). The fact that family 16 was relatively uninformative at this locus suggests that these results may have come primarily from other families in the collection. ESPA employs no correction for the nonindependence of sib pairs from sibships with three or more affecteds. However, an examination of allele sharing in individual families did not indicate that any single highly-affected sibship contributed disproportionately to the results. No other marker provided positive results in the UCSD/UBC families, and no evidence of linkage was obtained in the Amish or Icelandic samples.

As it was our hypothesis that the disease mutation was in DAT, and hence very close to the DAT markers, we tested our data for the presence of linkage disequilibrium, using a maximum likelihood-based version of the transmission disequilibrium test. These results are summarized in Table VII for the UCSD/UBC family set under diagnostic model 2 (BP + RD). No significant evidence of linkage disequilibrium was obtained for any of the five markers in our initial analysis, though there was a trend towards linkage disequilibrium for the marker HDAT-PCR1. Minisatellites have high mutation rates and have been implicated as disease-causing mutations [Krontiris, 1995]. In particular, a class of alleles in a given size range at the insulin 5′ VNTR has been demonstrated to contribute to the susceptibility to insulin-dependent diabetes mellitus. Based on this, we hypothesized that the size of the HDAT-VNTR1 allele might be associated with illness, even though no single specific allele was so associated. To test this, we pooled the HDAT-VNTR1 alleles into two groups based on size, such that each group had a frequency of approximately 50% in the combined family sample. Alleles of 5,500 bp or smaller were included in the group of smaller alleles, while those above 5,500 bp were in the larger allele group. Using these two created alleles, we obtained a maximum likelihood statistic of 3.87 (P = 0.024). Though not statistically significant, this does lend some suggestive support for the involvement of the VNTR in bipolar disorder.

TABLE VII. Transmission Disequilibrium Test in UCSD/UBC Families*

Marker	−2Ln(LR)	P
HDAT-*Taq*I	0.00	0.50
HDAT-VNTR1	0.513	0.23
HDAT-VNTR1 (two alleles)[a]	3.87	0.024
HDAT-PCR1	1.93	0.08
D5S392	1.13	0.143
D5S406	0.64	0.211

*These results employed diagnostic model 2 (bipolar + recurrent depression).
[a]HDAT-VNTR1 alleles were pooled into two groups, ≤ 5,500 bp and > 5,500 bp.

DISCUSSION

These data suggest the presence of a susceptibility locus for bipolar disorder near the DAT locus on chromosome 5p15.3. However, since the conventional criterion of a lod score of 3.0 is not reached, a statistically significant conclusion regarding linkage cannot be made. Furthermore, these results must also be qualified based on the number of tests conducted, and on arguments that even more stringent criteria should be employed for complex genetic disorders [Lander and Schork, 1994]. We chose to employ a variety of analytical methods because of the uncertainty regarding the mode of transmission of the disorder. Parametric methods (MLINK) clearly provide greater power when the mode of transmission is known. However, nonparametric methods (APM, ESPA, or TDT) may be more robust when the mode of transmission is unknown or heterogeneous. Such use of multiple methods may increase the risk of a false-positive. However, for this initial study, we felt it was more important to avoid falsely rejecting linkage.

Nevertheless, several factors argue that this region bears further examination in other samples. First, suggestive evidence of linkage was obtained at both multiple markers in this region, and in multiple families. The two families with the strongest positive lod scores, family 16 and the Amish family, are of very similar ancestry consistent with a common inherited risk locus. Second, consistent results were obtained using a variety of both parametric and nonparametric methods. Third, there is a compelling rationale for the involvement of the dopamine transporter.

We can conclude from these data that although linkage to DAT appears tight in some families, obligate crossovers occur in other families. This eliminates the hypothesis that the candidate gene DAT is causal in all families. However, it is possible that different mutations in DAT may exist, with differing patterns of transmission. Also, if DAT is a gene for bipolar disorder, it is likely to be one of many genes which contribute to the susceptibility to illness, and may operate in only a subset of families. A possible hint of such heterogeneity in our data set comes from suggestive evidence of linkage in family 16 to one marker (D18S35) in a region previously implicated by other investigators on chromosome

18 [Kelsoe et al., 1995; Stine et al., 1995; Berrettini et al., 1994]. Both between- and within-family heterogeneity is likely to confound detection of linkage, and may be reflected in the more positive results which we obtained using nonparametric methods, and the detection of positive lod scores under both dominant and recessive models. Yet our analyses using HOMOG failed to detect heterogeneity. This is likely due to the fact that our families were relatively small, and hence with limited power to detect heterogeneity.

Coon et al. [1993] reported on the only other study of the DAT locus in bipolar families. In their set of eight families they obtained strongly negative lod scores which excluded linkage to a 20-cM interval around DAT. However, their analyses were conducted under only one autosomal-dominant model of transmission. Furthermore, these families were ascertained in Utah, and though they were of primarily European ancestry, they may have represented a somewhat different subgroup than the two families of German/Swiss ancestry who yielded the highest lod scores in our data. Detera-Wadleigh et al. [1992] conducted a study of 24 RFLP markers spanning chromosome 5 in 14 bipolar pedigrees. Though overall their data excluded linkage to the entirety of chromosome 5, inspection of lod scores for individual families near the 5pter region indicated three families with lod scores in the range of 0.7–1.3 at the markers D5S10 and D5S12. D5S10 mapped to an interval between 0–9 cM proximal to D5S392, and D5S12 between 9–15 cM proximal to D5S392 [Murray et al., 1994]. In an 81-member subset of Amish pedigree 110, Pakstis et al. [1991] examined several RFLP markers in the 5p15.3 region. Though these data were largely negative, one marker, D5S12, was consistent with our data, yielding a maximum lod score of 0.50 at $\theta = 0.10$. Hence, there is some suggestion in the existing literature of soft evidence of linkage in this region in other families, as well as in a subset of the same Amish family we examined.

Our findings are intriguing in the light of a recent report by Cook et al. [1995] of association between DAT and attention deficit disorder (ADD). The attentional impairment and hyperactivity of this syndrome share some similarities with symptoms seen in mania [Biederman et al., 1991b]. An overlap between these disorders is further suggested by elevated rates of childhood ADD in adults with bipolar disorder [Winokur et al., 1993]. Furthermore, several epidemiological studies have suggested a possible genetic relationship between the disorders. Bipolar disorder has been reported to occur more frequently among parents of children with ADD as compared to normal controls [Biederman et al., 1991a]. Similarly, children of bipolar parents have been observed to have an elevated risk for ADD [Carlson and Weintraub, 1993].

The dopamine transporter has also been implicated by Gelernter et al. [1994] as a predisposing factor for paranoia and psychosis among chronic abusers of cocaine. In a case-control association study of chronic cocaine abusers, they found evidence of association of paranoia to the 40-bp 3′ repeat polymorphism in the DAT gene.

The theory that a defect in DAT results in an amphetamine-like dysregulation of dopamine release that predisposes to bipolar disorder is very appealing. However, caution should be observed in interpreting our findings. It is possible that although DAT led us to examine this region, the actual predisposing locus could instead be another nearby gene. Numerous genes are likely to reside in the region implicated by our data. One possible alternative candidate is the gene for a brain adenylate cyclase (ADCY2) which has been recently cloned by homology and mapped by in situ hybridization to 5p15.3 [Stengel et al., 1992]. Abnormalities in adenylate cyclase or other proteins involved in signal transduction have long been hypothesized to be involved in both bipolar disorder and the mechanism of action of lithium.

Though our results are not conclusive, they should provide encouragement for other investigators to examine this region in their samples. Confirmation of our results may then come through replication of linkage in independent family collections, or through demonstration of linkage disequilibrium. Association studies of DAT or ADCY2 would more specifically test each of these candidates. Lastly, studies are warranted which directly examine the DAT gene in individuals with bipolar disorder for coding sequence variants, splicing errors, or other abnormalities of gene structure and function which might predispose to illness.

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REFERENCES

Angrist B (1994): Clinical variations of amphetamine psychosis. In Cho AK, Segal DS (eds): "Amphetamine and Its Analogs." San Diego: Academic Press, pp 387–414.

Berrettini WH, Ferraro TN, Goldin LR, Weeks DE, Detera-Wadleigh S, Nurnberger JI, Gershon ES (1994): Chromosome 18 DNA markers and manic-depressive illness: Evidence for a susceptibility gene. Proc Natl Acad Sci USA 91:5918–5921.

Biederman J, Faraone SV, Keenan K, Tsuang MT (1991a): Evidence of familial association between attention deficit disorder and major affective disorders. Arch Gen Psychiatry 48:633–642.

Biederman J, Newcorn J, Sprich S (1991b): Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. Am J Psychiatry 148:564–577.

Boehnke M (1991): Allele frequency estimation from data on relatives. Am J Hum Genet 48:22–25.

Braverman MS (1985): An algorithm to improve the computational efficiency of genetic linkage analysis. Comput Biomed Res 18:24–36.

Byerley W, Hoff M, Holik J, Caron MG, Giros B (1993): VNTR polymorphism for the human dopamine transporter gene (DAT1). Hum Mol Genet 2:335.

Carlson GA, Weintraub S (1993): Childhood behavior problems and bipolar disorder—Relationship or coincidence? J Affective Disord 28:143–153.

Cook EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE, Leventhal BL (1995): Association of attention-deficit disorder and the dopamine transporter gene. Am J Hum Genet 56:993–998.

Coon H, Jensen S, Hoff M, Holik J, Plaetke R, Reimherr F, Wender P, *Leppert M, Byerley W* (1993): A genome-wide search for genes predisposing to manic-depression, assuming autosomal dominant inheritance. Am J Hum Genet 52:1234–1249.

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

Detera-Wadleigh SD, Berrettini WH, Goldin LR, Martinez M, Hsieh WT, Hoehe MR, Encio IJ, Coffman D, Rollins DY, Muniec D, Choi H, Wiesch D, Guroff J, Gershon ES (1992): A systematic search for a bipolar predisposing locus on chromosome 5. Neuropsychopharmacology 6:219–229.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991): "Touchdown" PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 19:4008.

Egeland JA, Hostetter AM (1983): Amish Study, I: Affective disorders among the Amish, 1976–1980. Am J Psychiatry 140:56–61.

Egeland JA, Sussex JN, Endicott J Hostetter AM, Offord DR, Schwab JJ, Allen CR, Pauls DL (1990): The impact of diagnoses on genetic linkage study for bipolar affective disorders among the Amish. Psychiatr Genet 1:5–18.

Endicott J, Spitzer R (1978): A diagnostic interview: The Schedule for Affective Disorders and Schizophrenia. Arch Gen Psychiatry 35: 837–844.

Gelernter J, Kranzler HR, Satel SL, Rao PA (1994): Genetic association between dopamine transporter protein alleles and cocaine-induced paranoia. Neuropsychopharmacology 11:195–200.

Giros B, Caron MG (1993): Molecular characterization of the dopamine transporter. Trends Pharmacol Sci 14:43–49.

Giros B, el Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, Caron MG (1992): Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. Mol Pharmacol 42:383–390.

Goodwin FK, Jameson KR (1990): "Manic-Depressive Illness." New York: Oxford University Press pp 614–615.

Helgason T (1979): Epidemeological investigations concerning affective disorders. In Schou M, Stromgren E (eds): "Origin, Prevention and Treatment of Affective Disorders." London: Academic Press, pp 241–256.

Jimerson DC (1987): Role of dopamine mechanisms in the affective disorders. In Meltzer HY (ed): "Psychopharmacology: The Third Generation of Progress." New York: Raven Press, pp 505–511.

Kelsoe JR, Ginns EI, Egeland JA, Gerhard DS, Goldstein AM, Bale SJ, Pauls DL, Long RT, Kidd KK, Conte G, Housman DE, Paul SM (1989): Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish. Nature 342:238–243.

Kelsoe JR, Kristbjarnarson H, Bergesch P, Shilling P, Hirsch S, Mirow AL, Moises HW, Helgason T, Gillin JC, Egeland JA (1993): A genetic linkage study of bipolar disorder and 13 markers on chromosome 11 including the D2 dopamine receptor. Neuropsychopharmacology 9:293–301.

Kelsoe JR, Sadovnick AD, Kristbjarnarson H, Bergesch P, Mroczkowski-Parker Z, Flodman PL, Rapaport MH, Mirow AL, Egeland JA, Spence MA, Remick RA (1995): Genetic linkage studies of bipolar disorder and chromosome 18 markers in North American, Icelandic and Amish pedigrees. Psychiatr Genet 5:17–18.

Kilty JE, Lorang D, Amara SG (1991): Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254:578–579.

Krontiris TG (1995): Minisatellites and human disease. Science 269:1682–1683.

Kuczenski R, Segal DS (1994): Neurochemistry of amphetamine. In Cho AK, Segal DS (eds): "Amphetamine and Its Analogs." San Diego: Academic Press, pp 81–114.

Lander ES, Schork NJ (1994): Genetic dissection of complex traits. Science 265:2037–2048.

Lathrop GM, Lalouel JM, Julier C, Ott J (1984): Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446.

Mirow AL, Kristbjarnarson H, Egeland JA, Helgason T, Gillin JC, Hirsch S, Kelsoe JR (1994): A linkage study of distal chromosome 5q and bipolar disorder. Biol Psychiatry 36:223–229.

Murphy DL, Brodie HK, Goodwin FK, Bunney WE (1971): Regular induction of hypomania by L-DOPA in "bipolar" manic-depressive patients. Nature 229:135–136.

Murray JC, Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Manion F, Quillen J, Sheffield VC, Sunden S, Duyk GM (1994): A comprehensive human linkage map with centimorgan density. Cooperative Human Linkage Center (CHLC). Science 265:2049–2054.

Ott J (1991): "Analysis of Human Genetic Linkage." Baltimore: Johns Hopkins University Press, pp 203–213.

Pakstis AJ, Kidd JR, Castiglione CM, Kidd KK (1991): Status of the search for a major genetic locus for affective disorder in the Old Order Amish. Hum Genet 87:475–483.

Ploughman LM, Boehnke M (1989): Estimating the power of a proposed linkage study for a complex genetic trait. Am J Hum Genet 44:543–551.

Risch N, Giuffra L (1990): Multipoint linkage analysis of genetically complex traits. Am J Hum Genet 47:197.

Sachs GS, Lafer B, Stoll AL, Banov M, Thibault AB, Tohen M, Rosenbaum JF (1994): A double-blind trial of bupropion versus desipramine for bipolar depression. J Clin Psychiatry 55:391–393.

Sandkuyl LA (1989): Analysis of affected sib pairs using information from extended families. Prog Clin Biol Res 329:117–122.

Schaffer AA, Gupta SK, Shriram K, Cottingham RW Jr (1994): Avoiding recomputation in genetic linkage analysis. Hum Hered 44: 225–237.

Shimada S, Kitayama S, Chien-Liang L, Patel A, Nanthakumar E, Gregor P, Kuhar M, Uhl G (1991): Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. Science 254:576–578.

Spielman RS, McGinnis RE, Ewens WJ (1993): Transmission test for linkage disequilibrium: The insulin gene region and insulin-dependent diabete mellitus (IDDM). Am J Hum Genet 52:506–516.

Spitzer R, Endicott J, Robins E (1978): Research diagnostic criteria: Rationale and reliability. Arch Gen Psychiatry 35:773–782.

Spitzer RL, Williams JBW, Gibbon M (1987): "Structured Clinical Interview for DSM-III-R (SCID)." New York: New York State Psychiatric Institute, Biometrics Research.

Stengel D, Parma J, Gannage MH, Roeckel N, Mattei MG, Barouki R, Hanoune J (1992): Different chromosomal localization of two adenylyl cyclase genes expressed in human brain. Hum Genet 90:126–130.

Stine OC, Xu J, Koskela R, McMahon FJ, Gschwend M, Friddle C, Clark CD, McInnis MG, Simpson SG, Breschel TS (1995): Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect. Am J Hum Genet 57:1384–1394.

Terwilliger JD (1995): A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic loci. Am J Hum Genet 56:777–787.

Vandenbergh DJ, Perisco AM, Uhl GR (1992a): A human dopamine transporter cDNA predicts reduced glycosylation, displays a novel repetitive element and provides racially-dimorphic TaqI RFLPs. Mol Brain Res 15:161–166.

Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, Uhl GR (1992b): Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 14: 1104–1106.

Weeks DE, Lange K (1988): The affected-pedigree-member method of linkage analysis. Am J Hum Genet 42:315–326.

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, Lathrop M (1992): A second-generation linkage map of the human genome. Nature 359:794–801.

Winokur G, Coryell W, Endicott J, Akiskal H (1993): Further distinctions between manic-depressive illness (bipolar disorder) and primary depressive disorder (unipolar depression). Am J Psychiatry 150:1176–1181.