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Brief Research Communication

Genetic Linkage Study of Bipolar Disorder and the Serotonin Transporter

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The serotonin transporter (HTT) is an important candidate gene for the genetic transmission of bipolar disorder. It is the site of action of many antidepressants, and plays a key role in the regulation of serotonin neurotransmission. Many studies of affectively ill patients have found abnormalities in serotonin metabolism, and dysregulation of the transporter itself. The human serotonin transporter has been recently cloned and mapped to chromosome 17. We have identified a *PstI* RFLP at the HTT locus, and here report our examination of this polymorphism for possible linkage to bipolar disorder. Eighteen families were examined from three populations: the Old Order Amish, Iceland, and the general North American population. In addition to HTT, three other microsatellite markers were examined, which span an interval known to contain HTT. Linkage analyses were conducted under both dominant and recessive models, as well as both narrow (bipolar only) and broad (bipolar + recurrent unipolar) diagnostic models. Linkage could be excluded to HTT under all models examined. Linkage to the interval spanned by the microsatellites was similarly excluded under the dominant models. In two individual families, maximum lod scores of 1.02 and 0.84 were obtained at D17S798 and HTT, respectively. However, these data overall do not support the presence of a susceptibility locus for bipolar disorder near the serotonin transporter.

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INTRODUCTION

The serotonin transporter gene (HTT) is a particularly interesting candidate for the genetic etiology of affective disorders because of its role both in the regulation of serotonergic neurotransmission, and in the mechanism of action of many antidepressant medications. By actively mediating the reuptake of serotonin into the presynaptic terminal, the transporter plays a key role in the regulation of synaptic serotonin levels. Abnormalities in serotonin metabolism have long been implicated in the pathophysiology of affective disorders. Some of these abnormalities include: decreased 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid of depressed patients [Asberg et al., 1976], decreased binding of radiolabeled imipramine in postmortem brains from suicide victims [Stanley et al., 1982], and decreased platelet reuptake of serotonin in depressed subjects [Tuomisto and Tukiainen, 1976]. Blockade of the transporter is key to the mechanism of action of a variety of antidepressant medications, including the new generation of selective serotonin reuptake inhibitors (SSRI).

The recent cloning of the human HTT gene has made possible investigations into its potential role in a variety of neuropsychiatric illnesses [Ramamoorthy et al., 1993; Lesch et al., 1993]. The gene belongs to a family of neurotransmitter transporter genes, which includes those for norepinephrine and dopamine, and has been mapped by in situ hybridization to chromosome 17q11.1–17q12 [Ramamoorthy et al., 1993]. We are currently surveying the genome for polymorphic loci genetically linked to bipolar disorder in families from three populations: the Old Order Amish, Iceland, and the general North American population. We now report our examination of the

HTT gene using a newly identified *Pst*I RFLP, and three nearby microsatellite markers.

MATERIALS AND METHODS

The ascertainment and diagnostic procedures employed in the Old Order Amish and Icelandic populations have been described in previous reports of our genome survey [Kelsoe et al., 1993; Mirow et al., 1994]. Old Order Amish pedigree 110, as studied in this report, was identical to the membership previously described, and included 118 subjects, 22 with bipolar disorder and 7 with recurrent unipolar depression. Three Icelandic pedigrees included 53 subjects, 11 with bipolar disorder, and 6 with recurrent unipolar depression.

The third group of families in this study included smaller families drawn from the general North American population, and ascertained primarily at two sites: San Diego and Vancouver. All subjects at each site were directly interviewed using the Structured Clinical Interview for DSM-3-R (SCID) [Spitzer et al., 1987], or the SADS-L modified for DSM-3-R (Family 16 only) [Endicott and Spitzer, 1978]. Diagnoses at each site were made by a best-estimate procedure using DSM-3-R criteria [American Psychiatric Association, 1987], in which the interview, medical records, and information from other family informants were reviewed by a panel of psychiatric clinicians, blind to genotypic data. All interviewers underwent an extensive training session for the SCID instrument, and reliability was monitored by independent and blind scoring of videotaped SCID interviews at both sites and between sites. Fourteen families were included in this general population family set. Of 127 members on whom both diagnoses and DNA were available, 35 of these had bipolar disorder, and 13 had recurrent major depression.

The HTT locus was examined using a *Pst*I RFLP which we have recently identified. This RFLP and genetic mapping of the HTT locus will be reported separately [Kelsoe et al., in preparation]. A 1-kb fragment containing the 3′ end of the gene was subcloned from the 2.5-kb full-length cDNA, hSERT [Ramamoorthy et al., 1993], and used as a probe. Five μg of genomic DNA were digested with *Pst*I, and the RFLP was detected as described previously [Kelsoe et al., 1993]. This *Pst*I RFLP and a PCR-based assay to detect it have also been described by Gelernter and Freimer [1994].

Three microsatellite markers (D17S783, D17S798, and D17S799) were chosen from the Généthon map of 1992 [Weissenbach et al., 1992] because of their location near the site to which HTT had been approximately mapped by in situ hybridization [Ramamoorthy et al., 1993]. Primers for these markers were obtained from Research Genetics (Huntsville, AL). These microsatellite markers were amplified and detected as described previously [Mirow et al., 1994].

Linkage analyses were conducted using the MLINK program from the FASTLINK package [Cottingham et al., 1993; Schaffer et al., 1994]. Analyses were conducted under both a narrow diagnostic model in which subjects with bipolar I, bipolar II, and schizoaffective disorder were considered affected, as well as a broader model which also included subjects with recurrent major depression. Three genetic models of transmission were considered, as illustrated in Table I. Age-dependent penetrance was modeled by five liability classes so as to increase linearly from the minimum at age 14 years, to the maximum at age 30 years. Allele frequencies were estimated from the families themselves. Heterogeneity analyses were conducted using HOMOG [Ott, 1991]. An "affecteds only" nonparametric analysis was conducted using the Affected Pedigree Member method already defined (APM) [Weeks and Lange, 1988]. In these analyses, the 1/sqr weighting function was employed, and statistical significance was estimated by simulation. The power of parametric analyses was estimated using SIMLINK [Ploughman and Boehnke, 1989].

RESULTS

Power analyses of our sample suggest that we are very unlikely to falsely exclude a common linked locus. Under assumptions of 50% heterogeneity and a tightly linked marker, our sample has a 52% probability of obtaining a lod >2, and a 33% probability of a lod >3. The probability of excluding an unlinked locus is 95% out to 5 cM. The two-point lod scores for affection status vs. HTT and the three microsatellite markers under the broad diagnostic model are summarized in Table II. No lod scores indicating statistically significant linkage to bipolar disorder were obtained under any model examined. For HTT itself, linkage could be excluded to at least 5 cM for each of the three genetic models and both diagnostic models. The maximum lod obtained for any single family under any of the models was 0.84 for family 16 in the UCSD/UBC family set under model 1, using the broad diagnostic definition at $\theta = 0$. Analyses under heterogeneity were uniformly negative under all models. APM analysis provided no support for linkage (statistic = 0.63, $P = 0.26$).

Similarly, the three nearby microsatellite markers yielded no statistically significant evidence of linkage. Only data from the broad diagnostic definition and genetic model 1 are presented in Table II, but no significant lods were obtained for any of the three markers under any of the models examined. Under model 1 and the broad diagnostic definition, linkage can be excluded to the entire 22-cM interval spanned by these three microsatellites. We have demonstrated HTT to map within this interval [Kelsoe et al., in preparation]. Linkage is similarly excluded to all three microsatellite markers under each of the other models, though with smaller exclusion intervals. The maximum lod for any individual family for these three markers was 1.02, obtained with D17S798 at $\theta = 0$ in family 10 of the UCSD/UBC family set under model 1 and the broad diagnostic definition. Analyses under heterogeneity

TABLE I. 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 the disease gene; f1, penetrance of AA, where A is the disease allele; f2, penetrance of Aa; f3, penetrance of aa.

TABLE II. Two-Point LOD Scores at HTT and Three Microsatellite Markers*

Locus	Model	θ 0.00	0.01	0.05	0.10	0.20	0.30	0.40
HTT	1	−11.916	−10.100	−6.631	−4.314	−1.806	−0.644	−0.169
	2	−6.235	−5.484	−3.644	−2.354	−0.988	−0.378	−0.119
	3	−4.509	−3.979	−2.721	−1.827	−0.867	−0.381	−0.119
D17S783	1	−15.805	−12.719	−7.975	−5.116	−2.275	−0.970	−0.311
D17S798	1	−25.530	−20.627	−13.125	−8.196	−3.154	−0.936	−0.100
D17S799	1	−11.138	−10.056	−7.640	−5.553	−2.794	−1.260	−0.444

* These analyses were conducted using a broad definition of phenotype, which includes bipolar I, bipolar II, schizoaffective, and recurrent major depression diagnoses as affected.

were negative for all three microsatellite markers under all models. APM, similarly, yielded no evidence for linkage. The APM statistics for D17S783, D17S798, and D17S799 were 1.59 ($P = 0.059$), 0.39 ($P = 0.35$), and −0.31 ($P = 0.60$), respectively.

DISCUSSION

These data indicate that HTT is not a common major locus for bipolar disorder in the populations which we examined. Though no other linkage study of HTT itself has been reported to date, these results are consistent with several reports of genome surveys which have failed to find evidence of linkage to markers in this general region on chromosome 17 [Berrettini et al., 1991; Coon et al., 1993]. Our results are also consistent with a recent report by Lesch et al. [1995], who sequenced HTT mRNA derived from platelets from 7 unipolar and 10 bipolar subjects and found no significant coding sequence variations.

Our results must be qualified based on the limitations of sample size and the models tested. If the mode of transmission of bipolar disorder varies significantly from the models examined, or if HTT is an uncommon disease locus or one of small effect, our power to detect a gene would be reduced. However, in summary, our data indicate that HTT is not a common major locus for bipolar disorder in the populations which we examined.

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