Nonlinkage of Bipolar Illness to Tyrosine Hydroxylase, Tyrosinase, and D_2 and D_4 Dopamine Receptor Genes on Chromosome 11

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Objective: *Previous linkage and allelic association studies using DNA polymorphisms, cosegregation of cytogenetic abnormalities with psychiatric illness, and assignment of genes involved in neurotransmitter metabolism suggested that chromosome 11 may harbor a gene predisposing to bipolar illness. The authors examined linkage in the families of 14 probands with bipolar illness, with the candidate genes tyrosine hydroxylase (TH), D_4 dopamine receptor (DRD4) at 11p15, tyrosinase (TYR) at 11q14-q21, and D_2 dopamine receptor (DRD2) at 11q22-q23, as well as with the c-Harvey-ras oncogene (HRAS) and insulin gene (INS), both located at 11p15, a region that previously showed linkage to bipolar illness.* *Method:* *The genetic data were analyzed with both lod score analysis (parametric) and affected-sib-pair analysis (nonparametric); both narrow and broad definitions of the clinical phenotype were used. Further influences of diagnostic uncertainties were accounted for by using diagnostic probability classes weighing the stability of each phenotype.* *Results:* *Two-point linkage results excluded close linkage of bipolar illness to each candidate gene; negative results were also obtained when the narrow definition of the clinical phenotype was used. Moreover, multipoint linkage analysis of HRAS and INS excluded the 11p15 region encompassing both DRD4 and TH. In agreement with the negative linkage results, affected-sib-pair analysis did not show preferential sharing of marker alleles at any of the candidate genes.* *Conclusions:* *The negative results obtained under different genetic models exclude a frequent role for DRD4, TH, TYR, and DRD2 in the pathogenesis of bipolar illness.*

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Bipolar illness is characterized by major depressive episodes alternating with phases of mania (bipolar I disorder) or hypomania (bipolar II disorder), and it affects 0.5% to 2.0% of the population. The bipolar spectrum diagnoses are defined as bipolar illness (bipolar I and II), the manic and depressive subtypes of schizoaffective disorder, recurrent unipolar illness, and major depressive disorders. Sometimes cyclothymia is also included, and minor depression is usually not.

Several lines of evidence suggest that a gene (or genes) on chromosome 11 may be implicated in the etiology of bipolar illness. Linkage of bipolar illness with the c-Harvey-*ras* oncogene (*HRAS*) and the insulin gene (*INS*), both located at 11p15, was reported in an Old Order Amish pedigree (1). The initial linkage, however, disappeared owing to bipolar spectrum diagnoses in at-risk individuals and lateral extensions of the core pedigree, suggesting that the initial linkage results were a spurious finding (2, 3). Nonetheless, a major gene for bipolar illness at 11p15 could not be excluded since extension of the core pedigree may have led to intrafamilial genetic heterogeneity and, consequently, false exclusion of linkage (2). Also, the possibility remained that the positive linkage data for the core pedigree rep-

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TABLE 1. Affective Disorders in Families of 14 Probands With Bipolar Illness

Family	Genera-tions	Proband Diagnosis	Family Members Studied	Family Members Affected: Total	Bipolar I	Bipolar II	Schizo-affective, Manic	Schizo-affective, Depressed	Major Depression	Recurrent Unipolar	Cyclo-thymia	Minor Depression
1	4	Bipolar I	11	6	2	2	0	0	0	2	0	0
2	2	Bipolar I	6	3	1	1	0	0	1	0	0	0
3	2	Bipolar I	9	5	2	0	0	0	0	2	1	0
4	3	Bipolar II	6	3	0	2	0	0	0	0	1	0
5	3	Bipolar I	9	6	2	1	0	0	2	1	0	0
6	2	Bipolar II	11	6	0	2	0	0	0	3	1	0
7	3	Bipolar II	6	3	0	2	0	0	0	1	0	0
8	3	Bipolar II	15	6	0	4	0	0	1	1	0	0
9	4	Bipolar II	33	12	1	1	1	1	7	1	0	0
10	3	Bipolar I	16	7	2	1	0	0	1	1	0	2
11	3	Bipolar I	10	6	1	2	0	0	1	1	1	0
12	4	Bipolar II	10	4	0	2	0	0	0	2	0	0
13	4	Bipolar I	16	10	1	0	0	0	3	4	0	2
14	3	Bipolar II	10	6	2	1	0	0	0	0	1	2
Total			168	83	14	21	1	1	16	19	5	6

resented the effect of a modifier gene (3). Candidate modifier genes are the gene for the D_4 dopamine receptor (*DRD4*), a high-affinity receptor for clozapine (4), and the gene for tyrosine hydroxylase (*TH*), the rate-limiting enzyme in catecholamine synthesis; both genes are close to *HRAS* and *INS* (5, 6). The modifier gene hypothesis is in part supported by a French study showing a significant association between *TH* and bipolar disorder (7).

Three chromosomal translocations involving 11q21-q25 were found cosegregating with psychiatric illness (8–10). This region harbors two potential candidate genes: the D_2 dopamine receptor (*DRD2*) and tyrosinase (*TYR*) genes. The D_2 dopamine receptor is one of the major sites of action of neuroleptic agents, such as haloperidol (11). Tyrosinase is not directly involved in neurotransmitter metabolism, but some families in which schizophrenia and schizoaffective disorder cosegregate with tyrosinase-negative oculocutaneous albinism have been described (12, 13).

A number of studies do not support the existence of a bipolar illness gene on chromosome 11 (for review, see 14). However, it has been documented that in linkage studies of complex diseases such as bipolar illness, spurious linkage findings may be produced more easily owing to phenotypic misclassifications and to misspecification of the disease model. For these reasons, we analyzed the families of 14 probands with bipolar I or bipolar II disorder for linkage to 11p15, including the candidate genes *TH* and *DRD4*, and to *TYR* and *DRD2*, at 11q14-q21 and 11q22-q23, respectively.

METHOD

Family Data

The families in this study were ascertained at the Erasme Hospital, Brussels, and included 12 families of Belgian ancestry, one Jewish Ashkenazi family (family 3), and one Italian family (family 8) (table 1). Two families (families 1 and 2) that were included in a previous study of linkage with the factor IX gene (15) were restudied. After giving informed consent, all probands, spouses, and available relatives were separately interviewed by an experienced clinician (K.M., V.D., D.H., L.S., or J.M.) using the Schedule for Affective Disorders and Schizophrenia—Lifetime Version (16), and diagnoses were based on the Research Diagnostic Criteria (17). The mean age at onset in the subjects with a bipolar spectrum diagnosis was 26.7 years (SD=8.9), and the range was 10–50 years.

DNA Analysis

All manipulations including isolation of genomic, plasmid, and phage DNA, digestion with restriction enzymes, Southern blot transfer of genomic DNA, and hybridization with radiolabeled plasmid or phage DNA were done according to standard procedures (18).

In our subjects, polymorphic alleles recognized by pHGTH4 (*TH*), pJ4.7 (*TH*), and λHD2G1 (*DRD2*) on *Pst*I, *Taq*I, and *Taq*I, respectively, were as described previously (7, 19). With phins310 (*INS*) on *Pvu*II we found multiple alleles, which we divided into three size classes: 2.2 kb or larger, between 1.0 and 0.7 kb, and 0.6 kb or smaller. With pUCEJ6.6 (*HRAS*) on *Taq*I we observed seven alleles with lengths of 4.4 kb, 3.7 kb, 3.5 kb, 3.3 kb, 3.2 kb, 2.9 kb, and 2.7 kb. With pB28 (*DRD4*) on *Hinc*II we detected six alleles with lengths of 6.0 kb, 5.8 kb, 4.2 kb, 4.1 kb, 4.0 kb, and 3.9 kb, resulting from a polymorphic 48-base-pair repeat sequence, described elsewhere (20).

Primers flanking the $(GA)_n$ and $(CA)_n$ polymorphisms near *TYR* and *DRD2*, respectively, and conditions of the polymerase chain reaction were as described previously (21, 22). The amplification product was analyzed on a 6% denaturating polyacrylamide gel.

Linkage Analysis

Lod scores were calculated by using the LINKAGE programs, version 5.1 (23), a bipolar illness gene frequency of 0.01, and a phenocopy rate of 0.0001. The female-male recombination ratio was set at 1.0, but no significant changes in the lod scores were obtained when we used published recombination ratios at 11p15 and 11q22-q23 (24). A disease penetrance of 0.91 in persons 60 years old or older was estimated with the LINKAGE program ILINK (25). We used the following penetrance values for each age class—15–19 years—0.10 (N=7), 20–24 years—0.26 (N=10), 25–29 years—0.39 (N=6), 30–34 years—0.54 (N=9), 35–39 years—0.61 (N=7), 40–44 years—0.70 (N=9), 45–49 years—0.75 (N=6), 50–54 years—0.82 (N=5), 55–59 years—0.87 (N=2), and ≥60 years—0.91 (N=14).

Different disease models were specified before the linkage analysis.

TABLE 2. Two-Point Lod Scores for Linkage of Bipolar Illness to Six Genes in Families of 14 Probands With Bipolar Illness

Gene and Model[a]	Informative Families	Recombination Fraction (θ)						Exclusion Limit θ (z=–2)
		0.00	0.01	0.05	0.10	0.20	0.30	
DRD4								
Model 1	6	–0.96	–0.87	–0.64	–0.47	–0.26	–0.14	
Model 2	10	–5.68	–4.27	–2.91	–2.03	–0.99	–0.46	0.10
Model 3	9	–0.89	–0.83	–0.64	–0.48	–0.28	–0.16	
HRAS								
Model 1	7	–4.31	–3.77	–2.56	–1.74	–0.85	–0.38	0.08
Model 2	12	–11.38	–8.59	–5.40	–3.61	–1.59	–0.58	0.18
Model 3	10	–5.90	–4.75	–2.96	–1.94	–0.91	–0.40	0.10
INS								
Model 1	8	–3.19	–2.36	–1.41	–0.88	–0.35	–0.12	0.03
Model 2	13	–9.13	–6.05	–3.35	–2.22	–1.18	–0.56	0.12
Model 3	12	–3.87	–3.08	–2.01	–1.36	–0.66	–0.28	0.05
TH								
Model 1	6	–3.67	–2.79	–1.82	–1.24	–0.61	–0.28	0.04
Model 2	12	–8.21	–5.30	–2.60	–1.30	–0.26	0.05	0.07
Model 3	10	–3.32	–2.47	–1.46	–0.91	–0.39	–0.17	0.03
TYR								
Model 1	8	–2.68	–2.25	–1.36	–0.82	–0.33	–0.13	0.02
Model 2	13	–10.83	–8.31	–4.94	–3.04	–1.15	–0.32	0.16
Model 3	10	–3.40	–2.94	–1.95	–1.28	–0.56	–0.22	0.05
DRD2								
Model 1	5	–2.13	–1.81	–1.18	–0.78	–0.35	–0.14	
Model 2	11	–6.53	–5.45	–3.75	–2.62	–1.34	–0.61	0.15
Model 3	9	–2.27	–1.87	–1.21	–0.82	–0.39	–0.17	0.01

[a]Model 1: bipolar I disorder and schizoaffective disorder—manic. Model 2: bipolar I disorder, schizoaffective disorder—manic, bipolar II disorder, schizoaffective disorder—depressed, unipolar illness, and major depression. Model 3: extension of model 2 in which patients with bipolar II disorder, schizoaffective disorder—depressed, unipolar illness, or major depression were assigned a diagnostic stability score based on number of episodes, number of symptoms during the most severe episode, and history of somatic treatment.

Models 1 and 2 were comparable to the classical narrow and broad phenotypic classifications used by Egeland et al. (1); model 1 included only patients with bipolar I disorder and patients with schizoaffective disorder—manic, and model 2 included patients with bipolar I disorder or schizoaffective disorder—manic plus patients with bipolar II disorder, schizoaffective disorder—depressed, unipolar illness, or major depression. Model 3 was an extension of model 2 in which the patients with bipolar II disorder, schizoaffective disorder—depressed, unipolar illness, or major depression were assigned a diagnostic stability score calculated on the basis of three clinical correlates: number of episodes, number of symptoms during the most severe episode, and a history of somatic treatment, as described by Rice et al. (26). Assuming that cases with a more stable diagnosis were more likely to be "genetic" cases, we calculated the odds of a genetic case versus a phenocopy. In our group, the 52 subjects with diagnoses other than bipolar I disorder and schizoaffective disorder—manic were grouped into a total of seven diagnostic probability classes, with odds of being genetic cases of 13.7 (N=1), 7.5 (N=1), 4.7 (N=9), 2.1 (N=14), 1.6 (N=1), 1.5 (N=21), and 1.1 (N=5) to 1, respectively. The phenotypes of the following individuals were excluded from the analysis: children aged 14 years or younger, noninterviewed adults, and patients with cyclothymia or minor depression. Affected spouses and their offspring were excluded from the analysis (in families 9, 11, and 13).

Marker allele frequencies and haplotype frequencies for haplotypes constructed for the *TH* and *DRD2* polymorphisms were calculated by using the 42 unaffected married-in individuals.

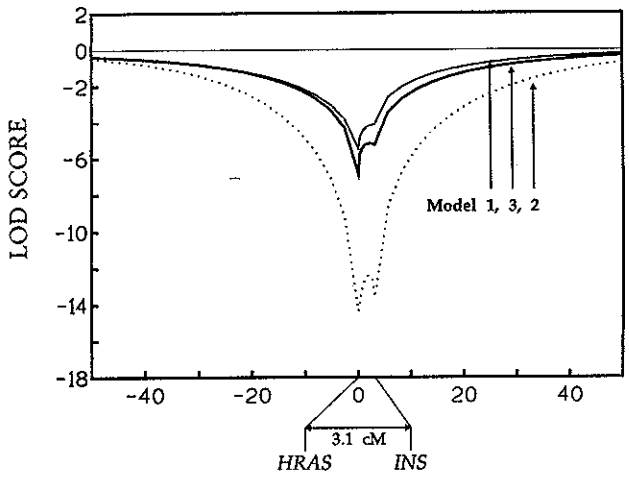
RESULTS AND DISCUSSION

Two-point lod scores for linkage of bipolar illness were calculated under models 1, 2, and 3 and are shown for the informative families in table 2. All markers gave negative lod scores under all models. From the lod score z=–2 we deduced the recombination fraction or exclusion limit θ representing the region on each side of the marker from which the disease gene can be significantly excluded. Under model 2, linkage with all markers was significantly excluded, with exclusion limits ranging from 0.07 to 0.18. Models 1 and 3 gave comparable lod scores, indicating that the bipolar II, schizoaffective—depressed, unipolar illness, and major depression phenotypes were not contributing much information in the linkage analysis. This can be explained by the fact that the majority of these patients had low diagnostic stability scores owing to lack of precise clinical information with respect to number of symptoms and episodes and requirement for medication. However, we could still exclude linkage of bipolar illness with *HRAS*, *INS*, *TH*, *TYR*, and *DRD2*, although the exclusion limits were smaller than under model 2. *DRD4* gave nonsignificant lod scores.

Three-point lod scores were calculated by using Haldane's mapping function with *HRAS* and *INS* fixed at 3.1 cM (6). Under all models, significant negative data were obtained (figure 1). Model 2 again gave the most negative results, while models 1 and 3 again gave similar results. The exact genetic locations of *DRD4* and *TH* are not known, but according to the most recent publications (5, 6) they are separated by a distance of 10.6 cM with *DRD4* located telomeric of *HRAS* and *TH* centromeric of *INS*. In models 1, 2, and 3 the exclusion limits of 20.1 cM, 50.8 cM, and 25.1 cM, respectively, comprised both *DRD4* and *TH*.

To examine the possibility that linkage of bipolar ill-

FIGURE 1. Three-Point Lod Scores for Linkage of Bipolar Illness to the Genes *DRD4, HRAS, INS, TH, TYR,* and *DRD2* in Families of 14 Probands With Bipolar Illness[a]



[a]Model 1: bipolar I disorder and schizoaffective disorder—manic. Model 2: bipolar I disorder, schizoaffective disorder—manic, bipolar II disorder, schizoaffective disorder—depressed, unipolar illness, and major depression. Model 3: extension of model 2 in which patients with bipolar II disorder, schizoaffective disorder—depressed, unipolar illness, or major depression were assigned a diagnostic stability score based on number of episodes, number of symptoms during the most severe episode, and history of somatic treatment.

ness to any of the candidate genes was confounded by the presence of asymptomatic gene carriers in our analysis, we also calculated the lod scores after eliminating the phenotype information of the unaffected subjects (age-dependent penetrance was zero). The results indicated that a substantial proportion of the exclusion data were derived from the unaffected subjects. However, the lod scores were still negative, suggesting nonlinkage rather than linkage. Also, in the linkage calculations we assumed genetic homogeneity (α=1) in our families. However, if genetic heterogeneity exists, multipoint linkage analyses with α=1 are more likely to exclude a disease gene from a linkage map (27). To test the robustness of our exclusion data we used HOMOG1 from the HOMOG package, version 2.51 (25), to calculate, from the three-point lod scores at α=1, lod scores based on different degrees of genetic heterogeneity. Linkage to *DRD4* was still excluded for α values of 0.75, 0.60, and 0.74 in models 1, 2, and 3, respectively. Linkage of bipolar illness to *TH* could not be excluded when heterogeneity was assumed in model 1. However, linkage to *TH* was excluded for an α of 0.66 and 0.80 in models 2 and 3, respectively.

As a safeguard against false negative findings due to misspecification of the disease model, we also analyzed our marker data by using the extended affected-sib-pair method (L.A. Sandkuijl, unpublished 1989 paper). In all affected sib pairs that were informative (i.e., allele segregation could be observed), the number of shared alleles was compared to the number of nonshared alleles. The null hypothesis of no linkage is rejected when the number of shared alleles is significantly higher than the number of nonshared alleles. Only model 2 gave informative results. In 79 affected sib pairs, the number of nonshared alleles was higher than the number of shared alleles for all markers.

Independent of the method used, negative linkage results were obtained with the candidate genes *DRD4*, *TH*, *TYR*, and *DRD2* under different genetic and phenotypic conditions, indicating that these genes do not play a frequent role in the pathophysiology of bipolar illness. Further, the absence of allele sharing among affected sibs indicated that none of these candidate genes is likely to act as a modifier gene in our bipolar subjects.

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