ORIGINAL ARTICLE

# Association Between the Tryptophan Hydroxylase Gene and Manic-depressive Illness

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**Background:** Genes encoding proteins involved in serotonergic metabolism are major candidates in association studies of mood disorders and suicidal behavior. This association study explores whether the tryptophan hydroxylase (*TPH*) gene, which codes for the rate-limiting enzyme of serotonin biosynthesis, is a susceptibility factor for manic-depressive illness, with or without a history of suicide attempts.

**Methods:** The *TPH* intron 7 A218C polymorphism was determined using a polymerase chain reaction–based method in DNA samples from 152 patients with bipolar disorder and 94 healthy control subjects.

**Results:** There was a significant association between *TPH* genotypes and manic-depressive illness. Among patients with bipolar disorder, no association was found between *TPH* alleles and suicidal behavior.

**Conclusions:** This result suggests the involvement of the *TPH* gene in susceptibility to manic-depressive illness. This preliminary result requires confirmation in further groups of patients and controls.

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THERE IS compelling evidence that abnormalities of serotonergic neurotransmission contribute to depression. The major serotonin (5-hydroxytryptamine [5-HT]) metabolite is 5-hydroxyindoleacetic acid (5-HIAA), and its concentration is low in the cerebrospinal fluid of a subgroup of patients with unipolar depression who attempted suicide by violent means.[1,2] Furthermore, platelet 5-HT uptake is diminished in patients with depression[3] and especially in patients with bipolar disorder.[4,5] Reduced binding of tritiated imipramine or tritiated paroxetine to brain 5-HT uptake sites and transporters has been found in patients with depression[6] and in suicide victims.[7]

Low 5-HIAA levels in cerebrospinal fluid, indicating reduced serotonergic function, also are associated with impulsive behavior.[8,9] In addition, among impulsive subjects, those with the lowest 5-HIAA concentrations have a history of suicide attempts.[10]

Although genetic factors have long been implicated in the etiopathogenesis of bipolar disorders[11] and to a lesser extent in that of suicidal behavior,[12-14] the mode of inheritance of manic-depressive illness and suicidal behavior is unclear.

The involvement of genetic factors in manic-depressive illness mostly has been investigated using linkage studies. Several chromosomal regions have been implicated, including 6pter-p24,[15] 13q13,[15] 15q11-qter,[15] 11p15,[16] Xp27,[17] Xq24-q27,[18] the pericentromeric region of chromosome 18,[19] and 21q22.3.[20] However, subsequent studies have failed to confirm some of these positive results, possibly because of genetic heterogeneity and complex modes of inheritance of major affective disorders. Thus, nonparametric strategies, which do not require any knowledge of the genetic parameters underlying the disease, may be more appropriate for identifying genes

*See also page 23*

involved in complex diseases. Recent association studies between candidate genes and manic-depressive illness suggest the implication of several genes of minor effect.[21-24] Although stratification bias can cause false-positive results, case-control studies using polymorphic markers close to or within candidate genes may be an appropriate method for detecting genetic susceptibility factors. Thus, despite this bias, case-control analyses of complex diseases (eg, essential hypertension and Alzheimer disease) have proved to be useful.

Candidate genes implicated in 5-HT metabolism already have been tested for as-

## PATIENTS AND METHODS

### PATIENTS AND CONTROLS

After giving informed consent, 152 patients with bipolar disorder (103 patients with bipolar I disorder and 49 patients with bipolar II disorder) and 94 healthy volunteers were included in this study. Patients and controls were all French (at least 3 grandparents born in France). Patients with bipolar disorder (84 women and 68 men) were recruited from consecutive admissions to the psychiatric unit and controls (37 women and 57 men) were blood donors from Hôpital Pitié-Salpêtrière, Paris, France, and Hôpital Corentin Celton, Issy-les-Moulineaux, France.

Patients and controls were interviewed by trained psychiatrists (F.B. and M.L.) with a French version of the Diagnostic Interview for Genetic studies.[36] Diagnosis with *DSM-III-R*,[37] Research Diagnostic Criteria, and history of suicide attempts were assessed with this instrument. Demographic and clinical characteristics of patients with bipolar disorder and controls are given in **Table 1**.

To minimize morbidity among subjects in the control group, only blood donors older than 35 years were included. Controls with a personal or family history of mood disorders or suicide attempt disclosed by interview were excluded.

Suicide attempts were classified as violent or nonviolent according to the criteria proposed by Asberg et al.[2] The criterion for suicide attempts was death intent requiring medical treatment at a hospital. Death intent and medical seriousness of the attempt were rated according to the 6-point scale of the Diagnostic Interview of Genetic Studies. Hanging attempts, use of firearms or knives, and jumping from heights were considered as violent attempts; drug overdoses were considered as nonviolent suicide attempts.

### LABORATORY METHODS

Because the 2 intron 7 TPH polymorphisms (A218C and A779C) are in complete disequilibrium,[32] we tested A218C, which is more easily revealed. Twenty-milliliter edetic acid blood samples were collected, and DNA was prepared from lymphocyte pellets by sodium dodecyl sulfate lysis, proteinase K digestion, phenol and chlorophorm extraction, and ethanol precipitation and was resuspended in TRIS buffer and edetic acid. For genotyping, target sequences were amplified from 200 ng of genomic DNA using polymerase chain reaction (PCR) in a 50-µL reaction volume using a Hybaid cycler (MWG-Biotech GmbH, Ebersberg, Germany). The PCR primers were HTHSSCPA,

5′-TTC AGA TCC CTT CTA TAC CCC AGA-3′;

and HTHSSCP5,

5′-GGA CAT GAC CTA AGA GTT CAT GGC A-3′;

in 1.5-mmol/L magnesium chloride, 50 mmol of potassium chloride, 10 mmol of TRIS buffer, and 20 pmol of each oligonucleotide, 2 U of *Taq* DNA polymerase (Brunschwig, Basel, Switzerland) and 200 mmol of each dNTP per tube. The PCR program was as follows: first cycle for 3 minutes at 95°C, 30 seconds at 58°C, and 1 minute at 72°C; and 29 cycles for 1 minute at 92°C, 30 seconds at 58°C, and 1 minute at 72°C. After amplification, PCR products were digested with 8 U of *BfaI* (BioConcept, Allschwil, Switzerland) overnight, then electrophoresed on a 1.4% agarose gel (Brunschwig). DNA was visualized by ethidium bromide staining and UV transillumination. The uncut amplicon is 918 base pairs (bp) long. After digestion, the "A" allele gives 860-bp and 58 (not seen in the gel)-bp fragments, and the "C" allele gives 615-, 245-bp, and 58 (not seen in the gel)-bp fragments.

### STATISTICAL ANALYSIS

Allelic and genotypic distributions between patients and controls were compared using the $\chi^2$ test. Odds ratios and 95% confidence intervals were calculated using the Woolf's method. The Armitage linearity tendency test[38] was used to identify any dose effect of a susceptibility allele. Differences were considered statistically significant at $P=.05$.

sociation with manic-depressive illness and suicidal behavior. Positive association between the monoamine oxidase A gene and manic-depressive illness have been reported.[24] This followed the report of a point mutation in the monoamine oxidase A gene in affected males, belonging to a single pedigree, who showed borderline mental retardation, and violent behavior.[25] Association between the 5-HT transporter gene and manic-depressive illness or suicide attempts remain controversial, because positive[26] and negative results have been obtained.[27,28] No association was found between $5\text{-HT}_{1A}$ receptor gene and bipolar disorder.[29]

The rate-limiting enzyme of 5-HT synthesis—tryptophan hydroxylase (*TPH*)—is a candidate gene for bipolar disorder and suicidal behavior. Tryptophan hydroxylase catalyzes the oxygenation of tryptophan to 5-hydroxytryptophan, which is then decarboxylated to form 5-HT. The human gene for TPH has been cloned,[30] and mapped on the short arm of chromosome 11 (11p14-p15.3).[31] Two biallelic polymorphisms have been identified in intron 7: A218C and A779C, disclosed by restriction fragment length polymorphism analysis and by single-strand conformational polymorphism analysis, respectively.[32] These 2 polymorphisms are in complete linkage disequilibrium in West European Caucasian controls.[32] Two other polymorphisms were disclosed by *AvaII* and *HinfI* restriction enzymes, using the human *TPH* copy DNA probe C2-38, but have not been accurately mapped.[30,33]

In a group of Finnish violent offenders meeting criteria for alcohol abuse, Nielsen et al[34] reported a significant difference in the conformational *TPH* polymorphism in intron 7 between subjects with and without a history of suicide attempts. In contrast, Abbar et al,[33] using *TPH* C2-38/*AvaII* polymorphism, did not find any significant difference between patients who attempted suicide and normal control subjects. No association was found between patients with bipolar disorder and controls with *TPH/AvaII* in a preliminary case-control study.[35]

To further explore the involvement of the *TPH* gene, we studied the *TPH* A218C polymorphism in a new sample of patients with bipolar disorder, some with and some without a history of suicide attempts, and in controls.

## RESULTS

The *TPH* genotypic distributions were in Hardy-Weinberg equilibrium among patients ($\chi^2$=0.13, *df*=2; *P*=.93) and controls ($\chi^2$=0.08, *df*=2; *P*=.96). There was an association between *TPH* genotype and manic-depressive illness (Table 1) ($\chi^2$=12.23, *df*=2; *P*<.02). The allelic distributions for patients and controls were significantly different ($\chi^2$=12.00, *df*=1; *P*=.001). The frequency of the rare *TPH* A allele in the control group (0.37) was consistent with that previously described in West European Caucasian controls (0.36).[32] The frequency of the A allele was higher in the patients with bipolar disorder (0.52). No difference between bipolar I and II disorder was found for A allele frequency (0.52 and 0.48, respectively). The odds ratio of bipolar disorder associated with 2 A alleles, at least 1 A allele, and 1 A allele only were 3.96 (95% confidence interval=1.76-8.94), 2.35 (95% confidence interval=1.34-4.12) and 1.96 (95% confidence interval=1.09-3.54), respectively.

No association was found between the *TPH* gene polymorphism (A218C) and suicidal behavior (presence vs absence of lifetime suicide attempt) in the patients with bipolar disorder. Among those who attempted suicide, no association was found for violent vs nonviolent suicide subgroups.

**Table 1. Demographic and Clinical Characteristics**

| Characteristic | Patients | Controls |
|---|---|---|
| M/F | 68/84 | 57/37 |
| Mean±SD age, y | | |
| At interview | 46.9±15.3 | 43.7±5.6 |
| At first episode | | |
| Bipolar I | 27.6±12.5 | . . .* |
| Bipolar II | 31.7±13.1 | . . . |
| At first hospitalization | | |
| Bipolar I | 33.6±13.9 | . . . |
| Bipolar II | 41.9±12.1 | . . . |
| Lifetime diagnoses, No. (%) | | |
| Family history of mood disorder | 73 (48) | 0 (0) |
| Alcohol abuse or dependence | 23 (15.1) | 1 (1.1) |
| Substance abuse or dependence | 16 (10.5) | 1 (1.1) |
| Panic disorder with or without agoraphobia | 23 (15.1) | 2 (2.1) |
| Agoraphobia without panic disorder | 12 (7.9) | 2 (2.1) |
| Social phobia | 15 (9.9) | 1 (1.1) |
| Obsessive compulsive disorder | 11 (7.2) | 1 (1.1) |
| At least 1 suicide attempt | 52 (34.2) | 0 (0) |

**No data are given here because control subjects, by definition, are not ill.*

## COMMENT

We report that a polymorphism in the intron 7 of the *TPH* gene is associated with manic-depressive illness. This finding seems to be robust, and discloses an effect that is consistent with a polygenic origin of bipolar disorder.

The risk of bipolar disorder is increased by the presence of at least 1 copy of the *TPH* A allele, and the risk is higher for *TPH* A–homozygous subjects. In our sample, there was a dose effect for the risk associated with the A allele for bipolar disorder as assessed by the linearity tendency test ($\chi^2$=12.19, *df*=1; *P*<.001).[38]

The allelic frequencies in our control population (**Table 2**) were similar to those reported by Nielsen et al[32] in West European Caucasian control populations. Our sample of patients with bipolar disorder was recruited with the same geographical criteria as controls, ie, Caucasian and of French origin for at least 2 generations. Despite these precautions, there may have been a stratification bias, and replication is required in other samples of patients with bipolar disorder, using other nonparametric methods.

It is unclear how the *TPH* A allele acts as a risk factor for bipolar disorder. The A218C *TPH* polymorphism is located in a potential GATA transcription-

**Table 2. Allelic and Genotypic Distributions and Frequencies of the Tryptophan Hydroxylase Gene Marker***

| | Genotypes | | | Alleles | |
|---|---|---|---|---|---|
| Variable | CC | AC | AA | C | A |
| Patients | | | | | |
| No. (a) | 34 | 79 | 39 | 147 | 157 |
| Freq±SD | 0.22±0.03 | 0.52±0.04 | 0.26±0.04 | 0.48±0.04 | 0.52±0.04 |
| Controls | | | | | |
| No. (b) | 38 | 45 | 11 | 121 | 67 |
| Freq±SD | 0.40±0.05 | 0.48±0.05 | 0.12±0.04 | 0.64±0.04 | 0.36±0.04 |
| No suicide attempt | | | | | |
| No. (c) | 19 | 52 | 16 | 90 | 84 |
| Freq±SD | 0.22±0.04 | 0.60±0.05 | 0.18±0.04 | 0.52±0.05 | 0.48±0.05 |
| At least 1 suicide attempt | | | | | |
| No. (d) | 11 | 24 | 17 | 46 | 58 |
| Freq±SD | 0.21±0.06 | 0.46±0.07 | 0.33±0.07 | 0.44±0.05 | 0.56±0.05 |
| Violent suicide attempt | | | | | |
| No. (e) | 2 | 8 | 4 | 12 | 16 |
| Freq±SD | 0.14±0.09 | 0.57±0.13 | 0.29±0.12 | 0.43±0.09 | 0.57±0.09 |
| Nonviolent suicide attempt | | | | | |
| No. (f) | 9 | 16 | 13 | 34 | 42 |
| Freq±SD | 0.24±0.07 | 0.42±0.08 | 0.34±0.08 | 0.45±0.06 | 0.55±0.06 |

**Statistical analysis: (a) vs (b) for genotypic distribution, $\chi^2$=12.23, df=2 (P=.002); (a) vs (b) for allelic distribution, $\chi^2$=12, df=1 (P<.001); (c) vs (d) for allelic distribution, $\chi^2$=1.46, df=1 (P=.22); (e) vs (f) for allelic distribution, $\chi^2$=0.03, df=1 (P=.86). Freq indicates frequency.*

factor binding site and therefore might affect *TPH* gene expression.[32] Alternatively, this polymorphism may be nonfunctionally significant and our results may indicate only linkage disequilibrium between the *TPH* A allele and a different mutation in the *TPH* gene, or another nearby gene. Although the other *TPH* intron 7 polymorphism (A779C SSCP) is located upstream from the 3 acceptor splice site, it has no functional significance because no aberrant splice product from the *TPH* gene has been detected.[32]

We did not find any association between a previously studied small sample of patients with bipolar disorder (N=70) and another restriction fragment length polymorphism of the *TPH* gene (*AvaII/C2-38*).[35] Because the location of the *AvaII* polymorphic site in the *TPH* locus is unknown, these discrepant results may be due to large distance between the 2 polymorphic sites (ie, the *AvaII* and the A218C polymorphism).

Possibly, a clinical or biological trait underlying manic-depressive illness could be associated with this *TPH* polymorphism. We, therefore, looked for an association between *TPH* polymorphism and suicide attempts. However, in our sample, there was no difference for A allele frequency between patients with bipolar disorder with and without a history of suicide attempts (0.56 and 0.48, respectively). This observation does not confirm the suggestion by Abbar et al[33] that carrying the U allele (A779C polymorphism), which is in strong disequilibrium with the A allele (A218C polymorphism), protects against suicidal behavior in patients with bipolar disorder.

Furthermore, in our sample of patients who attempted suicide, no difference was found for the A allelic frequency between violent and nonviolent attempt subgroups (0.57 and 0.55, respectively). Nevertheless, this negative result could be due to lack of power because of the small sample size. Our sample of patients with bipolar disorder who have attempted suicide (14 violent and 38 nonviolent attempts) provides a power of less than 20% for detecting a difference between frequencies of 0.55 and 0.57 (unilateral test).

In summary, *TPH* intron 7 polymorphism seems to be associated with violent behavior without a history of suicide attempts[34] and with manic-depressive illness. This *TPH* polymorphism might be associated with a trait related to violence and bipolar disorders rather than directly to these phenotypes themselves.

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