A Polymorphism in the Dopamine β-Hydroxylase Gene Is Associated with “Paranoid Ideation” in Patients with Major Depression

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**Background:** Increased dopaminergic activity may play a primary role in psychotic depression. Dopamine β-hydroxylase (DBH) catalyses the key step in biosynthesis of the neurotransmitter noradrenaline from dopamine, and low DBH activity is a possible risk factor for developing psychotic depression. An exon 2 polymorphism (DBH*444 g/a) of the DBH gene (DBH) is significantly associated with both serum and cerebrospinal fluid levels of DBH.

**Methods:** We determined the genotype of the DBH*444g/a polymorphism in a cohort of 164 patients with major depression and examined the association of this polymorphism with paranoid ideation, interpersonal sensitivity, and psychoticism on the Hopkins Symptom Checklist.

**Results:** Patients who possessed the A allele were significantly more likely to have higher scores for interpersonal sensitivity and paranoia than patients without the A allele (p = .004 and p = .048, respectively), suggesting that this allele may predispose patients to paranoia in major depression. In addition, we found an association between prolactin levels in men and DBH*444 g/a genotype such that homozygous G individuals displayed significantly higher levels than homozygous A or heterozygote individuals.

**Conclusions:** Depressed patients with the GG genotype of DBH have lower scores for interpersonal sensitivity and paranoid ideation. The GG genotype may be protective against the development of psychosis in the presence of a major depressive episode. Biol Psychiatry 2002;51:365–369 © 2002 Society of Biological Psychiatry

**Key Words:** Affective disorders, psychotic disorders, catecholamines, alleles, genes, prolactin

**Introduction**
There are at least two major issues of importance in the etiology of psychotic depression. First is the question of why only some depressed patients develop psychotic symptomatology. We favor the hypothesis of Winokur (1984) that a trait or propensity to psychosis is transmitted independently of the major affective illness and that this propensity is silent or not observed when the patient is in remission. Sands and Harrow (1994) similarly proposed a two-step process: 1) a vulnerability to psychosis and 2) the presence of concurrent affective symptoms. The second key issue is whether psychosis in depression is best conceptualized as an all-or-none trait, as implied by DSM-IV (delusional or nondelusional depression) or whether it is best conceptualized as a dimensional trait. For instance, there is little doubt that many depressed patients show varying degrees of paranoid ideation ranging from fleeting paranoid ideas to firmly held and fixed delusional paranoid ideas. A third issue is whether the neurobiological and neuropsychologic mechanisms are similar for varying types of psychotic symptoms, which may vary from paranoid delusions, somatic delusions, and nihilistic delusions to auditory hallucinations.

Schatzberg and Rothschild (1992) proposed that psychotic depression is a distinct subtype with increased levels of dopamine. This hypothesis is partially based on repeated observations that patients with psychotic depression have lower levels of dopamine-β-hydroxylase (E.C. 1.14.17.1; DBH), the enzyme that catalyzes the key step in conversion of dopamine to norepinephrine. Furthermore, DBH deficiency is associated with a lower dopamine-to-norepinephrine ratio (Robertson et al 1991). The gene encoding DBH (DBH), located on chromosome 9q34 (Craig et al 1988), appears to be a major quantitative trait locus controlling serum and cerebrospinal fluid (CSF) DBH activity (Cubells et al 1998; Wei et al 1997, 1998; Wilson et al 1988; Zabetian et al 2001). There are several polymorphisms in the DBH gene, including a single nucleotide polymorphism at cDNA position 444 in exon 2.
(Kobayashi et al 1989) that was recently found to be associated with differences in plasma and CSF levels of \( \text{DBH} \) (Cubells et al 1998).

Several studies have implicated low \( \text{DBH} \) activity as a risk factor for psychotic depression (Meltzer et al 1976; Meyers et al 1999; Mod et al 1986; Sapru et al 1989). Furthermore, in schizophrenic patients, low \( \text{DBH} \) levels appear to define a subset of patients with psychotic symptoms that have a better response to treatment with antipsychotic medication than in schizophrenic patients with high \( \text{DBH} \) levels (Sternberg et al 1983; van Kammen et al 1994).

In this article, our primary hypothesis was that a dimensional measure of “psychosis proneness,” as assessed by the Hopkins Symptom Checklist (Derogatis et al 1973) scales of paranoid ideation, interpersonal sensitivity, and psychoticism, would be related to the dopamine-\( \beta \)-hydroxylase genotype. After evaluation of this primary hypothesis, we compared the three genotypes on other measures to ensure that differences could not be explained by other variables.

Methods and Materials

Subjects

The depressed patients in this study were part of a larger study to examine predictors of antidepressant response (Joyce and Paykel 1989). Some data from these patients have been reported (Carter et al 2000b; Joyce et al, in press; Luty et al, in press). Depressed outpatients were recruited from a variety of sources and were entered into the study after giving written informed consent if a major depressive episode was the current principal diagnosis, they required treatment with an antidepressant drug, were free of any major medical illness (such as diabetes), and were of 18–64 years met the inclusion criteria for this study and provided satisfactory DNA samples.

Patient Assessment and Prolactin Measurement

After giving consent, patients attended for a detailed clinical and neurobiological assessment. This included clinical assessment by a psychiatrist with the Structured Clinical Interview for DSM-III-R (SCID; Spitzer et al 1992), the SCID-II for personality disorders (Spitzer et al 1992), the Hamilton (1960) and Montgomery (1979) depression rating scales, and the CORE mental state examination for psychomotor agitation and retardation (Parker et al 1994).

Patients completed a series of questionnaires including the Hopkins symptom checklist (Derogatis et al 1973), the Social Adjustment Scale (Weissman and Bothwell 1976), and Cloninger’s Temperament and Character Inventory (Cloninger 1987).

During the neurobiological evaluation, blood was obtained for DNA extraction, and, from a series of blood samples for neuroendocrine evaluation, an afternoon prolactin level was obtained. Prolactin was measured using the Beckman-Coulter Access Analyser, which uses a paramagnetic-particle, chemiluminescent immunoenzymatic two-site assay. The assay is standardized to the World Health Organization Third International Standard. Following the assessment, depressed patients were randomized to either nortriptyline or fluoxetine for acute treatment of their depression. All patients are being prospectively followed up for 5 years to describe their long-term outcomes.

DNA Extraction and Genetic Analysis

Genomic DNA was extracted from peripheral blood using the guanidium isothiocyanate method of Ciulla et al (1988); PCR-RFLP analysis of \( \text{DBH}\ast444 \) g/a was carried out using a modification of a previously described method (Cubells et al 1998). Each 25 L PCR contained 2 mmol/L \( \text{MgCl}_2 \), 0.625 (mol/L of each primer, 200 (mol/L dNTPs, 1 unit of Taq polymerase (Roche Biochemicals) and approximately 100 ng of genomic DNA. Primers were 5'-TCTTCTATGCCTGGAGCCCAGTGGCATGCCTTTGCT-3' and 5'-GACAGGAAAGGTACTGACATTCGGCACAG-3’. Thermal cycling was performed with an initial denaturation of 30 sec at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 60°C, 30 sec at 72°C, and a terminal extension of 2 min at 72°C. The PCR products were digested with EcoNI and analyzed by electrophoresis on 3% agarose–TBE gels consisting of 3:1 NuSieve GTG (EMC, Rockland, ME, USA) and UltraPure agarose (Gibco BRL). Ethidium bromide stained gels were digitally imaged and manually scored for genotypes. The PCR product was 207 bp in size. The \( \text{DBH}\ast444 \) A alleles did not digest with EcoNI, whereas G alleles digested to give 169 bp and 38 bp fragments.

Statistical Analysis

All data were entered into the relational database PARADOX and then transferred to the statistics program SYSTAT. Analyses included ANOVA, post hoc Tukey tests, \( t \) tests, chi-square, Pearson correlations, and multiple regression. In undertaking the statistical analyses, we were driven by our primary hypothesis that \( \text{DBH} \) polymorphisms would be associated with interpersonal sensitivity, paranoid ideation, and psychoticism on the SCL-90. For completeness, we also examined all other scales on the SCL-90. After finding differences, we ensured that these results could not be confounded by differing demographic or diagnostic sample characteristics. Although conservative, a Bonferroni correction of the SCL-90 subscales would suggest that a \( p \) value of 0.05/9 = 0.0056 would remain significant even with allowance for multiple tests.

Results

The \( \text{DBH}\ast444 \) g/a genotypes of 164 Caucasian depressed patients were established using a PCR-RFLP assay (Table 1). From these data, the A allele frequency of this sample
was estimated to be 0.48, and the G allele frequency was
0.52. These frequencies are similar to other published
studies on Caucasian populations (Cubells et al 1998). The
 genotype distribution is consistent with Hardy-Weinberg
equilibrium. We assume that the DBH allele frequency in
depressed patients is close to that in an unaffected popu-
lation because there is nothing to suggest that DBH
genotype per se is a risk factor for depression.

On the Hopkins symptom checklist, the D\textsubscript{A}/H\textsubscript{9252}H genotypes
were different with regard to measures of interpersonal
sensitivity and paranoid ideation, but not psychoticism;
however, on the other six scales of the Hopkins Symptom
Checklist, patients did not differ by genotype. There were
also no significant differences by D\textsubscript{A}/H\textsubscript{9252}H genotype in age,
gender, depression subtype, depression severity, or age of
onset of depression. The largest difference was on the
interpersonal sensitivity scale, where those with the ho-
mozygous GG genotype scored significantly lower on
interpersonal sensitivity than either those with the ho-
mozygous AA or heterozygous AG genotype. Even with a
Bonferroni correction, the lower interpersonal sensitivity
scores in those with the GG genotype would remain
significant.

We examined prolactin levels in these depressed pa-
tients by gender and DBH genotype (Table 2). As ex-
pected, women had higher prolactin levels than men;
however, within men a homozygous GG genotype is
significantly associated with higher prolactin levels. This
finding is consistent with the proposed higher functional
activity of the GG genotype (Cubells et al 1998) being
associated with low dopamine levels (and low interper-
sonal sensitivity), which are associated with disinhibition
of the inhibitory dopaminergic control of prolactin release.
Although the presence of an A allele of DBH is associated
with increased interpersonal sensitivity and lower prolac-
tin levels (due to higher dopamine secondary to lower
D\textsubscript{B}H functional activity), there was no relationship be-
tween prolactin levels and interpersonal sensitivity or
paranoid ideation.

After 6 weeks of treatment with either fluoxetine or
nortriptyline and marked symptomatic improvement, there
were no significant differences in interpersonal sensitivity
or paranoid ideation between the different D\textsubscript{A}/H\textsubscript{9252}H geno-
types. This similarity is not surprising and is consistent
with a depression-dependent expression of an independent
vulnerability or propensity to psychosis.

Discussion

The key finding of this study is that the presence of an A
allele of D\textsubscript{B}H in depressed outpatients is associated with
higher levels of interpersonal sensitivity and paranoid
ideation. If a Bonferroni correction is applied to these
results, then the association of D\textsubscript{B}H polymorphisms with

Table 1. Clinical Characteristics of Depressed Patients by Dopamine-\beta-Hydroxylase Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A/A</th>
<th>A/G</th>
<th>G/G</th>
<th>F or ( \chi^2 ) (df=2)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>37</td>
<td>84</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>33.0(11.3)</td>
<td>31.0(10.8)</td>
<td>32.4(12.6)</td>
<td>.46</td>
<td>ns</td>
</tr>
<tr>
<td>% female</td>
<td>51%</td>
<td>57%</td>
<td>58%</td>
<td></td>
<td>.99</td>
</tr>
<tr>
<td>% melancholia</td>
<td>49%</td>
<td>41%</td>
<td>51%</td>
<td></td>
<td>.98</td>
</tr>
<tr>
<td>% recurrent</td>
<td>62%</td>
<td>67%</td>
<td>61%</td>
<td></td>
<td>.94</td>
</tr>
<tr>
<td>% bipolar II</td>
<td>11%</td>
<td>10%</td>
<td>5%</td>
<td></td>
<td>1.10</td>
</tr>
<tr>
<td>Age onset MDD</td>
<td>24.0(11.7)</td>
<td>20.7(10.4)</td>
<td>22.3(11.1)</td>
<td>.117</td>
<td>ns</td>
</tr>
<tr>
<td>HDRS</td>
<td>20.7(4.1)</td>
<td>19.5(4.6)</td>
<td>19.6(4.4)</td>
<td>.94</td>
<td>ns</td>
</tr>
<tr>
<td>SCL Scales</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatization</td>
<td>1.2 (.6)</td>
<td>1.0 (.6)</td>
<td>1.0 (.6)</td>
<td>1.17</td>
<td>ns</td>
</tr>
<tr>
<td>Obsessive</td>
<td>2.0 (.7)</td>
<td>1.9 (.8)</td>
<td>1.7 (.9)</td>
<td>1.49</td>
<td>ns</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>1.8 (.9)</td>
<td>1.8 (.9)</td>
<td>1.3 (.7)</td>
<td>5.68</td>
<td>.004*</td>
</tr>
<tr>
<td>Depression</td>
<td>2.4 (.8)</td>
<td>2.4 (.7)</td>
<td>2.2 (.9)</td>
<td>1.51</td>
<td>ns</td>
</tr>
<tr>
<td>Anxiety</td>
<td>1.7 (.7)</td>
<td>1.5 (.6)</td>
<td>1.4 (.7)</td>
<td>2.05</td>
<td>ns</td>
</tr>
<tr>
<td>Anger</td>
<td>1.3 (.8)</td>
<td>1.3 (.8)</td>
<td>1.0 (.8)</td>
<td>2.52</td>
<td>ns</td>
</tr>
<tr>
<td>Phobia</td>
<td>.8 (.9)</td>
<td>.8 (.8)</td>
<td>.7 (.7)</td>
<td>.48</td>
<td>ns</td>
</tr>
<tr>
<td>Paranoid</td>
<td>1.2 (.9)</td>
<td>1.3 (.9)</td>
<td>.9 (.7)</td>
<td>3.08</td>
<td>.048*</td>
</tr>
<tr>
<td>Psychoticism</td>
<td>1.1 (.6)</td>
<td>1.1 (.6)</td>
<td>.9 (.5)</td>
<td>1.61</td>
<td>ns</td>
</tr>
</tbody>
</table>

Results are shown as % or mean (\pm SD). MDD, major depressive disorder; ND, nonsignificant; HDRS, Hamilton Depression Rating Scale; SCL, symptom checklist.
Tukey post hoc tests: \*GG vs AG, AA; \#GG vs AG.

Table 2. Prolactin Levels (IU/L) by Dopamine-\beta-Hydroxylase Genotype and Gender

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A/A</th>
<th>A/G</th>
<th>G/G</th>
<th>F</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>340 (126)</td>
<td>354 (113)</td>
<td>450 (191)</td>
<td>3.61</td>
<td>.032</td>
</tr>
<tr>
<td>Women</td>
<td>692 (320)</td>
<td>581 (267)</td>
<td>681 (299)</td>
<td>1.50</td>
<td>ns</td>
</tr>
</tbody>
</table>

Results are mean (SD).
interpersonal sensitivity remains significant, although the association with paranoid ideation would no longer be significant. On the Hopkins symptom checklist, all nine scales are positively correlated (0.27–0.74 in this study), with the highest correlation (0.74) being that between interpersonal sensitivity and paranoid ideation. The items on the interpersonal sensitivity scale tend to be focused on feeling uneasy with other people, whereas the paranoid ideation items tend to be focused on feeling that other people are watching, aware, or critical of the individual. This suggests the association of the A allele with interpersonal sensitivity is tapping the inner discomfort of the depressed person’s relationships with others, rather that the attribution that others are being critical or blaming.

This finding of higher interpersonal sensitivity in those with the A allele should be viewed in conjunction with the research on DBH in psychotic depression. In this outpatient sample, none of the depressed patients were diagnosed as having psychotic (delusional) depression. This suggests that the A allele, which is in fact present in about three quarters of patients, predisposes to interpersonal sensitivity and paranoid ideation. The items with the highest correlation (0.74) being that between interpersonal sensitivity and paranoid ideation. The items on the interpersonal sensitivity scale tend to be focused on feeling uneasy with other people, whereas the paranoid ideation items tend to be focused on feeling that other people are watching, aware, or critical of the individual. This suggests the association of the A allele with interpersonal sensitivity is tapping the inner discomfort of the depressed person’s relationships with others, rather that the attribution that others are being critical or blaming.

Our results are consistent with the observed association of the DBH*444g/a A allele and cocaine-induced paranoia (Cubells et al 2000). Taken together, these studies are evidence that DBH genotype is an independent risk factor for development of “psychosis” in the presence of an independent “challenge” to the brain (whether by drug or affective disturbance). The symptom results, and the corresponding prolactin levels in men, suggest that the critical genetic vulnerability is the presence or absence of the A allele of DBH*444g/a; however, because 75% of the population have an A allele, it may be more appropriate to suggest that the GG genotype is protective against the development of paranoia in the presence of affective disturbance or cocaine ingestion. This assumes that the DBH*444g/a polymorphism has a functional effect on activity or expression of DBH. It is not yet clear whether this is so. DBH*444g/a is a synonymous SNP (i.e., does not alter the reading frame of DBH), although it is located at a splice donor site and could potentially affect splicing of the transcripts; however, it is also possible that DBH*444g/a does not itself alter DBH function but is in linkage disequilibrium with a functional polymorphism. A promising candidate for such a functional polymorphism is the 1021C > T SNP recently identified in the 5′ flanking region of DBH (Zabetian et al 2001).

Our study does not address whether polymorphisms of DBH are related to other types of delusional symptoms in depression such as guilt or nihilism, nor does it address whether derogatory auditory hallucinations are also associated with polymorphisms of DBH. The results also need to be interpreted with the usual caution for association studies; however, there were sufficient a priori reasons for our hypothesis that genotypes associated with low DBH levels would be associated with greater psychotic symptoms.

In summary, we have shown that the presence of the A allele of DBH in depressed patients may confer an independently inherited vulnerability to psychosis, although it may be more appropriate to hypothesize that the GG genotype protects against the development of psychosis. Presumably, this is because the GG genotype is associated with higher DBH activity and hence lower dopamine, which may be a key protective mechanism against psychosis vulnerability. We also provide evidence of an association between DBH*444g/a and prolactin levels in men because homozygous G men had significantly elevated prolactin levels compared with homozygote A or heterozygote individuals. Finally, our results suggest that a dimensional approach of vulnerability to psychosis may be more useful than a categorical classification of delusional versus nondelusional.

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