Attention-Deficit/Hyperactivity Disorder Symptoms in Offspring of Mothers With Impaired Serotonin Production

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Context: Exposure to adverse events during prenatal and postnatal development, as well as serotonin deficiency, have been implicated in disturbances of mood and impulsivity, but the underlying mechanisms are unknown.

Objective: To investigate the long-term effects of an impaired serotonin synthesis on the developing human brain, we studied the effects of nonsynonymous mutations affecting tryptophan hydroxylase (TPH) enzymes responsible for serotonin production in maternal reproductive tissues (TPH1) and the brain (TPH2).

Design: Family-based case-control and functional studies of candidate genes.

Setting: Adult outpatients with attention-deficit/hyperactivity disorder (ADHD), their family members, and random control subjects were recruited across Norway.

Participants: Nine pedigrees with TPH1 and TPH2 mutation carriers were identified among 459 patients with ADHD and 187 controls. The TPH genes were then sequenced in 97 additional family members, and information about psychiatric diagnoses and symptoms was obtained from 606 controls, the 459 patients, and their relatives.

Main Outcome Measures: The effects of maternal vs paternal TPH1 mutations compared in all families.

Results: Nine different TPH1 and TPH2 mutations were found by sequencing in 646 individuals (1.0% and 0.2% allele frequency, respectively). In vitro studies showed that 8 TPH mutants had significantly impaired enzyme function. Family analysis of 38 TPH1 mutation carriers and 41 of their offspring revealed that offspring of mothers carrying TPH1 mutations reported 1.5- to 2.5-times-higher ADHD scores and related symptoms during childhood and as adults than did controls (P<10−6) or offspring of fathers with the corresponding TPH1 mutations (P<.001).

Conclusions: Impaired maternal serotonin production may have long-term consequences for brain development and increase the risk of ADHD-related symptoms and behavior in offspring. Replication studies are required to form conclusions about the clinical implications of mutations affecting serotonin biosynthesis.

Arch Gen Psychiatry. 2010;67(10):1033-1043

SEROTONIN (5-HYDROXYTRYPTAMINE) is a hormone and transmitter that performs a broad range of physiological functions in the human body. In addition to its transmitter function in the mature nervous system, serotonin has an important role during development, being involved in neurogenesis, neural migration, differentiation, and synaptogenesis.1 Mouse studies indicate that embryonic serotonin in early gestation is of maternal origin and that serotonin deficiency leads to growth retardation of the brain and other organs in the offspring.2,3 The early arrival of serotonin in embryonic brain structures may regulate ingrowth and development of other monoaminergic neurons, in particular dopamine terminals.4 Serotonin depletion in pregnant rats leads to decreased brain dopamine levels and increased locomotor activity in their adult offspring.5 Vertebrate genomes encode 2 different enzymes that are specifically involved in the biosynthesis of serotonin, that is, tryptophan hydroxylases 1 and 2 (TPH1 and TPH2), with slightly different struc-
ture and regulatory properties.\textsuperscript{6,8} Tryptophan hydroxylase 1 is found in the pineal gland and peripheral organs, including female reproductive tissues, whereas TPH2 is responsible for nearly all serotonin production in the brain.\textsuperscript{9,10} However, it has also been reported that significant amounts of TPH1 are expressed in various regions of the human brain, including the striatum and hippocampus.\textsuperscript{11} Selective elimination of Tph1 in mice is associated with an increased rate of malformations in their offspring, possibly mediated by an insufficient serotonin production in maternal reproductive tissues.\textsuperscript{2,3}

A dysfunctional serotonin neurotransmission has been implicated in human psychiatric disorders such as bipolar disorder, depression, anxiety, autism, schizophrenia, and attention-deficit/hyperactivity disorder (ADHD).\textsuperscript{12-16} Genetic variants within TPH1 (NCBI Entrez Gene 7166), TPH2 (NCBI Entrez Gene 121278), or a combination of these genes\textsuperscript{17} has been reported to be associated with all of these conditions in different clinical samples. However, although disturbances of dopamine signaling have received more attention, conclusive evidence of abnormalities within the serotonin or dopamine pathways in patients with ADHD is still lacking. The problems of identifying strong susceptibility genes by using common genetic variants in association studies may be due to inadequate statistical power, population stratification, or failure to address the appropriate clinical phenotypes and/or target genes. Recent whole genome association studies suggest that common single-nucleotide polymorphisms are unlikely to explain most of the familiar aggregation of common psychiatric disorders. Attention has therefore shifted toward a search for rare variants of potentially stronger effects.\textsuperscript{18} In addition to genetic factors, environmental agents also influence human phenotypes, including adult behavior, by their action in critical periods of embryonic development.\textsuperscript{19}

Although human data also suggest a connection between disturbed serotonin function and birth defects,\textsuperscript{20} the effect of an impaired serotonin synthesis on pregnancy outcome is unknown. To evaluate a possible causative role of serotonin deficiency in ADHD-related symptoms and behavior, we studied a large cohort of adult ADHD patients and multigenerational family members. By sequencing the TPH1 and TPH2 genes, we could show that children born to mothers with coding variants affecting TPH1 enzyme function have a high symptom load of inattention, hyperactivity, impulsivity, and other emotional problems.

**METHODS**

**SUBJECTS**

The patients in this study were adults (aged ≥18 years) with a clinical diagnosis of ADHD corresponding to DSM-IV criteria, recruited from all parts of Norway. Most of them were recruited from a national registry of adult ADHD patients in Norway from 1997 through 2005. To include patients diagnosed as having ADHD after 2003, additional patients were recruited directly from clinicians, using national guidelines based on the former registry assessment.

Controls were recruited from a random sample of the Norwegian population aged 18 to 40 years by using the National Public Registry. All of the sequenced controls were part of this sample. The total sample of 606 controls included 180 controls recruited from additional sources (students and friends of patients). The recruitment strategy and inclusion criteria of the patient and control samples have been described previously.\textsuperscript{21,22}

Family members of patients were invited by the patients to participate in the family study. The families included in the present study were families of probands with TPH1 mutations in which at least 3 family members agreed to participate. Seven families with a total of 86 members were included in the TPH1 analyses.

An informed consent based on detailed written information about the project was obtained from all patients, controls, and family members. The study was approved by the Norwegian Regional Medical Research Ethics Committee West institutional review board 3.

**TPH1 AND TPH2 SEQUENCING**

We extracted DNA from blood or saliva samples received from all study participants after informed consent. All coding exons and intron-exon regions of TPH1 were successfully sequenced in 187 population-derived controls and 457 adult ADHD patients. Similarly, TPH2 was successfully sequenced in 459 patients and 179 controls using a DNA analyzer (3730 series; Applied Biosystems, Foster City, California). An additional 9 patient samples and 4 control samples were excluded from analysis because sequence information was missing for more than 1 exon. Observers were blinded to patient groupings.

**EXPRESSION, PURIFICATION, AND ANALYSIS OF HUMAN TPH1 VARIANTS**

Human embryonic kidney cells (HEK293) were transfected with the pcDNA5/FRT-TPH1 and pcDNA5/FRT-TPH2 expression vector (wild-type [WT] and mutant forms) using lipofectamine (Gibco, Rockville, Maryland) as described by the manufacturer, and the TPH1 and TPH2 variants were expressed as previously described.\textsuperscript{8} The TPH1 messenger RNA (mRNA) levels in the HEK293 were measured as described in the online-only supplemental “eMethods” section (http://www.archgenpsychiatry.com).

Expression of maltose-binding protein TPH1 and TPH2 fusion proteins in Escherichia coli was performed as described previously for TPH2,\textsuperscript{23} except that for the TPH1 proteins, TOP10 competent cells (Invitrogen, Paisley, England) were used. The cell lysate was fractioned into soluble and insoluble parts by centrifugation at 10000g for 30 minutes. An equal volume of lysate buffer was added to the pelleted fraction and homogenized. Specific expression levels of fusion proteins in the soluble and pelleted fractions were determined by analyzing equal amounts (30 µg) by separation on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels that were stained with Coomassie blue or immunostained with anti-PH8 antibody.

Wild-type and mutant TPH1 and TPH2 were expressed and purified by a rapid batch method described previously for TPH2.\textsuperscript{24} Fusion proteins were eluted with 10mM maltose in 20mM HEPES (pH, 7.4), 400mM sodium chloride, 10% glycerol, and complete EDTA-free protease inhibitor cocktail (Roche Applied Science, Mannheim, Germany). Eluted fusion proteins were concentrated to approximately 1 mg/mL and analyzed after separation on 12% SDS-PAGE gel.
Coomassie blue–stained maltose-binding protein TPH1 and TPH2 fusion protein band intensities were integrated using a molecular imaging system (VersaDoc MP 4000; Bio-Rad Laboratories, Hercules, California), whereas immunostained protein bands were integrated using a multicolor DNA analysis system (Fluorimag; Amersham Biosciences, Sunnyvale, California). Protein bands from both methods were analyzed using commercially available software (Quantity One; Bio-Rad Laboratories). The activity and stability of TPH were assayed as described previously.\textsuperscript{24} Phosphorylation was performed as described previously.\textsuperscript{24} Typically, we used a 1:10 ratio of cyclic adenosine monophosphate–dependent protein kinase C-subunit to TPH for phosphorylation. Phosphorylation stoichiometry was determined by measuring phosphorus P\textsubscript{32} incorporation at various time points using a scintillation counter (TriCarb 2900TR; Packard Bioscience, Meriden, Connecticut) and by visualization using a phosphoimager (Bio-Rad Laboratories) after separation on 12% SDS-PAGE at various time points. Quantification of band intensity was performed using the Quantity One software (Bio-Rad Laboratories).

### MOLECULAR MODELING/PREDICTION ANALYSIS

The crystal structure of double-truncated TPH1 (PDB database, 1MLW; http://www.pdb.org/pdb/home/home.do) and the full-length model of TPH1 (PDB database, 1IN9; http://www.pdb.org/pdb/home/home.do) were used to analyze enzyme mutants for differences in atomic contacts, van der Waals overlaps, hydrogen bonding, and salt bridges using the WHAT IF protein structure analysis software.\textsuperscript{25} (Vriend group at the Centre for Molecular and Biomolecular Informatics, Nijmegen, the Netherlands).

### STATISTICAL METHODS

Descriptive statistics for clinical data were performed with SPSS statistical software (version 15.01; SPSS Inc, Chicago, Illinois). We used $\chi^2$ and Fisher exact tests for analyses of categorical variables, $t$ test for independent samples, and 1-way analysis of variance (ANOVA) for continuous variables. Associations between parental genotype and ADHD symptom severity with and without covariates were analyzed using multiple linear regression analyses.

### RESULTS

#### MUTATION SCREENING OF TPH1 AND TPH2

To determine the frequencies of functional variants in TPH1 and TPH2, their complete coding regions were first sequenced in a random sample of 187 Norwegian adults (Table 1). To examine whether TPH1 and TPH2 mutations were associated with ADHD, we obtained clinical information and sequenced these genes in 459 adult ADHD patients. In addition to previously reported common single-nucleotide polymorphisms, we found 5 novel (and 1 previously reported) missense mutations and 1 novel nonsense mutation in TPH1 (Table 1 and the eFigure). The total TPH1 mutation carrier frequency was similar in ADHD cases and controls (1.8% vs 2.1%). In TPH2, we found 1 previously reported and 1 novel missense mutation (2 individuals) in patients, compared with none in controls.

#### FUNCTIONAL CHARACTERIZATION OF MUTANT TPH1 PROTEINS

To determine the structural effects of the TPH1 mutations, a full-length model of the human enzyme was prepared using a combination of the crystal structure of truncated TPH1\textsuperscript{28} and homology and ab initio modeling (Figure 1). The model shows that the missense variants are distributed over all 3 enzyme domains. To examine the functional properties of the TPH1 protein variants, they were produced in E coli, and we analyzed their enzymatic activity, solubility, and stability as purified enzymes or in freshly prepared bacterial extracts. All variants were also transiently produced in HEK293 cells, and we measured their mRNA and protein abundance, protein solubility, and specific activity in the soluble fraction.

To determine whether the mutations affected protein expression levels, we calculated the amounts of TPH1 protein produced relative to mRNA levels. Some variability of protein expression was observed for the TPH1 mutants, but none of them were significantly different from the WT.
specific activity after purification (Figure 2F). High activity in freshly prepared bacterial extracts but low rate of inactivation could also explain why p.R142C has only 10% to 15% of the WT (Figure 2D). The increased stability in vivo and in vitro.24,27 Thus, the stability of the human TPH1 variants was also studied. Except for p.V177I, the catalytic domain and is completely inactive, all TPH1 variants (Figure 2C). The specific activities of pure bacterial homogenates, the protein solubility was intact for all TPH1 variants (Figure 2C). The specific activities of pure p.R142C, p.L274I, p.A300T, and p.H1410N were 10% to 58% of WT TPH1, whereas the activities of p.K54Q and p.V177I were comparable to that of the WT (Figure 2F). Further kinetic studies of the TPH1 missense variants showed that their Michaelis-Menten constant (Km) values for tryptophan or tetrahydrobiopterin were unaltered, but their specific activities (Vmax) values were reduced, corresponding to the reduced enzyme activities shown in Figure 2F. Although we did not observe significantly changed mRNA or protein expression by any of the TPH1 variants, we cannot exclude additional effects of the mutations at the transcriptional or translational level.

Most disease-related mutations of aromatic amino acid hydroxylases are missense variants with reduced enzyme stability in vivo and in vitro.24,27 Thus, the stability of the human TPH1 variants was also studied. Except for p.V177I, the purified enzyme variants had increased inactivation rates at 37°C, with apparent half-lives for p.A300T and p.H1410N of only 10% to 15% of the WT (Figure 2D). The increased rate of inactivation could also explain why p.R142C has high activity in freshly prepared bacterial extracts but low specific activity after purification (Figure 2F).

The activity of TPH1 and TPH2 is regulated by phosphorylation and binding to 14–3–3 proteins.25,26 To determine whether the missense variants of TPH1 had altered phosphorylation stoichiometry, the purified enzymes were phosphorylated with the catalytic subunit of cyclic adenosine monophosphate–dependent protein kinase. As shown in Figure 2E, all TPH1 variants were phosphorylated to approximately 1.5 mol phosphate per mole enzyme subunit, except for p.R145X, which incorporated only 0.7 mol phosphate/monomer. This indicates that the latter enzyme variant is lacking a phosphorylation site (eg, Ser260) in its C-terminal domain.28

To determine the possible effect of the novel TPH2 variant, p.D473N, we performed the same experiments as for the TPH1 variants. This variant had abundance and solubility similar to those of WT TPH2 but reduced specific activity (45%) (data not shown). Because we did not have access to family data, we did not pursue further studies on this mutation.

**CLINICAL FEATURES OF TPH1 AND TPH2 MUTATION CARRIERS**

As shown in Table 1, the frequency of TPH1 mutations was similar in the population-derived sample and the ADHD clinical sample. We found only 3 patients with TPH2 mutations and none in the control samples. By performing additional sequencing in 49 male and 48 female family members of persons with TPH1 or TPH2 mutations, we identified a total of 38 adults and 4 children (17 female and 25 male) who were heterozygous for TPH1 missense or nonsense mutations and 4 adults who had TPH2 mutations (eTable). According to our previous findings,6,24 the inactivating TPH2 mutation p.R303W appears to segregate with ADHD; however, family data were not available for the D473N mutation.

No obvious association of TPH1 mutations and a diagnosis of ADHD was apparent in the patients or controls. Persons with 1 malfunctioning TPH1 or TPH2 allele appeared to have normal gross development and life span; 4 mutation carriers were healthy at ages 70 to 85 years, which is consistent with the mild phenotypes of Tph1 and Tph2 knockout mice.9

To obtain more information about the effects of inactivating TPH1 or TPH2 mutations, all study members reported symptoms and psychiatric diagnoses related to ADHD, depression, and substance abuse.21,22 The adults reported childhood ADHD symptoms by using the 25-item Wender Utah Rating Scale (WURS; range, 0-100),30 present ADHD symptoms on the 18-item adult ADHD Self-Report Rating Scale (ASRS; range, 0-72),31 and bipolar spectrum symptoms on the Mood Disorder Questionnaire (MDQ; range, 0-13).32,33 On average, individuals with coding variants in TPH1 (n = 38) had moderately higher scores on childhood and adult ADHD symptoms (P = .03 and P = .07, respectively, t test) and bipolar symptoms (P = .22) than did persons with the WT enzymes (eTable). Stratification by sex showed a more pronounced effect among men than women. However, the observed differences disappeared when the analyses were repeated after exclusion of all ADHD probands, indicating that the presence of 1 malfunctioning TPH1 allele does not confer a strong risk of psychiatric symptoms.

**EFFECTS OF MATERNAL TPH1 MUTATIONS**

Because TPH1 is expressed in peripheral organs, including female reproductive tissues,34 and the maternal but not the paternal Tph1 genotype affects the offspring in
Figure 2. Biochemical properties of the wild-type (WT) and mutant tryptophan hydroxylase 1 (TPH1). A, Relative protein abundancy in transfected human embryonic kidney (HEK293) cells based on immunostaining and RNA levels. B, TPH1 activity in transfected HEK293 cell lysates. Levels of TPH1 protein were determined by immunostaining, and TPH activity of the soluble fractions of cell lysates was measured as described. The activity of A300T was significantly lower than that of the WT (*P<.05, t test). C, Relative solubility of TPH1 mutants. Soluble and insoluble fractions of *Escherichia coli* lysates were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie blue. D, Thermal stability of TPH1 WT and mutants at 37°C. The stability of p.K54Q, p.R142C, p.L274I, p.A300T, and p.I410N were significantly lower than that of the WT (*P<.05, t test). E, Phosphorylation of TPH1 WT and coding variants by cyclic adenosine monophosphate–dependent kinase. Upper panel, phosphorylation stoichiometry of purified maltose-binding protein (MBP)–TPH1 fusion proteins (*n*=3). Middle panel, purified MBP-TPH1 fusion proteins separated by SDS-PAGE and stained with Coomassie blue. Lower panel, phosphoimage of phosphorylated MBP-TPH1 fusion proteins separated by SDS-PAGE. The positions of molecular weight standards are indicated (left). F, Activity of MBP-TPH1 WT and mutants as purified proteins and in *E coli* lysates adjusted for enzyme amount after immunostaining of TPH1. Bars (whiskers) represent mean (SEM); ND, not determined. *Not detectable. †P<.05, ‡P<.005, ††P=.06.
mice, we hypothesized that an impaired maternal serotonin synthesis could affect brain development and behavior of human offspring. To correct for a possible recruitment bias resulting from an overrepresentation of persons with ADHD or related symptoms in relatives of TPH1 mutation carriers, we performed a pairwise comparison of the effects of maternal vs paternal TPH1 mutations for all mutations and families.

When comparing offspring of mothers with TPH1 mutations (n=20) with offspring of fathers with the corresponding TPH1 mutations (n=21) and a population-based control sample (assumed to represent the population distribution of TPH1 variants, n=606), we found a strong effect of parental TPH1 genotype. On average, the group with maternal TPH1 mutations reported 1.5- to 2.5-times-higher symptom levels of ADHD and bipolar disorder than did children of fathers with the TPH1 mutations or the population-derived control group (Table 2 and Figure 3). Using ANOVA, the 3 groups showed significant differences in current symptoms of ADHD (ASRS score, F = 10.1 [P = 5×10^{-3}]), childhood ADHD symptoms (WURS score, F = 15.2 [P = 3.7×10^{-2}]), and symptoms of bipolar disorder (MDQ score, F = 14.6 [P = 6.2×10^{-4}]). Data from an additional pairwise comparison of the 3 groups are shown in Figure 3.

As shown in Table 2, the age and sex distribution was similar in the maternal and paternal samples (Table 2), and sex-specific subanalyses of parental TPH1 mutation effect did not alter the results (data not shown). We could not find any signs of biased patient recruitment; that is, the same proportion of fathers and mothers (12 vs 10) carrying the mutations were recruited. Furthermore, a similar number of offspring (20 vs 21) were recruited from both groups. The proportion of family members consenting to participate was also similar for the 2 groups.

Scatterplots of ASRS scores within each family in Figure 4 illustrate that the effects of a maternal TPH1 mutation were observed across all families and mutations. Furthermore, the effect of a maternal TPH1 mutation remained essentially unchanged when parent probands with TPH1 mutations (4 mothers and 3 fathers) were excluded from the analyses (ASRS score, F = 4.8 [P = 0.008]; WURS score, F = 6.2 [P = 0.002]; MDQ score, F = 10.9×10^{-3} [P = 2×10^{-4}]). Experience with illegal drug use, which also may be considered an expression of impulsive behavior, was significantly different between individuals with the maternal vs paternal TPH1 mutations (47.4% vs 11.8%, respectively, who had ever tried illegal drugs [P = .03, t test]) (Figure 3D).

To further investigate the effect of parental TPH1 genotype and potential confounders on past or present symptoms in offspring, we performed a detailed comparison of demographic and clinical characteristics between individuals with mothers vs fathers with TPH1 mutations. As shown in Table 2, the age and sex distribution was similar in both groups. The proportion of family members consenting to participate was also similar for the 2 groups. Some individuals with mothers vs fathers with TPH1 mutations had a higher proportion of diagnosis of ADHD or related symptoms in relatives of TPH1 mutation carriers, we performed a pairwise comparison of the effects of maternal vs paternal TPH1 mutations for all mutations and families.

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Table 2. Linear Regression Analyses of Symptom Scores for Offspring of Mothers vs Fathers With TPH1 Mutations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mothers With TPH1 Mutations (n=20)</th>
<th>Fathers With TPH1 Mutations (n=21)</th>
<th>Linear Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Unadjusted β Value</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>28.9 (8.8)</td>
<td>33.3 (17.9)</td>
<td>.32</td>
</tr>
<tr>
<td>Female sex</td>
<td>8 (40.0)</td>
<td>12 (57.1)</td>
<td>.01</td>
</tr>
<tr>
<td>Mother smoking in pregnancy</td>
<td>13 (65.0)</td>
<td>6 (28.6)</td>
<td>.01</td>
</tr>
<tr>
<td>Formal ADHD diagnosis</td>
<td>8 (40.0)</td>
<td>3 (14.3)</td>
<td>.01</td>
</tr>
<tr>
<td>TPH1 mutation</td>
<td>13 (65.0)</td>
<td>11 (52.4)</td>
<td>.01</td>
</tr>
<tr>
<td>Symptom score, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WURS, total sum</td>
<td>35.4 (26.7)</td>
<td>17.7 (21.9)</td>
<td>17.7</td>
</tr>
<tr>
<td>ASRS, total sum</td>
<td>32.5 (13.8)</td>
<td>19.8 (11.1)</td>
<td>12.8</td>
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<tr>
<td>ASRS, inattentive score</td>
<td>17.8 (7.0)</td>
<td>11.9 (6.4)</td>
<td>5.96</td>
</tr>
<tr>
<td>ASRS, hyperactive/impulsive score</td>
<td>14.7 (7.9)</td>
<td>7.9 (5.2)</td>
<td>6.85</td>
</tr>
<tr>
<td>MDQ, sum</td>
<td>7.2 (5.8)</td>
<td>2.9 (4.3)</td>
<td>4.23</td>
</tr>
</tbody>
</table>

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ASRS, ADHD Self-Report Rating Scale; MDQ, Mood Disorder Questionnaire; TPH1, tryptophan hydroxylase 1; WURS, Wender Utah Rating Scale.

a Adjusted for age, sex, and maternal smoking in pregnancy.

b Score range, 0 to 100.

c Score range, 0 to 72.

d Score range, 0 to 13.
similar in offspring of mothers and fathers with TPH1 mutations, but maternal smoking during pregnancy was reported significantly more frequently in offspring of mothers with TPH1 mutations. Likewise, there was a nonsignificant trend that offspring of mothers with TPH1 mutations more frequently carried the TPH1 mutation themselves and had previously been formally diagnosed as having ADHD. According to multiple linear regression analyses, the effect of parental TPH1 genotype (β value) on the ASRS and WURS scores was almost unaltered after adjusting for age, sex, and maternal smoking during pregnancy. However, the differences in MDQ scores between the 2 groups were less apparent after this adjustment.

**COMMENT**

Herein we report the first sequencing of the human TPH1 and TPH2 genes in large nonclinical and adult ADHD samples, with special emphasis on the role of TPH1 protein variants. Because TPH1 is responsible for more than 90% of all serotonin production in peripheral tissues\(^3,7,9,35\) and can influence embryonic development,\(^3\) loss of TPH1 function could have a strong effect on human health. The total allele frequency of coding variants in TPH1 and TPH2 in Norway is approximately 1% and 0.2%, respectively. By extensive functional characterization of these variants, we have identified several loss-of-function mutations that are caused by decreased stability or by protein truncation (nonsense mutation). The in vitro activity and stability of coding variants of aromatic amino acid hydroxylases are generally well correlated with their in vivo enzymatic activity,\(^27\) indicating that individuals with heterozygous loss-of-function TPH1 mutations have an impaired serotonin biosynthetic capacity in peripheral tissues.

Although more than 99% of blood serotonin is stored in platelets, the free plasma serotonin is considered the active fraction. Mouse studies have shown that plasma serotonin levels in the offspring are determined by maternal Tph1 genotype and maternal plasma serotonin levels but not by whole-blood serotonin levels.\(^2\) Ideally, the plasma levels of serotonin in all TPH1 mutation carriers should be measured directly to estimate its effect on the developing embryos. However, because it is difficult to obtain plasma completely free of contami-
nating platelet serotonin and because serotonin levels fluctuate depending on dietary factors such as tryptophan intake, we considered that such measurements would not necessarily reflect the relevant maternal serotonin levels, for instance, during a critical gestational period. The serotonin transporter promoter polymor-

Figure 4. ADHD [attention deficit/hyperactivity disorder] Self-Report Rating Scale (ASRS) score within each family. The scatterplot was stratified by parental tryptophan hydroxylase 1 gene (TPH1) carrier status. Families are named by the type of TPH1 mutation (TPH1mut). One dot represents 1 person. Only individuals with completed ASRS scores (>15 years) are represented (total, N=36; maternal, n=19; paternal, n=17). Mean values (gray horizontal bars) and 95% confidence intervals (whiskers; for all families only) are shown.
phism 5-HTTLPR is another functional variant within the serotonergic system that has been reported to have a strong influence on brain morphologic features and function. However, the measured steady-state levels of serotonin or transporter function are not affected by this polymorphism, illustrating the problems of predicting the genotype-phenotype relationships and biological markers in complex disorders.

The TPH1 mutations in mothers seem to predispose to symptoms and behavior related to ADHD and affective disorder in their offspring, as demonstrated across different diagnoses and rating scales. As shown by the large variance in symptom load in different individuals with maternal TPH1 mutations (Figure 4), the clinical outcome probably depends on a sum of many different genetic or environmental factors in addition to variations in maternal serotonin levels.

Studies in humans and animals have indicated that female sex hormones may modify the expression of several serotonergic genes, increasing TPH1 and TPH2 mRNA and protein levels. Although the differences observed between offspring sexes in the present study were not significant, we cannot exclude a potential protective effect of female sex hormones in attenuating the impact of the TPH mutations by enhancing the expression of TPH. Clinical observations and experimental data indicate that many different factors, such as maternal stress experience, inadequate nutrition, or endocrine factors such as levels of thyroid hormones or glucocorticoids early in pregnancy also may contribute to neurodevelopmental disorders in the offspring through their influence on placental function and embryonic development.

We propose that the effect of serotonin deficiency depends on the maternal TPH1 status, independent of the patient’s own genotype. This mechanism of genetic transmission could contribute to the total observed heredity of psychiatric disorders but would be difficult to detect in conventional molecular genetic studies. Although our findings are in accordance with previous animal studies and accumulated clinical evidence, we note that the number of mutation carriers still is small and that statistical evidence of some of the results is only indicative of an effect. Thus, replication studies in other large samples and possibly also reanalyses of previous family-based studies are required to verify this finding.

Although TPH1 is mainly expressed in peripheral tissues, TPH1 variants have frequently been reported to be associated with psychiatric disorders. TPH1 is a strong candidate gene for schizophrenia, and both serotonin and melatonin have been suggested to be involved in the pathogenesis of autism spectrum disorders and Tourette syndrome. An association between low endogenous serotonin function and high alcohol preference has been reported and is consistent with the observed effect of TPH1 maternal genotype on reported illicit drug use in our study (Figure 3D). Thus, the effects of maternal TPH1 dysfunction are not limited to ADHD symptoms but may be linked to more general phenotypic traits, such as dysregulated impulsivity/inhibition, that are found in many psychiatric disorders and may be mediated by different transmitter systems.

We have not examined the functional role of the intrinsic markers used in previous association studies of TPH1, but our data support the idea that TPH1 plays a role in shaping human behavior and the possibility that some of the previously used single-nucleotide polymorphisms may have a functional role or could tag other nearby functional variants. In addition to a direct trophic role of maternal serotonin on embryonic development, the effect of maternal TPH1 genotype could be mediated by altered maternal nurturing behavior, as demonstrated in mice with disrupted serotonin neuronal differentiation.

Mouse studies have also indicated that maternal Tph1 could act in concert with embryonic Tph2 during brain development, and a combination of TPH1 and TPH2 risk alleles have been reported to increase the risk of bipolar disorder through genetic interaction. Although such an interaction in theory is possible, the very low allele frequency of TPH1 and TPH2 coding mutations (<1% for each gene) makes this combination very rare and would not influence the results of our study.

An important challenge when studying the effects of rare functional mutations is the need to sequence large population cohorts. Compared with conventional genetic association studies of frequent genetic markers, our sample of 38 TPH1 mutation carriers and 41 offspring of TPH1 mutation carriers is rather small. Based on the TPH1 mutation frequency estimate approximately equal to 1%, we would have to sequence a random population of 2000 individuals to obtain this sample size. However, because we had access to large multigenerational pedigrees, we were able to identify mutation carriers more efficiently and to systematically compare the effects of parental mutation carrier status within all families and for each mutation (Figure 4).

Our results may also have public health implications. Whether it is caused by genetic vulnerability, chronic inflammation, malnutrition, or other processes, maternal serotonin deficiency during pregnancy might predispose to neuropsychiatric disorders and cardiovascular illnesses. Recently, potent inhibitors of TPH1 and TPH2 activity have undergone clinical testing for use against functional gastrointestinal disorders problems that are most frequently reported in young female patients. Tryptophan hydroxylase inhibitors can block serotonin production in the periphery and in the brain. Our findings indicate that even 50% inhibition of TPH1 during pregnancy could have lasting behavioral effects on the offspring. However, further replication studies, preferably in larger samples, will be required to corroborate this relationship.

Submitted for Publication: October 27, 2009; final revision received February 23, 2010; accepted March 29, 2010.

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Author Contributions: Drs Halmøy and Johansson contributed equally to this study and should be considered co–first authors.
Financial Disclosure: None reported.

Funding/Support: This study was supported by the Research Council of Norway, Western Norway Regional Health Authority, and the University of Bergen.

Online-Only Material: The supplementary eMethods section, eFigure, and eTable are available at http://www.archgenpsychiatry.com.

Additional Contributions: We are grateful to the patients, controls, and family members who participated in this study. Guri E. Matte, BSc, Paal H. Borge, BSc, Sigrid Erdal, MSc, Sidsel E. Riise, BSc, Linda Sleire, MSc, and Ragnhild Nordengø, MSc, provided technical assistance.


**Correction**

Error in Byline and Author Affiliations. In the article “Support for the Vascular Depression Hypothesis in Late-Life Depression: Results of a 2-Site, Prospective, Anti-depressant Treatment Trial” by Sheline et al, published in the March issue of the *Archives* (2010;67[3]:277-285), there were errors in the byline and Author Affiliations. In the byline on page 277, the name Kathleen Welsh-Boehmer, PhD, should appear as Kathleen Welsh-Bohmer, PhD. On page 284, in the Author Affiliations, Dr Welsh-Boemer should appear as Dr Welsh-Bohmer.