Biochemical and Molecular Genetic Characteristics of the Severe Form of Tyrosine Hydroxylase Deficiency

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Background: Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in the biosynthesis of the catecholamines dopamine, norepinephrine, and epinephrine. Recently, mutations were identified in cases of autosomal recessive dopa-responsive dystonia and infantile parkinsonism. We describe a patient with severe symptoms and a new missense mutation in TH.

Methods: Relevant metabolites in urine and cerebrospinal fluid were measured by HPLC with fluorometric and electrochemical detection. All exons of the TH gene were amplified by PCR and subjected to single-strand conformation polymorphism analysis. Amplimers displaying aberrant migration patterns were analyzed by DNA sequence analysis.

Results: The patient presented with severe axial hypotonia, hypokinesia, reduced facial mimicry, ptosis, and oculogyric crises from infancy. The major metabolite of dopamine, homovanillic acid, was undetectable in the patient’s cerebrospinal fluid. A low dose of L-dopa produced substantial biochemical but limited clinical improvement. DNA sequencing revealed a homozygous 1076G→T missense mutation in exon 10 of the TH gene. The mutation was confirmed with restriction enzyme analysis. It was not present in 100 control alleles. Secondary structure prediction based on Chou-Fasman calculations showed an abnormal secondary structure of the mutant protein.

Conclusions: We describe a new missense mutation (1076G→T, C359F) in the TH gene. The transversion is present in all known splice variants of the enzyme. It produces more severe clinical and biochemical manifestations than previously described in TH-deficient cases. Our findings extend the clinical and the biochemical phenotype of genetically demonstrated TH deficiency.

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Tyrosine hydroxylase (TH, EC 1.14.16.2) catalyzes the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-dopa), the rate-limiting step in the biosynthesis of the catecholamines dopamine, norepinephrine, and epinephrine (Fig. 1). The iron-containing mixed function oxidase requires molecular oxygen and the cofactor tetrahydrobiopterin (BH4) for activity. TH is expressed mainly in specific brain areas and in the adrenal medulla (1).

A central role of TH for prenatal development and postnatal survival was indicated by the nonviability of TH-knockout mice (2). In humans, secondary impairment of TH enzymatic activity occurs in defects of BH4 synthesis and recycling, mostly referred to as variant phenylketonurias. The first indication of primary genetic TH deficiency (THD) in humans was provided in 1994 by Clayton et al. (3). To date, four different mutations have been described in six index cases from unrelated families. In two siblings, a point mutation in exon 11c.1141C→A (Q381K) (4, 5) and in another girl a point mutation in exon 5c.614T→C (L205P) (3, 6) have been identified. Recently, a missense mutation in exon 6c.698G→A (R233H) a “common” mutation in The Netherlands and a deletion...
delC291 in exon 3 could be identified in patients with autosomal recessive L-dopa-responsive infantile parkin-sonism. Patients were described as having autosomal recessive L-dopa-responsive dystonia, or Segawa syn-drome, or as L-dopa-responsive parkinsonism in infancy. Clinical symptoms of dystonia, hypokinesia, rigidity, and truncal hypotonia were reported to develop in early childhood. All patients showed marked clinical improvement on low doses of L-dopa together with the decarboxylase inhibitor carbidopa.

We identified a new mutation in a new case of THD with a very severe clinical and biochemical picture. The case extends both the biochemical and the clinical phenotype of the disease.

**Patient and Methods**

**CASE REPORT**

The boy was born prematurely (33rd week of gestation) to healthy consanguineous Italian parents. Severe respiratory distress complicated the perinatal period. Moderate hypotonia and swallowing difficulties were present since birth. Marked axial hypotonia, severe hypokinesia, and reduced facial mimicry increased over the first months of life. Prolonged diurnal periods of lethargy with increased sweating alternated with irritability and rare sporadic dystonic movements and prompted further investigation. The routine clinical chemistry investigations for neuro-metabolic disorders and the electroencephalogram were normal. Magnetic resonance imaging at 5 months of age revealed an unexpected degree of cerebral atrophy. A diagnosis of THD was suggested on the basis of cerebrospinal fluid (CSF) investigations of neurotransmitter metabolites, and therapy with a low dose of L-dopa (6 mg/kg body weight per day) together with the decarboxylase inhibitor carbidopa was initiated. After 10 months of treatment, there was only partial clinical improvement of axial tone, appearance of spontaneous movements, and reduced sweating. The child tolerated only a very gradual increase of medication, complicated by dose-dependent side effects, mainly hyperkinesia and irritability.

**BIOCHEMICAL INVESTIGATIONS**

The neurotransmitter metabolites 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) in CSF and 5-HIAA, HVA, and vanillylmandelic acid (VMA) in urine were measured with HPLC and electrochemical detection, and the metabolites 3-o-methyldopa and L-dopa in CSF and dopamine, epinephrine, and norepinephrine in urine were measured with HPLC and fluorometric detection. The CSF samples were collected according to a standardized protocol for lumbar puncture. The catecholamines were measured in an acidified 24-h urine. The analytical techniques used for the biochemical investigations recently have been described in detail.

**MUTATION DETECTION STUDIES**

Genomic DNA was extracted from leukocytes by standard methods. All exons of the TH gene were amplified by PCR. The amplimers obtained were subjected to single-strand confirmation polymorphism analysis by the Pharmacia Phast System. Running conditions for exon 10 were as follows: 12.5% polyacrylamide gel, 20 °C, 400 V, 5 mA, and 1 W (prerun at 100 V-h and separation at 135 V-h). The primers used for PCR amplification and sequence analysis of exon 10 were as follows: forward primer,
5'-GACCTCCCCTGAGCCGTGAG-3'; and reverse primer, 5'-GAGCAGGCAGCACACTTCACC-3'. Cycle sequencing of the coding and the noncoding strands of exon 10 was carried out by the Taq Dye Deoxy Terminator method in an ABI DNA sequencer (Applied Biosystems type 377). To confirm the mutation in genomic DNA, the 265-bp amplimers of exon 10 of the index patient and the parents were digested with the restriction enzyme _Ita_l, which spliced the wild-type allele seven times (fragments of 79, 59, 41, 33, 31, 10, 9, and 3 bp) and the mutant allele six times (fragments of 79, 59, 51, 33, 31, 9, and 3 bp).

**Mutation Nomenclature**

The reports by of Lüdecke and co-workers (4, 6, 10) and Knapskogg et al. (5) have used a nomenclature strategy based on human mRNA type 1. We have used a nomenclature strategy for indicating _TH_ mutations based on human mRNA type 4 as published by Nagatsu et al. (11). In the human mRNA type 1, a part of exon 1 and the full-length exon 2 are missing (11). This has consequences for the numbering of the exons, nucleotides, and amino acids. A table with the known mutations in the _TH_ gene comparing both nomenclature strategies has been published (8).

**Results**

**Biochemical Investigations**

Routine clinical investigations and investigations for neurometabolic disorders in our patient, including organic acids in urine and amino acids in urine, blood, and CSF, were all normal. Pterin concentrations in the urine were within the reference interval. Biopterin in the CSF was borderline increased together with a low normal dihydropteridine reductase activity in blood (Dr. N. Blau, Zurich, Switzerland), excluding a defect in _BH_4 biosynthesis.

Analysis of the CSF revealed a severe impairment of dopamine biosynthesis with undetectable HVA (lower limit of detection, 5 nmol/L) and a very low MHPG concentration (6% of the lower reference range; Table 1).

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**Table 1. Biochemical investigations in CSF and urine in our severe THD patient at the age of 1.9 years.**

<table>
<thead>
<tr>
<th>CSF concentration, nmol/L</th>
<th>Urine concentrationa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Index case</td>
</tr>
<tr>
<td>HVA</td>
<td>&lt;5</td>
</tr>
<tr>
<td>MHPG</td>
<td>2</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>176</td>
</tr>
<tr>
<td>HVA/5-HIAA ratio</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3-0-methyldopa</td>
<td>&lt;5</td>
</tr>
<tr>
<td>L-Dopa</td>
<td>&lt;5</td>
</tr>
<tr>
<td>VMA</td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
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</tbody>
</table>

* Concentrations in _μ_mol/mmol creatinine for HVA, 5-HIAA, and VMA; concentrations in nmol/mmol creatinine for dopamine, epinephrine, and norepinephrine.

b Reference ranges represent age-matched controls (9).
The concentrations of 5-HIAA, the end product of the serotonin pathway, and 3-0-methyldopa in CSF as well as the urinary excretion of vanillylactic acid, a metabolite of l-dopa, were within the appropriate reference intervals, which excluded aromatic l-amino acid decarboxylase deficiency and pointed to THD as the primary defect (12).

Urine analysis (Table 1) revealed a decreased concentration of HVA (6% of the lower reference range). The concentration of 5-HIAA deriving from serotonin and of VMA as the main metabolite of norepinephrine in the periphery were within the reference ranges. The concentrations of dopamine and epinephrine were in the lower reference range, and only the concentration of norepinephrine was decreased (15% of the lower reference range). The ratio of epinephrine to norepinephrine was increased (4.8; reference, 1). The excretion of the free metanephrines was very low; however, total normetanephrine and metanephrine were within the reference range.

After treatment with increasing doses of l-dopa (up to 6 mg/kg body weight per day) with decarboxylase inhibitor, the HVA concentration in the CSF increased but remained far below the reference range (32% of the lower reference range). In urine, the treatment led to an increase of HVA (48% of the lower reference range). The catecholamines norepinephrine and epinephrine and the ratio epinephrine/norepinephrine normalized, and dopamine increased (371% above the upper reference range) after treatment with l-dopa. Higher doses of l-dopa led to severe adverse clinical symptoms of irritability and violent, abrupt alternating flinging of the arms (ballism).

**Molecular Analysis**

Single-strand confirmation polymorphism analysis was carried out on all exons of the TH gene under at least two different conditions (temperature and gel type). Only exon 10 displayed an aberrant migration pattern in the patient (Fig. 2A) and in both parents (not shown). Direct sequencing revealed that the patient has a novel, homozygous missense mutation, 1076G→T (Fig. 2B). Both parents were heterozygous for this mutation. The finding of a homozygous mutation is in line with parental consanguinity. This transversion produces an amino acid exchange from cysteine to phenylalanine at codon 359 (C359F). The mutation abolishes an ItaI restriction site, producing a 51-bp fragment in the patient DNA instead of the 41- and 10-bp fragments in wild-type DNA (Fig. 3). The mutation was not found in 100 control alleles. In addition, the patient is homozygous for the common polymorphism V112M (10).

Secondary structure prediction according to Chou and Fasman (13) and Garnier et al. (14) predicted an extra turn in the secondary structure of the mutant protein (not shown). The mutation is present in all different splice variants known to be present for human TH (15). Human TH has seven cysteine residues. Six of the seven cysteine residues are conserved in other species (rat, bovine, and quail) (11), including the cysteine residue in our point mutation. The cysteine residues are located in the carboxy-terminal half of the enzyme where the catalytic domain is situated (11). The 20 amino acids around cysteine 359 are highly (90–100%) conserved among species (rat, bovine, and quail; Fig. 4). The mutant cysteine residue is one of five cysteine residues also conserved in the other human aromatic amino acid hydroxylases, tryptophan hydroxylase and phenylalanine hydroxylase (Fig. 4). The percentage of homology of the 20 amino acids surrounding this cysteine residue is 71% for human phenylalanine hydroxylase and 67% for human tryptophan hydroxylase. This part of the protein and the cysteine residue are conserved in other species (rat, bovine, and quail). The percentage of homology of the 20 amino acids around cysteine 359 is highly (90–100%) conserved among species (rat, bovine, and quail; Fig. 4). The mutation site C359F (C329F based on human mRNA type 1) is indicated by the box. Amino acids of rat, bovine, and quail TH; human phenylalanine hydroxylase (PAH); and human tryptophan hydroxylase (TPH) are indicated by dashes.
tyrosine residue therefore seem pivotal for enzymatic function of aromatic amino acid hydroxylases.

**Discussion**

THD has hitherto been described as a rare cause of autosomal recessive dopa-responsive dystonia or l-dopa-responsive infantile parkinsonism (4, 5, 7). The diagnosis was suspected in our patient because the concentration of HVA in the CSF was undetectable (<5 nmol/L) at the time of diagnosis. In previously described patients, HVA in the CSF was between 8% and 30% of the lower reference range (9) or 5% of the lower reference range (6). The biochemical results in the CSF together with the clinical picture indicated a severe deficiency of TH. The point mutation 1076G→T in the TH gene probably has a profound effect on the catalytic activity of the enzyme, and when present in the homozygous form, it does not seem to allow substantial residual enzymatic activity. The 1076G→T transition produced an amino acid change from cysteine to phenylalanine at codon 359 mRNA type 4 (codon 329 in mRNA type 1). TH is composed of two functional domains, i.e., a catalytic domain, which is located proximal to the C-terminal region, and a regulatory domain, which is located at the NH₂ terminus. The catalytic domain of the enzyme contains residues 188–456, and any truncation within these residues produces a protein that expresses extremely poorly and has no detectable activity (16). The six cysteine residues that are conserved in their positions in other species are located in the C-terminal half and form a catalytic domain. In addition, the amino acids around them are highly conserved among species. This suggests that these cysteine residues may play an important role in enzymatic function by keeping the conformation of the protein by means of intra- or intermolecular S-S bonds and/or by interacting with ferrous ion, which is an essential component for the catalytic action of TH (11). The C359F mutation seems to be the most severe disease-causing mutation described to date in THD. Our findings extend the biochemical phenotype of THD. Because HVA was undetectable in the CSF of our patient and MHPG was very low, both dopamine and norepinephrine biosynthesis are severely impaired in our patient. There seems to be hardly any flux through the catecholamine biosynthesis pathway in the brain. In all earlier studies on patients in whom a defect in TH was earlier studies on patients in whom a defect in TH was suspected in our patient because the concentration of HVA in the CSF was undetectable (<5 nmol/L) at the time of diagnosis. In previously described patients, HVA in the CSF was between 8% and 30% of the lower reference range (9) or 5% of the lower reference range (6). The biochemical results in the CSF together with the clinical picture indicated a severe deficiency of TH. The point mutation 1076G→T in the TH gene probably has a profound effect on the catalytic activity of the enzyme, and when present in the homozygous form, it does not seem to allow substantial residual enzymatic activity. The 1076G→T transition produced an amino acid change from cysteine to phenylalanine at codon 359 mRNA type 4 (codon 329 in mRNA type 1). TH is composed of two functional domains, i.e., a catalytic domain, which is located proximal to the C-terminal region, and a regulatory domain, which is located at the NH₂ terminus. The catalytic domain of the enzyme contains residues 188–456, and any truncation within these residues produces a protein that expresses extremely poorly and has no detectable activity (16). The six cysteine residues that are conserved in their positions in other species are located in the C-terminal half and form a catalytic domain. In addition, the amino acids around them are highly conserved among species. This suggests that these cysteine residues may play an important role in enzymatic function by keeping the conformation of the protein by means of intra- or intermolecular S-S bonds and/or by interacting with ferrous ion, which is an essential component for the catalytic action of TH (11). The C359F mutation seems to be the most severe disease-causing mutation described to date in THD.

Our findings extend the biochemical phenotype of THD. Because HVA was undetectable in the CSF of our patient and MHPG was very low, both dopamine and norepinephrine biosynthesis are severely impaired in our patient. There seems to be hardly any flux through the catecholamine biosynthesis pathway in the brain. In all earlier studies on patients in whom a defect in TH was genetically confirmed, there seemed to be some degree of residual TH activity. As evidenced by the CSF HVA concentrations in these patients, they all had the capability of synthesizing catecholamines to some extent. This also may explain the limited beneficial reaction to l-dopa in our patient. The side effects of very low l-dopa doses may be explained by receptor up-regulation. Iloprolide-Spect scanning, however, did not show evidence for dopamine D₃ receptor up-regulation.

The biochemical findings in our patient are in line with the obviously very severe clinical signs and symptoms that include structural abnormalities in the brain as observed in the magnetic resonance imaging. Therefore, our patient also extends the clinical phenotype of THD. THD in most cases reacts favorably to low-dose l-dopa therapy and is considered a treatable disease. Therefore, it is important to know the various possible clinical presentations of the disease. The present case illustrates that THD should be considered in all children with severe encephalopathy, especially when dominated by extrapyramidal signs and hypokinesia even when there are magnetic resonance imaging abnormalities in the central nervous system.

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**References**


