Mutations in the Cyclic Adenosine Monophosphate Response Element of the Tyrosine Hydroxylase Gene

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Tyrosine hydroxylase (TH) deficiency (OMIM 191290) is one cause of early-onset dopa-responsive dystonia. We describe seven cases from five unrelated families with dopa-responsive dystonia and low homovanillic acid in cerebrospinal fluid who were suspected to suffer from TH deficiency. Analysis of part of the TH promotor showed five homozygous and two heterozygous mutations in the highly conserved cyclic adenosine monophosphate response element. Our data suggest that, if no mutations are found in the coding regions of the gene in patients strongly suspected of TH deficiency, the search for pathogenic mutations should be extended to regulatory promotor elements.


Typically, TH deficiency becomes manifest by the end of the first year and is characterized by ptosis, inexpress...
sive faces, oculogyric crises, tremor, truncal hypotonia, and limb dystonia, and biochemically by a selectively decreased concentration of homovanillic acid (HVA) in cerebrospinal fluid (CSF). The definite diagnosis, however, depends on the demonstration of mutations in the TH gene. A number of different mutations in the coding sequence of the TH gene have been reported,\(^1,2\) including a “common” mutation in the Dutch population (c.698G>A; p.Arg233His)\(^3\) and a single base pair deletion in exon 3.\(^4\) In this report, we describe seven cases, clinically suspected to suffer from TH deficiency, with low CSF HVA levels and with a moderate-to-good response to L-dihydroxyphenylalanine (L-dopa) therapy (Table; see supplementary file online for case descriptions).

**Patients and Methods**

**Patients**

Clinical features of the patients studied here are described later in the Results.

**Cerebrospinal Fluid Analysis**

HVA and 5-hydroxy-indole acetic acid (5-HIAA) in CSF of Cases IV-1, IV-2, and V-1 were initially measured in Sydney, Australia. Repeat measurements of Cases IV-1 and IV-2 and initial measurement of Case II-1 were performed in Nijmegen, the Netherlands, according to previously described methods.\(^2\) CSF of the other patients was analyzed locally according to similar methodology.

**Tyrosine Hydroxylase Gene Analysis**

Genomic DNA was extracted from leukocytes by standard methods followed by polymerase chain reaction (PCR). The entire coding region of the TH gene was sequenced, including 211bp of the promotor region.\(^5\) Primers were designed to include the intron-exon boundaries as described elsewhere\(^6\) with the exception of modifications in the forward primers for exons 1, 6, 11, 13, and 14 (sequences available on request).

PCR amplifications were performed in a total volume of 50μl on an I-cycler (Bio-Rad, Veenendaal, the Netherlands). Each reaction mixture contained 200ng forward and reverse primer, 500μM of each deoxyribonucleoside triphosphate (dNTP), 50mM tris(hydroxymethyl)aminomethane-HCL buffer (pH 9.2), 16mM (NH\(_4\))\(_2\)SO\(_4\), 2.25mM MgCl\(_2\), 2% dimethylsulfoxide, 0.1% Tween 20, 1 unit AmpliTaq DNA Polymerase (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands), and 100ng genomic DNA. The PCR products were purified with the enzymes Exonuclease I and Shrimp Alkaline Phosphatase (Amersham Bioscience, Roosendaal, the Netherlands) and subsequently sequenced on an ABI PRISM 377 automated DNA sequencer using the BigDye Terminator v3.1 Cycle Sequencing Kit, according to the recommendations of the manufacturer (Applied Biosystems).

Mutations in the promotor region were all confirmed by restriction enzyme analysis by using AAT II (recognition site GACGT ↓ C; New England Biolabs, Beverly, MA). Restriction enzyme analysis of the exon 11 PCR fragment was performed with Nla III (recognition site CATG ↓; New England Biolabs). Restriction enzyme analysis of the exon 14 PCR fragment was performed with Bsp1407I (recognition site T ↓ GTACA; New England Biolabs).

Numbering of coding sequence mutations was according to GenBank reference sequence NM_199292 (TH isoform A) in which the A of the ATG transcription initiation codon is designated position as 1. Numbering of the promotor mutations was defined relative to the ATG initiation codon. According to this numbering, the cyclic adenosine monophosphate response element (CRE) resides between residues −67 and −74 directly upstream of the ATG initiation codon. Previously, a different numbering had been used, defining

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr)</th>
<th>HVA (nM)</th>
<th>Reference 5-HIAA (nM)</th>
<th>Reference 5-HIAA (nM)</th>
<th>HVA/5-HIAA</th>
<th>Reference Consensus Sequence</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>3</td>
<td>100</td>
<td>211–871</td>
<td>142</td>
<td>105–299</td>
<td>0.7</td>
<td>TGACGACA</td>
</tr>
<tr>
<td>II-1</td>
<td>7</td>
<td>69</td>
<td>350–550</td>
<td>129</td>
<td>100–225</td>
<td>0.5</td>
<td>TGATGTCG</td>
</tr>
<tr>
<td>III-1</td>
<td>11</td>
<td>83</td>
<td>220–560</td>
<td>114</td>
<td>90–237</td>
<td>0.7</td>
<td>TGACATCA</td>
</tr>
<tr>
<td>III-2</td>
<td>2</td>
<td>319</td>
<td>364–870</td>
<td>301</td>
<td>155–359</td>
<td>1.1</td>
<td>TGACATCA</td>
</tr>
<tr>
<td>IV-1</td>
<td>0.5</td>
<td>133</td>
<td>478–895</td>
<td>192</td>
<td>231–618</td>
<td>0.7</td>
<td>TGACATCA</td>
</tr>
<tr>
<td>IV-2</td>
<td>2</td>
<td>159</td>
<td>384–769</td>
<td>138</td>
<td>110–265</td>
<td>1.2</td>
<td>TGACATCA</td>
</tr>
<tr>
<td>V-1</td>
<td>1.1</td>
<td>100</td>
<td>429–789</td>
<td>200</td>
<td>156–275</td>
<td>0.5</td>
<td>TGACATCA</td>
</tr>
</tbody>
</table>

\(^a\)Boldface indicates mutation; wild-type control sequence: TGACGTCA.

\(^b\)Relative to the ATG start codon.


HVA = homovanillic acid; HIAA = 5-hydroxy-indole acetic acid.
the location of the CRE between residues −38 and −45 directly upstream of the transcription initiation site.7

Secondary Structure of Protein Prediction

Secondary protein structure prediction was performed using the software developed by the National Institute of Advanced Industrial Science and Technology (Tokyo, Japan; available at: http://mbs.cbrc.jp/papia-cgi/ssp_query.pl?query=seq). The final prediction of protein secondary structure was achieved by combining the results of five different prediction methods ("new joint" method). Conservation of amino acid sequences was performed by using the BLAST software (http://www.ncbi.nlm.nih.gov/BLAST).  

Results

Conventional sequence analysis of the 14 exons of the TH gene did not demonstrate any mutation in Cases I-1, III-1, III-2, IV-1, and IV-2. Novel mutations were found in exon 11 of the TH gene in Case II-1 (c.1159 C>A transversion), causing an amino acid substitution (p.Leu387Met), and a mutation in exon 14 in Case V-1 (c.1475 C>T, p.Pro492Leu). Both mutations affect amino acids in the TH enzyme that are highly conserved between different species (see Supplementary Table E1). Prediction of the secondary structure demonstrated the replacement of a β-sheet in the wild-type protein to a turn in the mutant p.Leu387Met protein. Similarly, the secondary structure of the protein changes from a random coil into a β-sheet in case of the mutant p.Pro492Leu protein. Finally, these amino acids were conserved in the four human amino acid hydroxylases (see Supplementary Table E1).

Because all seven cases displayed typical clinical features of TH deficiency, combined with a suggestive neurochemical pattern, that is, CSF HVA concentrations less than 50% of the lower reference range in six of seven cases with normal 5-HIAA concentrations, we decided to extend our search for mutations to intron-exon boundaries and part of the promotor region. Novel homozygous mutations were identified in the CRE (TGACGTCA) of the TH promotor in Cases I-1, III-1, III-2, IV-1, and IV-2, who are homozygous for the mutation at this base pair (−69 T>A). Site-directed mutagenesis of −69 T>A in the CRE octamer of the rat TH promotor, similar as the mutation observed in Case I-1, results in a reduction of basal TH gene transcription of 78%, which may thus explain the clinical picture of Case I-1.13 Site-directed mutagenesis resulted in a 90% reduction of basal expression by a −70 G>T mutation, providing an explanation for the reduced TH activity in Cases III-1, III-2, IV-1, and IV-2, who are homozygous for the mutation at this base pair (−70 G>A). Furthermore, the −71 C>T mutation, as observed in Case II-1, was found to cause a reduction of 90% of basal transcription of the TH promotor.15 Residual activity appeared significant in these patients, which likely corresponds to an especially good response to therapy and outcome compared with previously reported patient series.14 Mutations in the CRE site in several other genes, including human fibronectin,15 also strongly reduced the basal transcription of the gene, confirming the important role for the CRE site in gene transcription.

We observed the same promotor mutation (−70 G>A) in five cases from five families. Therefore, although TH deficiency is a rare disorder, this mutation may be regarded as relatively common. Because the affected children have different backgrounds (either from Turkish, Lebanese, or Fijian/Indian origin), it is not likely that there is a common founder for these mutations. We have previously identified a relatively common mutation in exon 6 (c.698G>A; p.Arg233His),3 suggesting that certain hotspots for pathogenic mutations exist in the TH gene and promotor.

Mutations in regulatory sites of genes are reported to comprise 1.2% of the total number of known mutations in human genes (Human Gene Mutation Database: http://www.hgmd.cf.ac.uk/ac/hahaha.php). Approximately 4,000 genes16,17 contain a CRE; but to the best of our knowledge, this is the first report of...
mutations in the CRE region leading to a human disorder. This suggests that mutations in the CRE of other genes, although not yet identified, may potentially be related to other familial syndromes where mutations in the coding regions of the responsible gene are not identified. We conclude that TH deficiency may be caused by mutations in the CRE of the TH promotor, and if mutations are not found in the coding regions of the TH gene in patients strongly suspected of TH deficiency, the search for pathogenic mutations should be extended to the CRE region.

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References


