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Brief Communication

A homozygous tyrosine hydroxylase gene promoter mutation in a patient with dopa-responsive encephalopathy: Clinical, biochemical and genetic analysis

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Abstract

We report a recessive mutation in the tyrosine hydroxylase gene (*TH*) promoter (c.1−71C>T), present at homozygosity in a patient with dopa-responsive encephalopathy. The change lies in a cAMP response element (CRE) and alters a binding site for the CREM transcription factor. Previous studies support that the CRE in the *TH* gene is essential for its transcription, suggesting that mutations within this consensus motif may cause an impairment of catecholamine biosynthesis and lead to a pathogenic phenotype.

Keywords: Tyrosine hydroxylase deficiency; Dopa-responsive encephalopathy; *TH* gene promoter; Mutation analysis; cAMP response element; CRE; Neurotransmission

Tyrosine hydroxylase (TH, EC 1.14.16.2) catalyses the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-dopa) and is the rate-limiting enzyme in the biosynthesis of the catecholamines dopamine, norepinephrine and epinephrine [1].

The human *TH* gene maps to 11p15.5, contains 14 exons and produces, at least, eight different mRNAs and protein products through alternative splicing and the differential usage of distinct transcription initiation sites [2–4]. In addition, TH levels, stability and activity are highly regulated by different posttranslational mechanisms that include phosphorylation, feedback inhibition by catecholamines, allosteric modulation of the enzyme activity and translational control [5].

Mutations in the *TH* coding region and splice sites have been associated with decreased enzymatic activity, determine TH deficiency and lead to a variety of clinical phenotypes including a recessive form of Segawa's syndrome [6], autosomal recessive L-dopa-responsive parkinsonism [7], recessive L-dopa-responsive dystonia (DRD) [8–11], L-dopa non-responsive dystonia or progressive early-onset encephalopathy [12]. Fourteen missense mutations, two deletions and one splice site mutation have been identified in the *TH* gene to date (Fig. 1).

The diagnosis of TH deficiency requires the quantification of biogenic amines and pterins in cerebrospinal fluid

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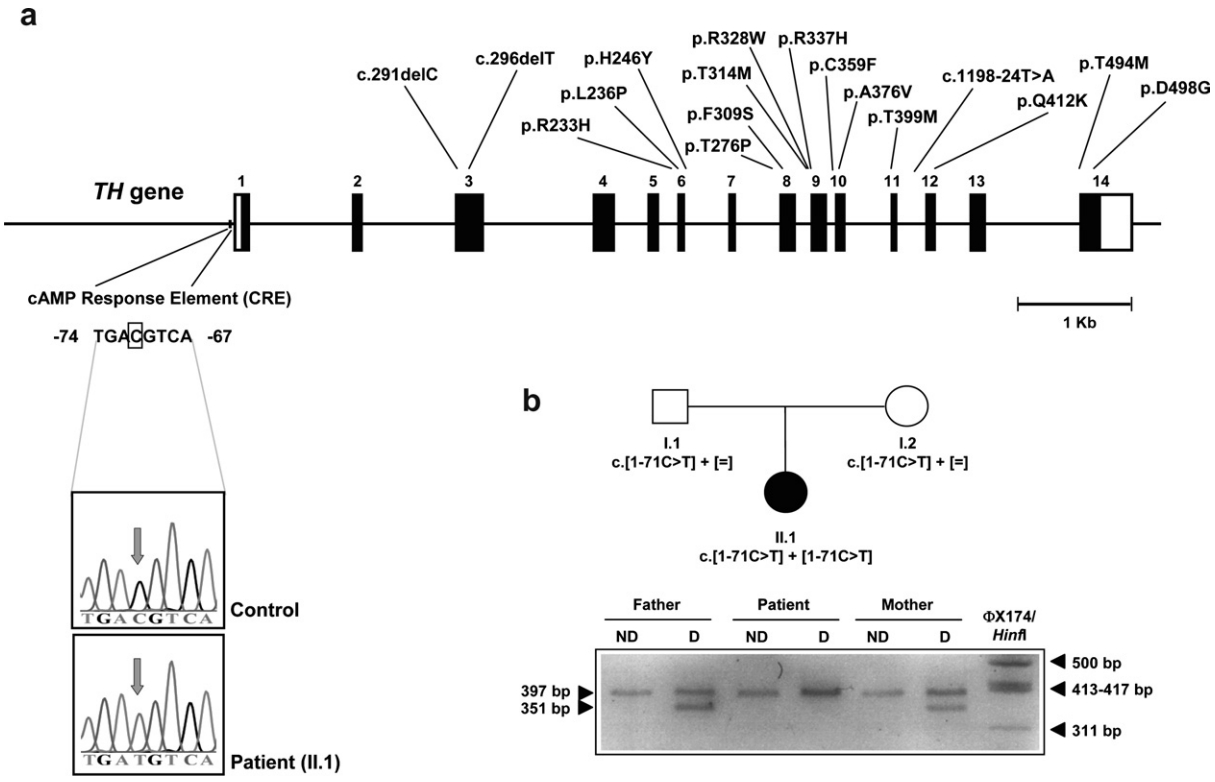

Fig. 1. Sequence and restriction analysis of a PCR product containing part of the promoter region of the *TH* gene. (a) On top, schematic representation of the gene, with the mutations previously found in patients with tyrosine hydroxylase deficiency. Exons are indicated as boxes, with the coding sequences in black. Below, sequence analysis of a healthy control and the patient, which shows a C to T transition within the cAMP response element (CRE). (b) Detection of the c.1−71C>T mutation in the patient, homozygous, and her parents, carriers, by AatII restriction analysis. ND, non-digested PCR product; D, digested product.

(CSF) and is characterized by low concentrations of the dopamine metabolites homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG). After the biochemical confirmation of the clinical suspicion, definite diagnosis is achieved by the screening of mutations in the *TH* gene. Several patients with clinical and biochemical evidences of TH deficiency, however, show no mutations in the *TH* coding region, which suggests a potential involvement of regulatory regions, such as the promoter or the 5′ and 3′ untranslated regions of the gene.

Here we report the first case with TH deficiency caused by a mutation in the *TH* gene promoter.

Patients and methods

Case report

A 2-year-old female was referred because of marked trunkal hypotonia and dystonic posturing of the limbs. Her perinatal history was unremarkable. There was no family history of a similar illness or consanguinity, although the parents were born in a small rural village in Extremadura, southern Spain. Her 7-year-old sister was healthy. On examination, at 6 months of age, the child showed marked trunkal hypotonia, clonic movements, tremor, dystonic posturing of the limbs and hypersalivation. She has a good contact. During early childhood, three cranial MRI including spectroscopy showed no abnormalities. EEG studies were normal.

She was referred to our centre at 2 years 5 months with the diagnosis of dystonic tetraparesis. Parents referred drooling and swallowing difficulties with solids. Language was absent, although she understood simple sentences. Marked hypomotility with facial hypomimia (mask face) was evident and she showed a dystonic posturing of the hands. She also manifested tremor and oculogyric crisis. She had severe trunkal hypotonia, with no head control and no stability to seat. Brisk rotulian reflexes and Achilles' tendon retractions were also present. Some symptoms were more prominent towards the end of the day, especially tremor and oculogyric crisis, and improve after sleeping.

Since a diagnosis was suspected on the basis of CSF biochemical data of neurotransmitter metabolites, therapy with low dose of L-dopa plus carbidopa (100/25) was initiated and gradually increased. At 6 months of treatment she was taking 6 mg/kg/day and no side effects were detected. Some symptoms clearly improved within weeks (Table S1) while others ameliorated moderately. Biochemical data before and after treatment are described in Table S1.

Biochemical analysis

CSF samples were collected early in the morning and stored following a previously reported protocol [13,14]. Biogenic amines (3-ortomethyldopa, MHPG, HVA and 5-hydroxyindoleacetic acid (5-HIAA)), and pterins (neopterin and biopterin) were analysed by HPLC with electrochemical and fluorescence detection procedures, as previously reported [14].

Samples were drawn in accordance with the Helsinki declaration. The study was approved by the local Ethics Committee and written informed consent was obtained from subjects.

Genetic study

We amplified and sequenced the *TH* coding regions, splice sites, 114 bp preceding the initiation codon and 354 bp following the stop codon of the *TH* gene in the patient using a set of ten primer pairs (Table S2). AatII

(New England Biolabs) restriction analysis of a PCR product including exon 1 and part of the promoter was performed to confirm the mutation in the patient, to genotype the parents and to screen 85 healthy controls. The restriction analysis was followed by electrophoresis on a 2% agarose gel and ethidium bromide staining. The mutation abolishes an AatII restriction site that is present only once in the 397-bp normal PCR product (normal pattern: 351 + 46 bp; mutant pattern: 397 bp).

Results

Biochemical analysis

Initial biogenic amines and pterin concentrations in CSF are reported in Table S3. A clear decrease in HVA concentration and HVA/5-HIAA ratio was observed, while the other metabolites analysed were normal, results compatible with a reduced synthesis of dopamine in the central nervous system due to a deficient function of the TH enzyme. Biogenic amines analysis in CSF after treatment still showed low HVA and HVA/HIAA ratio values, despite the obvious clinical improvement.

Genetic study

Complete sequencing of the *TH* gene revealed that the patient was homozygous for a novel putative disease-causing mutation in the promoter region (c.1−71C>T, see Fig. 1). The mutation is a C to T transition at position −71 with respect to the A of the translation initiation codon (corresponding to nucleotide 3163 of the RefSeq promoter sequence AY211521), and lies within the highly conserved cAMP response element (CRE), altering the binding site for the CREM transcription factor (TGACGTCA → TGATGTCA). Both parents were heterozygous for the mutation, which was not found in 170 chromosomes of healthy controls.

Discussion

To our knowledge, this is the first article reporting a mutation within the *TH* promoter in a patient with TH deficiency, although three other cases have been previously reported in an abstract form [15]. Previous studies have described mutations in the coding region or splice sites of the *TH* gene in patients with a wide range of clinical phenotypes caused by a TH deficiency (Fig. 1). Our index patient shows a severe clinical presentation of TH deficiency, moderate HVA deficiency and a good response to L-dopa therapy.

The *TH* promoter region contains numerous consensus binding sites for basal and neuron-specific transcription factors essential for the tissue-specific and differentiation-related expression [5,16–20]. The functional relevance of some of these sites and their involvement in the expression of the *TH* gene has been extensively studied. Among them, the cAMP response element (CRE), an octomer DNA motif 5′-TGACGTCA-3′ located at −74 to −67 bp upstream of the translation initiation codon, is not only responsible for the cAMP-mediated induction in response to environmental stimuli but is crucial for the basal transcription of the *TH* gene [21–23]. Interestingly, both deletional and site-directed mutagenesis analyses of the CRE motif in the rat *TH* gene showed that every nucleotide of the CRE motif is important for the transcriptional activity of the *TH* promoter and supports a causative role of the c.1−71C>T mutation located within the CRE sequence. Deletion of a 5′ region including 6 bp of the CRE resulted in a dramatic loss of *TH* transcription. In addition, replacement of C residue by T at −42 bp, that corresponds to the human c.1−71C>T mutation identified in our patient, diminished almost 90% of the basal and cAMP-mediated *TH* gene transcription [21], results that are in agreement with those reported elsewhere [24], where the C to G substitution at the same position of the rat *TH* promoter displayed a dramatic loss of both basal and induced transcriptional activity and showed reduced affinity to nuclear proteins.

In conclusion, all these data suggest that point mutations within the CRE motif lead to a severe impairment of the *TH* transcription and, consequently, of the catecholamine biosynthesis. Since some patients with biochemical evidence of TH deficiency show no mutations within the *TH* coding region or splice sites, the inclusion of the promoter region in the mutational screening may improve the genetic diagnosis of this disorder.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ymgme.2007.07.004.

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