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Tyrosine Hydroxylase Deficiency in Three Greek Patients with a Common Ancestral Mutation

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Abstract: **We present the clinical, biochemical, and molecular findings of three Greek patients with tyrosine hydroxylase (TH) deficiency. All patients presented with a severe clinical phenotype characterized by prominent motor delay, infantile parkinsonism, oculogyric crises, and signs of autonomic dysfunction. Cerebrospinal fluid analysis disclosed reduced dopamine metabolites and normal pterins. Response to levodopa was favorable though not dramatic. All patients were homozygous for a previously reported mutation (p.L236P). SNP haplotype analysis was consistent with a common ancestral mutation, thus indicating a founder effect in Greek patients with TH deficiency.** © 2010 Movement Disorder Society

Key words: tyrosine hydroxylase; infantile parkinsonism; oculogyric crisis; autonomic dysfunction; founder effect

INTRODUCTION

Tyrosine hydroxylase (TH, EC 1.14.16.2) is the rate-limiting step in the biosynthesis of catecholamines.[1] TH deficiency (MIM#605407) is a rare metabolic disorder; it has been reported in ~30 patients in the litera-

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TABLE 1. *Clinical features and biogenic amines concentrations (nmol/L) in CSF*

	Patient 1	Patient 2	Patient 3
Age at onset (mo)/Age at diagnosis (mo)	3/24	5/5	3/5
Symptoms at onset			
Normal acquisition of head control	+	+	+
Loss of head control followed by lack of motor acquisitions	+	+	+
Tremor	+	+	+
Oculogyric crises	+[a]	+	+[a]
Diurnal fluctuation/Sleep benefit	+	−	+
Autonomic dysfunction[b]	+	+	+
Sleep disturbance	−	−	−
Examination at the time of diagnosis			
Alert and irritable	+	+	+
Hypotonia	+	+	+
Ptosis/Hypomimia	+	+	+
Minimal spontaneous movements/Lack of axial control	+	+	+
Tremor	−	+	+
Dystonia[c]	+	+	+
Babinski sign	+	+	−
Hyperprolactinemia	+	+	+
CSF analysis			
HVA (normal range)[d]	50 (344–906)	31 (354–1328)	18.5 (354–1328)
HIAA (normal range)[d]	197 (170–490)	270 (217–1142)	235 (217–1142)
HVA/HIAA (normal range)[d]	0.25 (1.11–3.48)	0.11 (1.16–2.4)	0.08 (1.16–2.4)
MHPG (normal range)[d]	20 (20–80)	1.6 (30–124)	1.4 (30–124)
3OMD (normal range)[d]	10 (4–50)	4.6 (20–162)	10 (20–162)

[a]Associated with prominent dystonic posturing of limbs and trunk.
[b]Excessive sweating, increased upper respiratory secretions.
[c]Dystonic movements were observed when the infants were manipulated and stressed.
[d]Normal range of metabolite concentrations is age dependent.
HVA, homovanillic acid; HIAA, 5-hydroxyindoleacetic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; 3OMD, 3-*ortho*-methyldopa.

ture.[2–10] Clinical manifestations derive mainly from chronic dopamine deficiency in the developing brain.[9,11] Patients may present with a severe clinical phenotype characterized by lack of motor development, parkinsonism, dystonia, and oculogyric crises associated with autonomic and endocrine dysfunction.[2–11] Intermediate phenotypes also occur in TH deficiency and some patients may present with dopa responsive dystonia similar to Segawa disease.[12] Patients show a characteristic pattern of biogenic amine metabolites in CSF, with decreased homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) and normal pterins.[11] The treatment of choice in TH deficiency is levodopa (L-dopa), but response is heterogenous.[9,12]

We report herein three unrelated patients from Greece sharing a homozygous missense mutation in the *TH* gene, and we analyze the possibility of a common ancestral origin for this particular mutation in the Greek population.

PATIENTS AND METHODS

Patients

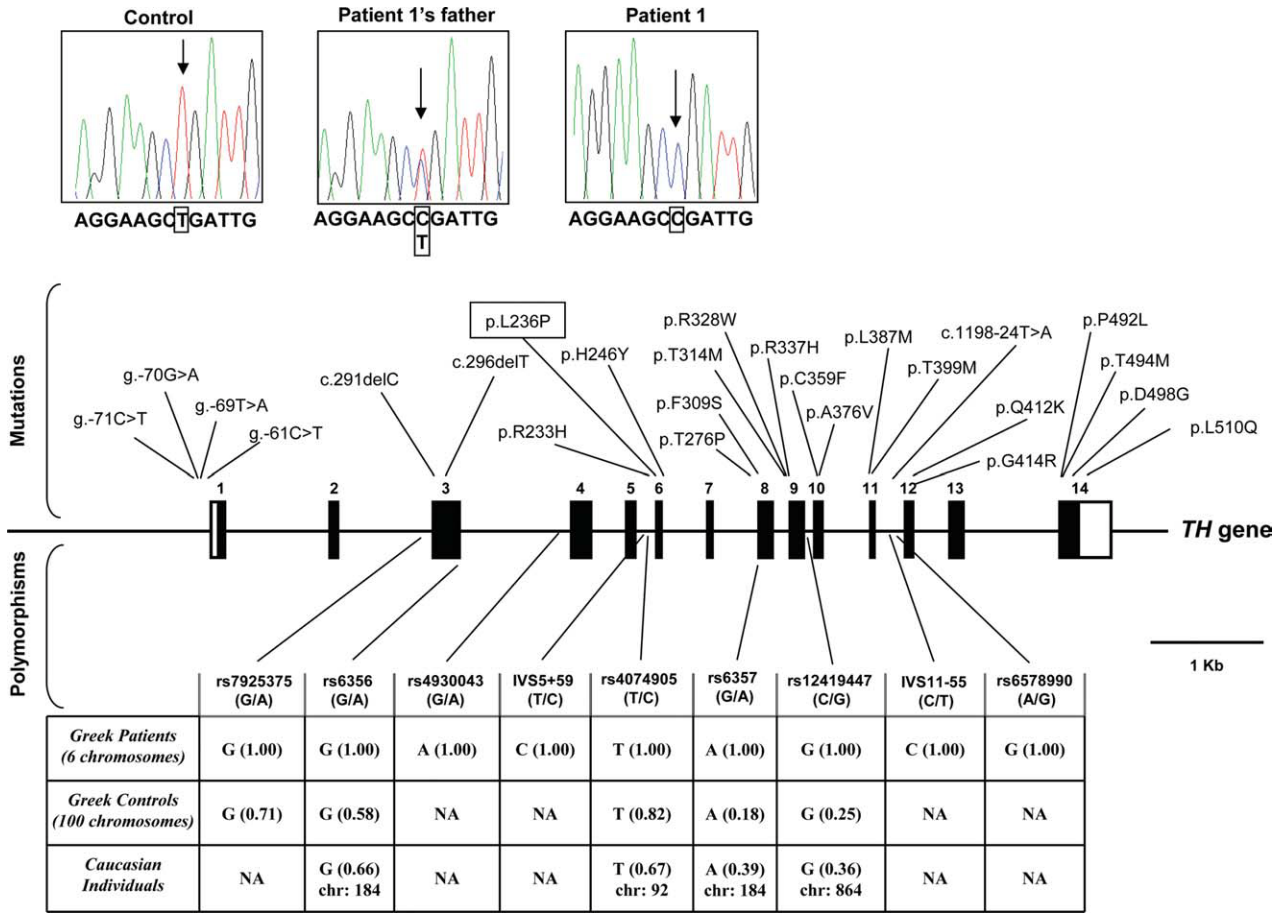
All patients were assessed clinically at Agia Sofia Hospital in Athens. They belonged to three unrelated families from different regions of Greece. Their parents were healthy and nonconsanguineous. All pregnancies and perinatal periods were uneventful. Their clinical presentation and physical exams are depicted in Table 1.

All patients were treated with L-dopa (0.5–1 mg/kg/day) and doses continue to be gradually titrated upwards according to tolerability. They all showed improvements in facial expression, symptoms of autonomic dysfunction, and started gradually to make motor progress (axial control and hand use). Drug-induced dyskinesias were observed in Patients 1 and 3, and they were managed with reduction of the L-dopa dose and slow gradual increase of the medication.

Biochemical Analysis

CSF biogenic amines [3-*ortho*-methyldopa (3OMD), MHPG, HVA, and 5-hydroxyindoleacetic acid (5-HIAA)] and pterins (neopterin and biopterin) were analyzed by HPLC in the Department of Clinical Biochemistry of the Sant Joan de Déu Hospital in Barcelona.[13]

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



	rs7925375 (G/A)	rs6356 (G/A)	rs4930043 (G/A)	IVS5+59 (T/C)	rs4074905 (T/C)	rs6357 (G/A)	rs12419447 (C/G)	IVS11-55 (C/T)	rs6578990 (A/G)
Greek Patients (6 chromosomes)	G (1.00)	G (1.00)	A (1.00)	C (1.00)	T (1.00)	A (1.00)	G (1.00)	C (1.00)	G (1.00)
Greek Controls (100 chromosomes)	G (0.71)	G (0.58)	NA	NA	T (0.82)	A (0.18)	G (0.25)	NA	NA
Caucasian Individuals	NA	G (0.66) chr: 184	NA	NA	T (0.67) chr: 92	A (0.39) chr: 184	G (0.36) chr: 864	NA	NA

FIG. 1. Top: Sequence analysis of a PCR product containing exon 6 of the *TH* gene in a healthy individual and in Patient 1 and her father. The identified mutation is p.L236P (c.707T>C). The protein numbering is based on sequence NP 954986.2. The cDNA sequence numbering is based on RefSeq sequence NM 199292 that corresponds to transcript variant 1, encoding the longest TH isoform (a), with nucleotide 20, the adenine of the ATG start codon, corresponding to position +1. Mutation nomenclature follows HGVS guidelines (www.hgvs.org). Bottom: Schematic representation of the *TH* gene, with previously reported mutations on top and the studied SNP polymorphisms below. Under the SNPs, we indicate the frequency of the allelic variants found in the Greek patients and controls screened by us and in Caucasian individuals form several databases: Centre d'Étude du Polymorphisme Humain (CEPH) (rs6356 and rs6357); Applied Biosystems AoC Caucasian database (rs4074905); and the birth records-based sample of Missouri twins (MOTWINS) (rs12419447). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Genetic Study

Genomic DNA was isolated from peripheral blood. Sequencing analysis of the *TH* gene was performed in the Department of Clinical Biochemistry of the Sant Joan de Déu Hospital in Barcelona. Further genetic studies were performed at the Department of Genetics of the University of Barcelona. We PCR-amplified and sequenced (ABI Prism, Applied Biosystems) the coding region, splice sites, 114 bp preceding the initiation codon, and 354 bp following the stop codon of the *TH* gene using a set of 10 primer pairs, as previously reported.[14] Putative disease-causing mutations as well as intronic and exonic polymorphisms were studied. *AluI* (New England Biolabs, Ipswich, MA) restriction analysis of a PCR product containing exons 5 and 6 (forward primer: 5′-GTAGGGGAGGCTGCTTCAA-3′; reverse primer: 5′-CTGGTGACAAGATGGGTCCT-3′) was performed to confirm the mutation identified in the patients and to screen 250 healthy controls (200 Spanish and 50 Greek). The restriction analysis was followed by agarose gel electrophoresis and ethidium bromide staining. The mutation abolishes a restriction site (normal pattern: 48 + 63 + 253 + 136 bp; mutant pattern: 48 + 63 + 389 bp).

Haplotype analysis, including nine polymorphic sites across the *TH* gene, was performed in the three patients. Five of these sites were also genotyped in 50 unrelated control individuals of Greek origin by PCR amplification and direct sequencing (Fig. 1). Three of these five variations (rs12419447, rs6357, rs4074905)

were representative of a haplotype block spanning a segment from introns 5 to 9, as defined by the *Haploview v4.1* software,[15] using the confidence intervals method[16] on the genotype data from the Greek controls. Estimation of haplotype frequencies was also performed by *Haploview v4.1*.

RESULTS

All patients showed decreased HVA and MHPG concentrations, low HVA/5-HIAA ratio, (Table 1) and normal pterin concentrations (data not shown). These findings were consistent with TH deficiency.

Mutational analysis of the *TH* gene revealed a previously reported[4] homozygous pathogenic mutation in exon 6 (c.707T>C) in all three patients causing a substitution of leucine for proline in residue 236 of the protein (p.L236P), located in the α2 domain (Fig. 1). Parents were heterozygous carriers of this mutation. Screening of 250 control subjects did not disclose the p.236P mutation.

We performed haplotype analysis of nine SNP polymorphisms along the gene revealing that all three patients carried the same variants at homozygosis in all the studied sites (Fig. 1). The scrutiny of five of these nine SNPs in 100 chromosomes form 50 Greek control individuals allowed estimation of haplotype frequencies in the general population. Ten haplotypes were identified with frequencies ranging from 0.0001 to 0.34, but the allele combination found in the patients (rs7925375G-rs6356G-rs4074905T-rs6357A-rs12419447G) was not among them, indicating that it represents a rare haplotype. Under the assumption that the frequency of the patients' haplotype in the Greek population is <0.01, the likelihood of independently encountering this haplotype in six chromosomes can be estimated as $<10\ e\ -12$, which strongly supports the hypothesis of a common origin for all the p.L236P alleles in opposition to a recurrent mutational event.

DISCUSSION

In this report, we present three patients with TH deficiency. All patients presented with a severe clinical phenotype consisting of infantile parkinsonism (hypokinesia and tremor), hypotonia, dystonia, and oculogyric crises. Features of autonomic dysfunction included ptosis, hyperhydrosis, and profuse nasal secretions; whereas, hyperprolactinemia was the only endocrine disturbance detected. The analysis of biogenic amine metabolites in CSF showed reduced levels of catecholamine metabolites and normal pterins. Treatment with L-dopa was followed by gradual improvement in motor and autonomic function in all patients.

TH activity cannot be measured in easily accessible tissues[11] and confirmation of diagnosis is based on molecular analysis. Our patients were found to be homozygous for the same missense mutation (p.L236P) that has been previously reported as p.L205P.[4] Expression studies in different systems by Ludecke et al. revealed that this mutation leads to normal TH RNA steady state levels but reduced protein levels.[4]

Correlation between residual enzyme activity and clinical severity has not been established in TH deficiency. In contrast, it appears that the concentration of HVA in CSF is indicative of phenotypic severity; HVA levels range from undetectable to 30% of the lower limit of the reference range in patients with severe phenotypes[4,7,8,17,18] and from 46 to 60% in patients with intermediate phenotypes.[10,19,20] HVA concentrations in our patients (5.2–14.5%) fell into the levels suggested for severe phenotypes of TH deficiency (Table 1). Interestingly, the highest HVA concentration in CSF was found in the eldest patients from our series (Patient 1), suggesting compensatory mechanisms of dopamine turnover. This idea is supported by the finding of a higher MHPG, the major product of norepinephrine and epinephrine, in this patient (Table 1).

In our patients, the clinical response to L-dopa therapy was favorable, but their motor progress is yet insufficient and it may be too soon to reach conclusions. Early initiation of therapy, gender, and tolerability to treatment have been proposed as prognostic factors in these disorders.[11,21–24] The latter is mainly represented by the occurrence of drug-induced dyskinesias that may prevent reaching therapeutic doses of dopaminergic medications.[24] In a TH knockout animal model, hypersensitivity to L-dopa and dopamine receptor agonists correlated with prominent locomotor hyperactivity.[25] Chronic replacement with L-dopa relieved this hypersensitivity. This phenomenon was also observed in Patients 1 and 3 from our series, and it was also reported in another patient with TH deficiency.[17]

Haplotype analysis across the *TH* gene in the six p.L236P alleles identified in the three Greek patients supported a common ancestral origin. In this regard, the fact that the haplotype context of all the mutated alleles is identical and extremely rare in the Greek population indicates that the probability of a recurrent mutational event is negligible. Supporting our findings, the original patient in whom this mutation was first reported was Greek.[4] A second patient with the same homozygous mutation has been reported, but his geographic origin was not stated.[9]

In summary, patients with TH deficiency harboring the p.L236P mutation at homozygosity show a severe phenotype with an early clinical presentation. A common origin for all the identified pL236P alleles in Greek patients in this study is strongly supported by our haplotype analysis.

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