

A New Tyrosine Hydroxylase Genotype with Orofacial Dyskinesia

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نمط جيني جديد للتيروسين هيدروكسيلييز مع خلل حركي فموي وجهي

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ABSTRACT: Tyrosine hydroxylase (TH) deficiency is a rare autosomal recessive and often treatable neurometabolic disorder with variable phenotypes. More than 20 pathological mutations have been identified in patients with TH deficiency. We report the case of a 10-month-old male patient who presented with developmental delay, hypotonia and oculogyric crises to the Salmaniya Medical Complex in Manama, Bahrain. At a later stage, he developed orofacial dyskinesia and tremors with hyper-reflexia and *clonus*. A magnetic resonance imaging scan of the brain showed mild atrophy with widened ventricles and genetic testing revealed a novel homozygous mutation (c.938G>T; p.Arg313Leu) in exon 9 of the *TH* gene. The patient showed a remarkable response to treatment using combined levodopa-carbidopa. In this case, the orofacial dyskinesia may be a specific clinical association unique to this novel mutation, which is the first to be described in Bahrain and the Middle East.

Keywords: Tyrosine Hydroxylase; Dopa-Responsive Dystonia; Case Report; Bahrain.

المخلص: نقص التايروسين هايدروكسيلييز هو اعتلال استقلابي عصبي وراثي نادر، غالباً ما يمكن علاجه، وله ظواهر سريرية متغيرة ومتعددة تم اكتشاف أكثر من 20 طفرة وراثية مرضية منوطة بهذه الحالات. في هذا التقرير نناقش حالة مريض قدم إلينا في سن العشرة شهور بأعراض شملت تأخر في النمو، رخاوة في توتر العضلات مع أزمات شخوص البصر. بعد ذلك، ظهرت عليه أعراض الحركات الإرادية في الفم والوجه مع اهتزازات و فرط في المنعكسات العصبية. أظهر تصوير الرنين المغناطيسي للدماغ ضمور خفيف مع اتساع في البطينين، وكشفت الاختبارات الجينية عن وجود طفرة جينية جديدة مع تغير متماثل (c.938G>T; p.Arg313Leu) في أكسون 9 على جين التايروسين هايدروكسيلييز. استجاب المريض استجابة ملحوظة للعلاج المركب بعقاري ال-ل-دوبا/ الكاربي دوبا. في هذه الحالة، حدث عرض الخلل الحركي الفموي الوجهي والذي قد يكون مؤشراً فريداً من نوعه لهذا التحور الجيني الجديد، وتعتبر هذه الحالة الأولى من نوعها في البحرين والشرق الأوسط.

مفتاح الكلمات: التايروسين هايدروكسيلييز؛ الخلل التوتري المستجيب للدوبا؛ تقرير حالة؛ البحرين.

TYROSINE HYDROXYLASE (TH) DEFICIENCY is a rare autosomal recessive neurometabolic disorder with variable phenotypes. It is almost exclusively caused by missense mutations in the *TH* gene and its promoter region on chromosome 11p15.5. In 1971, Castaigne *et al.* was the first to report this condition in two brothers.¹ Since then, it has been reported in 50 patients from 41 families worldwide.²

The only reported cases of TH deficiency in the Arab world were from Lebanon; the onset of hypokinesia, rigidity and dystonia was observed in patients under 12 months old. In these cases, all the patients showed a good clinical response to therapy with levodopa (L-dopa).^{3,4} This case is the first instance of TH deficiency among the Middle Eastern population in an infant carrying a novel mutation in the *TH* gene. The patient subsequently demonstrated an excellent response to treatment with L-dopa. Informed consent was obtained from the patient's parents for all tests and written consent was obtained for the publication of this case report.

Case Report

A 10-month-old boy was referred to the Department of Clinical Neurosciences at Salmaniya Medical Complex in Manama, Bahrain, with a three-month history of global developmental delay and excessive sleepiness. The mother's pregnancy had been uneventful. He was born at term with a birth weight of 3.4 kg (within the 25th percentile), a head circumference of 34 cm (within the 10th percentile) and a length of 50 cm (within the 25th percentile). His Apgar scores were 8 and 9 at one and five min, respectively. The postnatal period was uneventful. The patient was the first child of healthy consanguineous parents. There was no family history of neurological disorders.

A visual examination of the patient showed an alert infant who was able to fix, follow and smile responsively. He showed reduced facial expression but no *ptosis*. Full extra-ocular movements, normal saccades and no signs of nystagmus were observed. His optic *fundi* were normal. The patient had occasional episodes of uprolling of the eyes with head extension.

Table 1: Forward and reverse primers for amplifying the coding exons of the *tyrosine hydroxylase* gene

Exon	Forward primer (5'→3')	Reverse primer (5'→3')
1	tgtaaacgacggccagtTGGGGAGTGAAGGCAATTAG	caggaaacagctatgaccCCAACAGGGACTCAAACACC
2	tgtaaacgacggccagtCTTGGGCACTCAGAACCTTG	caggaaacagctatgaccCAGCTTCATCCTGAGCTTCCA
3	tgtaaacgacggccagtCTCAAAAACGTGCTCTCATCC	caggaaacagctatgaccAGCTGAGGCCTGAGACTCC
4	tgtaaacgacggccagtAAGAACGGGATCTGTGTGCT	caggaaacagctatgaccCTCCCCTACAAGACTTCCGG
5	tgtaaacgacggccagtTGCTTCAAGGTGGGTGACACA	caggaaacagctatgaccGGCTAAGGGTAGGGGATGTG
6	tgtaaacgacggccagtCTGCCCCTGATCACATCC	caggaaacagctatgaccTGGTGACAAGATGGGTCCCTC
7	tgtaaacgacggccagtCTTGTACCAGCATCAGTGC	caggaaacagctatgaccCCTGCAAGTCCCTCTTCTTC
8	tgtaaacgacggccagtCTAAAGCCACCAGCAAAAGC	caggaaacagctatgaccTACTGGGTGCACTGGAACAC
9 + 10	tgtaaacgacggccagtGCTACCGGGAAGACAATATCC	caggaaacagctatgaccAGCAGGCAGCACACTTAC
11 + 12	tgtaaacgacggccagtCTTCTCCCAAACAGAGCCTGA	caggaaacagctatgaccCCTGGAGGGGCTGCTGACA
13	tgtaaacgacggccagtCTTGTCTCTGCCAGTCCTC	caggaaacagctatgaccCTCAAGGCCAGAAGGAAGG
14	tgtaaacgacggccagtACCAGTTGGCTCAGAAAAGC	caggaaacagctatgaccAGGACAGGACCTCACCACAG

This occurred without a change in his sensorium, which is consistent with oculogyric crises. He was able to lift his head to 45 degrees while lying in a prone position. He had increased muscle tone in his four limbs, generally with brisk, deep tendon reflexes. The remainder of the examination was unremarkable.

Laboratory investigations showed normal serum amino acids, tandem mass spectrometry, urine organic acids, lysosomal enzymes and very long chain fatty acids. Renal, bone and liver function tests were also performed. Computed tomography (CT) and magnetic resonance imaging (MRI) scans showed signs of mild brain atrophy. An electroencephalogram (EEG) revealed intermittent bursts of sharp waves over the frontal regions.

In spite of ongoing rehabilitation therapy, the patient continued to regress; he became more hypotonic and developed tremors with *clonus* in both ankles. His speech and cognition progressed slowly. A physical examination at one-and-a-half years of age revealed prominent orofacial dyskinesia with drooling and, accordingly, he was started on a trial of combined L-dopa-carbidopa.

After three months of treatment, the muscle tone in his limbs improved and he began to walk with support. He also began to put words together to form short sentences. By the age of two, he was walking independently.

Dopa-responsive dystonia (DRD) was suspected

Table 2: Sequence analysis primer

	Primer
M13_Forward	TGTA AACGACGGCCAGT
M13_Reverse	CAGGAAACAGCTATGACC

clinically and this was subsequently confirmed by genetic analysis. Genomic deoxyribonucleic acid (DNA) from the patient and the mother was amplified for the gene encoding *TH* (National Center for Biotechnology Information reference sequence NM_199292.2) by means of a polymerase chain reaction (PCR). A PCR was performed using the AmpliTaq Gold[®] 360 Master Mix (Applied Biosystems[®], Thermo Fisher Scientific Inc., Carlsbad, California, USA) as described by the manufacturer. Forward and reverse primers are available in Table 1. The fragments that were obtained, including the individual exons, the splice donor site, and the splice acceptor site, were subjected to double-stranded DNA sequencing analysis on a 3730xl DNA Analyzer (Applied Biosystems[®]). The sequence analysis was performed using Sequencher DNA Sequencing Software, Version 4.8 (Gene Codes Corp., Ann Arbor, Michigan, USA) and SeqPilot Version 3.2.1.0 (JSI Medical Systems GmbH, Kippenheim, Germany) software. The sequence analysis primers are recorded in Table 2.

The sequence analysis identified a homozygous mutation, c.938G>T (p.Arg313Leu), in exon 9 of the *TH* gene in the proband; the patient's mother was heterozygous [Figures 1 A, B & C]. This change was predicted to be possibly pathogenic by the mutation prediction software, the Sorting Intolerant from Tolerant (SIFT) Tool Version 1.03 (J. Craig Venter Institute, Rockville, Maryland, USA). A substitution at position 313 from R to L was predicted to affect the protein function with a score of 0.01. The polyphen-2 mutation prediction software PolyPhen-2 showed that this amino acid change is probably damaging, giving a score of 0.987 (sensitivity 0.73, specificity

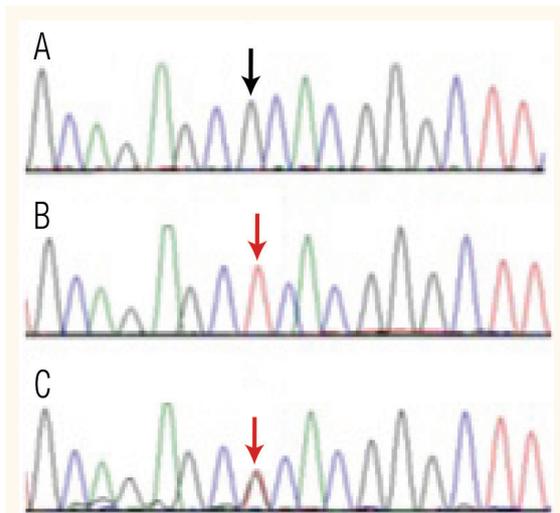


Figure 1 Panel A, B & C: Sequence profiles showing the c.938G>T mutation in exon 9 of the *tyrosine hydroxylase* (TH) gene. Panel A shows the normal genomic sequence, while panels B and C show the electropherograms corresponding to the genomic sequence of the proband and his mother, respectively. The vertical arrows point to the normal guanine nucleotide (A) and the variant thiamine nucleotide which was homozygous for the proband (B) and heterozygous for the mother (C).

0.96). The testing score of the Align-GVGD program (International Agency for Research on Cancer, Lyon, France) was C35; this score lies midway in the spectrum of output prediction classes (C0, C15, C25, C35, C45, C55, C65), with C65 being the most likely to interfere with function and C0 being the least likely. This mutation has not been reported in the Single Nucleotide Polymorphism Database, the Exome Variant Server or the Human Gene Mutation Database. At a later stage, the father was also screened and found to be a carrier of this novel mutation.

Discussion

DRD is largely caused by autosomal dominant mutations in the *GTP cyclohydrolase1* (*GCHI*) gene and, more rarely, by autosomal recessive mutations in the *TH* or *sepiapterin reductase* (*SPR*) genes.⁵ It was considered unlikely that the current patient had DRD caused by mutations in the *GCHI* gene, as DRD is an autosomal dominant disorder that presents in childhood at around six years of age.^{6–8} The current patient presented in infancy with delayed development and hypotonia that later became more pronounced. He developed fine tremors with orofacial dyskinesia in the second year of life which alerted the treating physicians to the more common diagnosis of TH deficiency.

TH-deficient DRD may present as pure dystonia or as a dystonia-plus syndrome, featuring oculogyric crises, mental retardation, Parkinsonism and other clinical features.⁹ TH deficiency has been divided into two phenotypes. The first is a progressive, hypokinetic-rigid syndrome with dystonia and an infantile onset, known as type A, and the second is a complex encephalopathy with a neonatal/early infancy onset, known as type B.² Generally, there is an overlap of clinical features between the two types and a variable response to treatment with L-dopa.¹⁰

The patient's presentation, clinical course and response to combined L-dopa-carbidopa was in keeping with a diagnosis of TH-deficient DRD type A.² Hypokinesia, bradykinesia and rigidity have typically dominated the neurological presentation in reported cases with dystonia often being less prominent,² as was the case for the current patient. However, other features, including *ataxia*, chorea, *ptosis* and symptoms of dysautonomia, were absent. The cognitive function of patients is reported to be improved in those who develop symptoms after their first year of life.² In addition, tremors and oculogyric crises are reported to be mild and rare.^{11–13} Oculogyric crises are present in cases of TH-deficient DRD type B and in the other DRD-plus syndromes caused by mutations in the *SPR* gene.⁵ Excessive sleepiness has been described in patients with TH-deficient DRD types A and B as well as in individuals with *SPR* gene mutations.^{2,14} Dyskinesia, on the other hand, has been reported mainly in TH-deficient DRD type B cases and is usually severe and unresponsive to treatment.² There has been no mention in the literature of orofacial dyskinesia in patients with TH-deficient DRD type A. Dyskinesias are generally reported as L-dopa-induced side-effects when the dosage is increased rapidly or the drug is prescribed for long periods. Therefore, the presence of orofacial dyskinesia in this case, prior to the patient's L-dopa treatment, may represent a mutation-specific phenotype that has not been described previously.

The differential diagnosis for psychomotor regression and dystonia includes early onset primary dystonia, early onset Parkinsonism and various other genetic and acquired disorders.

The patient did not undergo a lumbar puncture to assess the major metabolites of dopamine or homovanillic acid (HVA). In addition, tests for the urine pterins concentration and the dihydropteridine reductase activity in the blood were not performed. While these investigations help support the diagnosis of TH deficiency, they are not diagnostic.

Although the current patient had a mild form of TH deficiency, brain imaging revealed mild brain atrophy

with prominent cerebrospinal fluid spaces throughout the ventricular system. Similarly, a previous case report observed cerebral and cerebellar atrophy in an individual severely affected by TH deficiency.¹⁵

More than 20 pathological mutations (including a point mutation in the *TH* gene promoter region) have been reported in patients with TH deficiency. All of these individuals have been homozygous or compound heterozygous for *TH* gene mutations. Most mutations have been single nucleotide substitutions, the most common of which are c.698G>A (p.Arg233His) and c.707T>C (p.Leu236Pro).^{3,4,12,15–20} Single nucleotide deletions, resulting in frameshift mutations and protein truncation, have also been reported.^{18–20} Mutations in the highly conserved cyclic adenosine monophosphate (cAMP) response element, within the *TH* gene promoter region, have been found in six families with TH deficiency.^{3,15}

Both parents of the patient in this case report were found to be carriers of this mutation. They were subsequently offered genetic counselling to explain the ramifications of this genetic disorder.

Conclusion

Orofacial dyskinesia is an uncommon presentation, especially for the hypokinetic-rigid syndrome of TH deficiency. It may be specific to the novel homozygous mutation (c.938G>T; p.Arg313Leu) in exon 9 of the *TH* gene; this mutation is potentially unique to the Middle Eastern population.

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