INTERNATIONAL MEDICAL RESEARCH JOURNAL

LEVODOPA THERAPY RESULTED IN SIGNIFICANT MOTOR RECOVERY IN CHILDREN WITH TYROSINE HYDROXYLASE DEFICIENCY: A CASE SERIES

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ABSTRACT

Tyrosine hydroxylase (TH) deficiency (OMIM #191290) is an autosomal recessive disorder resulting from impaired biosynthesis of cerebral catecholamine neurotransmitters. It has a broad continuous clinical spectrum, manifesting from infancy to adulthood as progressive encephalopathy, as well as abnormal movements including dystonia and Parkinsonism. Early treatment with Levodopa may prevent these devastating manifestations. We hereby report three patients with TH deficiency, highlighting the challenges in diagnosing this rare condition, and the favourable therapeutic outcomes. The first patient is a male, presented with unexplained psychomotor delay, limb dystonia, truncal hypotonia, feeding difficulties and oculogyric crises since infancy; was initially diagnosed as cerebral palsy. At the age of 5, a genetic test revealed 2 pathogenic variants of TH gene: c.698G>A [p.(Arg233His)]; c.1293+5G>C. The second patient is the female sibling of the first patient; diagnosed at 3 years old upon family screening. The third patient is a female, who developed encephalopathy, and paroxysmal periods of lethargy alternated with irritability at 3 months of age. Diagnosis remained elusive even after consulting multiple doctors. A genetic test was finally performed at 7 months of age, and 2 pathogenic missense variants were identified in the TH gene: c.943G>A [p.(Gly315Ser)]; c.1196C>T [p.(Thr399Met)]. Treatment was started at a very low dose and gradually increased to the maximum effective dose of levodopa. During follow-up session, all of them showed significant motor recovery. The first patient, who is currently 9 years old, was able to ambulate with assistance. In addition, both the second patient (7 years of age) and the third patient (28 months of age) were able to walk independently. Hence, we concluded that TH deficiency is a treatable disorder if recognized early and should be considered in children with unexplained neurological symptoms.

KEYWORDS: M protein; Multiple myeloma; Immunofixation; Protein electrophoresis, Younger age

INTRODUCTION

Human tyrosine hydroxylase deficiency [TH deficiency; Online Mendelian Inheritance in Man (OMIM) #191290] is an autosomal recessive neurometabolic disorder due to mutations in the TH gene at the chromosome locus 11p15.5. Tyrosine hydroxylase catalyses the initial and rate-limiting step in the synthesis of catecholamine (Figure 1). TH deficiency causes impaired synthesis of cerebral catecholamine neurotransmitters, including dopamine, adrenaline (epinephrine), and noradrenaline (norepinephrine). TH deficiency has a broad continuous clinical spectrum. Furukawa and Kish (1) classified TH deficiency into three groups: dopa-responsive dystonia (DRD), infantile parkinsonism with motor delay, and progressive infantile encephalopathy. Willemsen et al. (2) described TH deficiency as type A or type B, where type A is a "progressive hypokinetic-rigid syndrome with dystonia" and type B is a "complex encephalopathy". There is an overlap between these various classifications, with type A similar to the DRD form and type B similar to the more severe progressive infantile encephalopathy (3). We hereby report three patients with TH deficiency from two families. We aim to highlight the challenges in diagnosing this rare genetic condition, and its therapeutic outcomes.

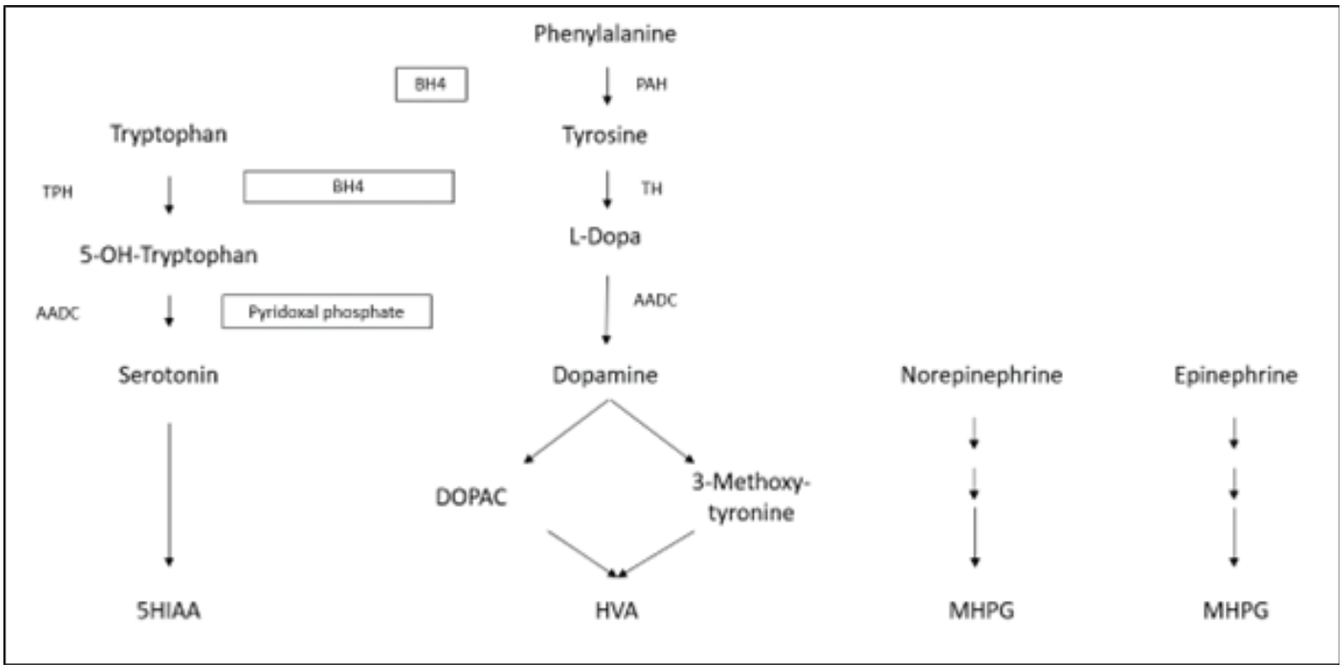

Figure 1: Simplified scheme of the biosynthesis and catabolism of serotonin and the catecholamines dopamine, norepinephrine and epinephrine. TPH = tryptophan hydroxylase; AADC = aromatic amino acid decarboxylase; PAH = phenylalanine hydroxylase; TH = tyrosine hydroxylase; BH4 = tetrahydrobiopterin; DOPAC = 3,4-dihydroxyphenylacetic acid.

Case series

Patient 1

Patient 1, a male, was delivered at term via a Lower Segment Caesarean Section (LSCS) at birth weight of 3.30 kg after an uncomplicated pregnancy. He was the first child of healthy, nonconsanguineous parents. He was a healthy baby up to 3 months of age, when his parents first observed that he became floppy and his developmental milestone became static. He had frequent oculogyric crises, described as episodic upward eye deviation with generalized limb spasms. These episodes occurred at intervals of approximately 3-4 days, and lasted up to 6 - 8 hours. He was diagnosed with cerebral palsy. He was then referred for a genetic consultation at 5 years old. Upon assessment, he was still unable to roll over and had no speech. He had truncal hypotonia and limb dystonia. He demonstrated hyperreflexia in the lower limbs but hyporeflexia in the upper limbs (Figure 3: a and g). The routine laboratory workup including plasma amino acid, dried blood spot acylcarnitine, urine organic acid, blood lactate, serum creatine kinase and karyotype were all normal. His serum prolactin was markedly elevated at 2,146 mU/L (Normal range: 102

- 496 mU/L). His Magnetic Resonance Imaging (MRI) of the brain was reported as normal. Parents refused the lumbar puncture procedure for neurotransmitters analysis in cerebrospinal fluid but agreed for whole exome sequencing (WES), which was performed using Ilumina Genomic at an accredited commercial genetic laboratory. The test revealed two heterozygous variants in the TH gene: c.698G>A [p(Arg233His)]; c.1293+5G>C. Variant c.698G>A in exon 6 is a missense variant that was previously reported as pathogenic, leading to a Arg233His amino acid change in the primary protein structure at position 233. Variant c.1293+5G>C (intron 12) was predicted to adversely affect the splicing. He was started on Levodopa (L-dopa, with a decarboxylase inhibitor) after his molecular result showed that he had TH deficiency. The Levodopa was initiated at 0.08 mg/kg/day and gradually titrated to 5 mg/kg/day over 12 months. He tolerated it well and started to show improvement in his motor development. His last clinical assessment at 9 years of age showed that he was able to stand and walk a few steps without support, able to form multisyllable words, and able to grip better although mild hypotonia and hyperreflexia were still present (Figure 3: d and i).

Patient 2

Patient 2 was the younger female sibling of Patient 1. She was born at 40 weeks of gestation via an elective LSCS with a birth weight of 3.2 kg. She was healthy until 3 months of age when she developed hypotonia after an episode of febrile illness. She also started to have oculogyric crises. She was seen at 3 years old as part of sibling screening after her brother was diagnosed with TH deficiency. Upon assessment, she shared similar clinical features as her brother: global developmental delay, generalized hypotonia, hyper reflexia and dystonia (Figure 3 b and g). Her serum prolactin level was 1,800 mU/L. A targeted genetic test was done and confirmed that she had the same TH variants c.698G>A and c.1293+5G>C;(Figure 2) as in her brother. Carrier status was confirmed in both parents. She was started on Levodopa at 0.1 mg/kg/day in three divided doses, which was increased gradually to 5.8 mg/kg/day over 12 months. She tolerated it well. Following that she started to exhibit social smiles and facial expressions and she was no longer experiencing an oculogyric crisis. During her last review at 7 years old, she was able to speak a few multisyllabic words. She was able to walk and use fork and spoon independently, but still needed some help to climb stairs (Figure 3: e and i). She only showed mild hypotonia and slightly increased deep tendon reflexes.

Patient 3

Patient 3, a female, was the first child of non-consanguineous parents. She was born at 40 weeks of gestation via emergency LSCS in view of prolonged labour with a birth weight of 2.72 kg. Her mother had a history of three previous miscarriages. Both parents had normal karyotype and were healthy with no significant family history. The antenatal period was uneventful with normal foetal movements. She presented with encephalopathy and paroxysmal periods of lethargy alternated with irritability at three months of age. She had a significant delay in motor milestones and intermittent blank stare with jerky movements of the lower limbs (Figure 3: c and j). Diurnal fluctuation of symptoms was noted. Her routine blood investigations, blood lactate, blood ammonia, thyroid function test, plasma amino acid, urine oligosaccharide, urine organic acids, urine purine and pyrimidine and karyotype were all normal. The results of electroencephalography (EEG) and cerebral MRI were normal. Her serum prolactin was elevated at 1,003 mIU/L. Diagnosis remained elusive after consulting multiple doctors. Whole genome sequencing was performed on genomic DNA using Ilumina next generation sequencing (NGS) at 7 months of age in an accredited commercial genetic laboratory. It identified two pathogenic missense variants in the TH gene. One heterozygous missense variant was reported in exon 8, c.943G>A [p(Gly315Ser)]; while another heterozygous missense variant was detected in exon 11, c.1196C>T [p(Thr399Met)]. She was started on Levodopa at a dose of 0.1 mg/kg/day; the dose was gradually increased to 11.5 mg/kg/day. She responded very well to treatment without significant adverse reactions. She demonstrated significant motor milestone recovery after the initiation of Levodopa treatment. During follow-up at two and half years old, she could walk, run, and climb staircase independently (Figure 3 h).

DISCUSSION

TH deficiency is a rare autosomal recessive neurometabolic disorder caused by mutations in the TH gene that is most likely under-diagnosed worldwide. The broad spectrum of disease manifestations ranging from mild movement disorder to a life-threatening, neurological disorder, makes it difficult and challenging to be recognized clinically. Cerebrospinal fluid (CSF) neurotransmitters analysis will reveal the biochemical hallmark of TH deficiency: the decreased level of metabolites of dopaminergic neurotransmitter

homovanillic acid, a normal concentration of metabolites of serotonin neurotransmitter 5-hydroxyindolacetic acid, and a normal level of pteridine metabolites, neopterin, and biopterin in the cerebrospinal fluid (4). Measurement of these neurotransmitter metabolites in CSF requires a carefully controlled method of collection and sample handling as well as parental consent for lumbar puncture (5). Early diagnosis of TH deficiency can only be made by CSF neurotransmitter analysis or molecular genetic testing. Urine neurotransmitter screening is neither specific nor sensitive for the diagnosis of TH deficiency. Parental refusal for this invasive procedure and lack of awareness on the availability of molecular genetic testing had further delayed the diagnosis in Patient 3. Furthermore, there are more than 6,300 reported genetic diseases with molecular basis known in 2022 (http://omim.org/statistics/entry) and it is impossible for a doctor to know the clinical phenotype of each of them. Rare genetic disease is a difficult field in medical practice, but with rapidly evolving and expanding genomic technologies, as well as available and searchable databases, it has become possible to end the diagnostic odyssey earlier today, and will probably be much easier in the future (6). This is clearly demonstrated in our case series. It took 5 months to 5 years from the onset of symptoms and signs for these three patients to obtain a definitive diagnosis. The utilisation of genomic tests ended their diagnostic odyssey early, enable accurate diagnosis and management of a rare monogenic disease and potentially saved them from more devastating effects of this disease.

With regards to our patients' clinical phenotype, all three of them had an onset of symptoms at 3 months of age. All demonstrated overt motor milestone delay since infancy. The patients in our case series had signs of dopamine deficiency such as hypokinesia, dystonia, oculogyric crises, and diurnal fluctuation; similar to previous reports (1,2). According to the classification proposed by Willemsen et al. (2), Patient 1 and 2 clinical phenotype fits the description of TH deficiency type A, whereas Patient 3 has TH deficiency type B. All our patients had elevated levels of serum prolactin. Dopamine suppresses the release of prolactin. Dopamine deficiency will therefore cause hyperprolactinemia. Hyperprolactinemia was found in 50% of the severe cases of TH deficiency (7). This could

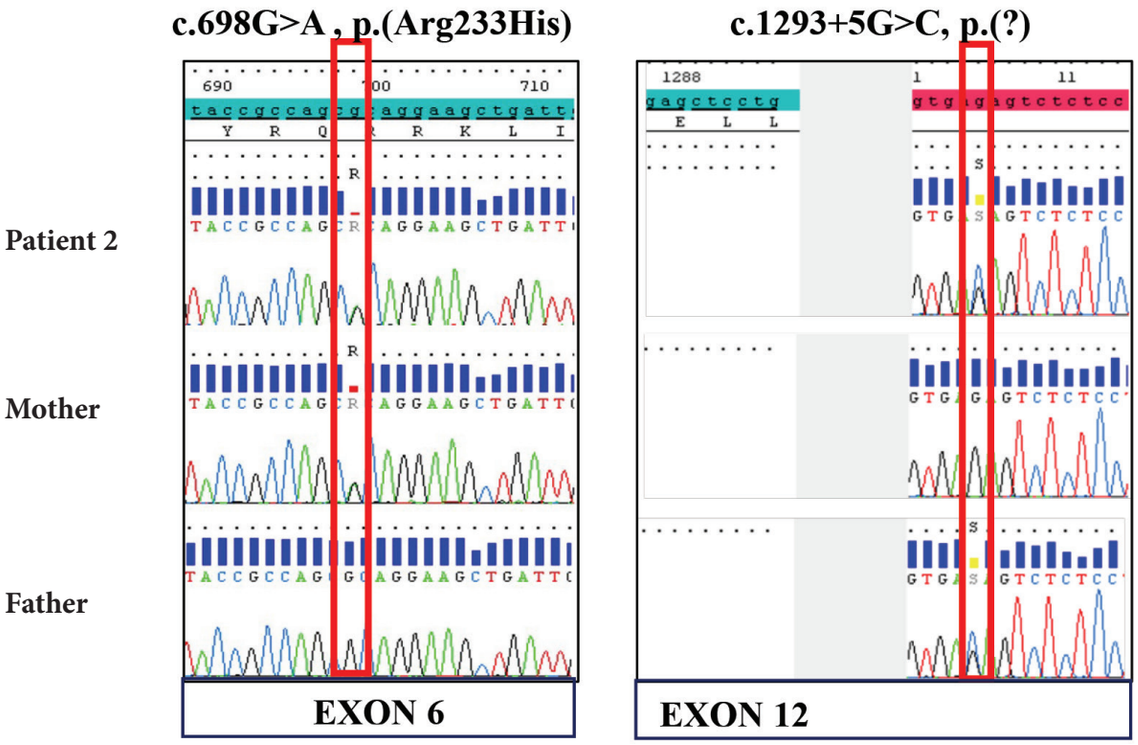

Figure 2: Sequence electropherogram of Patient 2 showed compound heterozygous mutations identified in exon 6 and intron 12 of TH gene indicated in the red box. Genetic testing carried out on parental samples detected the variants are in the trans phase thus confirmed the autosomal recessive inheritance.

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be a useful biomarker to alert clinicians to consider TH deficiency in patients with unexplained developmental delay and hypotonia. Levodopa dosage in TH patients is usually 3-10mg/kg/day. The initial dose is usually lower, as tolerated by patient. Some individuals with the severe form of TH deficiency are hypersensitive to Levodopa and are prone to intolerable dyskinesias at the initiation of the therapy. This hypersensitivity warrants the use of very low initial doses of levodopa (7). Patients who are able to tolerate L-dopa, exhibit drastic improvements, as seen in Patients 2 and 3. Early detection and treatment of TH deficiency will result in improved outcomes. In general, Type A patients respond better to treatment than Type B patients. Treatment has to be started early in the critical period of development when the brain's plasticity is at its highest.

CONCLUSION

Our case series has demonstrated that TH deficiency is a treatable disorder when detected early. Hence, it should be considered in all children with unexplained neurological symptoms, especially movement disorders. A high index of clinical suspicion is needed so that TH deficiency will be considered early in the differential diagnoses and included in the biochemical and molecular screening.

ETHICAL DECLARATIONS

The Medical Research and Ethics Committee, Ministry of Health Malaysia has determined that this case series study was exempted from requiring further review/ approval (NMRR ID-22-00483-YRK). Parental written consent had been obtained for the use of all the photographs.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest involved in this study. Tests conducted at the commercial genetic laboratory were paid by the parents themselves. There was no third-party sponsor involved in this study.

ACKNOWLEDGEMENTS

We would like to thank the Director General of Health Malaysia for his permission to publish this article. We also want to acknowledge the laboratory support conferred by the technicians at Institute for Medical Research (IMR), Kuala Lumpur, Malaysia.

Table 1: Clinical summary of three TH-deficient patients

Patient	**1**	**2**	**3**
Gender	Male	Female	Female
Fetal distress	No	No	No
Age of onset	3 months	3 months	3 months
Age at diagnosis	5.5 years	3 years	8 months
Family History			
	Yes	Yes	No
Presenting Clinical Features			
Developmental Delay	Yes	Yes	Yes
Truncal Hypotonia	yes	Yes	Yes
Sign of Dopamine/ Norepinephrine Deficiency			
Oculogyric Crisis	Yes	Yes	Yes
Ptosis	Yes	Yes	No
Profuse oropharyngeal secretion	No	No	Yes
Paroxymal sweating	No	No	Yes
Choreoathetosis	No	No	No
Tremor	Yes	Yes	Yes
Diurnal Fluctuation	No	No	Yes
TH mutation	c. 698G>A [p.(Arg233His)]; c.1293+5G>C (splice site)	c. 698G>A p.(Arg233His); c.1293+5G>C (splice site)	c.943G>A [p.(Gly315Ser)]; c.1196C>T [p.(Thr399Met)]
Serum Prolactin (mU/L) (Normal range: 102 - 496 mU/L)	2,146	1,800	1,003
CSF neurotransmitters	Not done	Not done	Not done
Medication			
L-Dopa			
Initial Dose, mg/kg/day (age)	0.08 mg/kg/day (5y)	0.12 mg/kg/day (3y)	1 mg/kg/day (8m)
Maximum dose, mg/kg/day (age)	5 mg/kg/day (8y)	5.8 mg/kg/day (5.5y)	11.5 mg/kg/day (2y)
Current dose, mg/kg/day (age)	5 mg/kg/day (9y)	4.4 mg/kg/day (7y)	11 mg/kg/day (2yr3m)
Motor Outcome			
Age of walking independently	Yes (9 years)	Yes (6 years)	Yes (2 years)

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