COMMENT

Dopa-responsive dystonia in Bulgarian patients: report of three cases

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Dopa-responsive dystonia (DRD) comprises a group of rare autosomal inherited neurotransmitter disorders characterized with childhood or adulthood onset. We report three cases of DRD. Two boys (1.5-year-old and 1.3-year-old) were diagnosed with TH deficiency and found to have compound heterozygous missense variants in the *TH* gene. For the first patient p.Arg202His and the p.Leu205Pro in the *TH* gene, were reported. In the second patient were revealed p.Thr373Met and p.Arg202His variants in the same gene. The third patient, a 10-years old boy was diagnosed with GCH1 deficiency due to heterozygous pathogenic variant (p.Lys224Arg) in the *GCH1* gene. The diagnosis of DRD was determined by whole exome sequencing (Patient 1) and whole genome sequencing (Patients 2 and 3). Here, we describe the first two patients with TH deficiency in Bulgaria and one with GCH1 deficiency. We also review the molecular mechanism of the disorder and summarized the reported pathogenic or likely pathogenic variants in the *TH* and *GCH1* genes. The disorder has broad clinical and genetic heterogeneity which is often misdiagnosed. Our aim is to improve awareness for the DRD, especially in Bulgaria because early diagnosis is essential for the better prognosis and therapy outcome.

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INTRODUCTION

Dopa-responsive dystonia (DRD) was originally reported in 1971 by Dr. Masaya Segawa and described as "hereditary progressive basal ganglia disease". It comprises a group of autosomal inherited neurotransmitter disorders including GTP cyclohydrolase I deficiency, 6-pyruvoyl-tetrahydropterin synthase deficiency, sepiapterin reductase deficiency, dihydropteridine reductase deficiency, tyrosine hydroxylase deficiency and aromatic L-amino acid decarboxylase deficiency, which may appear during childhood or adulthood [1, 2]. There is clinical spectrum of symptoms ranging from generalized dystonia with marked diurnal fluctuations, autonomic disturbances with progressive hypokinetic-rigid phenotype to progressive infantile encephalopathy [3–6]. In 1998, Jeon S et al. proposed the definition of DRD as a syndrome of selective nigrostriatal dopamine deficiency caused by genetic defect in the dopamine synthetic pathway without nigral cell loss [7].

Dopamine, noradrenaline and serotonin are monoamine neurotransmitters implicated in different cognitive processes and controlling a number of physiological, emotional and behavioral functions such as modulation of psychomotor function, cardiovascular, respiratory control, sleep mechanisms, hormone secretion, body temperature and pain. They are synthesized from amino acids through various enzyme catalyzed reactions [4, 8, 9] (Fig. 1).

Pathogenic variants in *GCH1* are associated with the most common form of dystonia. The gene is located on chromosome 14q13 and encodes GTP cyclohydrolase 1 enzyme [4, 10]. GCH1 deficiency affects catecholamines (dopamine, noradrenaline and adrenaline) pathway and serotonin synthesis leading to deficiency of the metabolites 5-HVA and 5-HIAA [4, 8]. The DRD associated with GCH1 deficiency could follow either autosomal dominant or autosomal recessive inheritance. The autosomal dominant form is usually manifested with the classic phenotype without hyperphenylalaninemia, the clinical presentation includes action leg dystonia with diurnal fluctuations, typically progressing to segmental or generalized dystonia with remarkable levo-dopa response [11]. The autosomal recessive form is expressed with more severe neurologic clinical presentation (convulsions, psychomotor retardation, truncal hypotonia and autonomic dysfunction) [12].

Pathogenic variants in the *TH* gene are associated with autosomal recessive tyrosine hydroxylase deficiency (THD) which is extremely rare condition with a prevalence of 1-9/1 000 000 and broad range of clinical features. The *TH* gene located on the 11p15 chromosome is composed of 14 exons and encodes the tyrosine hydroxylase enzyme [13]. The enzyme is essential and rate-limiting for the catecholamine production and it's not involved in serotonin synthesis which results in normal CSF levels of 5-HIAA in THD patients [10, 14]. The condition is classified into two types:

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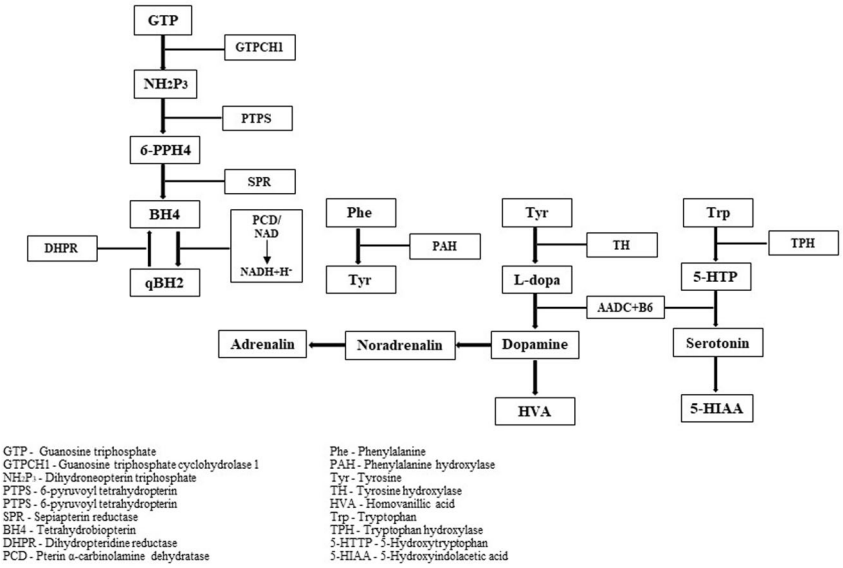

Fig. 1 The precursors of dopamine and serotonin are synthesized from tyrosine and tryptophan by the enzyme tyrosine and tryptophan hydroxylase. Tetrahydrobiopterin (BH4) and pyridoxal 5′-phosphate (B6) are necessary as cofactors. De novo biosynthesis of BH4 starts from guanosine triphosphate (GTP), involved enzymes are GTP cyclohydrolase 1 (GTPCH) encoded by the gene *GCH1* (OMIM: 600225); pyruvoyl-tetrahydropterin synthase (PTPS), encoded by the *PTS* gene (OMIM: 612719) and sepiapterin reductase (SPR) encoded by the *SPR* (OMIM: 182125). The recycling of the BH4 synthesis from qBH2 is driven by two enzymes: pterin-4a[1]carbinolamine dehydratase (PCD) encoded by the *PCBD* gene (OMIM 126090) and dihydropterin reductase (DHPR) encoded by the *QDPR* (OMIM 612676) [8, 10]. BH4 is an essential cofactor for PAH, tyrosine and tryptophan hydroxylases. Tyrosine hydroxylase catalyzes the conversion of L-dopa from tyrosine, and tryptophan hydroxylase is involved in 5-HTP (5-hydroxytryptophan) synthesis from tryptophan. Pyridoxal 5′-phosphate (B6) together with aromatic l-amino acid decarboxylase (AADC) transforms L-dopa to dopamine and 5-HTP to serotonin (5-HT). The release of dopamine and serotonin is followed by a rapid metabolization to HVA and 5HIAA, whose levels can be measured in the patient's cerebrospinal fluid. Dopamine is also converted into catecholamines noradrenaline and adrenalin that play major roles in autonomic nervous system [8–10].

type A (onset in infancy and early childhood, with mild to progressive dystonia and usually with good effect of L-dopa treatment) and type B (onset in neonatal and early infancy, delays in achieving motor milestones, disfunction of autonomous nervous system and poor or no response to L-dopa treatment), but recently it was recognized that patients present with clinical spectrum [10].

We report three male patients with complex childhood-onset clinical phenotype. Two of them are compound heterozygous carriers of missense variants in the *TH* gene, and in the third patient heterozygous likely pathogenic variant in the *GCH1* gene was detected.

Clinical features

Patient 1 was introduced to our laboratory in 2020, when he was 1.5-year-old boy. He was born as a second child, from uncomplicated pregnancy and delivery at term. Since the age 5-month axial hypotonia was noticed, from the 9th month dystonic and oculogyric crises occurred, and the sleep rhythm was disturbed. Episodic phases of lethargy and semiptoses noticed. Due to paroxysmal events, diagnosis of epilepsy was suspected and the boy was treated by valproic acid, repitend and synthetic ACTH, without favorable effect. EEG showed nonspecific findings, with slow delta-theta rhythm. Repeated head MRI showed normal findings with description of temporal left arachnoid cyst 26 x14mm. NGS for epileptic encephalopathies was done, the findings came negative. He had a healthy 6-year-old sister and no family history for DRD.

Patient 2 was a 1.3-year-old boy at the time of admission, he was born from the first pathologic pregnancy with infection at the maternal-fetal interface. At the age of 3-months he started experience irritability, jerky movements and developed generalized rigidity. He appeared hypokinetic with masked facies and no head control, oculogyric crises, rigidity of the limbs and hypotonia. EEG and MRI of the CNS showed nonspecific findings. The urine metabolic screening, MLPA for the most common microdeletion and microduplication disorders and Sanger sequencing of mitochondrial DNA (mtDNA) were done, the results were normal. His parents are healthy and there was no family history of DRD.

Patient 3 was adopted at the age of 1.6-year and had normal neurological and physical development. His symptoms started at the age of 10-years and have worsened over time. Focal hand dystonia (changes in the handwriting), slurred speech, staggering, kinetic hand tremor, gait and postural abnormalities were reported. He started experiencing vomiting, headache, memory impairments and fatigue. The condition progressed, leaving him helpless and dependent on those around him in everyday life. EEG, MRI and CT of the CNS showed nonspecific findings. Molecular genetic testing of spinocerebellar ataxia types 1, 2, 3, 6 and 8, whole genome arrayCGH, Sanger sequencing of mitochondrial DNA (mtDNA), urine metabolic screening, neoplasm sequencing panel (NGS) and WES were done, the findings were negative. The medical records of his biological parents were unavailable.

MATERIALS AND METHODS

DNA was extracted from K2EDTA blood samples according to the manufacturer protocols (QIAamp Blood kit manual) from all three patients after receiving a signed informed consent. For the first patient whole exome sequencing was performed on a BGISEQ-500/MGI-2000 platform followed by targeted sequencing of the exon 5 of the *TH* gene (NM_000360.4) (Chr 11: 2,189,091 - 2,189,169) for his family, using an Illumina MiSeq platform. The second and third patients were examined through whole genome sequencing on a BGISEQ-500 platform. Targeted bioinformatic

Table 1. Summary of the sequencing data.

Patient	Method	Initial bases on target (Mb)	Average sequencing depth on target (X)	Fraction of target covered > = 10x (%)
1	WES	60.46	188.87	99.70
2	WGS	59.5[a]	40.74	98.29
3	WGS	412.45[a]	44.53	99.18

[a]The target region size.

analysis of the WES and WGS data has been made followed by the classification according to the Standards and Guidelines for the Interpretation of Sequence Variants by the ACMG [15]. The summary of the whole exome and whole genome sequencing statistic data is reported on Table 1.

RESULTS

The patient 1 was a compound heterozygous carrier of two pathogenic variants in the *TH* gene, the second patient was compound heterozygous carrier of one pathogenic and one likely pathogenic variant in the same gene. Pathogenic and likely pathogenic variants in the *TH* gene are associated with tyrosine hydroxylase deficiency. The third patient was a heterozygous carrier of a likely pathogenic variant in the *GCH1* gene, associated with an autosomal dominant form of dopa-responsive dystonia. Identified variants are described in Table 2 corresponding to the recommendations by HGVS nomenclature [16].

Patient 1 is the first patient diagnosed with tyrosine hydroxylase deficiency in Bulgaria. He inherited the p.Arg202His variant from his healthy mother and the p.Leu205Pro from his healthy father. His healthy older sister is a heterozygous carrier of the p.Leu205Pro. The variant c.1118 C > T in the tyrosine hydroxylase gene identified in the patient 2 is reported in ClinVar as "variant with unknown significance" (ClinVar ID: 1403045). The variant is located in a critical and well-established functional domain (active site of an enzyme) (PM1), it is present in population databases with very low population frequency (gnomAD 0.0004017%) (PM2). This is a missense variant in *TH* gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease (PP2), in silico analysis supports that this missense variant has a deleterious effect on the gene or gene product (PP3) and our patient had a well-defined clinical presentation for DRD (PP4) [15]. In summary, we classified the variant c.1118 C > T in the *TH* gene according to the currently available evidence as "likely pathogenic". His parents were tested in another laboratory and they refused access to the results. The third patient was adopted at the age of 1.6-year and there is no available information about his inheritance status.

DISCUSSION

Dopa-responsive dystonia is rare, severe and often treatable neurometabolic disorder. The population rate in Europe ranges from 1/1,000,000-1/200,000. TH deficiency is less frequent than GCH1 deficiency and fewer than 100 patients have been reported worldwide. To our knowledge, patients 1 and 2 are the first two reported patients with THD in Bulgaria. We don't have information about another reported patient with autosomal dominant GTP cyclohydrolase 1 deficiency.

In humans, tyrosine hydroxylase has four isoforms (hTH1–hTH4) derived from an alternative splicing of the gene, they differ in amino acid sequence in their N-terminal region [17]. The protein consists of four subunits, each one comprises regulatory ACT domain, N-terminal tail with different length, catalytic and oligomerization domain [18–20]. The catalytic domain is located in the C-terminal area and the regulatory domain in the N-terminal domain. The amino acid sequence of the gene is highly conserved among different species [18–20]. Isoform 1 (hTH1) is the most conserved and contains 14 exons with open reading frame of 1491 bp [10, 21]. The TH is expressed in monoaminergic neurons and adrenal gland [22]. In patients with DRD level of the tyrosine hydroxylase in the putamen and nigrostriatal dopaminergic neurons is severely decreased [5, 23]. Homozygous and compound heterozygous pathogenic or likely pathogenic variants in the gene cause THD. To date, 85 unique pathogenic and likely pathogenic variants in the *TH* gene are reported in the ClinVar database (Supplementary material). Among them, there are 13 nonsense, 21 frameshift, 22 splice sites variants. No homozygous or compound heterozygous pathogenic truncating variants have been reported. Functional analysis in knock-out mice demonstrates gestational lethality due to norepinephrine deficiency [24, 25]. This data indicates that the catecholamines are essential for the embryonic and postnatal development in many species and the complete loss of TH activity is incompatible with life [9]. Two variants are reported in the protein promotor region, functional analysis with site-directed mutagenesis in rats showed decreased basal TH expression [26, 27]. Based on the reported functional analysis results, it can be assumed that when a diagnosis of THD is suspected, the genetic analysis should include not only the exons, but also the introns and the promotor regions. There are 19 reported missense pathogenic variants with subsequent partial loss of enzyme activity and four large deletions with length > 50 bps. Two "hot spot" missense variants can be defined: c.698 C > A and c.707 T > C. According to gnomAD v2.1.1 database the pathogenic variant with the highest allele frequency is c.364 C > T (MAF in East Asian population = 0.0007172).

The human *GCH1* gene encodes the GTP cyclohydrolase 1 which is essential for tetrahydrobiopterin (BH4) biosynthesis as a main cofactor for three aromatic acid monooxygenases: phenylalanine, tyrosine and tryptophan hydroxylase [25]. GCH1 deficiency leads to decreased tyrosine hydroxylase protein in the nigrostriatal dopamine neuron and clinically is manifested with DRD phenotype [28, 29]. In 1992 Togari et al. successfully isolated full length cDNA clones encoding human GTP cyclohydrolase 1 from a human liver cDNA library and their results demonstrate that, in humans, GTP cyclohydrolase I is encoded by at least three different mRNA, they differ in amino acid sequence and 3'UTR [30]. The longest cDNA encodes the active enzyme (250 amino acids) and catalyze the conversion of GTP into dihydroneopterin triphosphate, the potential function of the inactive mRNA's remains unknown [31]. Dopa-responsive dystonia due to GCH1 deficiency is inherited as an autosomal recessive or autosomal dominant trait with reduced penetrance and heterogenous clinical presentation. The clinical heterogeneity can be explained as a result of the difference in the level of the enzyme activity, which is dependent of location and type of variants in the *GCH1* gene [32].

Pathogenic variants in the *GCH1* are the most common cause for DRD. To date, there are 87 unique pathogenic and likely pathogenic variants in the *GCH1* in the ClinVar database. Among them, 11 are nonsense, 18 frameshift, 16 splice sites variants, 29 missense and ten large deletions with length >50 bps. According to gnomAD v2.1.1 database the pathogenic variant with the

Table 2. Identified variants in the patients.

P	Gene	Gene locus	Amino acid alteration	Genotype	Population frequency (gnomAD v.2.1.1)	ACMG criteria	ACMG classification	ClinVar ID	Diagnose/ Inheritance	Ref.
1	*TH*	c.605 G > A	p.Arg202His	het	0.0001063	PS3, PM2, PP2, PP3, PP5	Pathogenic	12327	Segawa syndrome/AR	[22]
		c.614 T > C	p.Leu205Pro	het	0.00001228	PS3, PM1, PM2, PM3, PP2, PP5	Pathogenic	12325		[9]
2	*TH*	c.605 G > A	p.Arg202His	het	0.0001063	PS3, PM2, PP2, PP3, PP5	Pathogenic	12327	Segawa syndrome/AR	[16–19]
		c.1118 C > T	p.Thr373Met	het	0.000004017	PM1, PM2, PP2, PP3, PP4	Likely pathogenic	1403045		N/A
3	*GCH1*	c.671 A > G	p.Lys224Arg	het	0.0003856	PS4, PM2, PP1, PP2, PP5	Likely pathogenic	9283	DRD/AD	[23, 28]

highest allele frequency is c.671 A > G (MAF in Latino/Admixed American population = 0.0008748).

All variants in the *TH* and *GCH1* gene reported in ClinVar are described in Supplementary material.

Dopa-responsive dystonia is a biochemical disorder influencing neurotransmitters synthesis, with the majority of patients experiencing good response to L-dopa treatment. There are very few studies that present the gene mutation frequency and genotype-phenotype correlation in DRD patients and so far, the genotype cannot predict the phenotype [8, 10]. The phenotype plasticity in both TH and GCH1 deficiency typically correlates with the severity of the enzymatic defect [33, 34]. Here, we report three undiagnosed patients with neurologic features before the genetic testing. As a result of WES and WGS data analysis, two of them were diagnosed with THD due to compound heterozygous missense variants in the tyrosine hydroxylase (*TH)* gene. In the third patient we revealed heterozygous missense variant in the GTP cyclohydrolase 1 (*GCH1)* gene resulting in autosomal dominant form of DRD.

Genetic testing, like WES and WGS has become rapidly integrated into clinical practice, worldwide. The implementation of WES/WGS in the overall healthcare system in Bulgaria is in an early phase. In Bulgaria, diagnostic genetic testing has been offered since the early 1980s, with the first genetic laboratories being opened at university hospitals in the country (in Sofia, Plovdiv, Pleven, Varna and Stara Zagora). Until now, this network of university laboratories carries out essential activities at the national level, such as mass neonatal screening and prenatal screening and diagnosis of congenital anomalies and genetic diseases. State genetic laboratories traditionally offer cytogenetic analysis, some also molecular-cytogenetic (FISH), but only recently in three of the laboratories it is possible to perform next-generation sequencing (of gene panels) for diagnostic purposes, and only one of the laboratories can offer a WES and whole genome sequencing. Nevertheless, many patients and families with rare diseases walk a difficult path to reach a diagnosis and eventual treatment.

There are different obstacles that slow the application of the genomic technologies in every day clinical practice, including evaluation and interpretation of genomic data, deficiency of clinical genetic professionals, inequitable access to laboratories with professional expertise and the lack of financial support for the most of the patients.

The aim of our report is to improve awareness of DRD, because the rarity and the clinical diversity of the disorder can lead to late diagnosis. Neurotransmitter analysis along with genetic testing should be the first-tier tests when dystonia is suspected, because it is potentially treatable disorder and the diagnostic delay can affect the patient's treatment outcome [35].

DATA AVAILABILITY

The dataset analyzed during the current study are publicly available in ClinVar database. Also, may be available from the corresponding author following an application to and with approval from the local ethics committee.

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AUTHOR CONTRIBUTIONS

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. All patients participated voluntarily. The participants provided written informed consent to participate in this study.

ADDITIONAL INFORMATION

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