Dopa-Responsive Dystonia in Han Chinese Patients: One Novel Heterozygous Mutation in GTP Cyclohydrolase 1 (*GCH1*) and Three Known Mutations in *TH*

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Background:
This study aimed to clarify the diagnosis and expand the understanding of dopa-responsive dystonia (DRD).

Material/Methods:
Relevant data from clinical diagnoses and genetic mutational analyses in 3 Han Chinese patients with sporadic DRD were collected and analyzed. Protein structure/function was predicted.

Results:
One novel mutation of c.679A>G (p.T227A) in *GCH1* and 3 known mutations of c.457C>T (p.R153X), c.739G>A (p.G247S), and c.698G>A (p.R227H) in tyrosine hydroxylase (*TH*) have been found and predicted to be damaging or deleterious. All of the mutations were localized in conserved sequences. The iterative threading assembly refinement (I-TASSER) server generated three-dimensional (3D) atomic models based on protein sequences from the novel nonsense mutation of c.679A>G (p.T227A) in *GCH1,* which showed that residue 227 was located in the GCH1 active site.

Conclusions:
Patients carrying different non-synonymous variants had remarkable variation in clinical phenotype. This study expands the spectrum of genotypes and phenotypes of DRD in the Han Chinese ethnicity, provides new insights into the molecular mechanism of DRD, and helps the diagnosis and treatment of DRD.

MeSH Keywords:
Dopamine Agonists • Dystonic Disorders • GTP Cyclohydrolase

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Background

Neurotransmitter diseases should be considered in any child or adult presenting with symptoms of unexplained seizure, dystonia, tremor, spastic paraplegia, ataxia, or other ocular movement disorders [1]. Dopa-responsive dystonia (DRD) is one of the neurotransmitter diseases and a heterogeneous group of movement disorders, clinically characterized by childhood or adolescent onset of the disease, sometimes associated with concurrent or subsequent Parkinsonism. Typically, DRD presents in mid-childhood (age 5–6 years) with dystonia affecting the gait (feet, legs, and trunk). Females are more commonly affected than males and the symptoms of female patients are more severe than those of male patients [2]. Symptoms show diurnal variation in about 75% of cases and a dramatic therapeutic and sustained response to low doses of levodopa (L-dopa) without any adverse effects [3–5]. The prevalence of DRD is reported to be 0.5–1.0 per million [6], but currently there is no statistical prevalence data of this disease in the Han Chinese ethnicity. DRD is considered to be caused by mutations in the genes encoding the enzymes involved in dopamine and tetrahydrobiopterin (BH4) biosynthesis [7]. A candidate gene approach has identified GTP cyclohydrolase 1 (*GCH1*) (NCBI Reference Sequence: NM_001024024.1) as the disease gene in DRD [6]. It is mainly caused by mutations of the *GCH1* gene, with a mostly autosomal dominant inheritance with sex-related reduced penetrance. *GCH1* is located on chromosome 14 and is composed of 6 exons of 477 bp, 110 bp, 56 bp, 32

bp, 85 bp, and 2127 bp, and spans approximately 30 kb of genomic DNA (GenBank accession numbers: Z30952, U19256, U19257, U19258, U19259, D38602). It is more rarely caused by mutations in the tyrosine hydroxylase (*TH*) gene and the sepiapterin reductase (*SPR*) gene with recessive inheritance [8,9]. *GCH1* deficiency (Segawa disease) is the most common neurotransmitter disorder in most populations. The *GCH1* codes for the enzyme guanosine triphosphate cyclohydrolase (GTPCH1), which is the first and rate-limiting enzyme in the synthesis of tetrahydrobiopterin and is an essential cofactor for tyrosine (and phenylalanine and tryptophan) hydroxylase and thus dopamine synthesis [6,10]. In contrast, mutations in *TH* (autosomal recessive Segawa disease, MIM #605407), which lead to deficiency in TH, are extremely rare and affect the rate-limiting step in catecholamine biosynthesis [11] (Figure 1).

Three sporadic Han Chinese patients with DRD are described, which expands the previously described phenotype. By using next-generation sequencing, 1 novel heterozygous mutation in *GCH1* and 3 known mutations in *TH* were detected.

Material and Methods

Patients

Three sporadic Han Chinese patients were admitted to our clinic of Shanghai Children's Hospital from 2014 to 2017. They met
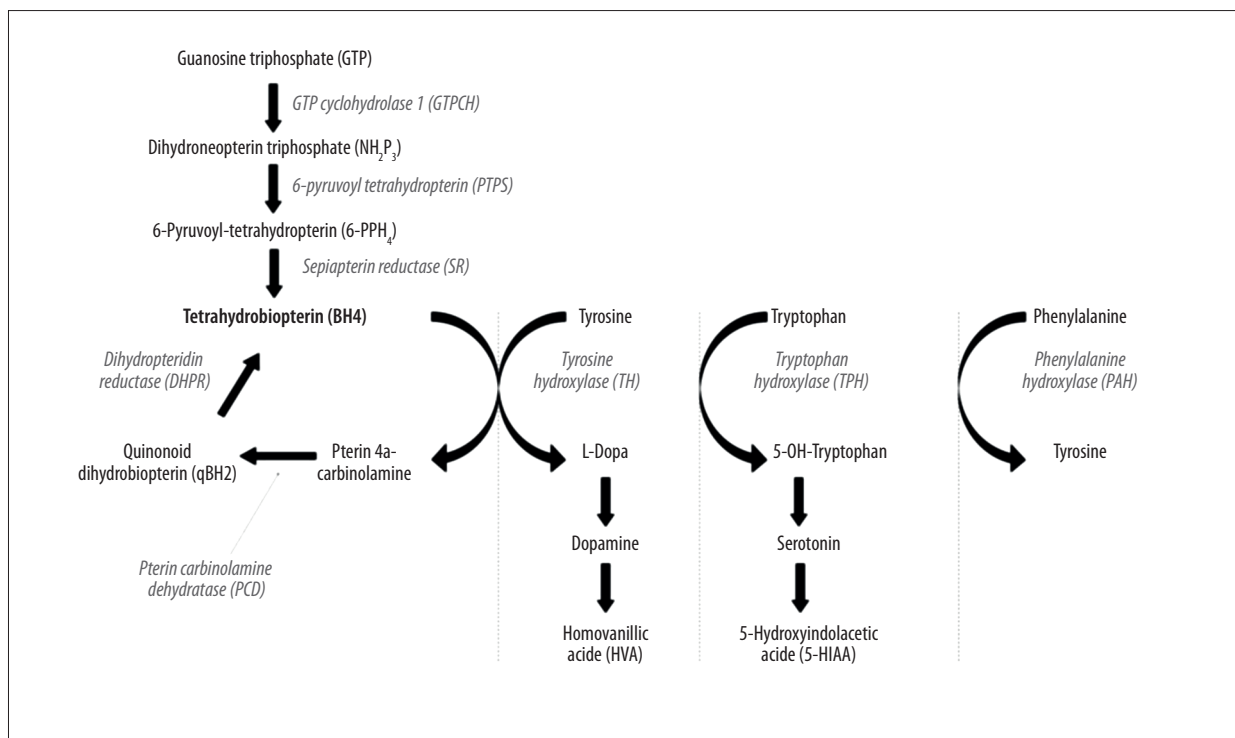

Figure 1. Metabolic pathway of BH4, dopamine, serotonin, and tyrosine.

the established criteria for DRD. All of the patients' perinatal and neonatal courses were normal. They had no definite family history of DRD. Metabolic screening of urine and plasma revealed no abnormality. The concentrations of homovanillic acid (HVA) in cerebrospinal fluid were normal in one patient, lower in another patient, and not tested in the third patient. The 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), pterin, tyrosine, and 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid revealed no abnormality in the 2 tested patients. Cranial magnetic resonance imaging and electroencephalogram showed no abnormalities. The symptoms of these patients were improved after levodopa treatment. All probands' parents were studied to investigate inheritance of mutations.

Variation detection

On admission, venous blood samples were obtained from the patients as well as from their unaffected parents. Next-generation sequencing (NGS) was performed on all of the samples. Genomic DNA samples were sonicated, followed by hybridization with the NimbleGen 2.0 probe sequence capture array of Roche (*http://www.nimblegen.com/products/seqcap/ez/v2/index.html*) to enrich the exon DNA (Joy Orient, China). Libraries were first tested for enrichment by quantitative polymerase chain reaction (qPCR) and for size distribution, and the concentration was determined using the Agilent Bioanalyzer 2100. The samples were sequenced on the Illumina Hiseq2500. Two parallel reactions were performed for each sample. Data filtering, mapping, and variant detection were applied. Exon-enriched DNA was sequenced on the Illumina hiseq2500 platform according to the manufacturer's instructions (Illumina). Raw image files were processed using the BclToFastq (Illumina) for base calling and generating the raw data. Low-quality variations were filtered out using the quality score ≥20 (Q20). The sequencing reads were aligned to the NCBI human reference genome (hg19) using BWA. Samtools and Pindel were used to analyze the single-nucleotide polymorphism (SNP) and the indexing of the sequence. Lastly, data analysis was performed as follows: synonymous changes and SNPs with the minor allele frequency (MAF) higher than 5% were removed (*http://www.ncbi.nlm.nih.gov/projects/SNP*); non-synonymous changes were filtered using SIFT software (*http://sift.jcvi.org*); and the function of mutated genes and their relationship to CMS were further analyzed.

Protein function prediction for gene mutations

To predict the effect of amino acid substitutions, we performed *in silico* analysis using the SIFT/PROVEAN (*http://sift.jcvi.org*) and Polyphen-2 (*http://genetics.bwh.harvard.edu/pph2*) web software. SIFT Score ranges from 0 to 1. The amino acid substitution is predicted as damaging if the score is ≤0.05, and tolerated if the score is >0.05 (J. Craig Venter Institute, USA). The variant is predicted to be deleterious if the PROVEAN score is ≤–2.5), and neutral if the score is >–2.5 (J. Craig Venter Institute, USA). Polyphen-2 prediction outcomes can be "probably damaging", "possibly damaging", or "benign".

Conserved sequence analysis

UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly was used to analyze whether the mutation locations on both alleles were in conserved sequences.

Protein structure prediction on the iterative threading assembly refinement (I-TASSER) server for the nonsense mutation

The I-TASSER Suite pipeline consists of 4 general steps: threading template identification, iterative structure assembly simulation, model selection and refinement, and structure-based function annotation. The server is available at *http://zhanglab.ccmb.med.umich.edu/I-TASSER*.

Ethical considerations

Since we wanted to further clarify the genetic cause and diagnosis, the consultants/registrars in the Department of Pediatric Neurology informed the parents about the purpose of the DNA analysis. The samples from the children and their parents were studied after obtaining informed consent from the parents. This work was approved by the Ethics Committee of Shanghai Children's Hospital.

Results

Clinical manifestations of the patients (Table 1).

Patient 1 was admitted to our clinic at the age of 6 years. His lower limbs were affected, leading to walking difficulties, with various associated symptoms, including difficulty climbing stairs, backward verbal communication, and poor hopping ability. The diurnal variation was obvious. He was much better in the morning than in the evening. Muscular tone was increased with hyperreflexia, especially in lower limbs. Dystonic posture of the legs was evident when walking forward, but it was improved when walking backward. The Babinski sign was positive. No tremors or oculogyric crisis appeared. Lumbar puncture was refused. Achilles tendon separation and nerve roots rhizotomy were conducted at ages 3 and 4 years, but with poor results. His parents and sister were unaffected. In summary, he had early psychomotor delay and dystonia, without tremors or oculogyric crisis. Currently, he shows a good response to levodopa (4 mg/kg/d, bid), together with recovery training. Spasticity was ameliorated after the treatment with levodopa.

Table 1. Clinical features of the 3 patients.

	Patient 1	Patient 2	Patient 3
Sex	Male	Female	Female
Age of visiting	6 years old	14 months	26 months old
Gene mutation	c.679A>G (p.T227A) in *GCH1*	c.457C>T (p.R153X) and c.739G>A (p.G247S) in *TH*	c.698G>A (p.Arg233H) in *TH*
Family history	No	No	No
Dystonia and its nature	Muscular tone was increased with hyperreflexia, especially in lower limbs	Muscular tone was decreased	Muscular tone was slightly increased
Posture or movements	Difficulty walking, climbing stairs, and hopping	Motor regression, cannot sit up at the age of 14 months old, weakness of extremities	An abnormal gait, difficult in balancing, unstable in standing or walking on tiptoes
Accompanying signs and symptoms	Speech was limited to single words, no tremors or oculogyric crisis	No tremors or oculogyric crisis	Bradykinesia, no tremors or oculogyric crisis
The diurnal variation	Obvious	Observed	Observed
Response to levodopa	Good	Moderate	Good

Table 2. CSF metabolite concentrations in 2 patients with tyrosine hydroxylase deficiency before treatment (concentrations in nmol/L).

Case (sex)	Age	HVA	5-HIAA	HVA/5-HIAA	MHPG
Patient 2 (F)	14 mo	352	250	1.4	35
Patient 3 (F)	26 mo	395	222	1.7	38
Methods and reference range [20,21]		384–769	110–265	1.8–4.4	35–64

Patient 2 was examined in our clinic at the age of 14 months. She showed normal development until the age of 6 months, after which she has presented motor regression. Now, at the age of 14 months, she cannot sit up. She showed weakness of the extremities and decreased muscular tone. Dystonic posture of the legs was evident when walking forward, but it was not improved when walking backward. No tremors were observed and tendon reflex was normal. She can say "dad" and "mom". Diurnal variation was observed. HVA was lower in cerebrospinal fluid while MHPG, pterin, tyrosine, and 5-HIAA were normal (Table 2). Her parents are unaffected. The electromyogram results were normal at the regional hospital. In summary, she had early psychomotor regression and early dystonia, without tremors or oculogyric crisis. At present, she has shown a moderate response to levodopa (1.25 mg/kg/d, tid).

Patient 3 was referred to our clinic at the age of 26 months. She walked with an abnormal gait, unsteadily at 16 months and with difficulties in balancing. She was unstable standing, walked on tiptoes, and fell easily. One month ago, she showed bradykinesia together with instability in holding, a slight jitter, and weakness in the lower extremities. Her muscular tone was slightly increased. She cannot sit or stand up for any length of time. No tremors appeared. Diurnal variation showed up but was not very obvious. She can express herself in a few words. HVA, MHPG, pterin, tyrosine, and 5-HIAA in cerebrospinal fluid were normal (Table 2). Her mother previously had 2 spontaneous abortions, and both her parents and her brother were unaffected. There was no consanguinity or genetic bond between the parents. In summary, she had psychomotor delay, early dystonia, and abnormal gait, without tremors or oculogyric crisis. Currently, the patient has a good response to levodopa (1 mg/kg/d, tid).

Gene sequencing analysis (Figure 2)

Gene mutation analysis of patient 1 revealed a novel heterozygous missense mutation of c.679A>G (p.T227A) in exon 6 of *GCH1*. This mutation was detected in the proband's father but not in his mother.
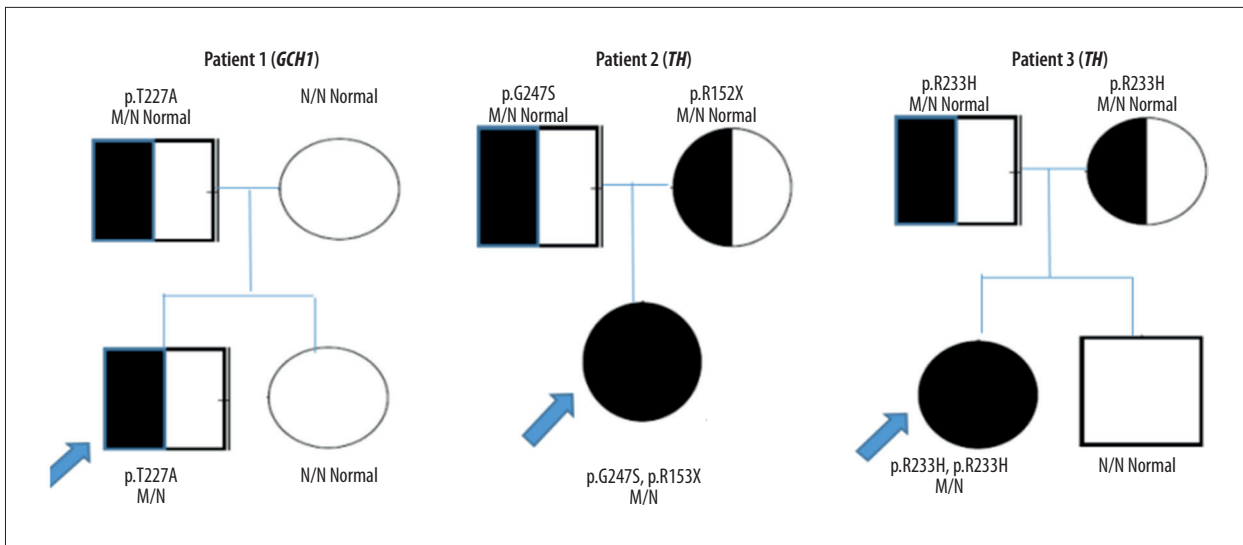
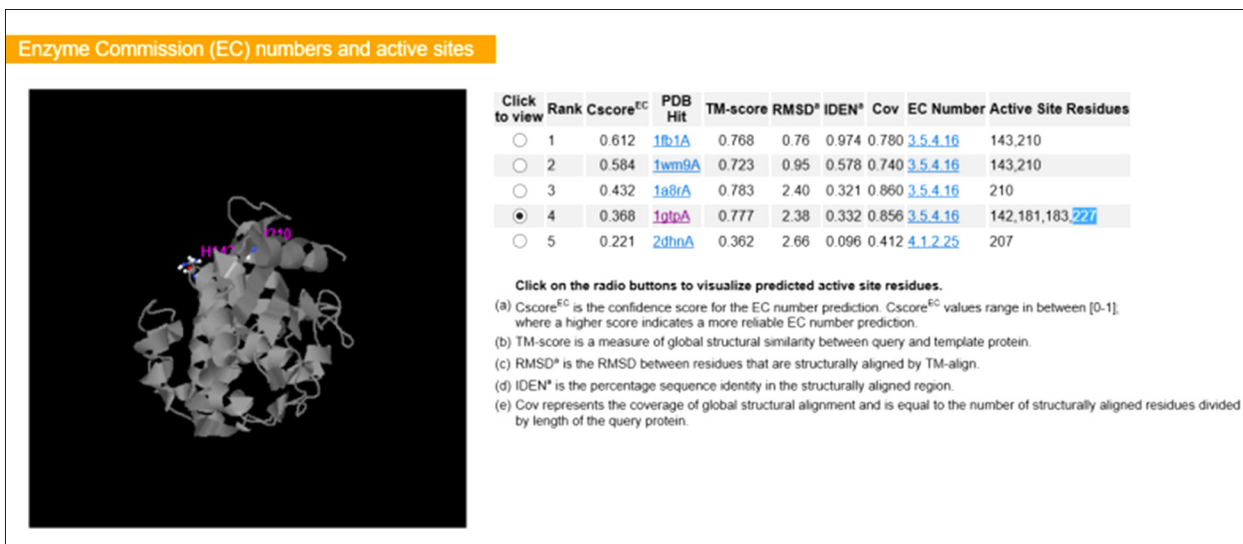**Figure 2.** Pedigree of the 3 sporadic patients.

Figure 3. I-TASSER predicts that residue 227 is located in GCH1 active site.

Patient 2 had 2 known compound heterozygous mutations of c.457C>T (p.R153X) in exon 4 and c.739G>A (p.G247S) in exon 7 of *TH*. One was a nonsense mutation inherited from her mother and the other was a missense mutation inherited from her father.

Gene mutation analysis of patient 3 showed 2 known homozygous mutations of c.698G>A (p.Arg233H) in exon 6 of *TH*. They were inherited from her father and mother, respectively.

Protein function prediction of the gene mutations

The novel missense mutation of c.679A>G (p.T227A) in *GCH1* and 3 known mutations of c.457C>T (p.R153X), c.739G>A (p.G247S), and c.698G>A (p.Arg233H) in *TH* were predicted to be damaging/deleterious by the SIFT/PROVEAN and Polyphen-2 web software.

Conserved sequence analysis

We used the UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly, which showed that the mutations of c.679A>G (p.T227A) in *GCH1*, c.457C>T (p.R153X), c.739G>A (p.G247S), and c.698G>A (p.Arg233H) in *TH* were located in conserved sequences.

Protein structure prediction on the iterative threading assembly refinement (I-TASSER) server for the nonsense mutation (Figure 3)

The iterative threading assembly refinement (I-TASSER) server generated three-dimensional (3D) atomic models based on protein sequences from the novel nonsense mutation of

c.679A>G (p.T227A) in *GCH1,* which showed that residue 227 was located in the GCH1 active site.

Discussion

Heterozygous mutations in *GCH1* are the underlying cause of more than half of the cases of autosomal dominant DRD. Homozygous mutations in *GCH1* have been recognized as a rare cause of hyperphenylalaninemia. Major symptoms include mental retardation, convulsions, disturbance of muscle tone, abnormal movements, difficulties in feeding, hypersalivation, hyperthermia in the absence of infections, and hyperphenyl-alaninemia refractory to a phenylalanine-restricted diet [12]. Patient 1 had a missense mutation of c.679A>G (p.T227A) in exon 6 of *GCH1* inherited from his father. Threonine was substituted by alanine. UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly predicted that it was in a conserved sequence. Protein function prediction of this mutation *in silico* software showed it to be damaging/deleterious. I-TASSER predicts that residue 227 is located in the GCH1 active site. According to the characteristics of child-onset dystonia, a moderate level of neurologic symptoms and a normal phenylalaninemia level, patient 1 belonged to the autosomal dominant classification of *GCH1* deficiency.

Tyrosine hydroxylase (*TH*, EC 1.14.16.2) catalyzes the hydroxylation of l-tyrosine to l-dihydroxyphenylalanine (L-dopa), the rate-limiting step in the biosynthesis of the catecholamines dopamine, norepinephrine, and epinephrine. In humans, secondary impairment of *TH* enzymatic activity occurs in defects of BH4 synthesis and recycling, mostly referred to as variant phenylketonurias [13]. The clinical manifestations of tyrosine hydroxylase deficiency (THD) are variable, ranging from early-onset lethal disease to mild Parkinson disease-like symptoms appearing in adolescence [14]. Willemsen et al. [15] reviewed the clinical and biochemical data on 36 patients and the literature describing phenotypes and genotypes of THD. They proposed categorizing the patients into 2 subgroups – THD type A and B – based on clinical features. Type A was defined as a progressive extrapyramidal movement disorder with onset in infancy or childhood. Type B is a more severe, complex encephalopathy with onset in the neonatal period or early infancy [15]. But to date, there is no exact relationship between genotype and phenotype of clinical syndrome caused by a mutation in *TH*. It was reported that *TH* was responsible for typical DRD or L-Dopa-responsive Parkinsonism [16]. Patient 2 had compound heterozygous mutations of c.457C>T (p.R153X) and c.739G>A (p.G247S), which were located in a conserved sequence. One was a nonsense mutation, which was first reported in a case of a homozygous mutation by Chi et al. [17] and was definitely deleterious. The other was Glycine substituted by Alanine. Protein function prediction *in silico* analysis showed it was damaging/deleterious.

The functional analysis of this mutation by Fossbakk et al. [14] showed impaired enzyme activity. According to the characteristics of infancy-onset dystonia, moderate level of neurologic symptoms, patient 2 belonged to type A TH deficiency. Patient 3 had homozygous mutations of c.698G>A (p.Arg233H), which is "common" in the Netherlands [18]. This mutation resulted in an amino acid change from arginine to histidine at codon 233. The substituted arginine-233 residue was highly conserved, which was detected by the UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly. In the paper by Fossbakk et al. [14], functional analysis of the mutant p.Arg233H showed that the residual activity was reduced to 14%, which was considered to affect the normal function of the TH protein and was predicted to be damaging/deleterious by *in silico* analysis. According to the clinical manifestations, patient 3 would also be categorized as type A TH deficiency. Catecholamine metabolites, including HVA, in cerebrospinal fluid in patient 2 weere lower than in patient 3, and symptoms of patient 2 appeared to be worse than in patient 3. Severity in TH-DRD was obviously associated with the residual enzymatic activity. The CSF levels of catecholamine metabolite were within normal range in patient 3, but HVA and MHPG were close to the lower reference range limit, which may be related to testing error or severity of disease.

In all cases, the parents of affected subjects were examined and found to be free of neurological symptoms; this absence of symptoms reflected either the well-described reduced penetrance of DRD, or the requirement, in some cases, of 2 gene mutations to cause disease [19].

Conclusions

In summary, patient 1, with *GCH1* mutation, had early psychomotor delay and dystonia, without tremors or oculogyric crisis, and had a good response to levodopa. Patient 2, with *TH* mutations, had early psychomotor regression and early dystonia, without tremors or oculogyric crisis, and had a moderate response to levodopa. Patient 3, with *TH* mutations, had psychomotor delay, early dystonia, and abnormal gait, without tremors or oculogyric crisis, and had a good response to levodopa. In conclusion, this study expands the spectrum of genotype and phenotype of DRD in people of Han Chinese ethnicity, provides new insights into the molecular mechanism of DRD and the search for new, targeted treatment strategies, and to find stable compounds that may reverse the negative effects of the mutations on the enzyme activity. Further large-scale case studies are needed to establish the genotype-phenotype correlation of DRD.

Conflict of interest

None.

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