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Novel *GCH-1* Mutation in Chinese Families with Dopa-responsive Dystonia (Segawa Disease)

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Abstract: The wide array of clinical manifestations of dopa-responsive dystonia (DRD) or Segawa disease and the prevalence of numerous DRD-associated mutations in guanosine triphosphate cyclohydrolase 1 (*GCH1*) gene makeDRD diagnosis challenging. Methods: In this study, we assessed the clinical and genetic characteristics of two Chinese families with 3 DRD probands. Clinical assessment of DRD-related symptoms was conducted for all participants. All 6 exons of *GCH1* were assessed by genetic analyses for individuals with heteroduplex DNA as compared that of controls. Results: The median DRD-onset age was 24 years and the female to male ratio of DRD patients was 8:1. Six out of eight (75%) patients responded to levodopa therapy. The data indicated that the *GCH1* sequence had a novel point mutation resulting in T to C transition at position 80 in exon 1 of the cDNA sequence (c.80T>C), which resulting in an amino acid change (L27P) of *GCH1* in the probands and their mother in the first DRD family. Conclusion: A novel *GCH1* mutation (c.80T>C) was identified in the DRD patients in the first family. Our findings indicate that both clinical symptom assessment and genetic testing should be employed for improving DRD diagnosis.

Keywords: Dopa-responsive Dystonia, Guanosine Triphosphate Cyclohydrolase, *GCH1*, Chinese

1. Introduction

Dopa responsive dystonia (DRD) or Segawa disease is an inherited progressive dystonia with marked diurnal fluctuation. DRD is a rare hereditary disease with a prevalence of 0.5–1 per million individuals [1]. The hallmark of DRD is a dramatic and sustained response to relatively small doses of levodopa. DRD is most commonly inherited in an autosomal dominant manner and is caused by a mutation in guanosine triphosphate cyclohydrolase 1 *(GCH1)* gene, which encodes an enzyme that catalyzes the rate-limiting step in the synthesis of tetrahydrobiopterin (BH4). BH4 serves as an essential cofactor for tyrosine hydroxylase and is involved in converting L-tyrosine to levodopa [2]. Thus, mutations in *GCH1* could lead to disruption in levodopa synthesis. To date, more than 100 mutations in *GCH1* gene have been reported among different populations. In this study, the clinical features and *GCH1* sequences of Chinese familial patients with DRD were analyzed.

2. Methods

2.1. Study Subjects

This study was approved by the Ethics Commission of

General Hospital of Chinese PLA. Informed consent was obtained from all study participants. Of the two participating Chinese families, three proband patients (1-IV-17, 1-IV-18, 2-IV-3) fulfilled the previously described criteria for DRD [3]. The three proband patients and their healthy parents/relatives (1-III-15, 1-III-16, 1-III-11, 1-III-18, 1-III-5, 1-III-6, 1-IV-8, 2-IV-3, 2-IV-7, 2-IV-14, 2-III-11, 2-III-1, and 2-III-2) were included in the study. The clinical data obtained for all study participants were reviewed and re-examined.

Blood samples were collected from all participants for performing DNA analysis. DNA was sequenced to detect mutations and one novel mutation was further analyzed to determine whether the variation was a causal mutation or a neutral polymorphism. We screened 300 genetically unrelated healthy controls with the same ethnic background for these variations by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [4].

Mutations were identified in the eight patients affected in at least two generations from the two families (Family 1 and Family 2) (Figures 1 and 2). In most cases, the affected members presented with typical DRD features, which included lower limb weakness, stiffness, and dystonia. Both genetic analysis data and clinical presentation data were analyzed together.

2.2. Clinical Symptom Assessment

Patient 1-IV-17 was a 3-year-old girl. Her symptoms included lower leg weakness and were present from birth to the age of 3 years. Typically, her limb stiffness symptoms began in the afternoon and became worse in the evening. Her condition progressively worsened but her disease pathophysiology was restricted to her bilateral lower limbs. She had difficulty in walking and was previously misdiagnosed with cerebral palsy. Her condition was finally diagnosed as DRD in our hospital and her brother was also found to have the same symptoms (1-IV-18) and was diagnosed with the same condition. Treatment with levodopa (187.5 mg/day) for 3 days led to improvement of her symptoms. The patient has continued the L-dopa treatment at the aforementioned dosage.

Patient 1-IV-18 was a 6-year-old boy and was the brother of the patient 1-IV-17. At age 5, he began walking slightly unsteadily. One year later, he experienced lower limb stiffness with diurnal fluctuations. However, his arms were not affected. Weakness and stiffness of the legs were the major symptoms that progressed slowly until his latest assessment. Brisk tendon reflexes were observed in his legs; however, Babinski signs were negative. Treatment with a low dose of L-dopa (187.5 mg/day) led to symptom resolution. The patient experienced marked reduction in dystonia after initially developing transient levodopa induced-dyskinesia. The symptoms improved gradually.

Patient 2-IV-3 was a 24-year-old woman. At age 14, she suffered from gait disturbance characterized by leg stiffness after exercise. The stiffness would completely resolve after resting. Two years later, she experienced postural tremor of the limbs and trunk, and the lower limb stiffness worsened.

For the last 8 years, before she was diagnosed with DRD, she was unable to walk without assistance and was misdiagnosed with spastic paralysis. Her symptoms disappeared after administration of low dose of L-dopa (187.5 mg/day) for 1 week. The L-dopa treatment was recommenced from then on. She did not experience any discomfort or side effects.

2.3. DNA Extraction and Analysis

DNA was extracted from the whole blood of the participants using DNeasy Blood & Tissue Kits (Qiagen, Valencia, CA). The DNA fragments spanning 6 exons of the *GCH1* gene were amplified using polymerase chain reaction (PCR) using oligodeoxynucleotides. Each fragment was purified and subsequently analyzed by direct sequencing [4]. The resultant sequence data were compared with the published human gene sequences in the National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov) to determine sequence differences. Phylogenetic conservation of the mutation sites was analyzed by aligning amino-acid sequences of several species (retrieved from the Entrez protein NCBI database) using the Clustal X 2.0.12 program [5].

3. Results

3.1. The Clinical Features of Patients with Familial DRD

On the basis of the case history and clinical findings, a diagnosis of DRD was made. The median onset age was 24 years and the ratio of female to male of DRD patients was 8:1. Two probands in the first family childhood-onset and were misdiagnosed with cerebral palsy. In addition to the classic signs of dystonia (3/8), diurnal weakness and stiffness (7/8) was the main symptom present. After beginning levodopa therapy, both patients exhibited a subjective and objective improvement in their clinical symptoms. All the affected patients in the two families showed typical DRD symptoms, including dystonia of the lower limbs. Some afflicted family members also reported muscle fatigue. In the 8 affected patients, 6 patients (75%) were initially prescribed 62.5 mg of levodopa 3 times daily. Maintenance doses ranging between 187.5–250 mg/d were required to achieve the adequate symptomatic relief. The subjective response to treatment was assessed at both 6-month and 12-month follow-up visits. Patient 1-IV-18 reported good response to therapy and experienced marked reduction in dystonia after beginning levodopa treatment. However, he initially presented transient levodopa induced-dyskinesia, followed by gradual recovery. Other patients did not experience treatment-related adverse effects. All symptoms improved in patient 1-IV-17, patient 1-IV-8, and patient 2-IV-3. Clinical examination revealed a marked improvement in dystonic and parkinsonian features in patients 2-IV-7 and 2-IV-14 (Figures 1 and 2, and table).

3.2. GCH1 Mutations in DRD Patients

Genetic analysis was performed for all eight affected patients and the mutation in the coding region of the *GCH1* gene on chromosome 14q22.1-14q22.2 was assessed. All six

exons of *GCH1* gene were amplified for the assessment. Interestingly, we detected a point mutation (T to C transition) at position 80 in exon 1 of the cDNA sequence (c.80T>C) (Figure 3) and the mutation predicted an amino acid change (L27P) in *GCH1* of patient 1-IV-17 and patient 1-IV-18. Their mother (1-III-15) had c.80T>C mutation; however, their father did not have the mutation. The sequence variation was not detected in the 300 healthy controls.
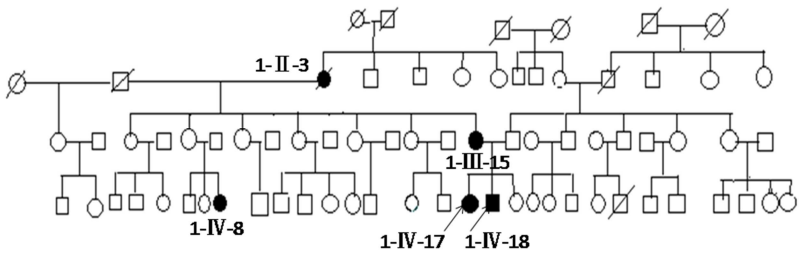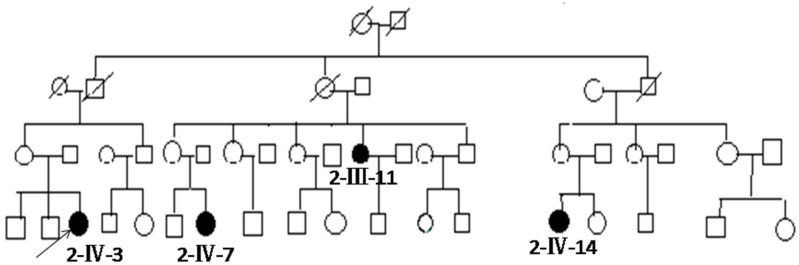

Figure 1. Pedigree of Chinese family (1) with dopa-responsive dystonia.

Arrow indicates proband. Shaded symbols represent those with dopa-responsive dystonia (DRD). Clear symbols indicated unaffected individuals. The patients 1-III-15, 1-IV-8，1-IV-17, and 1-IV-18 had the novel mutation.

Arrow indicates proband. Shaded symbols represent those with DRD. Clear symbols indicate unaffected individuals. None of the patients had the mutation.

Figure 2. Pedigree of Chinese family (2) with DRD.
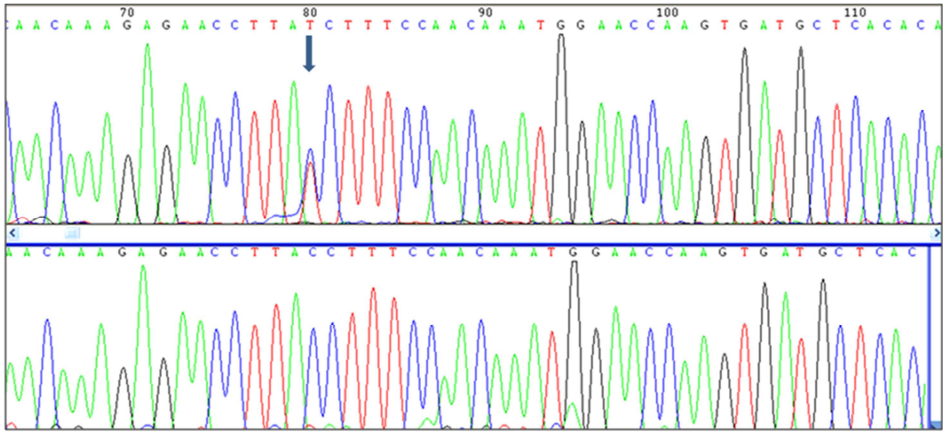
Mutation is shown in the upper half, and the corresponding normal sequences are shown below. c.80T>C (L27P).

Figure 3. Chromatograms of the guanosine triphosphate cyclohydrolase 1 (GCH1) mutation identified in this study.

Table 1. Clinical and genetic characteristics of dopa-responsive dystonia (DRD).

ID	Age at onset (ys)	sex	Clinical presentation	Initial diagnosis	levodopa dose	*GCH1* gene mutation
1-IV-18	6	M	generalized dystonia and gait disorder	cerebral palsy	187.5mg/d	c.80T>C
1-IV-17	3	F	could not walk from birth to 3ys	cerebral palsy	187.5mg/d	c.80T>C
1-IV-8	14	F	weakness and stiffness in the legs	spastic paralysis	250mg/d	c.80T>C
1-III-15	40	F	weakness and pain in the legs after exercise	no diagnosis	Not treated	Not tested
1-II-3 (died)	46	F	easily tired muscles	unknown	Not treated	Not tested
2-IV-3	14	F	dystonia and weakness in the legs	spastic paralysis	187.5 mg/d	no
2-IV-7	17	F	diurnal stiffness and dystonia in the legs	Parkinson's disease	187.5 mg/d	no
2-IV-14	30	F	easily tired muscles and slightly stiffness	dystonia	250 mg/d	no.
2-III-11	42	F	fatigue	unknown	Not treated	no

Guanosine triphosphate cyclohydrolase 1 (*GCH1*).

4. Discussion

DRD is characterized by childhood-onset with symptoms including lower limb dystonia with diurnal fluctuation. Previous reports indicate that the condition responds well to small doses of levodopa. The prevalence is higher in females than in males [6]. In our study, the median onset age was 24 years (because the patient in Family 2 may be diagnosed late) and the ratio of female to male was 8:1. The higher prevalence of DRD in female subjects indicates that gender-related factors may influence the expression of *GCH1* gene [6].

DRD is a remediable dystonia and early diagnosis can be beneficial. However, DRD patients present a wide range of clinical presentations and the disorder is commonly misdiagnosed as cerebral palsy, spastic paralysis, focal dystonia, and juvenile Parkinson's disease [7-11]. The dramatic response of patients to L-dopa treatment underlines the importance of considering DRD in the differential diagnosis of patients presenting with a phenotype resembling cerebral palsy or other disorders. In our study, two childhood-onset patients from the first family were previously misdiagnosed with cerebral palsy. After receiving L-dopa treatment, they showed a dramatic improvement. The majority of the childhood-onset DRD patients included in this study were previously misdiagnosed with cerebral palsy, and the patients with adult-onset DRD were previously misdiagnosed with spastic paralysis or Parkinson's disease. The classic signs of dystonia accompanied with diurnal weakness and stiffness of the lower limbs were the primary distinguishing symptoms that indicated DRD. Next, the genetic sequences of the GTP-cyclohydrolase I (*GCH1*) gene were assessed for patients that were diagnosed with DRD on the basis of their symptoms.

The *GCH1* gene, which maps to 14q22.1–22.2, has been shown to be involved in DRD development [12]. Our data indicate that there was c.80T>C mutation in the translated portion of the *GCHI* gene of familial DRD patients. These data were in agreement with those of other groups [12-14]. As reported previously, each family afflicted with DRD (Segawa disease) has a distinct mutation in *GCH1* gene [15]. In our study, the two childhood-onset patients in the first family were found to have c.80T>C mutation in the coding region. However, no *GCH1* mutation was found in the Family 2, further experimentation should be future studies including more familial DRD. Since their preliminary symptoms were in the lower limbs and the symptoms had childhood-onset, we speculate that the patients did not have sporadic DRD.

To date, more than 100 mutations in *GCHI* gene have been associated with DRD (Human Gene Mutation Database, http://www.hgmd.org). The mutation found in our study (c.80T>C) has not yet been reported. The mutation led to a change from ATC to ACC at the mRNA level. The nucleotide change might interfere with the translation of *GCH1* mRNA and could reduce the expression of the *GCH1* enzyme in the central nervous system. We speculate that the *GCH1* dysfunction caused by c.80T>C (L27P) is similar to that caused by a previously reported mutation [16]. The L82P mutations are conserved in humans, rat, mouse, and chicken. They are located at the turn of the helix-turn-helix structure of the *GCH1* molecule. Since the G90V mutation at the turn of the helix-turn-helix has been reported to cause a dominant-negative effect [17], it is possible that the L27P mutation located at a similar position could impair enzyme activity via a similar mechanism. Because of the low prevalence of DRD and high variation in *GCH1* mutations, the diagnosis of DRD on the basis of clinical manifestations is challenging. Thus, identification of the commonly prevalent mutations associated with DRD would be helpful in the development of a genetic screening assay for DRD.

5. Conclusion

Our findings have identified a novel *GCH I* gene mutation in DRD patients. Our findings also indicate that in addition to the clinical features, genetic testing should also be employed for diagnosing and treating DRD.

Disclosure

Conflict of Interest

All the authors do not have any possible conflicts of interest.

References

[1] Segawa M: Hereditary progressive dystonia with marked diurnal fluctuation. Brain Dev 2011; 33: 195-201.

[2] McGraw-Hill: Disorders of tetrahydrobiopterin and related biogenic amines. The Metabolic and Molecular Bases of Inherited Diseases 8th ed. New York, NY; 2001: 1725-1776.

[3] Abeling NG, Duran M, Bakker HD, Stroomer L, Thöny B, Blau N, Booij J, Poll-The BT. Sepiapterin reductase deficiency an autosomal recessive DOPA-responsive dystonia. Mol Genet Metab 2006; 89: 116-120.

[4] Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. Clustal W and Clustal X version 2.0. Bioinformatics 2007; 23: 2947-8.

[5] Ichinose H, Ohye T, Matsuda Y, Hori T, Blau N, Burlina A, Rouse B, Matalon R, Fujita K, Nagatsu T. Characterization of mouse and human GTP cyclohydrolase I genes. J Biol Chem 1995; 270: 10062-10071.

[6] Hwu WL, Wang PJ, Hsiao KJ, Wang TR, Chiou YW, Lee YM. Dopa-responsive dystonia induced by a recessive GTP cyclohydrolase I mutation. Hum Genet 1999; 105: 226-230.

[7]   Furukawa Y, Lang AE, Trugman JM, Bird TD, Hunter A, Sadeh M, Tagawa T, St George-Hyslop PH, Guttman M, Morris LW, Hornykiewicz O, Shimadzu M, Kish SJ. Gender-related penetrance and GTP-cyclohydrolase I gene mutations in dopa-responsive dystonia. Neurology 1998; 50: 1015-1020.

[8]   Bandmann O, Marsden CD, Wood NW: Atypical presentations of dopa-responsive dystonia. Adv Neurol 1998; 78: 283-290.

[9]   Tassin J, Dürr A, Bonnet AM, Gil R, Vidailhet M, Lücking CB, Goas JY, Durif F, Abada M, Echenne B, Motte J, Lagueny A, Lacomblez L, Jedynak P, Bartholomé B, Agid Y, Brice A. Levodopa-responsive dystonia: GTP cyclohydrolaseI or parkin mutations? Brain 2000; 123: 1112-1121.

[10]   Kong CK, Ko CH, Tong SF, Lam CW. Atypical presentation of dopa-responsive dystonia: generalized hypotonia and proximal weakness. Neurology 2001; 57: 1121-1124.

[11]   Chaila EC, McCabe DJ, Delanty N, Costello DJ, Murphy RP. Broadening the Phenotype of Childhood-Onset Dopa-Responsive Dystonia. Arch Neurol 2006; 63: 1185-1188.

[12]   Cao L, Zheng L, Tang WG, Xiao Q, Zhang T, Tang HD. Four Novel Mutations in the *GCH1* Gene of Chinese Patients with Dopa-Responsive Dystonia. Mov Disord 2010; 25: 755-783.

[13]   Nutan Sharma, Ioanna A. Armata, Trisha J. Mutation in 5'upstream region of GCHI gene causes familial dopa-responsive dystonia. Mov Disord 2011; 26: 2140-2141.

[14]   Li-hua Yu, Hua-yong Zhou, Fa-yun Hu. Two novel mutations of the GTP cyclohydrolase I gene and genotype–phenotype correlation in Chinese Dopa-responsive dystonia patients. European Journal of Human Genetics 2012; 12: 239-245.

[15]   Segawa M, Nomura Y, Yukishita S, Nishiyama N, Yokochi M. Is phenotypic variation of hereditary progressive dystonia with marked diurnal fluctuation/dopa-responsive dystonia (HPD/DRD) caused by the difference of the locus of mutation on the GTP cyclohydrolase 1 (*GCH-1*) gene? Adv Neurol 2004; 94: 217-223.

[16]   Tamaru Y, Hirano M, Ito H, Kawamura J, Matsumoto S, Imai T, Ueno S. Clinical similarities of hereditary progressive/dopa responsive dystonia caused by different types of mutations in the GTP cyclohydrolase I gene. J Neurol Neurosurg Psychiatry 1998; 64: 469-473.

[17]   Hirano M, Yanagihara T, Ueno S. Dominant negative effect of GTP cyclohydrolase I mutations in dopa-responsive hereditary progressive dystonia. Ann Neurol 1998; 44: 365-371.