SHORT REPORT

Phenocopies in a large GCH1 mutation positive family with dopa responsive dystonia: confusing the picture?

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Background: Dopa responsive dystonia (DRD) is a disorder characterised by childhood onset dystonia but a wide range of clinical presentations has now been described.
Objective: To study a large Canadian family with presumed DRD.
Methods: The clinical features of the family were collected before molecular genetic mutational analysis.
Results: All nine individuals in whom a clinical diagnosis of DRD was definite or probable were heterozygous for a GCH1 gene deletion. However, eight of nine possibly clinically affected members did not carry the GCH1 mutation.
Conclusions: Great care must be taken in diagnosing DRD even in families with the classic phenotype, because of potential phenocopies of the disease.

D opa responsive dystonia (DRD) is an inherited disorder with a broad range of clinical symptoms and signs that typically respond well to low doses of dopaminergic drug treatment.[1–3] The wide range of clinical presentations has now been well reported, but confirming the diagnosis either biochemically or genetically remains difficult. In the majority of cases where the gene defect has been found, it has involved a mutation in the GTP cyclohydrolase I gene (GCH1).[3 4]

METHODS

After clinically diagnosing DRD in the proband, attempts were made to interview, examine, and obtain blood samples from as many family members as possible. We were able to do this in 41 subjects (including two offspring of III-19 and III-20). Subjects were considered to be *definitely* affected if they had symptoms of DRD as well as unequivocal dystonia or parkinsonism on examination; they were considered *probably* affected if they had typical symptoms of DRD or parkinsonism

with possible signs of dystonia or parkinsonism on examination; they were considered *possibly* affected if they gave a typical or suggestive history for DRD but did not have evidence of dystonia or parkinsonism on examination. Also included in the latter category were asymptomatic individuals with definite dystonia or parkinsonism on examination. Individuals were considered clinically asymptomatic if they had neither symptoms of DRD nor signs of dystonia or parkinsonism on examination. All histories and examinations were performed before determination of genetic status.

Molecular genetics analysis

Polymerase chain reaction (PCR) primers for amplification of the six exons in GCH1 and the amplification conditions were the same as previously described.[5 6] Amplified DNA fragments were directly sequenced with the same primers as for amplification in three definitely affected members (II-8, III-17, III-18).[6 7] A heterozygous 18 base pair deletion in exon 1 of GCH1 was identified and has previously been reported.[8] Because the deletion mutation abolishes a *Sac*I cleavage site in exon 1 of this gene, restriction site analysis for *Sac*I was performed in all the family members whose DNA samples were available. All carriers identified were heterozygous in terms of the GCH1 mutation.

RESULTS
Definitely affected

Clinical assessment indicated that six individuals were definitely affected and all these carried the mutant allele (fig 1; table 1). One (II-8), the only one presenting with parkinsonism, had onset at age 56 years; all the others had onset before five years. All but one had clear diurnal fluctuations. All complained of gait disturbance and all had equinovarus posturing of the feet (although a remote history of polio may have been responsible for this in the patient with adult onset parkinsonism). Nocturnal or exercise induced leg pains occurred in four, and foot deformities characterised by a high
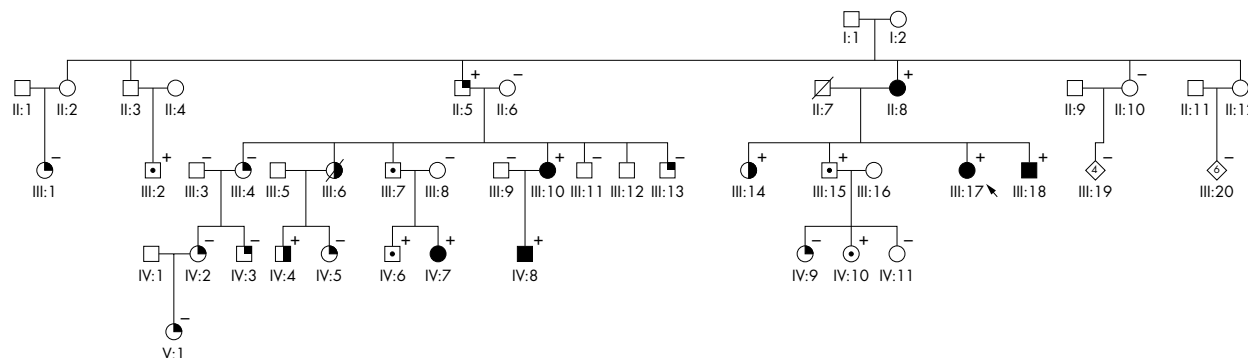
Figure 1 Kindred with dopa responsive dystonia. Filled symbols, clinically definitely affected; empty symbols, not affected; half filled symbols, clinically probably affected; quarter filled symbols, clinically possibly affected; circle in square, asymptomatic carrier; + and −, positive or negative for GCH1 mutation.

Table 1 Clinical features of dopa responsive dystonia

Identification No	Sex	Age at onset (years)	Age when last seen (years)	Presentation	Initial diagnosis	On examination	Levodopa/ carbidopa response and dose (mg/d)	GTP cyclohydrolase I gene mutation
Definitely affected								
II-8	F	56	72	Gradual stiffening of limbs and incoordination of movements following head trauma	Parkinson's disease	Leg short secondary to polio; mild bradykinesia and dyskinesia both hands (on treatment)	Yes (100/25)	+
III-10	F	1-2	39	Toe walking progressing to gait difficulties with falls	Cerebral palsy	Dystonia and bradykinesia right arm and legs	Yes (200/50)	+
III-17	F	2	45	Progressive gait difficulty and poor balance with marked diurnal variation	Cerebral palsy	Dystonia and bradykinesia all limbs	Yes (200/50)	+
III-18	M	Child-hood	33	Mild fatigue and leg pains that limited walking	DRD	Exercise induced, bradykinesia and dystonia of legs	Yes (400/100)	+
IV-7	F	1	18	Toe-walker progressing to leg pains and inversion of her left foot	DRD	Dystonia left foot; bradykinesia lower limbs	Yes (150/37.5)	+
IV-8	M	3	16	Toe-walking, leg pains at night	Spastic diplegia	Bradykinesia and dystonia of legs	Yes (100/25)	+
Probably affected								
III-6	F	5	40	Pain in right leg when walking; toe walking at end of day		Mild bradykinesia in both feet and right hand	Facial spasms, an increase in stiffness (100/25)	Obligate carrier; DNA sample not analysed due to contamination
III-14	F	10	43	Scoliosis, then 8 years later "pressure in legs" from walking	Scoliosis	Questionable bradykinesia in feet after walking; scoliosis	?	+
IV-4	M	6	16	Toe walking and inversion of foot when tired; resolved at age 13?	Extensive investigations as child but no specific diagnosis made	Slight inversion of left foot after prolonged running	An increase in stiffness (100/25)	+
Possibly affected								
II-5	M	–	69	None		Mild increase tone all limbs and mild but definite bradykinesia of both legs	Not tried	+
III-1	F	20	52	Muscle cramps and pains in legs especially at night; improved with levodopa		Normal	Initially improved (subjective) (100/25)	–
III-4	F	6?	46	Cramping right hand after few minutes writing	Writer's cramp	Pen tight after writing few sentences; no abnormal posturing	No effect (300/75)	–
III-13	M	13	32	Walking a long distance he reported he would walk up on the toes of his left foot, the foot would invert and his leg would hurt	DRD	Brisk reflexes, unsustained clonus at ankles, questionably increased tone in his left leg. Examination after five minutes of walking was unchanged	Initially improved (subjective) (100/25)	–
IV-2	F	?	27	Pain in calf when walking one block, more readily at end of day; boots deformed because of this		Normal	Initially improved (subjective) (200/50)	–
IV-3	M	11	13	Pains in thighs and calves with exercise and at night, cramping in hands with prolonged writing		Mild postural and kinetic tremor	Not tried	–
IV-5	F	12	15	Back and leg pains precipitated by walking		Questionable in turning of left leg on walking	Not tried	–
IV-9	F	1	20	Feet in turned as young child – improved with bracing; pain in feet if walks more than one hour		Normal	Not tried	–
V-1	F	1	3	Toe walking in evening, leg pains at night		Normal	Not tolerated (100/25)	–

DRD, dopa responsive dystonia.

arch and shortened foot were noted in three (one of whom had also had polio). These foot deformities were also noted in two probably affected patients (IV-4, III-14) who carried the mutation, and in one of the possibly affected individuals (IV-5) who was gene negative. Four definitely affected individuals had had unnecessary orthopaedic procedures (a total of 11 operations, including Harrington rods for scoliosis), done before their diagnosis of DRD. Writing dystonia (4/6), postural tremor (5/6), scoliosis (3/6), poor balance (5/6), and

brisk reflexes (5/6) were all common findings in this group. These individuals all elected to have treatment and all received levodopa/carbidopa. One individual (III-18) in this group, plus one in the probable group (III-6 gene positive), described a wearing off effect late in the day, although these persons were not examined at these times. One patient experienced transient dyskinesias (III-10) but these responded to a lowering of the dose of drug treatment, and one (II-8) continues to have mild levodopa/carbidopa dyskinesias on 100/25 mg/day.

Probably affected

The three individuals who were clinically diagnosed as probably affected all carried the gene mutation (individual III-6 is an obligate carrier but her DNA sample could not be amplified owing to contamination).

Individual IV-4 at the age of six years old, would, when tired, develop plantar flexion and inversion of his left foot. By the age of 10, the symptoms occurred with exercise as well, and were associated with painful cramps in the foot. When he was 13, the symptoms abated. Examination at age 16 was normal with the exception of minimal inversion of his left foot with exercise and an asymmetry of his feet. He tried levodopa/carbidopa at one stage at a dose of 50/12.5 twice daily, but felt that his entire foot was more stiff during treatment and so discontinued it. He was classified as being probably rather than definitely affected as he did not have unequivocal signs of dystonia on examination.

Individual III-14 developed "pressure" in her legs from walking and pains in her legs at night at the age of 18. She had developed scoliosis at the age of 10. She was diagnosed as probably affected owing to the presence of mild bradykinesia on foot tapping, demonstrated on examination after prolonged walking.

Individual III-6 at the age of five was noted to toe walk and later developed pain in her right leg at the end of the day, which has persisted. Levodopa/carbidopa 50/12.5 mg/day resulted in spasms of her face lasting 30 minutes. Subsequent doses resulted in an increase in pain and stiffness in her legs and feet. On examination she had questionable bradykinesia of both feet and her right hand and she was therefore classified as being probably affected.

Possibly affected

Of nine individuals considered possibly affected, one carried the gene mutated allele (II-5). Individual II-5 is an obligate carrier as he has a daughter who is definitely clinically affected. He did not report any clinical symptoms compatible with dystonia or parkinsonism but on examination it was felt that he had rigidity and bradykinesia.

The eight remaining possibly affected individuals described symptoms of either exercise induced or late day pain or cramping of at least one extremity, yet had no signs of dystonia or parkinsonism on examination. Of the eight gene negative individuals, five had been tried on levodopa. Three initially felt improved, one did not tolerate the treatment, and one noticed no effect. Selected case summaries are included below to illustrate the clinical spectrum of the possibly affected DRD individuals and some of the difficulties associated with making a diagnosis.

Individual V-1 was two years old when her family was first seen. She was not cooperative for examination. Her mother reported that she was an intermittent toe walker since she first began walking. This tended to be worse in the evening or after exercise, and she often developed severe leg pains which interfered with sleeping. She was tried on both levodopa/carbidopa and levodopa/benseraside, but both resulted in hyperactivity and had to be discontinued. Trihexiphenidyl resulted in a marked improvement in her symptoms. When it was discontinued her nocturnal leg cramps returned and so it was restarted, with similar improvement. On subsequent review, the family reported that in retrospect the child walked on her toes mainly following visits by her aunt (III-10) and cousin (IV-8), who were definitely affected before they had received levodopa.

Her mother (IV-2) also was diagnosed as having possible DRD. Since childhood, she had complained of pain in the right calf with associated stiffness and eversion of the foot. This would come on within walking one block and more readily towards the end of the day. Interestingly, she stated that the problem only happened if she was walking outdoors. She also noted that the left foot turned out but it was not painful. She was given a trial of levodopa/carbidopa and once she reached a dose of 200/50 mg/day she was able to walk without any pain at all, although she noted that she still walked on the sides of her feet. Her examination was normal despite her symptoms.

The grandmother of V-1 (III-4) was also felt to be possibly affected. She reported excessive cramping after a few minutes of writing with her right hand. She also reported that she wore out her shoes unevenly on the sides. There was no diurnal variability of these symptoms. On examination she gripped the pen excessively after a few minutes but had no abnormal posturing of her hand. She felt no improvement with levodopa treatment.

Individual III-13, first seen at 26 years old, had an operation at age 13 to remove a chipped bone after being kicked. Ever since then, whenever he was tired or walked a long distance he reported he would walk up on the toes of his left foot, the foot would invert and his leg would hurt. He had very brisk reflexes, unsustained clonus at his ankles, and questionably increased tone in his left leg. Examination after five minutes of walking was unchanged. The remainder of his neurological examination was normal. He initially reported that treatment with levodopa/carbidopa 100/25 mg/day resulted in a complete resolution of his symptoms. However, on further follow up he was no longer taking the treatment and was not any worse.

Four asymptomatic individuals (III-2, III-15, IV-6, IV-10) were also found to carry the mutation, and one individual, who was not seen, was an obligate carrier (III-7). III-7 was not known to have any symptoms and functioned normally according to his family. The ages when last seen of the four asymptomatic individuals ranged from 15 to 42 years.

DISCUSSION

This family manifests the wide spectrum of features possible in DRD, as well as unexpected pitfalls with the clinical diagnosis.[9] [10] In recent years this already broad clinical spectrum has been expanded to include individuals with atypical or unusual presentations.[11–17] Within our family, two individuals were originally felt to have cerebral palsy by their treating physicians, and one also had scoliosis as her initial symptom. These atypical cases further compound the difficulty of making a clinical diagnosis of DRD.

A wide variety of mutations in GCH1 has been reported as the cause of DRD.[1] [5] [18] Furthermore, mutations in the tyrosine hydroxylase gene have also been found to cause a DRD-like clinical picture, with autosomal recessive inheritance.[12] [19] Genetic confirmation therefore requires sequencing of the entire coding region of GCH1 and tyrosine hydroxylase genes as a minimum, and yet the underlying genetic defect may still be missed. Cerebrospinal fluid analysis for total neopterin (the byproducts of the GTP cyclohydrolase I reaction) and oral phenylalanine loading have both been reported useful but are cumbersome and not readily available.[20] [21] Thus the diagnosis of DRD largely remains a clinical one, and yet there are no accepted standard clinical diagnostic criteria.

The response to levodopa is a possible way of confirming the diagnosis. In this family, many of the gene negative individuals reported that their symptoms were improved, at least initially, but not as dramatically as in the definitely affected individuals. Thus a dramatic response might indicate gene positive status. However, two gene positive individuals experienced an increase in stiffness or pain during levodopa treatment and thus did not benefit from this treatment.

None of the possibly affected individuals with a typical history of DRD turned out to carry the mutation. Therefore, unless signs of either parkinsonism or dystonia are seen on examination, the diagnosis of DRD is "improbable" *v* "possible." The age of onset may be helpful in trying to assess the likelihood that an individual has the young onset form of DRD (that is, the later the disease onset, the less likely that the individual is truly affected by the disease).

Reduced penetrance is common for many genetic forms of dystonia, as in torsion dystonia (DYT1) and inherited myoclonus dystonia (DYT11), where the penetrance can be less than 50%.[22–24] In this kindred, five individuals carried the mutated gene yet had no clear symptoms of the disease and no findings typical of DRD on examination. Individuals who are identified as "asymptomatic carriers" of DRD are at risk of developing parkinsonism later in life. These individuals should be counselled to be aware of this possibility and informed that their parkinsonism is not caused by a neuro-degenerative process but by a partial enzyme deficiency, and hence is easily treated and has a good prognosis.

The many unnecessary operations and the suffering experienced by several members of this family stress the importance of keeping DRD high on the differential diagnosis of childhood onset dystonia. However, the occurrence of DRD phenocopies in the family also suggests that great care must be taken in making the diagnosis of DRD, even within families with classic disease. Indeed, our group has reported another family in which there was DRD and isolated scoliosis and where the latter feature was not caused by the GCH1 mutation.[16]

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