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A De Novo Mutation in *MTND6* Causes Generalized Dystonia in 2 Unrelated Children

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Abstract

Dystonia is often associated with the symmetrical basal ganglia lesions of Leigh syndrome. However, it has also been associated with mitochondrial *ND* mutations, with or without Leber hereditary optic neuropathy. The m.14459G>A mutation in *ND6* causes dystonia with or without familial Leber hereditary optic neuropathy. We report heteroplasmic 14459G>A mutations in 2 unrelated children with nonmaternally inherited generalized dystonia and showing bilateral magnetic resonance imaging lesions in nucleus pallidus and putamen. Both children have reached their teenage years, and they are intellectually active, despite their motor problems.

Keywords

dystonia, early onset, relatively normal lives, mitochondrial DNA, *ND6* gene

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Numerous mutations in the 7 mitochondrial DNA-encoded subunits of complex I give rise to a variety of mitochondrial diseases, most inherited maternally and some manifesting as homoplasmic or heteroplasmic. The commonest disorder, Leber hereditary optic neuropathy, is due to 3 mutations in *ND1*, *ND4* (mostly homoplasmic), or *ND6*.[1] Leigh syndrome is usually associated with mutations in *ND1* and, predominantly, in *ND3* and *ND5*, which are often expressing the "mitochondrial encephalomyopaty lactic acidosis and stroke-like episodes" phenotype.

Two pathogenic mutations in *MTND6* have been reported, the m.14484T>C change typical of Leber hereditary optic neuropathy and the unique change (m.14453G>A) associated with severe mitochondrial encephalomyopaty lactic acidosis and stroke-like episodes.[2] A common pathogenic mutation (m.14487T>C) was associated with early- or later onset Leigh syndrome[3]; optic atrophy, ptosis, and intractable epilepsy[4]; progressive generalized dystonia; and bilateral striatal necrosis.[5] A second mutation (m.14459G>A) was associated with Leber hereditary optic neuropathy alone[6] or with maternally inherited Leber hereditary optic neuropathy and dystonia/spasticity.[6-8]

We report here 2 unrelated children of Italian–American descent, who harbor heteroplasmic levels of the m.14459G>A *MTND6* mutation and do not have optic neuropathy. They have diffuse dystonia and show magnetic resonance imaging signs of bilateral striatal necrosis but they are bright and live reasonably normal lives, despite motor limitations.

Patients' Reports

Patient 1 is a 16-year-old boy who carried since infancy a clinical and radiological diagnosis of Leigh syndrome, and he has been compromised for several years by generalized dystonia. His dystonia affects primarily his leg posture although the arms are also involved, and he has received significant

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symptomatic relief with Botox injections. Academically, he does well and he is competitive in chess. He uses a power wheelchair, which increases his exploration of space.

Family history is noncontributory: The mother is healthy, and he is the only child. Physical examination shows an alert and cooperative boy. He has mild right upper eyelid ptosis and normal ocular fundi. He has prominent lumbar lordosis and mild scoliosis.

Examination of his head and heart, lungs, and abdomen is negative. He is disabled from a motor point of view. Tendon reflexes give 1+ responses bilaterally, and plantar responses are indeterminate. He is taking clonazepam and amitriptyline. He is suggested to take N-acetylcysteine as an antioxidant for the glutathione pathway.

Patient 2 is a 12-year-old boy who presented with gait difficulties and dystonia at 4 years of age. Soon thereafter, he used a rolling walker with infrequent falls. His family history shows that both parents are healthy as is his 2-year younger brother. He took occupational therapy 3 times a week, physical therapy 4 times a week, and horseback riding once a week. Physical examination showed a tall and well-appearing child, and examination of his head, chest, abdomen, and skin was normal. He has no scoliosis.

Neurological examination showed an attentive child who makes nice eye contact and speaks well, is cooperative, and cognitively bright. He had mild bilateral ptosis but full extraocular movements. He had a thin muscle bulk but normal tone and good strength. He walks on a wide base, with dystonic posturing of his arms and legs and he would fall without a walker. Deep tendon reflexes are diffusely absent. Magnetic resonance images show hyperintensity in the striatum bilaterally on T2, fluid-attenuated inversion recovery, and diffusion-weighted imaging sequences.

Methods

Tissues

We obtained muscle and skin biopsies from both probands; skin biopsy from the mother of patient 2; and blood, urinary sediment and buccal smears from probands and their mothers.

Histochemical and Biochemical Analyses

Muscle biopsies were analyzed with standard histological and histochemical stainings. Respiratory chain enzyme activities were measured spectrophotometrically as described previously in 10% of muscle extracts.[9]

Molecular Studies

Total DNA was extracted from skeletal muscle, skin fibroblasts, blood, urinary sediment, and buccal smears using Puregene DNA Isolation Kit reagents (Qiagen Sciences, Valencia, California) according to the manufacturer's recommended protocol. Whole-genome amplification was accomplished by REPLI-gmtDNA kit (Qiagen). For patient 1 and his mother, whole mitochondrial genome sequencing was performed by long-range polymerase chain reaction (PCR) using 3 overlapping primer sets that amplify the entire mitochondrial DNA, using 100 ng of input DNA for each reaction. The cycling conditions for all reactions were (1) 95°C for 2 minutes; (2) 95°C for 15 seconds; (3) 68°C for 7 minutes; (4) repeat step 2 29 times; and (5) final extension for 12 minutes. Direct sequencing of amplified fragments was performed in an ABI Prism 3130 XL Genetic Analyzer using Big Dye Terminator Cycle Sequencing Reaction Kits (Perkin-Elmer Applied Biosystems, Foster City, California) using appropriate primers. Heteroplasmy level in this patient was determined using restriction fragment length polymorphism analysis, mitochondrial DNA was amplified by PCR using forward (m.14352-13371) and reverse (14529-14509) primers and was digested with the *Tsp*R1 restriction endonuclease.

For patient 2, TruSeq next-generation sequencing methodology on MiSeq platform was employed to interrogate all 16-kb mitochondrial genome and to detect the mutations and heteroplasmy levels. All reagents and supplies were obtained from Illumina Inc (San Diego, California).

Results

Histochemical analysis of muscle at age 4 in patient 1 showed rare atrophic fibers, and some fibers had increased staining with succinate dehydrogenase and cytochrome c oxidase, suggesting no clear evidence of mitochondrial disorder, but specific defects of complex I or complex III.

Biochemical analysis in skeletal muscle of patient 1 showed a marked increase in citrate synthase activity, and all respiratory chain enzymes except complex II appear decreased when referred to citrate synthase: complex I, 58% normal; complex III, 69% normal; and complex IV, 70% normal. Biochemical analysis in muscle of patient 2 showed only a partial defect of complex I (72% of normal) referred to a normal amount of citrate synthase.

A single mutation, m.14459G>A in *ND6* of mitochondrial DNA, was detected by restriction fragment length polymorphism, in the proportion of 87% in skeletal muscle of patient 1 and 61% heteroplasmy in muscle of patient 2 (Figure 1). Heteroplasmy in blood was 69% in patient 1 and 53% in patient 2. The heteroplasmy level in the urinary sediment was 91% and in buccal mucosa 89% in patient 1, but in patient 2 the heteroplasmy level was 59% in fibroblasts. In contrast, restriction fragment length polymorphism showed no heteroplasmy in urinary sediment or buccal mucosa from patient 1's mother or in urinary sediment and fibroblasts from patient 2's mother, both asymptomatic. The lack of mutation in urinary sediments and in buccal smear or cultured skin fibroblasts in both mothers tends to exclude maternal inheritance but rather to postulate a de novo mutation and totally negative family histories in previous generations or in the younger brother of patient 2.

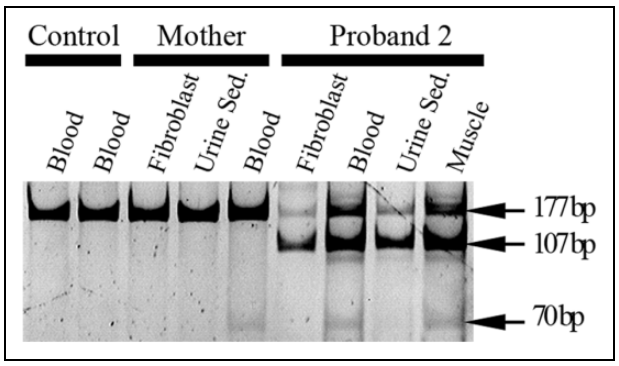

Figure 1. Polymerase chain reaction-restriction length polymorphism analysis of the m.14459G>A mutation using a mismatch primer. The mutant sequence introduces a *Tsp*RI site yielding additional 107 and 70-bp fragments, whereas the wild-type sequence lacks the *Tsp*RI site and yields only the 177-bp fragment.

Discussion

The first report of this mutation[7] was associated with Leber hereditary optic neuropathy and dystonia in a fifth-generation Hispanic Native American family, and the variant m.14459G>A was not detected in any Native American mitochondrial DNA denoted with haplogroup D.[7] It was further discovered that the mother in the third generation had a 73% heteroplasmic mutation in her blood, but all her descendants had more severe pediatric dystonia and showed essentially homoplasmic blood mutation.

Four more families present heterogeneous clinical presentations, including Leber hereditary optic neuropathy in all but one and early-onset dystonia in a few children or young adults carrying the mutation either homoplasmic or heteroplasmic in blood or in skeletal muscle (Table 1). One family has complicating symptoms (but no Leber hereditary optic neuropathy) due to associated signs of neurofibromatosis in 2 members.[8]

Dystonia was a prominent symptom rarely associated with Leber hereditary optic neuropathy in any of the above-mentioned patients, starting usually early in childhood and showing hyperintensities by brain magnetic resonance imaging affecting mostly the putamina, the caudate nuclei, or the globi pallidi.

However, the same mutation caused severe Leigh syndrome in 2 siblings who died at 6 years and 10 months, and the first had dystonic posturing in addition to extreme hypotonia, spasticity, and seizures.[11] A third unrelated girl died at 8 months of age with virtually homoplasmic levels of the mutation in her blood, muscle, liver, and fibroblasts. However, her mother and maternal grandmother had undetectable blood levels of the mutation, suggesting that the mutation had arisen de novo in the patient.[11]

We are trying here to understand how this mutation can cause 3 distinct conditions: a severe and rapidly fatal Leigh syndrome, a mixed dystonia with Leber hereditary optic neuropathy either present in the patient or in a family member, and a ''benign'' early-onset dystonia without cognitive dysfunction.

First, we have to deal with tissue-specific mutation load. The 3 children with fatal infantile Leigh syndrome had homoplasmic mutation in all their tissues, readily explaining the widespread severity of complex I deficiency.

How could the often-cited homoplasmic situation explain Leber hereditary optic neuropathy? Homoplasmy in blood is often interpreted as being tissue spread, but how could the optic nerve be exquisitely affected? The curious observation of the isolated dystonia in our 2 separate children could depend on the high mutation loads in muscle (87% and 61%), which were not approaching the homoplasmy of patients with Leigh syndrome. However, it is reasonable to think that approximately homoplasmic levels of the mutation were present in selected basal ganglia, that is, the globus pallidus and the putamen.

A second question is the pathogenic role of the de novo mutations. Appearance of de novo mitochondrial DNA mutations had raised a serious problem, incriminating low mutation loads in asymptomatic mothers of children with Leigh syndrome or other mitochondrial DNA-related diseases. However, it had long been found by clinical scientists that as many as 20% of 50 patients carrying mitochondrial DNA mutations in *ND* subunits revealed de novo heteroplasmic mutations (more commonly T>C or G>A changes), especially affecting *ND4*, *ND5*, and *ND6*.[12,13]

On the basis of these data, we encounter a unique de novo m.14459G>A mutation occurring in 2 young men starting with dystonia in infancy and becoming generalized in their teen years. They had bilateral abnormalities of their basal ganglia, suggesting the dismal diagnosis of Leigh syndrome, which spared them from hypotonia, weakness, bulbar signs, and delayed cognitive development. On the contrary, both young men were and still are interested in intellectual activities. Their main problem is their dystonic gait and the shaking of their arms.

Therapy for dystonia has had some benefit in patient 1 using Botox injections in his arm muscles, but administration of clonazepam and amitriptyline is questionably useful. Patient 2 is been treated with parabenzoquinone EPI-743, a respiratory chain enhancer used in patients with Leigh syndrome. Both patients can be considered candidates to undergo deep brain stimulation of the globus pallidus internus, which has been considered a safe and efficient treatment for medically refractory dystonia,[14] especially in patients from 4 to 18 years of age.[15] It would be interesting to peruse carefully the brain magnetic resonance images in both patients, checking especially hyperintensities in the globus pallidus and putamen.

Two clinically identical siblings were reported with pure dystonia, who harbored a m.11778G>A mutation[16] in *MTND4*, typically associated homoplasmically with Leber hereditary optic neuropathy. These siblings carried the maternally inherited mutation in muscle at similarly heteroplasmic (86%) levels. A 22-year-old woman presented at 17 months with dystonia manifesting as extension, internal rotation, and tremor in both legs. She had normal early development and walked independently at 12 months. Dystonia increased in frequency and duration and forced her to use a wheelchair, but at 16 years, she obtained excellent grades in British examinations.

Table 1. Clinical Heterogeneity in Patients with the m.14459G>A Mutation in *ND6*.

Clinical Presentation	Homo-Heteroplasmy	Brain Magnetic Resonance Imaging	Ref
Leber hereditary optic neuropathy in mother	Blood heteroplasmy (73%)		7
Pediatric dystonia in all her descendants	Blood homoplasmy		
Leber hereditary optic neuropathy in 42-year-old mother	Blood heteroplasmy (50%)	Bilateral caudate	10
Leber hereditary optic neuropathy+ dystonia in a 19-year old daughter	Blood homoplasmy		
Dystonia in a 3-year-old and 13-year-old girl	Muscle heteroplasmy (50%)	All basal ganglia	
Dystonia (+pseudobulbar palsy) in a 3-year-old girl	Mucle homoplasmy	Bilateral putamina	8
Mentally retarded 2-year-old brother with neurofibromatosis type 1	Blood homoplasmy	Neurofibromas	
Cutaneous stigmata of neurofibromatosis type 1 in the 35-year-old mother			
No Leber hereditary optic neuropathy in any maternal relatives			
Dystonia+spasticiy in a 45-year-old woman	Blood heteroplasmy (34%)	Bilateral putamina	6
Leber hereditary optic neuropathy in her 49-year-old brother	Blood heteroplasmy (18%)		
Stroke in the 79-year-old mother	Blood heteroplasmy (4%)		
Leigh syndrom-death at 6 years in first sibling	Fibroblast homoplasmy	All basal ganglia	11
Leigh syndrome-death at 10 months in second sibling	Muscle, liver homoplasmy		
Leigh syndrome-death at 8 months	Tissue homoplasmy		
Generalized dystonia	Tissue heteroplasmy	Pallidus, putamen	p.w.
Generalized dystonia	Tissue heteroplasmy	Palliidus putamen	p.w.

Magnetic resonance imaging of her brain showed striking changes in both putamina. Her brother had a very similar clinical history, excluding, like his sister, any visual impairment. Likewise, his brain magnetic resonance imaging at the age of 19 years revealed symmetric putaminal necrosis. He achieved notable academic success.[16]

Dystonia has been recently classified as a network disorder, in which sensorimotor basal ganglia and associated thalamocortical circuits can have a key role in the physiopathology of dystonia.[17]

Author Contribution

YGK, JC, AN, and JL performed the molecular work documenting the mutation; DCD and KE contacted the families; MH and DCD provided clinical care; HOA and SDM wrote the paper, and SDM led the research.

Declaration of Conflicting Interests

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Ethical approval

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