A Novel Intronic Mutation in the DDP1 Gene in a Family With X-linked Dystonia-Deafness Syndrome

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**Background:** X-linked dystonia-deafness syndrome (Mohr-Tranebjaerg syndrome) is a rare neurodegenerative disease characterized by hearing loss and dystonia. So far, 7 mutations in the coding region of the DDP1 gene have been described. They consist of frameshift, nonsense, missense mutations or deletions.

**Objective:** To investigate the presence of mutations in the DDP1 gene in a family with dystonia-deafness syndrome.

**Design:** Seven members belonging to 2 generations of a family with 2 affected subjects underwent genetic analysis. Mutational screening in the DDP1 gene was made through DNA direct sequencing.

**Results:** We found an intronic mutation in the DDP1 gene. It consists of an A-to-C substitution in the position −23 in reference to the first nucleotide of exon 2 (IVS1-23A>C). The mutation was present in 2 affected men and their respective unaffected mothers, whereas it was absent in the healthy men from this family and in 90 healthy controls.

**Conclusions:** Intronic mutations in the DDP1 gene can also cause X-linked dystonia-deafness syndrome. In our case, the effect of the mutation could be due to a splicing alteration.

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**REPORT OF CASES**

**PATIENT III-1**

This 31-year-old man had had normal motor and mental development until he was 4 years old. Then, a neurosensorial hearing loss, which progressed to total deafness in a few years, was diagnosed. At the age of 11 years, he began to have focal dystonia of the right hand while writing. Several months later, he developed a dystonic posture in the right foot and painful retrocollis and torticollis to the right. Two years later, he showed a predominantly right-side hemidystonia. At age 18 years, the dystonic process became generalized, affecting the 4 limbs and painful retrocollis and torticollis to the right. Two years later, he showed a predominantly right-side hemidystonia. The symptoms progressed during the ensuing years. At age 18 years, the dystonic process became generalized, affecting the 4 limbs and axial, cervical, and cranial muscles. At age 24 years, he began to complain of loss of vision. On examination, he showed retrocollis with frequent neck spasms to the right side, which sometimes were associated with head movements.
with mouth opening and eye closure. He also showed backward trunk spasms and dystonia in the 4 limbs. Ocular motility was normal. He was totally deaf but did not seem to have any significant cognitive impairment. Visual evoked potentials did not show cortical responses. Cranial magnetic resonance imaging results were normal, and cervical magnetic resonance imaging disclosed multiple disk herniations. Causes of secondary dystonia were ruled out. He received multiple treatments, including intrathecal baclofen, local botulinum toxin, left pallidotomy, and right pallidal stimulation, but none of them was successful.

**PATIENT III-3**

This 29-year-old man is a cousin of patient III-1 (Figure 1). At the age of 11 years, he was diagnosed as having a 30% bilateral hearing loss. When he was 20 years old, he complained of tremor in the upper limbs and experienced irritability and compulsive behaviors. Then, he underwent psychiatric evaluation and was diagnosed as having an impulse control disorder. On examination, he showed mild generalized dystonic signs, predominantly affecting the right arm and cervical muscles, mild dysarthria, and impaired postural reflexes. Postural and action myoclonus-like movements were observed in the upper limbs. The neuropsychological assessment revealed a mild frontosubcortical dysfunction. Cranial magnetic resonance imaging results were normal.

### METHODS

After informed consent was obtained, genomic DNA of family members was extracted from leukocytes using the QIAamp DNA blood minikit (Qiagen, Hilden, Germany). The **DDP1** exons were amplified by polymerase chain reaction using the primers and conditions previously described.2 The polymerase chain reaction products were purified using the Gel Band Purification Kit (Qiagen), and both sense and antisense strands were subsequently sequenced using Dye Terminator Cycle Sequencing Ready Reaction (Perkin Elmer, Foster City, Calif) and run on an ABI Prism automatic DNA sequencer (Perkin Elmer).

In a second step, the **DDP1** gene was also investigated in 90 healthy controls through restriction fragment length polymorphism using the endonuclease *Hinfl*. The digestion products were run on a polyacrylamide gel (concentration, 10%; Tris-borate-ethylenediaminetetraacetic acid [TBE] × 1) at 350 V for 3 hours and silver stained by standard procedures.

### RESULTS

After sequencing the **DDP** gene of the patients, we found only an intronic change in the position −23 A-to-C mutation relative to exon 2 of the **DDP** gene using the *Hinfl* restriction enzyme. 1 indicates heterozygote genotype corresponding to a healthy female subject of this family (a mother of the proband); 2, control subject without the mutation; 3, affected male carrier of the mutation; bp, base pair; and kb, kilobase.

[Figure 1. Family pedigree with dystonia-deafness syndrome. Arrows indicate proven mutation carriers; closed squares, affected men; open squares, unaffected men; open circles, unaffected women; N, absence of the mutation; question marks, status unknown; and slashes, deceased.]

[Figure 2. Partial sequence of the **DDP** gene intron. The A-to-C change is marked by an arrow. A, Normal sequence. B, Mutated sequence.]

[Figure 3. Restriction fragment length polymorphism analysis of the position −23 A-to-C mutation relative to exon 2 of the **DDP** gene using the *Hinfl* restriction enzyme. 1 indicates heterozygote genotype corresponding to a healthy female subject of this family (a mother of the proband); 2, control subject without the mutation; 3, affected male carrier of the mutation; bp, base pair; and kb, kilobase.]

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In addition, the mutation was not detected by endonuclease restriction analysis in 90 healthy controls, supporting the fact that this mutation is not a common polymorphism.

We report the first intronic mutation in the DDP gene causing X-linked DDS in a family. This mutation cosegregated with the disease and was absent in healthy controls. Similar to results from previous reports, the inheritance pattern of the disease was recessive, as female carriers did not have any clinical expression of the disease. Until now, only the 108delG mutation has been associated with a dominant pattern, with female carriers showing dystonic features without deafness.

Phenotype variability of the disease in families with the same mutation has previously been reported. One of our patients had a severe clinical picture dominated by total hearing loss and severe generalized dystonia, as well as loss of vision. However, his cousin only had mild hearing loss and dystonia, and behavioral disturbances. It is difficult to explain why the same mutations give rise to different phenotypes, but this effect has been observed in other dystonic syndromes, such as primary torsion dystonia associated with mutations in the DYT1 gene. In this disease, patients usually develop an early-onset generalized dystonia, but asymptomatic carriers or patients with late-onset focal dystonia have been reported. However, in contrast with X-linked DDS, primary torsion dystonia shows an autosomal dominant inheritance pattern with incomplete penetrance. In this disease, phenotype variability could be due to gene dosage effect related to haploinsufficiency, as has been reported in other diseases, such as familial Parkinson disease. The mechanisms underlying phenotype variability in X-linked DDS are unknown, but other environmental or, more likely, genetic factors or protein interactions could be involved. It is known that DDP1 is a translocase of the inner mitochondrial membrane protein that interacts with several components from the mammalian mitochondrial import system for proteins and other metabolites. Interactions of DDP1 protein with other proteins in the mitochondria, like Tim13, could play a role in the disease expression.

Previous mutations in the DDP1 gene cause protein disruption because of deletions, frameshift or nonsense mutations resulting in protein truncation. This suggests that major changes in the peptide are necessary to cause the disease. In our case, the presence of the mutation in the affected subjects and its absence in the nonaffected members of the family support the conclusion that this mutation has a pathogenic role. It is well known that intronic mutations can cause protein dysfunction because of an abnormal RNA splicing, as could be in our case. However, further studies are necessary to clarify the role of intronic mutations in X-linked DDS.

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