Sensorineural Hearing Loss and Mondini Dysplasia Caused by a Deletion at Locus DFN3

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Objective: To study a family with inner ear malformations and sensorineural hearing loss.

Design: Clinical, radiological, and genetic study of the members of a family with different degrees of sensorineural hearing loss.

Results: The males in the family manifested profound congenital hearing loss with severe inner ear malformations, while the only affected female had progressive hearing loss that had begun during puberty. Computed tomography showed inner ear malformations in both males, with enlarged internal auditory meatus and Mondini dysplasia. Genetic analysis disclosed a microdeletion at the locus DFN3 on chromosome X.

Conclusion: A familial Mondini dysplasia is associated to a microdeletion at the deafness locus DFN3.


The incidence of severe hearing loss in newborns is 1 per 1000 live births, a rate that increases considerably in the case of children with risk factors for hearing loss. At least 50% of cases of profound hearing loss can be attributed to genetic causes. Of these, 30% are associated with other disorders and, thus, are referred to as cases of syndromic hearing loss. The remaining 70% are cases of nonsyndromic hearing loss, up to 80% of which result from autosomal recessive inheritance, 10% to 20% from autosomal dominant inheritance, and 2% to 3% from X-linked inheritance.

DFN3-type hearing loss constitutes approximately 50% of sex-linked nonsyndromic deafness. It is characterized by sensorineural deafness with or without conductive component with stapes fixation. When the latter is treated surgically, there can be leakage of perilymph and cerebrospinal fluid, demonstrating an abnormal communication between the subarachnoid and the perilymphatic spaces that may be located at the level of the cochlear aqueduct or in the depths of the internal auditory canal.

Males inheriting this disorder manifest severe deafness, while carrier females may have a progressive mild to moderate hearing loss.

The DFN3 locus was mapped to the Xq13-q21 region. In the evolutionary conserved homologous region of the murine X chromosome, it had been mapped POU3f1, a gene that encodes a transcription factor that is expressed during embryonic development in the brain, the neural tube, and the otic vesicle at 15 to 17 days after conception. The map position and the temporal and spatial expression pattern of POU3f1 rendered its human homologue (POU3F4) on Xq21 an excellent candidate gene for DFN3. POU3F4 was cloned, and the finding of nonsense and missense mutations in POU3F4 in several independent patients with DFN3 validated the hypothesis.

Mondini dysplasia, described in 1791 by Carlo Mondini, is one of the most commonly diagnosed inner ear malformations. Morphologically, it is characterized by a helical cavitation of the otic mesenchyme, which leads to the abnormal development of the cochlea in which only the basal turn is clearly identified, while the remaining turns show variable degrees of development but never reach normal proportions.

We report herein a DFN3-linked pedigree with sensorineural hearing loss and Mondini dysplasia.

RESULTS

CASE 1

A 17-year-old boy had had bilateral profound hearing loss since childhood. He also had epilepsy, hyperkinetic syndrome, and goiter. The results of the physical exploration study were normal. Thyroid hormone levels (triiodothyronine, thyroxine, and thyrotropin) and the results of the
**PATIENTS AND METHODS**

**Figure 1** shows the pedigree described in this report.

**CLINICAL STUDY**

The clinical history and otorhinolaryngological examination were completed for each of the patients numbered in the pedigree. Audiological examination consisted of pure-tone audiometry at frequencies ranging from 125 to 8000 Hz in airway and from 250 to 4000 Hz in boneway (Clinical Audiometer, model AC4; Interacoustics, Assen, Denmark). In patients 2 and 3, the vestibular function was studied by means of electroneystagmography (Biologic Navigator, Focus version 4.5; Micromedical Technologies, Chatham, Ill). High-resolution computed tomography findings were similar to those observed in the preceding patient (Figure 2, A). Caloric stimulation revealed bilateral vestibular areflexia. Computed tomography findings were normal. The results of computed tomography scan were normal, without dilation of vestibular aqueduct. The thyroid function studies to determine thyrotropin, triiodothyronine, and thyroxine levels and perchlorate discharge test were also normal. Pure-tone audiometry disclosed only the presence of bilateral residual hearing (Figure 2, A). Caloric stimulation revealed bilateral vestibular areflexia. Computed tomography showed marked malformations of cochlea; only the basal turn was developed, the upper coils forming a common cavity. The internal auditory meatus was dilated and the bone between its lateral end and the basal turn of the cochlea was absent (Figure 3 and Figure 4). The vestibule was enlarged and the semicircular canals were underdeveloped. The vestibular aqueduct was enlarged. The cochlear aqueduct and facial nerve canal were normal.

**CASE 2**

A 24-year-old woman, the sister of patient 1, had had progressive hearing loss since puberty. Pure-tone audiometry demonstrated the existence of sensorineural hearing loss in the right ear, which was more marked at high frequencies, and residual hearing in the left ear. Figure 5 shows the audiograms in the past 6 years of evolution. The clinical study disclosed the presence of goiter; the remaining findings were normal. The results of computed tomography scan were normal, without dilation of vestibular aqueducts. The thyroid function was normal.

**CASE 3**

A 23-year-old man had had profound hearing loss since birth. The results of otorhinolaryngological examination were normal; pure-tone audiometry disclosed the presence of bilateral profound hearing loss (Figure 2, B). A complete electroneystagmography was done; there was no evidence of nystagmus on caloric stimulation. The computed tomography findings were similar to those observed in the preceding patient (Figure 6).

**CASE 4**

In an 18-year-old woman, the sister of patient 3, the results of physical examination were normal. Pure-tone audiometry demonstrated mild sensorineural hearing loss (Figure 2, C).

**CASES 5 AND 6**

Two asymptomatic female siblings, 13 and 11 years old, had normal results of physical and audiological examinations.
GENETIC STUDY

The deafness inheritance pattern in this family suggests that it resulted from an X-chromosome alteration. Yet, the association of sensorineural deafness, Mondini dysplasia, and goiter in some members of the family raised the possibility that patients were affected by Pendred syndrome. We began the study of the family by genotyping all individuals of the family for markers D7S523 and D7S2420, which flank on the telomeric and centromeric side, respectively, the Pendred gene located on chromosome 7q31.1,12 The analysis of haplotypes excluded segregation of deafness with this locus (data not shown).

Then we genotyped the individuals for several polymorphic markers evenly distributed along the X-chromosome. The analysis of the results excluded linkage to loci DFN2,13 DFN4,14 and DFN6.15 However, a positive lod score of 1.51 at a recombination frequency of 0.000 was found for markers DXS441 and DXS1225, suggesting that the deafness locus in this family was located at Xq21 (see haplotypes in Figure 1), where the DFN3 locus maps.6,7

Since point mutations within the protein coding sequence of POU3F4 are responsible for about half of the familial DFN3 cases,16 we sequenced the gene of male patients 1 and 3, as indicated in the “Patients and Methods” section, finding the gene intact. Then we examined these patients for the presence of sequence-tagged sites of the region encompassing POU3F4. We found that some of them were absent from the affected males, ie, they were not amplified by polymerase chain reaction (Figure 7). The results indicate that a deletion of a chromosomal region centromeric to POU3F4 was responsible for the deafness in the family. The deletion spans at least 1200 kilobases (kb) and extends from a site located 250 kb distant from POU3F4 toward the centromere (Figure 7).

In the family described herein, 2 of 3 individuals with sensorineural hearing loss also had goiter. Our results demonstrate that this association of deafness and goiter is merely circumstantial and does not represent a case...
of Pendred syndrome. First, the locus for deafness in this family was not linked to markers flanking the Pendred syndrome gene (PDS) on 7q31. Second, goiter in Pendred syndrome is caused by a defect in iodine organification. This is not the case in this family, since the perchlorate discharge tests were negative. Finally, the finding of goiter was confined to 2 siblings, suggesting that it is probably a hereditary trait, but in any case segregating independently of deafness.

Genetic analysis indicated that the hearing loss in this family segregated with polymorphic markers of the Xq21 region, where the DFN3 locus is located. This locus has been shown to be responsible for deafness in more than 25 families. In about 50% of cases, the underlying defects were small mutations that inactivated the regulatory gene POU3F4.16 In the remaining cases, deletions encompassing the gene and/or the region centromeric to the gene, or a duplication-inversion of this centromeric region, were identified.9,16 The finding of rearrangements keeping POU3F4 intact, like the deletion observed in the family described herein, suggests that the Xq21.1 region contains another deafness gene or, alternatively, a regulatory element that controls the expression of POU3F4.16

The DFN3 type of deafness was first characterized as a mixed and progressive hearing loss with congenital fixation of the stapedial footplate and leakage of perilymph and cerebrospinal fluid.4,18 Yet, in most cases, the
sensorineural component is so important that the conductive component, if any, remains masked. This is the case with the family described herein, as shown in Figure 2. Indeed, the hearing loss was profound and congenital in male individuals.

High-resolution computed tomography scanning in axial and coronal planes showed malformations of the temporal bones in the 2 young deaf cousins we examined. Specifically, they manifested features that appear to be common to all patients with DFN3, the dilatation of the internal acoustic meatus and an abnormally wide communication between the internal acoustic meatus and the basal turn of the cochlea.5,19 In addition, both cousins had a Mondini dysplasia with a normal cochlear basal turn, the upper coils forming a common cavity as originally described by Mondini.10,18 To our knowledge, only 1 other family with DFN3 affecting cochlear anatomy has been described.20 In the affected males of that pedigree, the cochlear hypoplasia involved the upper coils and the upper part of the basal turn with an abnormal columella, and was defined as a Mondini-like dysplasia. The vestibular and cochlear aqueduct were normal. A deletion of similar size, at the same location described in this article, was responsible for this phenotype.20

Mondini dysplasia is believed to represent the arrest of embryonic development at about 7 weeks of gestation. This dysplasia is not specific to a single type of deafness, since it has been associated with several syndromes that include hearing loss, most often Pendred syndrome. The Mondini dysplasia also has been reported as an isolated finding in nonsyndromic cases,10,21 and families with congenital sensorineural hearing loss with autosomal dominant inheritance22 and presumed autosomal recessive inheritance23 have been described, but in none of these cases was the genetic defect identified.

Audiograms were obtained from the 4 females of the third generation (the others were not accessible to the clinical study). Among them, the oldest one (aged 24 years) displayed a mild sensorineural hearing loss, contrasting with the profound deafness in males. This sex difference has also been observed in other DFN3 families as well as in the other forms of X-linked deafness: DFN2,13 DFN4,14 and DFN6.15 This fact may be explained by taking into account the mechanism of lyonization, ie, the random inactivation of 1 of the 2 X chromosomes in women, which takes place in very early stages of embryonic development. This mechanism results in gene dosage compensation in females with respect to males.24 Thus, males are constitutively hemizygous for genes on the X chromosome, while females are functionally hemizygous; for this reason, diseases transmitted by X-linked inheritance can be expressed in women, but in a milder form than in men because of compensation by normal, functional X chromosomes in cells in which the altered chromosome happens to be inactivated. No hearing loss was noted in the youngest female carrier, presumably because of their young age. However, given that all of them inherited the haplotype at risk, their hearing state should be carefully monitored in the future.

We emphasize the finding of a Mondini dysplasia associated with the DFN3 locus. It suggests that POU3F4 or another proximal gene may be involved in the first stage of the otic vesicle differentiation. Further clinical char-

References
