Nonsyndromic X-linked hearing loss

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1. ABSTRACT

To date, 135 loci and 50 genes have been identified as causes of nonsyndromic hearing loss. Until recently, four loci (DFN2, DFN3, DFN4, and DFN6) had been implicated in nonsyndromic X-linked hearing loss; however, a new classification (DFNX1-5) has been proposed to reduce confusion in the terminology. The different types of nonsyndromic X-linked hearing loss demonstrate various clinical features in terms of the onset and progressiveness of hearing loss, pattern of audiogram, and the presence or absence of inner ear malformations. In addition to the POU3F4 gene, which was the first gene identified as causing nonsyndromic X-linked hearing loss, a second gene, PRPS1, has recently been identified to be the causative gene of DFNX1 (DFN2). This study reviews the new classification system, as well as the clinical features, molecular genetics, and developmental pathogenesis of different forms of nonsyndromic X-linked hearing loss.

2. INTRODUCTION

Congenital hearing loss is one of the most common sensory disorders in humans, affecting approximately one in 1,000 newborns (1). More than half of congenital hearing loss is due to genetic causes, and 70% of these cases are nonsyndromic. To date, 135 loci and 50 genes have been identified as causes of nonsyndromic hearing loss (2). X-linked hearing loss is clinically and genetically heterogeneous, and may present as part of a syndrome or in a nonsyndromic form, accounting for 1-5% of nonsyndromic genetic hearing loss (3). Until recently, four loci (DFN2, DFN3, DFN4, and DFN6) had been implicated in nonsyndromic X-linked hearing loss, but a new classification has been proposed to reduce confusion in the terminology. DFNX1-4 have been designated to the four existing loci and a new locus, DFNX5, has also been categorized as an X-linked nonsyndromic locus to describe the locus for X-linked auditory neuropathy (AUNX) (2-3).
Nonsyndromic X-linked hearing loss

Table 1. Classification and clinical features of nonsyndromic X-linked hearing loss

<table>
<thead>
<tr>
<th>Locus</th>
<th>Original</th>
<th>OMIM</th>
<th>Location</th>
<th>Gene</th>
<th>Type of hearing loss</th>
<th>Severity</th>
<th>Frequency</th>
<th>Age at onset</th>
<th>Progression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFNX1</td>
<td>DFN2</td>
<td>#304500</td>
<td>Xq22-24</td>
<td>PRPS1</td>
<td>SNHL</td>
<td>Severe–profound</td>
<td>All</td>
<td>Congenital</td>
<td>Stable</td>
<td>(9)</td>
</tr>
<tr>
<td>DFNX2</td>
<td>DFN3</td>
<td>#304400</td>
<td>Xq21.1</td>
<td>POU3F4</td>
<td>Mixed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFNX3</td>
<td>DFN4</td>
<td>%c.300030</td>
<td>Xp21.2</td>
<td>-</td>
<td>SNHL</td>
<td>Severe–profound</td>
<td>All</td>
<td>Congenital</td>
<td>Progressive</td>
<td>(4)</td>
</tr>
<tr>
<td>DFNX4</td>
<td>DFN6</td>
<td>%c.300066</td>
<td>Xp22</td>
<td>-</td>
<td>SNHL</td>
<td>Mild–Severe</td>
<td>High–All</td>
<td>First decade</td>
<td>Progressive</td>
<td>(40)</td>
</tr>
<tr>
<td>DFNX5</td>
<td>AUNX</td>
<td>%c.300614</td>
<td>Xq23-27.3</td>
<td>-</td>
<td>SNHL</td>
<td>Mild–Profound</td>
<td>Low–All</td>
<td>10–16 years</td>
<td>Progressive</td>
<td>(43)</td>
</tr>
</tbody>
</table>

In addition to the POU3F4 gene, which was the first gene identified as causing nonsyndromic X-linked hearing loss, a second gene, PRPS1, has recently been identified as the causative gene of DFNX1 (DFN2) (4-5). Considering the changes in the classification and discovery of a new causative gene, this paper will review the clinical and genetic aspects, as well as the molecular and developmental pathogenesis of nonsyndromic X-linked hearing loss.

3. CLASSIFICATION

Before genetic data were available, nonsyndromic X-linked hearing loss had been classified into several types according to audiologic characteristics and the age of onset of hearing impairment (6). Later, genetic analysis of the families led to the determination of seven loci (DFN1-7) for various forms of nonsyndromic X-linked hearing loss exhibiting different audiologic and clinical characteristics. However, after the initial designation, two loci (DFN5 and DFN7) were withdrawn. In addition, postlingual progressive hearing loss that had been assigned to the DFN1 locus was later reclassified as a syndromic form of X-linked hearing loss (Mohr-Tranebjaerg syndrome), because neurological degenerations, including dystonia, ataxia, optic atrophy leading to blindness, and mental deterioration, were observed in adult life (7). Mohr-Tranebjaerg syndrome was found to be caused by mutations in the TIMM8A/DDP (deafness/dystonia peptide) gene, located on Xq22 (8). Therefore, only four loci (DFN2, DFN3, DFN4, and DFN6) remain to be included in nonsyndromic X-linked hearing loss. To avoid confusion in nomenclature, the currently known four X-linked loci have been recently reclassified (2-3). The new classification designates DFNX1-4 to DFN2, DFN3, DFN4, and DFN6, respectively, and adds DFNX5 for nonsyndromic X-linked auditory neuropathy, previously known as AUNX (Table 1). We herein describe the clinical and molecular characteristics of nonsyndromic X-linked hearing loss, as well as the underlying pathogenesis of different types of the disease.

4. CLINICAL CHARACTERISTICS AND MOLECULAR GENETICS

4.1. DFNX1 (DFN2)

Four families with DFNX1 (DFN2) have been described. All affected members of the families exhibit pure sensorineural hearing loss, but the onset, progressiveness, and affected frequencies differ slightly among the families (Table 1). The first family, described by Tyson et al. (9) was a four-generation British-American family in which affected males exhibited profound congenital sensorineural hearing loss, involving all frequencies, and obligate females had mild to moderate sensorineural hearing loss, primarily in the high tones. The other three families showed postlingual progressive sensorineural hearing loss. Manolis et al. (10) reported an American family that included affected males who exhibited progressive hearing loss more severe in the low and mid frequencies and obligate female carriers who had mild low tone hearing loss. The third family, reported by Cui et al. (11), was a Chinese family with progressive sensorineural hearing involving all frequencies. Recently, Liu et al. (5) analyzed another large Chinese family presenting with progressive severe to profound hearing loss with a flat-shaped audiogram.

Linkage analysis in the DFN2 families has mapped the DFN2 locus to chromosome Xq21-22. Recently, Liu et al. (5) identified the PRPS1 gene as the causative gene for DFNX1 (DFN2); DNA sequence analysis of the four DFNX1 (DFN2) families revealed four different missense mutations in the PRPS1 gene. PRPS1 encodes phosphoribosylpyrophosphate synthetase 1 (PRPS-1), which catalyzes the reaction of ribose-5-phosphate with ATP to synthesize phosphoribosylpyrophosphate (PRPP), which is essential for the de novo synthesis of purine, pyrimidine, and pyridine nucleotides (12). Two of the missense mutations (c.193G>A and c.259G>A) were located in exon 2, while the others (c.869 T>C and c.916G>A) were located in exon 7 of the PRPS1 gene (5).

4.2. DFNX2 (DFN3)

DFNX2 (DFN3) is the most common form of X-linked hearing loss, accounting for up to 50% of cases (13). The clinical features are often quite typical in terms of audiologic and radiologic findings, making the diagnosis rather easy in most cases. Congenital mixed hearing loss with progression of the sensorineural component to profound hearing loss is most commonly seen in affected males. However, there have been reports of cases presenting with pure sensorineural hearing loss, in which the conductive component is assumed to be masked by the sensorineural component (14-15). The air-bone gap is usually larger in the lower frequencies, while bone...
Somatic mosaicism

Table 2. Intragenic mutations of the POU3F4 gene

<table>
<thead>
<tr>
<th>Domain</th>
<th>Nontruncating</th>
<th>Truncating</th>
</tr>
</thead>
<tbody>
<tr>
<td>POU-specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>domain</td>
<td>p.Glu203Ser (27)</td>
<td>p.L200X (26)</td>
</tr>
<tr>
<td></td>
<td>p.Ser228Leu (25)</td>
<td>p.L208X (26)</td>
</tr>
<tr>
<td></td>
<td>p.Glu236Asp (20)</td>
<td></td>
</tr>
<tr>
<td>POU-homeodomain</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Arg230Glu (20)</td>
<td>p.Ser288fs (22)</td>
</tr>
<tr>
<td></td>
<td>p.Ile285Asn (20)</td>
<td>p.Ser288fs (20)</td>
</tr>
<tr>
<td></td>
<td>p.Pro303Ser (22)</td>
<td>p.Val289fs (14)</td>
</tr>
<tr>
<td></td>
<td>p.Ile308Asn (20)</td>
<td>p.Lys299fs (4)</td>
</tr>
<tr>
<td></td>
<td>p.Ser310del (16)</td>
<td>p.Ile308fs (20)</td>
</tr>
<tr>
<td></td>
<td>p.Ala312Val (18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Arg233Giv* (24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Asn285Thr (22)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Arg239Gly (23)</td>
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</tr>
<tr>
<td></td>
<td>p.Arg239Pro (16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Arg330Ser (24)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Lys334Glu (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Pro216del (22)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Ala116fs (16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Ser98X (20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.Ile128fs (26)</td>
<td></td>
</tr>
</tbody>
</table>

Somatic mosaicism

conduction thresholds are higher in the higher frequencies (16). During stapes surgery performed to correct the conductive component of hearing loss, a heavy outflow of perilymph (termed perilymphatic gusher) occurs when the footplate of the fixed stapes is opened, which may result in further aggravation of inner ear dysfunction (17). Symptoms of vestibular dysfunction were not characteristically detected in the affected males, although systematic vestibular function tests were not performed in many of the patients.

Another pathognomonic finding of DFNX2 (DFN3) is the characteristic temporal bone anomalies seen on high-resolution temporal bone computed tomography (CT). Modiolus deficiency and a fistulous connection between the internal auditory canal and the basal turn of the cochlea are the most important anomaly, which has been termed incomplete partition type III (18-19). The interscalar septa between the basal/middle turn and middle/apical turn are often present, distinguishing this form of cochlear anomaly from incomplete partition type II, which has a cystic appearance in the middle and apical turns of the cochlea (16, 19). The distal portion of the internal auditory canal is often widened, and a dysplastic vestibule, as well as widening of the labyrinthine segment of the facial canal, has also been reported (18).

In obligate female carriers, approximately 30-40% show mild to moderate sensorineural or mixed hearing loss, usually presenting in adulthood and progressing with time (20). A majority of the female carriers revealed normal temporal bone CT findings, but some of the cases with hearing loss demonstrated dilatation of the internal auditory canal without inner ear malformations (18, 20). In one report, full-blown clinical features of DFNX2 (DFN3) were identified in two sisters in a family where 11 male relatives exhibited normal hearing and no apparent temporal bone abnormalities (21). The lack of pedigree or genetic data in this report precludes explanation regarding the genetic background in this family, but non-random inactivation of the normal gene on the X-chromosome in favor of the mutated allele or a dominant form of X-linked inheritance may be considered.

The causative gene of DFNX2 (DFN3) has been identified as the POU3F4 gene, which belongs to a superfamily of POU domain transcription factors characterized by two DNA binding domains, a POU-specific domain and a POU homeodomain (4). Various types of mutations, involving the POU3F4 gene, have been identified in more than 80% of patients showing the clinical characteristics of DFNX2 (DFN3) (22). To date, 29 intragenic mutations in the POU3F4 gene have been identified, including 15 missense mutations, 12 truncating mutations, and 2 in-frame deletions (Table 2) (4, 14, 16, 20, 22-26). Most of the missense mutations are clustered in one of the DNA binding domains, more frequently in the POU homeodomain. The two in-frame deletions, p.Phe201_Lys202del and p.Ser310del, are located in the alpha1 helix of the POU-specific domain and in the middle of the alpha2 helix of the POU homeodomain, respectively (16, 27).

In addition to the intragenic mutations, partial or complete deletions of the POU3F4 gene, as well as deletions, inversions, and duplications of the proximal region of the POU3F4 coding sequence have been reported. Six deletions (20, 22, 28) and one paracentric inversion (29) involve the POU3F4 gene itself, while twelve deletions or inversions are located upstream of the POU3F4 coding sequence (22, 28, 30). The size of the deletions encompassing the POU3F4 gene ranges from 530 kb (25) to 16 Mb (28). Some patients with deletions of large segments of DNA manifested additional symptoms other than hearing loss, such as hypogonadism (13), mental retardation (31), behavioral problems (13, 31), and hypotonia (28). These manifestations may be the result of a contiguous gene syndrome involving more than one gene within the deleted segment.

All deletion mutations located upstream of the POU3F4 gene, with the exception of the mutation in one family (II/7) (32), overlap an 8-kb region approximately 900 kb proximal to the POU3F4 gene, where an otic enhancer element is suspected to reside (31). A recent study by Ahn et al. (33) identified an otic enhancer region using a reporter construct, including a 3,370-bp genomic region, which encompasses an evolutionarily conserved region within the 8-kb overlapping segment located 920-kb upstream of the POU3F4 open reading frame. Naranjo et al. (30) reported that a family having a 3,902 bp-sized deletion, almost completely overlapping the enhancer region identified by Ahn et al. (33), indeed exhibited typical phenotypes of DFNX2 (DFN3). Naranjo et al. (30) also identified three other otic enhancers in a 1-Mb region upstream of POU3F4 capable of activating POU3F4 expression in the developing otic vesicle. Furthermore, one reported deletion (II/7) includes neither the POU3F4 gene nor the known enhancer region 920-kb upstream of POU3F4 (32), indicating that multiple cis-regulatory elements may be involved in POU3F4 expression during inner ear development (30-31). Analysis of the clinical data from families with deletion mutations of POU3F4 failed to
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demonstrated a clear correlation between severity of phenotype and the size or location of the deletion (30).

4.3. DFNX3 (DFN4)

The DFNX3 (DFN4) locus has been linked to profound congenital sensorineural hearing loss in two families. The first family, reported by Lalwani et al. (34), was an American family which exhibited profound congenital sensorineural hearing loss of all frequencies in affected males and mild to moderate high frequency sensorineural hearing impairment in carrier females. Temporal bone CT was normal in this family. A linkage analysis revealed that the mutation in this family was linked to the Xp21.2 region, partially overlapping the DMD (Duchenne muscular dystrophy) locus. Since these patients did not manifest symptoms of DMD, it was hypothesized that the causative gene may be located in the intronic regions or upstream of the dystrophin gene, or that there may be a mutation in an unidentified cochlea-specific promoter of the dystrophin gene (34).

Pfister et al. (35) described a Turkish family in which affected males had profound sensorineural hearing loss at birth and carrier females showed stable mild to moderate hearing loss in all frequencies, appearing in the second decade of life. No abnormalities were found on temporal bone CT, and vestibular function tests were normal. The mapping results of the second family narrowed the linked region to be entirely within the DMD locus at Xq21.2, suggesting that the gene responsible for DFNX3 (DFN4) may be either an allele of DMD or a mutation in a DMD nested gene (35). As with the first family, there was no sign of muscular dystrophy, and serum creatinine phosphokinase levels were normal in this family. However, an electroretinogram (ERG) revealed decreased b-wave amplitude in dark adaptation, a finding that is also seen in DMD patients, providing evidence that dystrophin dysfunction may be responsible for hearing loss (36). Hearing loss has not been reported in patients with classic DMD, and animal studies using mdx mice, a mouse model for human DMD, have yielded controversial results regarding auditory function. A study evaluating hearing in mdx mice after noise exposure showed that mdx mice exhibit normal hearing, as compared to control mice in quiet conditions, whereas significant increases in threshold and latencies were observed in auditory brainstem responses after noise exposure for 1 month (37-39). The role of dystrophin in auditory processing and the exact molecular mechanism underlying hearing loss in DFNX3 (DFN4) families have yet to be elucidated.

4.4. DFNX4 (DFN6)

In 1996, del Castillo et al. (40) reported a four-generation Spanish family, including ten affected males and seven affected females. Affected males exhibited postlingual bilateral sensorineural hearing loss detected at school age (5-7 years), which progressed to severe or profound hearing loss involving all frequencies. The affected females revealed bilateral moderate sensorineural hearing loss mainly in the high frequencies, usually presenting in the 4th decade of life. The locus for this family was mapped to Xp22, but the responsible gene causing hearing loss in DFNX4 (DFN6) has not been identified. TBL1X located at Xq22.3 has been implicated in hearing impairment in ocular albinism with late-onset sensorineurual deafness (OASD; MIM 300650), but differences in the onset and severity of hearing loss, as well as the non-overlapping location of the two loci for OASD and DFNX4 (DFN6) make it unlikely that TBL1X is the causative gene for DFNX4 (DFN6) (41).

4.5. DFNX5 (AUNX1)

Nonsyndromic auditory neuropathy can be classified into AUNA for autosomal dominant loci, AUNB for autosomal recessive loci, and AUNX for X-linked loci. The loci for the X-linked form of auditory neuropathy (AUNX) have recently been proposed to be included in the classification of nonsyndromic X-linked hearing loss as DFNX5. A single Chinese family exhibiting X-linked recessive inheritance of an auditory neuropathy phenotype has been identified as AUNX1 (42-43). In this family, hearing loss appeared around adolescence (10-16 years) and progressed from mild lower frequency hearing loss to severe to profound hearing loss affecting all frequencies. Additionally, the speech discrimination was poor. Auditory brainstem responses and stapedial reflexes were abnormal, while otoacoustic emissions demonstrated normal or partially normal findings (42). After the onset of hearing loss, three of the affected males were later diagnosed with diffuse peripheral sensory neuropathy with normal motor nerve conduction (43). Linkage analysis of this family mapped the AUNX1 locus to a 28-Mb sized region at Xq23-q27.3 (43). This interval overlaps two loci for X-linked Charcot-Marie-Tooth disease (CMTX) associated with hearing loss, CMTX4 and CMTX5, which map to Xq24-q26 and Xq21.32-q24, respectively (44-46). Considering that DFNX5 (AUNX1) patients also develop diffuse peripheral sensory neuropathy in addition to hearing loss, designation of AUNX1 as X-linked nonsyndromic hearing loss DFNX5 may be incorrect; there is a possibility that AUNX1 may be an allelic disorder of CMTX disease. Several genes, such as SLC6A14, GRIA3, and SOX3, located within the AUNX1 locus, have also been suggested as candidate genes; however, further investigation is needed to identify the causative gene for AUNX1 (43).

5. STRUCTURAL AND FUNCTIONAL ANALYSIS OF MUTATIONS IN THE CAUSATIVE GENES

5.1. DFNX1 (DFN2) – PRPS1

DFNX1 (DFN2) has been found to be caused by the phosphoribosylpyrophosphate synthetase 1 (PRPS1) gene (MIM 311850), which belongs to the phospho-ribosylpyrophosphate (PRPP) synthetase family that consists of three highly conserved genes: PRPS1, PRPS2 (MIM 311860), and PRPSIL1 (MIM 611566) (12). Although PRPS2 and PRPSIL1 have not been associated with any disease entity, missense mutations in PRPS1 are implicated in four syndromes, including PRS-1 superactivity (MIM300661) (47), CMTX5 (Rosenberg-Chutorian syndrome) (MIM 311070) (46), Arts syndrome (MIM 301835) (48), and nonsyndromic X-linked sensorineural hearing loss DFNX1 (DFN2) (MIM 304500) (5). PRPS1 encodes phosphoribosylpyrophosphate
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POU3F4, most of which are clustered in the POU homeodomain, affect amino acid residues that are either directly in contact with the DNA or highly conserved among the different classes of POU proteins (55-56). Molecular modeling of POU3F4, using the known crystal structure of POU2F1/Oct-1 as a template, has demonstrated that different types and locations of intragenic mutations can have severe effects on the structural stability of the protein, thereby reducing the DNA binding ability (16). The p.R329P mutation, which is located in the third alpha-helix of the POU homeodomain that is crucial for protein-DNA interactions, resulted in destabilization of two important salt bridges, as well as a series of hydrophobic contacts that may be detrimental to the alpha-helical structure. Another intragenic mutation, p.S310del, that results in deletion of a single amino acid positioned in the second alpha-helix of the POU homeodomain, caused a shift in one position of the subsequent residues in the helix so that several hydrophobic interactions were disturbed and a polar residue became buried in the hydrophobic core. These modeling results demonstrate that mutations in the amino acid residues that are in direct contact with the DNA (p.R329P) and those that are not in direct contact with DNA but that contribute to the overall stability of the protein (p.S310del) can severely affect the DNA-protein interaction of the POU3F4 protein (16). Biochemical assays confirmed that the DNA binding ability and transcriptional activity were completely abolished with different types of intragenic mutations, including a missense mutation, a single amino acid deletion, and a truncating mutation (p.R329P, p.S310del, and p.Ala116fs, respectively), as compared to wild-type POU3F4 proteins, indicating that the hearing loss in DFNX2 (DFN3) is due to the functional null of POU3F4 protein (16). The failure of mutant POU3F4 proteins to inhibit normal transcriptional activity of the wild-type proteins excluded the possibility of a dominant-interfering mechanism of the mutant proteins being responsible for the clinical phenotypes in DFNX2 (DFN3) (16).

5.2. DFNX2 (DFN3) – POU3F4

POU3F4 (POU domain, class III, transcription factor 4) is a member of a superfamily of POU domain transcription factors, characterized by a bipartite DNA-binding domain consisting of a 75-amino acid POU-specific domain and a 60-amino acid POU homeodomain, which are tethered by a variable linker of 15-30 residues (50). The DNA-binding domains have helix-turn-helix structures that have been shown to bind to a consensus binding site, 5' -CAATATGCTAAT-3', and the POU-specific domain cooperates with the POU homeodomain to enhance binding affinity and specificity (51-52). POU3F4 and its murine ortholog Pou3f4 play an important role in regulating many developmental processes in the inner ear and the central nervous system, where they are mainly expressed (53-54). In the inner ear, Pou3f4 is crucial for patterning of the mesenchymal compartment (53), but the downstream target gene involved in inner ear development has not yet been identified.

Since the various types of POU3F4 mutations show no clear correlation to the degree or type of hearing loss and temporal bone abnormalities, loss-of-function of POU3F4 has been clinically suspected to be the cause of hearing loss in DFNX2 (DFN3) patients. Nuclear magnetic resonance and crystallographic studies of POU2F1/Oct-1 have revealed that the identified missense mutations of POU3F4, most of which are clustered in the POU homeodomain, affect amino acid residues that are either directly in contact with the DNA or highly conserved among the different classes of POU proteins (55-56). Molecular modeling of POU3F4, using the known crystal structure of POU2F1/Oct-1 as a template, has demonstrated that different types and locations of intragenic mutations can have severe effects on the structural stability of the protein, thereby reducing the DNA binding ability (16). The p.R329P mutation, which is located in the third alpha-helix of the POU homeodomain that is crucial for protein-DNA interactions, resulted in destabilization of two important salt bridges, as well as a series of hydrophobic contacts that may be detrimental to the alpha-helical structure. Another intragenic mutation, p.S310del, that results in deletion of a single amino acid positioned in the second alpha-helix of the POU homeodomain, caused a shift in one position of the subsequent residues in the helix so that several hydrophobic interactions were disturbed and a polar residue became buried in the hydrophobic core. These modeling results demonstrate that mutations in the amino acid residues that are in direct contact with the DNA (p.R329P) and those that are not in direct contact with DNA but that contribute to the overall stability of the protein (p.S310del) can severely affect the DNA-protein interaction of the POU3F4 protein (16). Biochemical assays confirmed that the DNA binding ability and transcriptional activity were completely abolished with different types of intragenic mutations, including a missense mutation, a single amino acid deletion, and a truncating mutation (p.R329P, p.S310del, and p.Ala116fs, respectively), as compared to wild-type POU3F4 proteins, indicating that the hearing loss in DFNX2 (DFN3) is due to the functional null of POU3F4 protein (16). The failure of mutant POU3F4 proteins to inhibit normal transcriptional activity of the wild-type proteins excluded the possibility of a dominant-interfering mechanism of the mutant proteins being responsible for the clinical phenotypes in DFNX2 (DFN3) (16).
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periodic mesenchyme ventral to the otic vesicle starting from 10.5 days post coitus (dpc) which corresponds to when the initial condensation of the periotic mesenchyme begins (57). At stages later than 11.5 dpc, when the entire otic capsule undergoes mesenchymal condensation, *Pou3f4* gene expression is detected around the entire otic capsule until 13.5 dpc (57-58). From 15.5 to 18.5 dpc, *Pou3f4* expression is down-regulated in the otic capsule and becomes restricted to prospective otic fibrocytes in the spiral limbus and spiral ligament (58). Given these expression patterns of *Pou3f4*, it is assumed that *Pou3f4* plays a critical role in the morphogenesis of the mesenchymal component of the developing inner ear, especially the otic capsule and otic fibrocytes.

The temporal bone abnormalities seen in DFNX2 (DFN3) patients, including cochlear modiolar deficiency, fistulous connection between the cochlear basal turn and internal acoustic canal, and dilatation of the distal internal acoustic canal, are suspected to arise at 25-47 days of gestation in humans; this roughly corresponds to 10.0-13.5 dpc in mouse development when *Pou3f4* is strongly expressed in the developing otic capsule (59-61). Phippard *et al.* (57) analyzed the subcellular localization of *Pou3f4* proteins during otic capsule development and found that *Pou3f4* remains nuclear in the regions of the otic capsule that eventually develop into the mature bony labyrinth, whereas *Pou3f4* shifts from a nucleus to perinuclear position in those regions that will cavitate to form acellular regions in the temporal bone, such as the scala tympani, scala vestibule, and the internal acoustic canal. These data provide evidence that *Pou3f4* plays a critical role in patterning of the temporal bone during inner ear development. Indeed, detailed morphologic study of mutant mice containing a null mutation of the *Pou3f4* gene, generated by homologous recombination in embryonic stem cells, demonstrated enlargement of the internal auditory canal, constricttion of the superior semicircular canal, and hypoplasia of cochlear structures derived from the otic mesenchyme, including the spiral limbus, the scala tympani, and otic fibrocytes (53). In addition, abnormalities in structures where *Pou3f4* expression is not detected, such as coiling defects of the cochlea or malformation of the stapes, suggest the involvement of *Pou3f4* in the epithelial-mesenchymal and mesenchymal-mesenchymal signaling during inner ear development (53). In a different mouse model, *POU3F4del-J* mice that carry a genomic deletion including the *Pou3f4* gene, histologic analysis of the cochlear mid-modiolar section revealed absence of the modiolus and partial defects in the interscalar septa of the cochlea, which closely resembled the cochlear anomaly seen in DFNX2 (DFN3) patients (62). Stapes fixation that is exhibited in DFNX2 (DFN3) patients is not observed in the animal models; however, detailed morphologic examinations of the stapes demonstrated more flattened and polygonal shape of the stapes footplate and thinner crus in the mutant animals (53). Functional studies on the middle ear sound conduction of *Pou3f4* knockout mice revealed loss of sensitivity in the mid-range frequencies of the velocity function measured at the umbo (63). Although manifestations of stapes abnormalities are not entirely similar in DFNX2 (DFN3) patients and *Pou3f4*-deficient mice, it is thought that abnormal development of the oval window that is derived from the otic capsule where *POU3F4* (or *Pou3f4* in mice) is expressed leads to abnormal mobility or morphology of the stapes resulting in conductive component of hearing loss. Also, increased outward perilymphatic pressure forced on the stapes footplate due to the fistulous connection between the cochlea and the internal auditory canal may contribute to the disturbance of sound conduction into the inner ear.

In addition to the otic capsule defects that lead to the conductive component of hearing loss, abnormalities of the cochlear lateral wall are believed to account for progressive sensorineural hearing loss in DFNX2 (DFN3) patients. Minowa *et al.* (64) observed severe ultrastructural defects in spiral ligament fibrocytes and reduced endocochlear potential in *Pou3f4*-deficient mice generated by targeted mutagenesis. Further investigation using *Pou3f4*-deficient mice demonstrated that immunoreactivities for Cx26, Cx31, Na-K-ATPase, and the Na-K-C1 cotransporter, which are essential for potassium recycling and ionic homeostasis in the cochlea, were severely reduced in type I, II, and V fibrocytes (65-66). Also, *Aqp1* which is expressed in type III fibrocytes that normally line the inner surface of the otic capsule, was distributed broadly throughout the spiral ligament (62). When the onset and pattern of cochlear lateral wall defect were analyzed in *Pou3f4del-J* mice, despite normal compartmentalization of the spiral ligament mesenchyme, differentiation of the compartmentalized mesenchyme into specific otic fibrocytes was completely blocked in the absence of *Pou3f4* function, causing lack of protein expression important for ionic homeostasis (62). As for the stria vascularis that is composed of 3 cell types of different origins, specification was completed in the absence of *Pou3f4* function, yet the expression of Kir4.1 channels in the strial intermediate cells, essential for endocochlear potential generation, was lost afterwards (62). These observations reveal that *Pou3f4* deficiency causes defects in both otic fibrocytes and stria vascularis at different developmental stages by different pathological mechanisms, which may contribute to loss of endocochlear potential leading to progressive sensorineural hearing in DFNX2 (DFN3) patients.

7. SUMMARY AND PERSPECTIVE

Five loci and two nuclear genes have been identified to be associated with nonsyndromic X-linked hearing loss. A new nomenclature (DFNX1-5) has been proposed to classify the loci for nonsyndromic X-linked hearing loss in order to reduce confusion resulting from withdrawal or recategorization of several loci since the initial classification. More than 50% of nonsyndromic X-linked hearing loss cases are diagnosed as DFNX2 (DFN3) caused by mutations in the *POU3F4* gene, which can be easily identified due to the pathognomonic temporal bone CT findings and confirmed via mutational analysis of the *POU3F4* gene, composed of a single exon. The phenotypes of DFNX2 (DFN3) are suspected to be due to mutations in the *POU3F4* gene, leading to a functional null of the protein rather than a gain of ectopic function.
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Developmental defects in the mesenchymal compartment of the inner ear, including the otic capsule and spiral ligament fibrocytes in the cochlear lateral wall, are associated with conductive and progressive sensorineural hearing loss in DFNX2 (DFN3) patients. The recent discovery of a new gene, PRPS1, as the cause of DFNX1 (DFN2), has provided further understanding of the underlying genetic basis for nonsyndromic X-linked hearing loss. Although defects involving the cochlear hair cells where PRPS1 is expressed are suspected to be related to hearing loss in DFNX1 (DFN2), more investigation is needed to elucidate the pathologic mechanism of the clinical phenotype. Identification of more families exhibiting hearing loss segregating with the DFNX3 or DFNX4 locus will help clarify clinical and genetic characteristics, which may lead to the discovery of new causative genes involved in nonsyndromic X-linked hearing loss.

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**Abbreviations:** CT: computed tomography; DMD: Duchenne muscular dystrophy; PRPP: phosphoribosyl pyrophosphate; PRS-I: phosphoribosylpyrophosphate synthetase 1; R5P: ribose-5-phosphate

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