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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 heterogenous, 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).

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

Locus		OMIM	Location	Gene	Type of hearing loss	Severity	Frequency	Age at onset	Progression	Reference
New	Original									
DFNX1	DFN2	#304500	Xq22-24	*PRPS1*	SNHL	Severe~ profound	All	Congenital	Stable	*(9)*
							Low, Mid	Postlingual	Progressive	*(10)*
							All	Postlingual	Progressive	*(11)*
							All	Postlingual	Progressive	*(5)*
DFNX2	DFN3	#304400	Xq21.1	*POU3F4*	Mixed	Severe~ profound	All	Congenital	Progressive	*(4)*
DFNX3	DFN4	%300030	Xp21.2	-	SNHL	Severe~ profound	All	Congenital	Stable	*(34),(35)*
DFNX4	DFN6	%300066	Xp22	-	SNHL	Mild~ Severe	High→All	First decade	Progressive	*(40)*
DFNX5	AUNX	%300614	Xq23-27.3	-	SNHL	Mild~ Profound	Low→All	10~16 years	Progressive	*(43)*

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) (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 identification 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 AUNX1 (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 (PRS-I), which catalyzes the reaction of ribose-5-phosphate with ATP to synthesize phosphoribosyl pyrophosphate (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

Table 2. Intragenic mutations of the *POU3F4* gene

Domain	Nontruncating	Truncating
POU-specific domain	p.Phe201_Lys202del (27)	p.Gln203fs (4)
	p.Ser228Leu (25)	p.L208X (26)
	p.Thr230Ile (23)	p.Leu217fs (4)
	p.Glu236Asp (20)	
POU-homeodomain	p.Arg282Gln (20)	p.Ser288fs (22)
	p.Ile285Asn (20)	p.Ser288fs (20)
	p.Pro303Ser (22)	p.Val289fs (14)
	p.Ile308Asn (20)	p.Lys299fs (4)
	p.Ser310del (16)	p.Ile308fs (20)
	p.Ala312Val (14)	
	p.Leu317Trp (4)	
	p.Arg323Gly[1] (24)	
	p.Asn328Thr (22)	
	p.Arg329Gly (23)	
	p.Arg329Pro (16)	
	p.Arg330Ser (24)	
	p.Lys334Glu (4)	
Other		p.Pro66X (22)
		p.Ala116fs (16)
		p.Ser98X (20)
		p.Gly128fs (26)

[1]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). Modiolar 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

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 sensorineural 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 *PRPS1L1* (MIM 611566) (12). Although *PRPS2* and *PRPS1L1* 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

synthetase 1 (PRS-I), which is involved in catalyzing the synthesis of phosphoribosyl pyrophosphate (PRPP) from ATP and ribose-5-phosphate (R5P). Since PRPP is essential for the *de novo* synthesis of purine, pyrimidine, and pyridine nucleotides, mutations in *PRPS1* affect vital cell functions, including nucleic acid synthesis, energy metabolism, and cellular signaling (12). PRS-I consists of two domains with a similar sandwich-like alpha/beta structure that are connected by two short, flexible loops. The active sites of human PRS-I, as seen by crystallization, comprises the binding sites for ATP and R5P; the ATP binding site is primarily composed of three structural elements, including the flexible loop (residues Phe92-Ser108), the PPi (pyrophosphate)-binding loop (residues Asp171-Gly174), and the flag region (residues Val30-Ile44 of an adjacent subunit) (49). Structural analysis of the mutant and wild type proteins revealed that two of the missense mutations identified in DFNX1 (DFN2) families, p.D65N and p.A87T, may induce conformational changes in the ATP binding site, resulting in a reduction in ATP binding efficiency. The p.I290T mutation is located at the beginning of loop22, one of the flexible loops connecting the two domains, which may alter the enzymatic activity of PRS-I. Another mutation, p.G306R that is positioned near the allosteric site of PRS-I, has been predicted to affect the regulatory mechanism of the enzyme (5). Functional analysis performed by measuring PRS-I enzymatic activity in erythrocytes demonstrated a significant reduction of PRS-I activity in affected males having the p.D65N mutation, when compared to unaffected males and unrelated healthy controls (5). PRS-I activity detected in cultured skin fibroblasts from affected males was also significantly decreased, as compared to female carriers and unaffected males (5).

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).

6. DEVELOPMENTAL PATHOGENESIS

6.1. DFNX1 (DFN2)

The expression of *Prps1* has been observed in hair cells, Claudius cells, and the greater epithelial ridge of murine cochlea, and also in vestibular hair cells at embryonic day 18.5 (5). By postnatal day 6, *Prps1* was expressed in spiral ganglion cells, in addition to the hair cells and Claudius cells, while expression in the greater epithelial ridge was decreased. Considering the expression pattern of *Prps1* in the hair cells and the absence of distortion products otoacoustic emissions in DFNX1 (DFN2) patients, the main pathology leading to hearing loss is currently suspected to be of hair cell origin (5). Further investigation is required to identify the mechanism of how *PRPS1* mutations lead to hearing loss in DFNX1 (DFN2).

6.2. DFNX2 (DFN3)

The expression patterns and developmental roles of Pou3f4, the mouse homolog of POU3F4, in the inner ear have been studied in detail using mouse models for DFNX2 (DFN3). The *Pou3f4* gene is initially detected in the

periotic 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 nuclear 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, constriction 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-Cl 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.

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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9. REFERENCES

1. N. E. Morton: Genetic epidemiology of hearing impairment. *Ann N Y Acad Sci* 630, 16-31 (1991)

2. G. Van Camp, R. J. H. Smith: Hereditary Hearing Loss Homepage. URL:http://hereditaryhearingloss.org.

3. M. B. Petersen, Q. Wang, P. J. Willems: Sex-linked deafness. *Clin Genet* 73, 14-23 (2008)

4. Y. J. de Kok, S. M. van der Maarel, M. Bitner-Glindzicz, I. Huber, A. P. Monaco, S. Malcolm, M. E. Pembrey, H. H. Ropers, F. P. Cremers: Association between X-linked mixed deafness and mutations in the POU domain gene POU3F4. *Science* 267, 685-688 (1995)

5. X. Liu, D. Han, J. Li, B. Han, X. Ouyang, J. Cheng, X. Li, Z. Jin, Y. Wang, M. Bitner-Glindzicz, X. Kong, H. Xu, A. Kantardzhieva, R. D. Eavey, C. E. Seidman, J. G. Seidman, L. L. Du, Z. Y. Chen, P. Dai, M. Teng, D. Yan, H. Yuan: Loss-of-function mutations in the PRPS1 gene cause a type of nonsyndromic X-linked sensorineural deafness, DFN2. *Am J Hum Genet* 86, 65-71 (2010)

6. W. Reardon, H. R. Middleton-Price, L. Sandkuijl, P. Phelps, S. Bellman, L. Luxon, M. E. Pembrey, S. Malcolm: A multipedigree linkage study of X-linked deafness: linkage to Xq13-q21 and evidence for genetic heterogeneity. *Genomics* 11, 885-894 (1991)

7. L. Tranebjaerg, C. Schwartz, H. Eriksen, S. Andreasson, V. Ponjavic, A. Dahl, R. E. Stevenson, M. May, F. Arena, D. Barker, *et al.*: A new X linked recessive deafness syndrome with blindness, dystonia, fractures, and mental deficiency is linked to Xq22. *J Med Genet* 32, 257-263 (1995)

8. H. Jin, M. May, L. Tranebjaerg, E. Kendall, G. Fontan, J. Jackson, S. H. Subramony, F. Arena, H. Lubs, S. Smith, R. Stevenson, C. Schwartz, D. Vetrie: A novel X-linked gene, DDP, shows mutations in families with deafness (DFN-1), dystonia, mental deficiency and blindness. *Nat Genet* 14, 177-180 (1996)

9. J. Tyson, S. Bellman, V. Newton, P. Simpson, S. Malcolm, M. E. Pembrey, M. Bitner-Glindzicz: Mapping of DFN2 to Xq22. *Hum Mol Genet* 5, 2055-2060 (1996)

10. E. N. Manolis, R. D. Eavey, S. Sangwatanaroj, C. Halpin, S. Rosenbaum, H. Watkins, J. Jarcho, C. E. Seidman, J. G. Seidman: Hereditary postlingual sensorineural hearing loss mapping to chromosome Xq21. *Am J Otol* 20, 621-626 (1999)

11. B. Cui, H. Zhang, Y. Lu, W. Zhong, G. Pei, X. Kong, L. Hu: Refinement of the locus for non-syndromic sensorineural deafness (DFN2). *J Genet* 83, 35-38 (2004)

12. A. P. de Brouwer, H. van Bokhoven, S. B. Nabuurs, W. F. Arts, J. Christodoulou, J. Duley: PRPS1 mutations: four distinct syndromes and potential treatment. *Am J Hum Genet* 86, 506-518 (2010)

13. I. Bach, H. G. Brunner, P. Beighton, R. H. Ruvalcaba, W. Reardon, M. E. Pembrey, S. D. van der Velde-Visser, G. A. Bruns, C. W. Cremers, F. P. Cremers, *et al.*: Microdeletions in patients with gusher-associated, X-linked mixed deafness (DFN3). *Am J Hum Genet* 51, 38-44 (1992)

14. M. Bitner-Glindzicz, P. Turnpenny, P. Hoglund, H. Kaariainen, E. M. Sankila, S. M. van der Maarel, Y. J. de Kok, H. H. Ropers, F. P. Cremers, M. Pembrey, *et al.*: Further mutations in Brain 4 (POU3F4) clarify the phenotype in the X-linked deafness, DFN3. *Hum Mol Genet* 4, 1467-1469 (1995)

15. Q. J. Wang, Q. Z. Li, S. Q. Rao, Y. L. Zhao, H. Yuan, W. Y. Yang, D. Y. Han, Y. Shen: A novel mutation of POU3F4 causes congenital profound sensorineural hearing loss in a large Chinese family. *Laryngoscope* 116, 944-950 (2006)

16. H. K. Lee, M. H. Song, M. Kang, J. T. Lee, K. A. Kong, S. J. Choi, K. Y. Lee, H. Venselaar, G. Vriend, W. S. Lee, H. J. Park, T. K. Kwon, J. Bok, U. K. Kim: Clinical and molecular characterizations of novel POU3F4 mutations reveal that DFN3 is due to null function of POU3F4 protein. *Physiol Genomics* 39, 195-201 (2009)

17. W. E. Nance, R. Setleff, A. McLeod, A. Sweeney, C. Cooper, F. McConnell: X-linked mixed deafness with congenital fixation of the stapedial footplate and perilymphatic gusher. *Birth Defects Orig Artic Ser* 07, 64-69 (1971)

18. P. D. Phelps, W. Reardon, M. Pembrey, S. Bellman, L.

Luxom: X-linked deafness, stapes gushers and a distinctive defect of the inner ear. *Neuroradiology* 33, 326-330 (1991)

19. L. Sennaroglu, S. Sarac, T. Ergin: Surgical results of cochlear implantation in malformed cochlea. *Otol Neurotol* 27, 615-623 (2006)

20. S. Marlin, M. P. Moizard, A. David, N. Chaissang, M. Raynaud, L. Jonard, D. Feldmann, N. Loundon, F. Denoyelle, A. Toutain: Phenotype and genotype in females with POU3F4 mutations. *Clin Genet* 76, 558-563 (2009)

21. E. Papadaki, P. Prassopoulos, J. Bizakis, S. Karampekios, H. Papadakis, N. Gourtsoyiannis: X-linked deafness with stapes gusher in females. *Eur J Radiol* 29, 71-75 (1998)

22. F. P. Cremers, F. R. Cremers, H. Kremer: POU3F4 and mixed deafness with temporal defect (DFN3). In: Inborn errors of development: the molecular basis of clinical disorders of morphogenesis. Eds: C. J. Epstein, R. P. Erickson, A. Wynshaw-Boris, *Oxford University Press*, New York (2008)

23. R. A. Friedman, Y. Bykhovskaya, G. Tu, J. M. Talbot, D. F. Wilson, L. S. Parnes, N. Fischel-Ghodsian: Molecular analysis of the POU3F4 gene in patients with clinical and radiographic evidence of X-linked mixed deafness with perilymphatic gusher. *Ann Otol Rhinol Laryngol* 106, 320-325 (1997)

24. Y. J. de Kok, C. W. Cremers, H. H. Ropers, F. P. Cremers: The molecular basis of X-linked deafness type 3 (DFN3) in two sporadic cases: identification of a somatic mosaicism for a POU3F4 missense mutation. *Hum Mutat* 10, 207-211 (1997)

25. A. P. Vore, E. H. Chang, J. E. Hoppe, M. G. Butler, S. Forrester, M. C. Schneider, L. L. Smith, D. W. Burke, C. A. Campbell, R. J. Smith: Deletion of and novel missense mutation in POU3F4 in 2 families segregating X-linked nonsyndromic deafness. *Arch Otolaryngol Head Neck Surg* 131, 1057-1063 (2005)

26. H. K. Lee, S. H. Lee, K. Y. Lee, E. J. Lim, S. Y. Choi, R. K. Park, U. K. Kim: Novel POU3F4 mutations and clinical features of DFN3 patients with cochlear implants. *Clin Genet* 75, 572-575 (2009)

27. H. Hagiwara, Y. Tamagawa, K. Kitamura, K. Kodera: A new mutation in the POU3F4 gene in a Japanese family with X-linked mixed deafness (DFN3). *Laryngoscope* 108, 1544-1547 (1998)

28. M. H. Song, H. K. Lee, J. Y. Choi, S. Kim, J. Bok, U. K. Kim: Clinical evaluation of DFN3 patients with deletions in the POU3F4 locus and detection of carrier female using MLPA. *Clin Genet* (2010)

29. Y. J. de Kok, G. F. Merkx, S. M. van der Maarel, I. Huber, S. Malcolm, H. H. Ropers, F. P. Cremers: A duplication/paracentric inversion associated with familial X-linked deafness (DFN3) suggests the presence of a regulatory element more than 400 kb upstream of the POU3F4 gene. *Hum Mol Genet* 4, 2145-2150 (1995)

30. S. Naranjo, K. Voesenek, E. de la Calle-Mustienes, A. Robert-Moreno, H. Kokotas, M. Grigoriadou, J. Economides, G. Van Camp, N. Hilgert, F. Moreno, B. Alsina, M. B. Petersen, H. Kremer, J. L. Gomez-Skarmeta: Multiple enhancers located in a 1-Mb region upstream of POU3F4 promote expression during inner ear development and may be required for hearing. *Hum Genet* (2010)

31. Y. J. de Kok, E. R. Vossenaar, C. W. Cremers, N. Dahl, J. Laporte, L. J. Hu, D. Lacombe, N. Fischel-Ghodsian, R. A. Friedman, L. S. Parnes, P. Thorpe, M. Bitner-Glindzicz, H. J. Pander, H. Heilbronner, J. Graveline, J. T. den Dunnen, H. G. Brunner, H. H. Ropers, F. P. Cremers: Identification of a hot spot for microdeletions in patients with X-linked deafness type 3 (DFN3) 900 kb proximal to the DFN3 gene POU3F4. *Hum Mol Genet* 5, 1229-1235 (1996)

32. I. Huber, M. Bitner-Glindzicz, Y. J. de Kok, S. M. van der Maarel, Y. Ishikawa-Brush, A. P. Monaco, D. Robinson, S. Malcolm, M. E. Pembrey, H. G. Brunner, *et al.*: X-linked mixed deafness (DFN3): cloning and characterization of the critical region allows the identification of novel microdeletions. *Hum Mol Genet* 3, 1151-1154 (1994)

33. K. J. Ahn, F. Passero, Jr., E. B. Crenshaw, 3rd: Otic mesenchyme expression of Cre recombinase directed by the inner ear enhancer of the Brn4/Pou3f4 gene. *Genesis* 47, 137-141 (2009)

34. A. K. Lalwani, J. R. Brister, J. Fex, K. M. Grundfast, A. T. Pikus, B. Ploplis, T. San Agustin, H. Skarka, E. R. Wilcox: A new nonsyndromic X-linked sensorineural hearing impairment linked to Xp21.2. *Am J Hum Genet* 55, 685-694 (1994)

35. M. H. Pfister, F. Apaydin, O. Turan, M. Bereketoglu, V. Bylgen, U. Braendle, H. P. Zenner, A. K. Lalwani: A second family with nonsyndromic sensorineural hearing loss linked to Xp21.2: refinement of the DFN4 locus within DMD. *Genomics* 53, 377-382 (1998)

36. M. H. Pfister, F. Apaydin, O. Turan, M. Bereketoglu, V. Bilgen, U. Braendle, S. Kose, H. P. Zenner, A. K. Lalwani: Clinical evidence for dystrophin dysfunction as a cause of hearing loss in locus DFN4. *Laryngoscope* 109, 730-735 (1999)

37. E. M. Raynor, M. J. Mulroy: Sensorineural hearing loss in the mdx mouse: a model of Duchenne muscular dystrophy. *Laryngoscope* 107, 1053-1056 (1997)

38. D. A. Pillers, N. M. Duncan, S. J. Dwinnell, S. M. Rash, J. B. Kempton, D. R. Trune: Normal cochlear function in mdx and mdx(Cv3) Duchenne muscular dystrophy mouse models. *Laryngoscope* 109, 1310-1312 (1999)

39. T. J. Chen, S. S. Chen, D. C. Wang, Y. L. Hsieh:

Increased vulnerability of auditory system to noise exposure in mdx mice. *Laryngoscope* 112, 520-525 (2002)

40. I. del Castillo, M. Villamar, M. Sarduy, L. Romero, C. Herraiz, F. J. Hernandez, M. Rodriguez, I. Borras, A. Montero, J. Bellon, M. C. Tapia, F. Moreno: A novel locus for non-syndromic sensorineural deafness (DFN6) maps to chromosome Xp22. *Hum Mol Genet* 5, 1383-1387 (1996)

41. M. T. Bassi, R. S. Ramesar, B. Caciotti, I. M. Winship, A. De Grandi, M. Riboni, P. L. Townes, P. Beighton, A. Ballabio, G. Borsani: X-linked late-onset sensorineural deafness caused by a deletion involving OA1 and a novel gene containing WD-40 repeats. *Am J Hum Genet* 64, 1604-1616 (1999)

42. Q. Wang, R. Gu, D. Han, W. Yang: Familial auditory neuropathy. *Laryngoscope* 113, 1623-1629 (2003)

43. Q. J. Wang, Q. Z. Li, S. Q. Rao, K. Lee, X. S. Huang, W. Y. Yang, S. Q. Zhai, W. W. Guo, Y. F. Guo, N. Yu, Y. L. Zhao, H. Yuan, J. Guan, S. M. Leal, D. Y. Han, Y. Shen: AUNX1, a novel locus responsible for X linked recessive auditory and peripheral neuropathy, maps to Xq23-27.3. *J Med Genet* 43, e33 (2006)

44. J. M. Priest, K. H. Fischbeck, N. Nouri, B. J. Keats: A locus for axonal motor-sensory neuropathy with deafness and mental retardation maps to Xq24-q26. *Genomics* 29, 409-412 (1995)

45. H. J. Kim, S. H. Hong, C. S. Ki, B. J. Kim, J. S. Shim, S. H. Cho, J. H. Park, J. W. Kim: A novel locus for X-linked recessive CMT with deafness and optic neuropathy maps to Xq21.32-q24. *Neurology* 64, 1964-1967 (2005)

46. H. J. Kim, K. M. Sohn, M. E. Shy, K. M. Krajewski, M. Hwang, J. H. Park, S. Y. Jang, H. H. Won, B. O. Choi, S. H. Hong, B. J. Kim, Y. L. Suh, C. S. Ki, S. Y. Lee, S. H. Kim, J. W. Kim: Mutations in PRPS1, which encodes the phosphoribosyl pyrophosphate synthetase enzyme critical for nucleotide biosynthesis, cause hereditary peripheral neuropathy with hearing loss and optic neuropathy (cmtx5). *Am J Hum Genet* 81, 552-558 (2007)

47. O. Sperling, G. Eilam, Brosh Sara Persky, A. De Vries: Accelerated erythrocyte 5-phosphoribosyl-1-pyrophosphate synthesis. A familial abnormality associated with excessive uric acid production and gout. *Biochem Med* 6, 310-316 (1972)

48. A. P. de Brouwer, K. L. Williams, J. A. Duley, A. B. van Kuilenburg, S. B. Nabuurs, M. Egmont-Petersen, D. Lugtenberg, L. Zoetekouw, M. J. Banning, M. Roeffen, B. C. Hamel, L. Weaving, R. A. Ouvrier, J. A. Donald, R. A. Wevers, J. Christodoulou, H. van Bokhoven: Arts syndrome is caused by loss-of-function mutations in PRPS1. *Am J Hum Genet* 81, 507-518 (2007)

49. S. Li, Y. Lu, B. Peng, J. Ding: Crystal structure of human phosphoribosylpyrophosphate synthetase 1 reveals a novel allosteric site. *Biochem J* 401, 39-47 (2007)

50. W. Herr, R. A. Sturm, R. G. Clerc, L. M. Corcoran, D. Baltimore, P. A. Sharp, H. A. Ingraham, M. G. Rosenfeld, M. Finney, G. Ruvkun, *et al.*: The POU domain: a large conserved region in the mammalian pit-1, oct-1, oct-2, and Caenorhabditis elegans unc-86 gene products. *Genes Dev* 2, 1513-1516 (1988)

51. K. F. Malik, J. Kim, A. L. Hartman, P. Kim, W. S. Young, 3rd: Binding preferences of the POU domain protein Brain-4: implications for autoregulation. *Brain Res Mol Brain Res* 38, 209-221 (1996)

52. A. K. Ryan, M. G. Rosenfeld: POU domain family values: flexibility, partnerships, and developmental codes. *Genes Dev* 11, 1207-1225 (1997)

53. D. Phippard, L. Lu, D. Lee, J. C. Saunders, E. B. Crenshaw, 3rd: Targeted mutagenesis of the POU-domain gene Brn4/Pou3f4 causes developmental defects in the inner ear. *J Neurosci* 19, 5980-5989 (1999)

54. J. M. Mathis, D. M. Simmons, X. He, L. W. Swanson, M. G. Rosenfeld: Brain 4: a novel mammalian POU domain transcription factor exhibiting restricted brain-specific expression. *Embo J* 11, 2551-2561 (1992)

55. J. D. Klemm, M. A. Rould, R. Aurora, W. Herr, C. O. Pabo: Crystal structure of the Oct-1 POU domain bound to an octamer site: DNA recognition with tethered DNA-binding modules. *Cell* 77, 21-32 (1994)

56. K. Okamoto, M. Wakamiya, S. Noji, E. Koyama, S. Taniguchi, R. Takemura, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, M. Muramatsu, *et al.*: A novel class of murine POU gene predominantly expressed in central nervous system. *J Biol Chem* 268, 7449-7457 (1993)

57. D. Phippard, A. Heydemann, M. Lechner, L. Lu, D. Lee, T. Kyin, E. B. Crenshaw, 3rd: Changes in the subcellular localization of the Brn4 gene product precede mesenchymal remodeling of the otic capsule. *Hear Res* 120, 77-85 (1998)

58. M. O. Trowe, H. Maier, M. Schweizer, A. Kispert: Deafness in mice lacking the T-box transcription factor Tbx18 in otic fibrocytes. *Development* 135, 1725-1734 (2008)

59. C. Piussan, A. Hanauer, N. Dahl, M. Mathieu, C. Kolski, V. Biancalana, S. Heyberger, V. Strunski: X-linked progressive mixed deafness: a new microdeletion that involves a more proximal region in Xq21. *Am J Hum Genet* 56, 224-230 (1995)

60. L. Sennaroglu, I. Saatci: A new classification for cochleovestibular malformations. *Laryngoscope* 112, 2230-2241 (2002)

61. K. Theiler: The house mouse: atlas of embryonic development, Second Edition. *Springer*, New York (1989)

62. M. H. Song, S. Y. Choi, L. Wu, S. K. Oh, H. K. Lee, D.

J. Lee, D. B. Shim, J. Y. Choi, U. K. Kim, J. Bok: Pou3f4 deficiency causes defects in otic fibrocytes and stria vascularis by different mechanisms. *Biochem Biophys Res Commun* 404, 528-533 (2011)

63. D. S. Samadi, J. C. Saunders, E. B. Crenshaw, 3rd: Mutation of the POU-domain gene Brn4/Pou3f4 affects middle-ear sound conduction in the mouse. *Hear Res* 199, 11-21 (2005)

64. O. Minowa, K. Ikeda, Y. Sugitani, T. Oshima, S. Nakai, Y. Katori, M. Suzuki, M. Furukawa, T. Kawase, Y. Zheng, M. Ogura, Y. Asada, K. Watanabe, H. Yamanaka, S. Gotoh, M. Nishi-Takeshima, T. Sugimoto, T. Kikuchi, T. Takasaka, T. Noda: Altered cochlear fibrocytes in a mouse model of DFN3 nonsyndromic deafness. *Science* 285, 1408-1411 (1999)

65. A. P. Xia, T. Kikuchi, O. Minowa, Y. Katori, T. Oshima, T. Noda, K. Ikeda: Late-onset hearing loss in a mouse model of DFN3 non-syndromic deafness: morphologic and immunohistochemical analyses. *Hear Res* 166, 150-158 (2002)

66. E. M. Braunstein, E. B. Crenshaw Iii, B. E. Morrow, J. C. Adams: Cooperative Function of Tbx1 and Brn4 in the Periotic Mesenchyme is Necessary for Cochlea Formation. *J Assoc Res Otolaryngol* 9, 33-43 (2008)

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

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