On the pathophysiology of DFNA9 : effect of pathogenic variants in the COCH gene on inner ear functioning in human and transgenic mice

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Abstract

DeaFNess Autosomal Dominant 9 (DFNA9) is a dominant hereditary non-syndromic form of progressive sensorineural hearing loss often associated with vestibular dysfunction. DFNA9 is caused by pathogenic variants in the COCH gene. This gene encodes for cochlin, a protein that is abundantly expressed in the spiral ligament and spiral limbus of the inner ear but the function of cochlin is still not fully understood. There are 22 known pathogenic variants located in different domains of the *COCH* gene that can cause DFNA9, all expressing slightly different phenotypes. It is believed that *COCH* mutations affect the intracellular trafficking of cochlin which could explain the characteristic pathology seen in temporal bones of DFNA9 patients. This pathology involves a widespread accumulation of acellular eosinophilic deposits throughout the labyrinth. To gain a better understanding of the pathology underlying DFNA9, different mouse models were developed.

The objective of this review is to describe the different pathogenic variants in the *COCH* gene and their effect on intracellular trafficking, associated phenotypes and histopathological findings in both patients and mouse models.

Key words: DFNA9, COCH gene, pathogenic variants, spiral ligament, histopathology

INTRODUCTION

Hearing impairment is the most frequent sensory deficit in the human population, affecting 440 million people worldwide.[1] DeaFNess Autosomal Dominant 9 (DFNA9) is a hereditary non-syndromic form of progressive sensorineural hearing loss with a high frequency onset. Vestibular symptoms are often associated with DFNA9 but the penetrance of these symptoms is variable among patients, ranging from minimally affected to suffering from vertigo or imbalance.[2-4] Mutations causing DFNA9 are located in the *COCH* gene. This gene encodes for cochlin, a protein that is abundantly expressed in the spiral ligament and spiral limbus of the inner ear. The function of cochin is not fully understood but it has been reported to assist in structural support, sound processing and maintenance of balance within the inner ear.[2, 5] Studies performed in mice suggest that cochlin is also involved in the regulation of macrophage activation, recruitment of immune cells and cytokine production.[6, 7] Cochlin expression has also been demonstrated in the eye and, upon overexpression, is associated with a higher intraocular pressure both in humans and mouse.[8]

Pathological studies of the temporal bones of DFNA9 patients have revealed diffuse degeneration of the spiral ligament and the spiral limbus. In addition, accumulation of acellular eosinophilic deposits has been observed throughout the labyrinth.[9, 10] In order to gain a better understanding in the pathology of *COCH* mutations, different mouse models have been created. Morton et al. developed a mouse model carrying the G88E mutation, one of the mutations causing DFNA9.[3] In addition, a knockout mouse model for the Coch protein ($Coch^{-/-}$) was developed by Makishima et al.[11]

In this review report, our objective is to describe the different pathogenic variants in the *COCH* gene and their associated phenotypic and histological consequences, both in patients and mouse models.

LOCALIZATION OF THE COCH PROTEIN IN THE INNER EAR

Cochlin is an abundant protein in the inner ear where is it expressed in the spiral ligament, spiral osseous and spiral limbus of the inner ear as outlined in Figure 1.

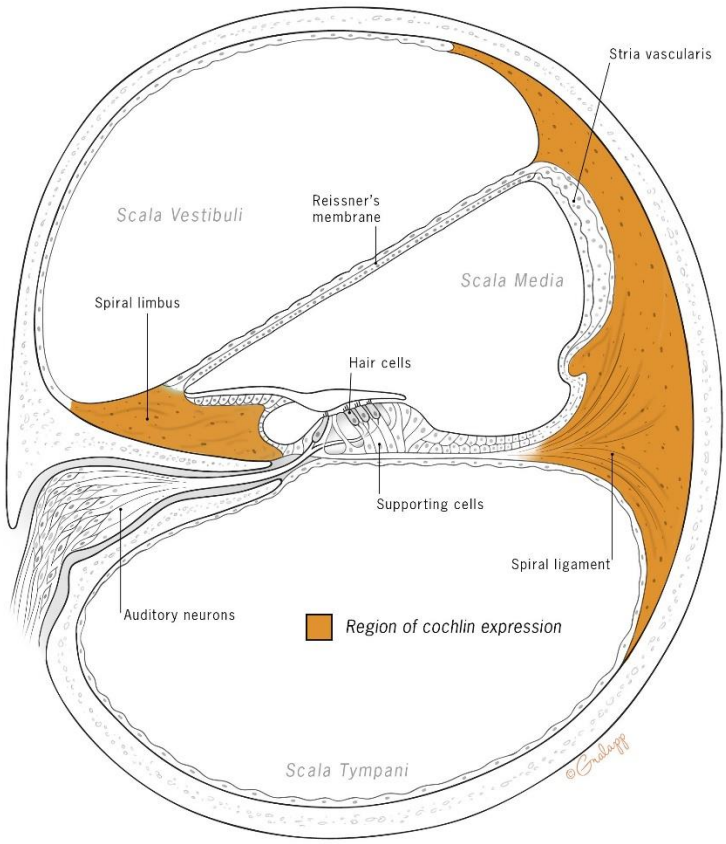

Figure 1. Cochlin expression in the inner ear.

In the vestibular labyrinth, cochlin expression is observed in the area of the stromal fibrocytes in the cristae [12] as can be seen in Figure 2. Expression of the COCH protein is also very prominent in the middle ear, more specific in the incudomallear and incodustapedial joints and the pars tensa of the tympanic membrane (TM).[13] Besides the ear, cochlin is expressed in lower levels in the spleen, lymph nodes, thymus, cerebellum and eye.[8]

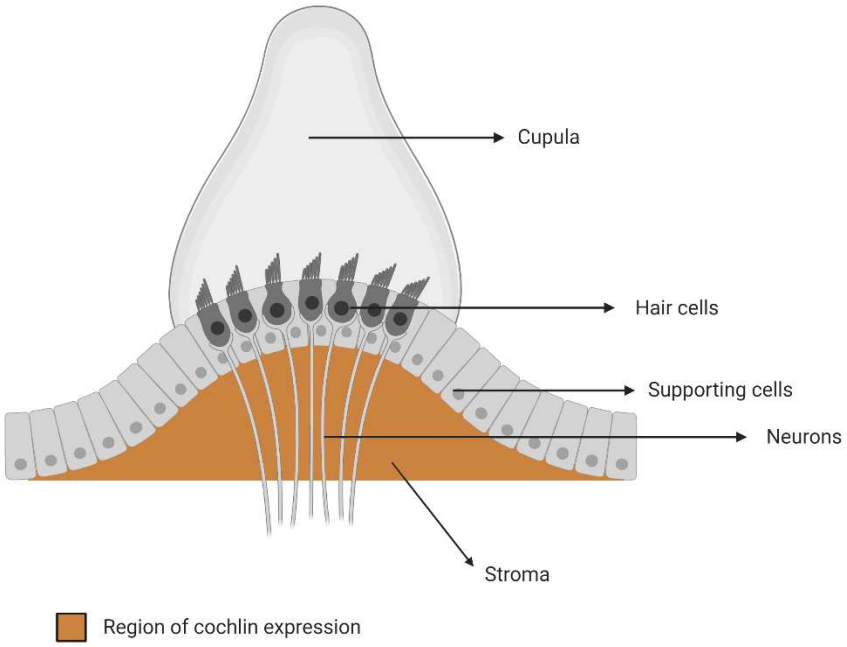

Figure 2. Cochlin expression in the vestibular organ.

INTRACELLULAR TRAFFICKING OF NORMAL COCH PROTEIN

As assessed by several overexpression studies, the COCH protein was observed in the endoplasmic reticulum (ER) and Golgi network adjacent to the nucleus, indicating that cochlin is transported and processed through the ER/Golgi secretory pathway. This is further supported by the observation that cochlin is glycosylated in its mature secreted form in the culture media of transfected cells.[14-16] Glycosylation is indeed important to allow trafficking of immature proteins through different cellular compartments to reach their destination or to function properly. Glycoproteins that are incorrectly folded or assembled will not be exported from the ER or Golgi, but instead will be destroyed by the proteasome or rescued by chaperone that aid to achieve correct folding.[15] The COCH protein has two consensus sites for asparagine linked (N linked) glycosylation, one is at amino acid residue 100 in the LCCL domain, the other at amino acid residue 221 in the vWFA1 domain.[15]

STRUCTURE AND FUNCTION OF COCHLIN

The *COCH* gene is located at the long arm of chromosome 14 and encodes for cochlin. This protein contains the following domains: an N-terminal signal peptide (SP), an LCCL (Limulus factor C, cochlin, lung gestational protein) domain, two vWFA domains (von Willebrand factor A-like) and two short intervening domains (ivd), as shown in Figure 3.[2, 3, 16]

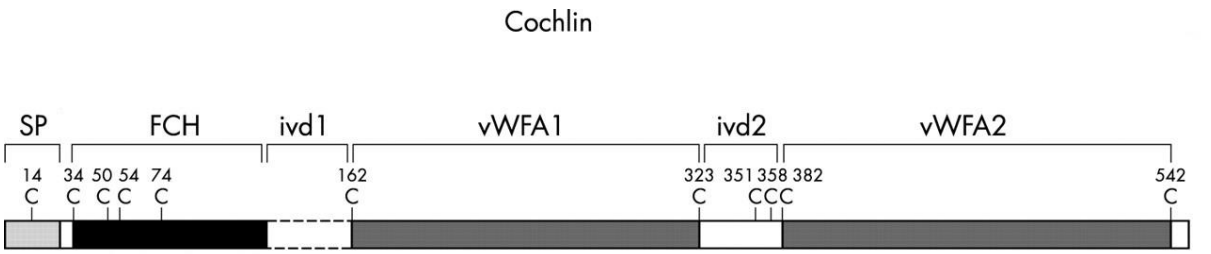

Figure 3. Structure of the COCH gene and positions of all cysteine residues. This figure is reproduced from Robertson et al.[14]

The ***LCCL domain***, consisting of a central alfa helix wrapped by two beta sheets, has strong homology with Factor C, an endotoxin-sensitive serine proteinase involved in the immune response in the horseshoe crab Limulus where it functions as an antibacterial peptide.[17] In the spleen, the cochlin p8 (non-glycosylated LCCL domain) and p18 (glycosylated LCCL domain) isoforms are released in the blood during infection and inflammation to regulate local cytokine production, recruitment of immune effector cells and bacterial clearance. Post-translational cleavage of cochlin is conducted by aggrecanase-1, a member of the Adamts protein family.[7] Several studies have identified cochlin as an important modulator of immune responses, specifically by follicular dendritic cells in the spleen and lymph nodes.[6,7] In the spleen, collagen VII is a direct interaction partner of cochlin. Mutations in the *COL7A1* gene, encoding for collagen VII, causes Recessive Dystrophic Epidermolysis Bullosa (RDEB), a skin fragility disorder often associated with drastically elevated susceptibility to bacterial colonization of skin.[18] It has been hypothesized that collagen VII and cochlin are essential in the regulation of antibacterial immunity. This hypothesis is further supported by the observation that loss

of collagen VII in mice evoked loss of cochlin in the lymph conduits resulting in the inability to activate immune cells and therefore an increase in bacterial colonization. Systemic administration of the LCCL domain of cochlin reactivated macrophages and led to normalization of bacterial colonization of the skin.[18]

Another study [6] investigated the role of the LCCL domain in the inner ear. During bacterial infection the N-terminal LCCL is cleaved by aggrecanase 1 and secreted into the scala tympani of the inner ear. There is a growing body of evidence that cochlin mediates the segregation and entrapment of invading bacteria through direct interaction, as well as enhances the recruitment of inflammatory cells and production of cytokines in the restricted scala tympani space where bacterial pathogens are aggregated. This enriched innate immunity in the inner ear protects essential auditory structures, such as the organ of Corti.[6]

The **vWFA2 domain** of cochlin has a structure similar to other vWFA domains: a central beta-sheet of 6 strands, flanked by 3 and 4 helices. It has a metal ion-dependent adhesion site (MIDAS) motif, which plays an important role in structural stability and ligand binding in vWFA domain-containing proteins.[14] The vWFA domains are believed to be involved in maintaining the structure of the extracellular matrix due to their affinity for type I, type II and type IV collagens.[6, 7, 11]

Dominant-negative pathogenic variants in the *COCH* gene can cause the autosomal dominant disorder DFNA9. In contrast to DFNA9, DNFB110 is the autosomal recessive variant caused by inactivating variants.[19] Today, we know of six families that carry loss-of-function mutations in the *COCH* gene. These patients have congenital moderate sensorineural hearing loss that is not associated with vertigo or balance problems. However, one subject homozygous for the p.R98X variant had vestibular dysfunction similar as observed in DFNA9 patients.[20] An overview of the different loss-of-function mutations affecting the *COCH* gene is given in Table 1.[19,20,21]

Table 1. Different loss-of-function mutations in the *COCH* gene causing DFNB110.

Mutation	Ethnicity	Reference
L39X	Iranian	(Mehregan et al., 2019)
R91G	Middle Eastern	(Booth et al., 2020)
R98X	Belgian/Moroccan	(JanssensdeVarebeke et al., 2018)
K147X	European	(Booth et al., 2020)
V191R	European	(Booth et al., 2020)
E211X	Pakistani	(Booth et al., 2020)

DFNA9 patients will often express symptoms that meet the clinical criteria for Meniere's disease (MD) including vertigo spells, hearing loss, tinnitus or aural fullness, which may lead to a diagnostic delay.[22] Despite the fact that MD patients do not carry pathogenic variants in the *COCH* gene, it is observed that in the vestibular end organs from definite MD patients cochlin is upregulated when compared to normal human vestibular end organs.[22,23] The upregulation of cochlin is associated with a downregulation of collagen IV. It is proposed that an altered cochlin and extracellular matrix (ECM) protein expression may play a role in MD. As the most consistent temporal bone finding in MD patients is endolymphatic hydrops, it is possible that alterations in ECM proteins may lead to a disruption in water and ion homeostasis in the inner ear, contributing to the pathophysiology of MD.[23]

Cochlin is also associated with Autoimmune Inner Ear Disease (AIED), a rare disease accounting for less than 1% of all cases of hearing impairment or dizziness.[24] AIED is characterized by a rapidly progressive, often fluctuating, bilateral sensorineural hearing loss over a period of weeks to months.[23] Significantly higher serum levels of anti-cochlin antibodies have been found in AIED patients compared with patients with noise- or age-related hearing loss and healthy controls suggesting an active immune response to the COCH protein.[24, 25] Also, T-cell responses to cochlin have been reported in AIED patients implicating cochlin in the pathogenesis of AIED.[25]

The exact function of cochlin is not fully understood but as mutations in the *COCH* gene can cause DFNA9 and DFNB110, which are characterized by progressive sensorineural hearing loss and vestibular dysfunction, it is believed that cochlin is critical for maintaining auditory function.[6] Accumulation of cochlin in the eye has been demonstrated to be associated with glaucoma.[8] As both the anterior eye chamber and the perilymph space of the ear contain fluid, it is speculated that cochlin may have a function in maintaining the shear stress and ion homeostasis of these fluids by interacting with collagen II to build up the ECM.[6, 7, 8, 11] In the vestibular organ, cochlin is believed to have a role in the structural homeostasis of the vestibule by acting in concert with the bundles of fibrillar collagen II.[24]

MUTATIONS IN THE COCH GENE CAUSING DFNA9

There are twenty-two known pathogenic variants in the *COCH* gene causing DFNA9. Most of these heterozygous mutations are located in the LCCL domain but there are a few mutations affecting the vWFA domains.[12]

MUTANT COCHLIN: INTRACELLULAR

An overview of the different DFNA9 mutations and their effect on intracellular processes is provided in Table 2.

Mutant cochlin containing the p.P51S, p.V66G, p.G87W, p.G88E, p.P89H, p.I109T, p.W117R, p.A119T, p.F121S, p.V123E or p.V140del mutations, all located in the LCCL domain, display localization in the ER and Golgi complex similar to wildtype cochlin.[15, 17] Cochlins with mutations in the vWFA domains are localized in the ER, but not in the Golgi complex, indicating a failure of transportation from the ER to the Golgi complex.[15] The cell biological consequence of such mutations is depicted in Figure 4.

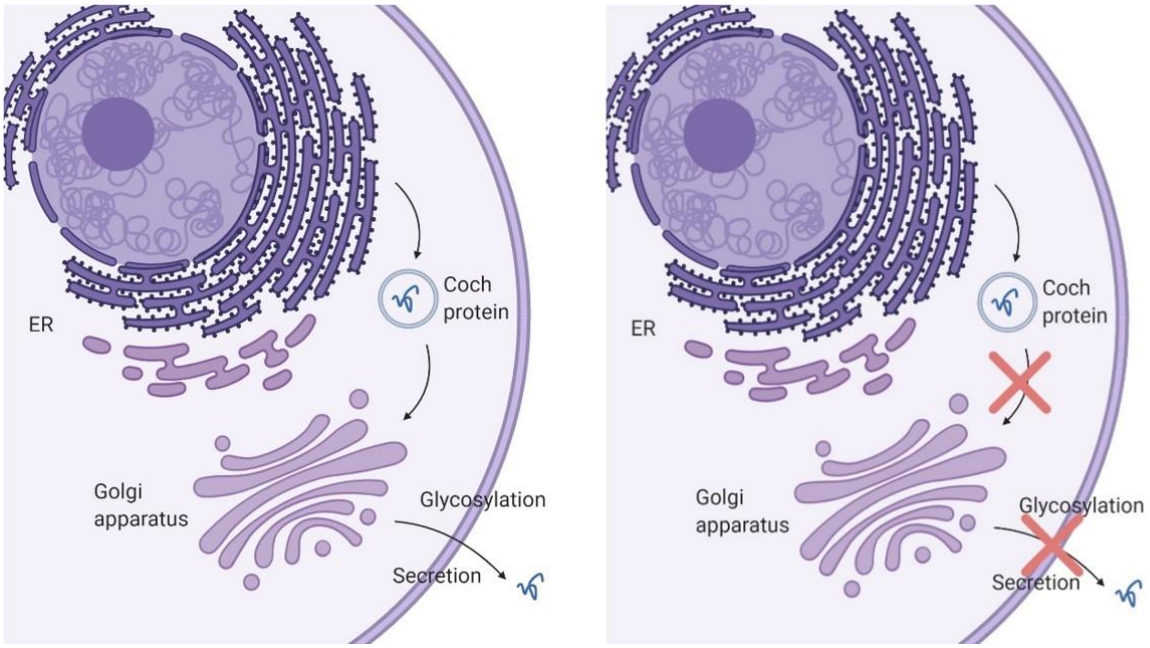

Figure 4. In the left panel the intracellular trafficking and secretion of normal COCH protein is illustrated. In the right panel the intracellular trafficking and secretion of mutant COCH protein with mutations in the vWFA domains is illustrated.

The p.V104del, p.I109T, p.F121S, p.C126Y, p.A487P, p.F527C, p.C542F and p.C542Y mutant cochlin proteins are found to accumulate intracellularly and were completely absent in the culture media of transfected cells. This finding, based on overexpressing studies, indicates that these mutant cochlins are not secreted.[15]

In contrast, the p.G87W, p.P89H and p.A119T mutations, located in the LCCL domain, were detected in culture media similar to wildtype cochlin.[15] The mutations located in the LCCL domain that were not detected in the culture media additionally formed larger-sized cochlin aggregates of 130 kDa, which is representative for a dimeric appearance. The cochlins affected by mutations in the vWFA domains were detected as multimeric cochlins (>130 kDa). These large-sized cochlins are believed to be the result of the presence of unpaired cysteines supporting aggregation through disulfide bonds.[15, 17] The p.I109T mutation, located in the LCCL domain, creates an additional N-glycosylation linked consensus site resulting a slightly larger cochlin compared to the other cochlins.[15, 17, 26]

As described above, mutations in the LCCL domain do not affect intracellular protein trafficking, but instead lead to protein misfolding. Mutant proteins have been observed to form stable dimers and oligomers in vitro. [15, 17] Moreover, their presence can stabilize wildtype cochlin and lead to its non-natural oligomerization.[27] The accumulation of these aggregates over a longer time course will eventually lead to the typical pathology observed in DFNA9 patients.[17, 28] Mutations in the *COCH* gene act via a mechanism of non-haploinsufficiency, i.e. only one normal allele is needed to enable normal development of the cochlea and is sufficient for (maintenance of) normal hearing.[11] Two findings are highly relevant and support this hypothesis: (1) heterozygous *Coch*$^{+/-}$ mice do not exhibit sensorineural hearing loss [29] and (2) human carriers of one *COCH* null allele without sensorineural hearing loss (i.e. a nonsense variant in a consanguineous family) have been identified.[21]

Mutations in the COCH gene can cause misfolding and conformational changes in the Coch protein which may hid the binding site for aggrecanase 1, making them less susceptible for cleavage.[6,7] One study investigated cleavage of the LCCL domain in the presence of the p.P51S, p.V66G, p.G88E, p.I109T, p.W117R, p.V123E and p.C162Y mutations. Cleavage of the LCCL domain by aggrecanase 1, in response to inflammation of the inner ear, was impaired in all mutations.[17] Impaired cleavage of the LCCL domain in response to inflammation could cause an accumulation of endotoxin in the inner ear over several decades. Accumulation of these endotoxins could slowly destroy the cochlea and other structures, eventually contributing to the hearing loss seen in DFNA9 patients.[17]

PHENOTYPE OF THE DIFFERENT PATHOGENIC VARIANTS IN THE COCH GENE

An overview of the different *COCH* mutations and their associated phenotypic appearance is summarized in Table 3.

The p.V66G, p.A119T, p.F121S, p. C162Y, p.I399_a404del, p.A487P, p.F527C, p.C542F and p.C542Y mutations have an earlier age of onset (average 2nd-3th decade) than usually observed in DFNA9 patients where the normal age of onset is around the 4th-5th decade of life.[4, 5, 14, 28, 30, 31, 32] All these mutant cochlins, except for those with the p.V66G, p.F121S, A119T, p.I399_A404del and

p.C542F mutations, are known to form high-molecular-weights aggregates in cells.[4, 5, 14, 28, 30, 31,32] The formation of these aggregates in combination with a failure to secrete cochlin results in an earlier onset of hearing loss in DFNA9 patients with vWFA domain mutations.[15] Although in general the amount of accumulated mutant cochlin is negatively correlated with the age of onset, the presence of other causative or environmental factors cannot be excluded as one individual has been reported with a mutation in the LCCL domain, P89H, displaying unilateral congenital hearing loss.[33] Furthermore, it is observed that in patients carrying mutations in the LCCL domain the sensorineural progressive hearing loss is accompanied by vestibular dysfunction, while mutations in the vWFA domains predominantly cause sensorineural hearing loss.[15, 17]

Comparison to mouse models

In human DFNA9 patients, pathological variants in the *COCH* gene are generally found in heterozygous form (except for some homozygous carriers of the p.P51S variant in Belgium) who have an earlier age of onset[34]). The mouse model carrying the heterozygous mutation ($Coch^{G88E/+}$) as well as the homozygous mutation ($Coch^{G88E/G88E}$) were developed and presented with a different phenotype.[3] In $Coch^{G88E/G88E}$ mice, vestibular function was impaired, starting at the age of 7 months, when compared to $Coch^{G88E/+}$ and $Coch^{+/+}$ mice. In contrast, hearing thresholds of $Coch^{G88E/+}$ and $Coch^{G88E/G88E}$ mice were significantly elevated compared to $Coch^{+/+}$ mice at 21 months old.[3, 29]

Additionally, a mouse model knockout for the Coch protein was developed. In patients, there are a few loss-of-function mutations in the COCH gene leading to functional *COCH* knockouts, causing DFNB110. These patients suffer from congenital moderate sensorineural hearing loss.[19,21] In contrast to the human situation, the mouse knockout for the *Coch* gene only has elevated ABR thresholds at the age of 21 months at the highest frequency tested (41.2 kHz). Mice with one functional allele express hearing thresholds similar to wildtype littermates. $Coch^{-/-}$ mice express vestibular dysfunction starting from the age of 9 months while $Coch^{+/-}$ mice remain unaffected.[29]

HISTOPATHOLOGY OF THE DIFFERENT COCH MUTATIONS CAUSING DFNA9

An overview of the histopathological consequences observed in different *COCH* mutations is summarized in Table 4.

In the middle ear of human DFNA9 affected temporal bones carrying the V66G mutation, aggregates are present in both the incudomalleal and incudostapedial joints as well as in the lamina propria layer of the pars tensa of the tympanic membrane.[12] Another study investigating the histopathology in the middle ear of an L114P carrier, observed the same pathology, i.e. the deposition of a mixture of basophilic and eosinophilic amorphous material in the incudomallear and incudostapedial joint and near the umbo of the TM.[9]

For the p.L114P, p.V66G, p.G88E, p.P51S and p.W177R mutations, the temporal bones of affected individuals were examined to investigate pathology in the vestibular system and the cochlea.[9, 10, 12, 35]

In the vestibular system of all affected individuals there was diffuse, severe degeneration of the stroma of all maculae and cristae with marked loss of cellularity and replacement by an eosinophilic, acidophilic homogenous ground substance. Moreover, there was a mild to severe loss of hair cells in all cristae and maculae as well as a loss of 50% of Scarpa ganglion cells. The eosinophilic deposition is also seen in the cribose area of the vestibular nerve.[9, 10, 12, 35]

One study found a thickening of the walls of the vestibular membranous labyrinth and perilymphatic trabecular meshwork. Furthermore, there was formation of new bone and fibrous tissue within a semicircular canal, and a collapse and distortion of the walls of the membranous labyrinth.[9]

In the cochlea there is a widespread a diffuse degeneration of the spiral ligament, spiral osseous and the spiral limbus, with marked loss of cellularity and replacement by an eosinophilic, uniformly staining.[35] The deposits are particularly prominent in the more medial parts of the ligament underlying the stria vascularis and in the area of the insertion of the ligament into the basilar

membrane.[10] The stria vascularis is generally preserved as well as the tectorial membrane and the Reissner membrane. Investigation of the temporal bones of an individual carrying the V66G mutation revealed a change in the supportive structure of the pathological spiral ligament compared to the normal spiral ligament characterized by a marked excess of a branched microfibrillar/filamentous structure that fills up the extracellular spaces and the absence of major fibrillar type II collagen bundles.[31] Throughout the organ of Corti there is a variable loss of inner and outer hair cells. A severe and diffuse loss of the peripheral processes of the spiral ganglion cells has been observed while the cell bodies of the spiral ganglion show a variable degree of loss. The loss of spiral ganglion cells seems to be most prominent in the basal turn of the cochlea.[10, 12, 31, 35] The typical DFNA9 histopathology seen in the temporal bones of a patient carrying the L114P mutation is illustrated in Figure 5.[9]

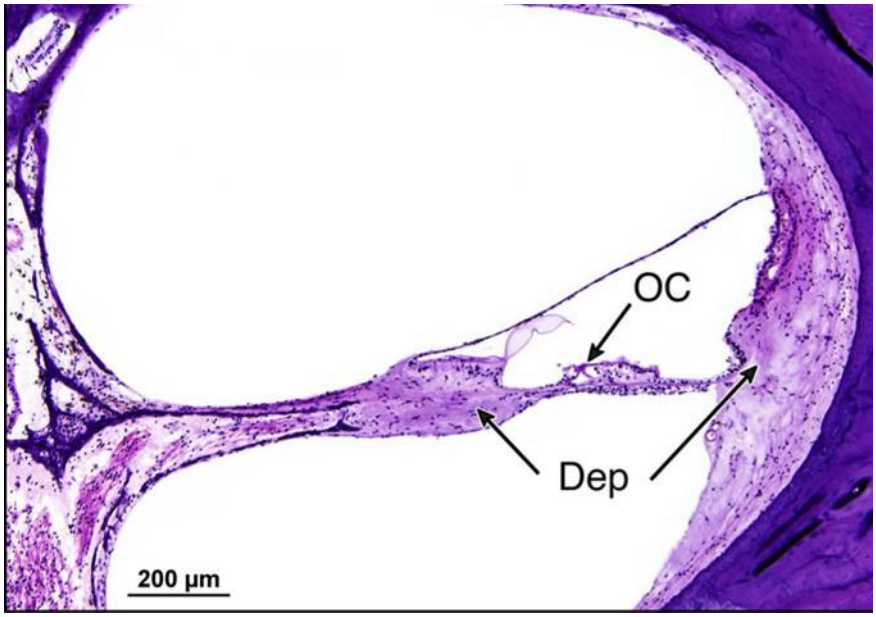

Figure 5. Histopathology seen in the temporal bone of an DFNA9 affected individual. An amorphous deposit (Dep) is noted in the spiral ligament (SL), distal end of the osseous spiral lamina (OSL), and at the base of the limbus (L). This figure is reproduced from Burgess et al.[9]

Comparison to mouse models

In mice, histopathology of *Coch*$^{G88E/G88E}$ mice (at one year, 21 months and 26 months old) revealed some deterioration of type IV fibrocytes of the spiral ligament but this was also observed in the wildtype littermates. Furthermore, this mouse model did not demonstrate the eosinophilic deposits characteristic of DFNA9.[3,29] Histopathology of *Coch*$^{-/-}$ mice sacrificed at 21 months of age did not reveal any significant histological changes: neither hair cell loss, nor obvious changes in morphology of accessory structures of the cochlear duct, including the spiral ligament, stria vascularis, limbus or tectorial membrane. Also, no fibrillar deposits where observed.[29]

Another study studied the middle ears from 12 months old *Coch*$^{+/+}$, *Coch*$^{-/-}$ and *Coch*$^{G88E/G88E}$ mice.[12] It was revealed that acellular deposits are present in both the incudomallear and incudostapedial joints in *Coch*$^{G88E/G88E}$ middle ears similar to the pathology observed in human DFNA9-affected temporal bones. There is, however, a difference in appearance as in human DFNA9 affected bones a mixture of eosinophilic and basophilic deposits is present while in the *Coch*$^{G88E/G88E}$ mouse model deposits appear to have only eosinophilic staining.[9, 10, 12, 31, 35]

DIFFERENCES BETWEEN HUMAN AND MICE DFNA9 HISTOPATHOLOGY

In the temporal bones of patients carrying pathological mutations in the *COCH* gene causative for DFNA9, a loss of cellularity in the spiral ligament, spiral osseous and spiral limbus is typically observed.[9, 10, 12, 31, 35] In these regions, there is a deposit of eosinophilic aggregates. Some loss of inner and outer hair cell throughout the organ of Corti has been observed as well as a loss of nearly 50% of spiral ganglion cells and dendritic fibers. In the vestibular organ atrophy of the neuroepithelium of the cristae, sacculi and maculae has been demonstrated. Similar to what has been identified in the cochlea, eosinophilic deposits are found in the same regions and there is loss of hair cells and Scarpa neurons.[9, 10, 12, 31, 35] However, in the $Coch^{G88E/G88E}$ mouse model, this typical DFNA9 pathology is not present in the inner ear of 12-, 21- and 26-month-old mice despite sever impairment of hearing and vestibular function at the oldest ages.[3,29] In contrast, the pathology found in the middle ear of human patients carrying the V66G mutations and 12-month-old $Coch^{G88E/G88E}$ mice is highly similar.[12] Absence of eosinophilic deposits in the inner ear of $Coch^{G88E/G88E}$ mice at the same age when middle ear pathology is present suggests a difference in onset and progression of deposit formation in the middle and inner ear. In the $Coch^{-/-}$ mouse model *Coch* staining and pathology is completely absent supporting a dominant negative effect involved in DFNA9 pathology.[12]

Yao et al. discovered that when mutant cochlin was injected into the murine inner ear, mice were nearly deaf one week after injection although some of them recovered slightly 4 weeks after injection.[31] They observed changes in the thickness of the stria vascularis in mice injected with mutant cochlin compared to mice injected with wildtype cochlin. In the spiral ligament an extensive loss of fibrocytes type I to IV was observed after a single injection of mutant cochlin but fibrocytes in the spiral limbus were largely spared.[26] The pathology observed after injection of mutant cochlin in the inner ear is similar to the pathology found in DFNA9 patients.[9, 10, 12, 31, 35] Absence of this pathology in $Coch^{G88E/G88E}$ mice, even at 26 months of age, suggests that the pathology found in DFNA9

is the result of an accumulation of mutant protein over a longer time course and this disease progression seems to be slower in mice compared to humans.[3]

CONCLUSION

In this review we compared the intracellular processes, different phenotypes and histopathology observed in different pathological variants in the *COCH* gene causing DFNA9 and DFNB110. Mutations in the LCCL domain of the *COCH* gene will not affect the intracellular trafficking and secretion but will lead to misfolding of the LCCL domain. Mutations in this domain give rise to progressive sensorineural hearing loss associated with vestibular dysfunction. In contrast, mutations located in the vWFA domains will cause an earlier onset of disease due to their effect on intracellular trafficking and secretion of cochlin leading to intracellular aggregate formation. Hearing and vestibular function were impaired in $Coch^{G88E/G88E}$ mice similar to the phenotype observed in DFNA9 patients. Although there is a clear difference in phenotype between mutations localized in the LCCL domain and the vWFA domains, it is important to notice that DFNA9 patients carrying the same mutations can have different phenotypes indicating that our understanding of genetic diseases in incompletely understood. Examination of DFNA9 affected temporal bones revealed atrophy of the regions were *COCH* expression is abundant, in addition to deposits of an eosinophilic substance observed in these regions. In the $Coch^{G88E/G88E}$ mice this pathology was already observed in the middle ear at 12 months of age but was still absent in the inner ear at 26 months of age, suggesting a slower progression. Absence of the pathology in $Coch^{-/-}$ mice suggests that a dominant negative mechanism is involved in DFNA9 pathology.

Table 2. Overview of mutations causing DFNA9 and their effect on intracellular and post-translational processes.

Reference	Domain	Mutation	Intracellular trafficking	Secretion	Protein misfolding	N-linked glycosylation	Post-translational cleaving
(Choi et al., 2013)	LCCL	G38D	N/A	N/A	N/A	N/A	N/A
(de Kok et al., 1999) (Fransen et al, 2001)	LCCL	P51S	Golgi and ER	Normal	Misfolding, dimers	Normal	Impaired
(Khetarpal, 2000)	LCCL	V66G	Golgi and ER	Normal	Misfolding, dimers	Normal	Impaired
(Chen et al., 2013)	LCCL	G87V	N/A	N/A	N/A	N/A	N/A
(Bae et al., 2014) (Collin et al., 2006)	LCCL	G87W	Golgi and ER	Normal	Misfolding	Normal	N/A
(Kemperman et al., 2005)	LCCL	G88E	Golgi and ER	Normal	Misfolding, dimers	Normal	Impaired
(Bae et al., 2014), (Dodson et al., 2012)	LCCL	P89H	Golgi and ER	Normal	Normal	Normal	N/A
(Bae et al., 2014), (Nagy et al., 2004)	LCCL	V104del	Golgi and ER	Not secreted	Misfolding, dimers	Normal	N/A
(Kamarinos et al., 2001)	LCCL	I109N	N/A	Normal	N/A	N/A	N/A
(Bae et al., 2014), (Pauw et al., 2007)	LCCL	I109T	Golgi and ER	Not secreted	Misfolding, dimers	Extra N-glycosylation site	Impaired
(Robertson et al., 1998)	LCCL	L114P	N/A	N/A	N/A	N/A	N/A
(Robertson et al., 1998), (Liepinsh et al., 2001)	LCCL	W117R	Golgi and ER	Normal	No misfolding	N/A	Impaired
(Bae et al., 2014), (Usami et al., 2003)	LCCL	A119T	Golgi and ER	Normal	No misfolding	Normal	N/A
(Bae et al., 2014), (Hildebrand et al., 2010)	LCCL	F121S	Golgi and ER	Not secreted	Misfolding, dimers	Normal	N/A
(Jung et al., 2015)	LCCL	V123E	Golgi and ER	Normal	No misfolding	Normal	Impaired
(Bae et al., 2014), (Gao et al., 2013)	vWFA1	C162Y	ER not Golgi	Impaired	Aggregates	Normal	Impaired
(Gallant et al., 2013)	vWFA2	I399_A404del	N/A	N/A	No aggregates	N/A	N/A
(Bae et al., 2014), (Street et al., 2005)	vWFA2	A487P	N/A	Not secreted	Aggregates	Normal	N/A
(Yuan et al., 2008)	vWFA2	M512T	N/A	N/A	No aggregates	N/A	N/A
(Cho et al., 2012)	vWFA2	F527C	Golgi and ER	Impaired	Aggregates	N/A	N/A
(Street et al., 2005)	vWFA2	C542F	Golgi and ER	Normal	N/A	N/A	N/A
(Yuan et al., 2008)	vWFA2	C542Y	N/A	Not secreted	Aggregates	N/A	N/A

Table 3. Overview of mutations causing DFNA9 and their phenotype. Mouse mutations are indicated in grey.

Reference	Species	Domain	Mutation	Ethnicity	Age of onset hearing loss	Vestibular disorder	Tinnitus
(Choi et al., 2013)	Human	LCCL	G38D	Korean	N/A	N/A	No
(de Kok et al., 1999) (Fransen et al, 2001)	Human	LCCL	P51S	Dutch	4th to 6th decade	Instability	No
(Khetarpal, 2000)	Human	LCCL	V66G	USA	2nd decade	Ataxic gait	No
(Chen et al., 2013)	Human	LCCL	G87V	Chinese	4th decade	Vertigo, tendency to fall	Yes
(Collin et al., 2006)	Human	LCCL	G87W	Dutch	4th decade	Instability, vertigo, tendency to fall	No
(Kemperman et al., 2005)	Human	LCCL	G88E	Dutch	4th to 6th decade	Instability, vertigo, tendency to fall	No
(Jones et al., 2011) (Robertson et al., 2008)	Mouse	LCCL	*Coch*$^{G88E/+}$	/	21 months	None	N/A
(Jones et al., 2011), (Robertson et al., 2008)	Mouse	LCCL	*Coch*$^{G88E/G88E}$	/	21 months	Elevated VesP thresholds starting from 7 months	N/A
(Dodson et al., 2012)	Human	LCCL	P89H	USA	Congenital	N/A	No
(Nagy et al., 2004)	Human	LCCL	V104del	Hungarian	4th decade	Severe vertigo, nausea, vomiting	No
(Kamarinos et al., 2001)	Human	LCCL	I109N	Australian	4th to 5th decade	Unsteady, unable to walk in the dark	No
(Pauw et al., 2007)	Human	LCCL	I109T	Dutch	4th decade	Instability, vertigo, tendency to fall	No
(Burgess et al., 2016)	Human	LCCL	L114P	Korean	N/A	N/A	No
(Robertson et al., 1998)	Human	LCCL	W117R	USA, Korean	3th to 5th decade	none	No
(Usami et al., 2003)	Human	LCCL	A119T	Japanese	3th decade	Recurrent dizziness/vertigo	No
(Hildebrand et al., 2010)	Human	LCCL	F121S	USA	2nd to 3th decade	Balance problems, vertigo, positional nystagmus, dizziness	Yes
(Jung et al., 2015)	Human	LCCL	V123E	Korean	4th to 5th decade	None	No
(Gao et al., 2013)	Human	vWFA1	C162Y	Chinese	2nd decade	None	No
(Gallant et al., 2013)	Human	vWFA2	I399_A404del	USA	3th decade	None	Yes
(Street et al., 2005)	Human	vWFA2	A487P	Italian	2nd decade	N/A	No
(Yuan et al., 2008)	Human	vWFA2	M512T	Chinese	4th decade	None	No
(Cho et al., 2012)	Human	vWFA2	F527C	Korean	3th decade	None	No
(Street et al., 2005)	Human	vWFA2	C542F	USA	2nd decade	None	No
(Yuan et al., 2008)	Human	vWFA2	C542Y	Chinese	2nd to 5th decade	None	Yes

(Jones et al., 2011), (Makishima et al., 2005)	Mouse	Knockout	*Coch*$^{-/-}$	/	21 months	Elevated VsEP thresholds starting at 13 months	N/A
(Jones et al., 2011), (Makishima et al., 2005)	Mouse	One functional allele	*Coch*$^{+/-}$	/	No hearing loss	Elevated VsEP thresholds starting at 21 months	N/A

VsEP, vestibular sensory-evoked potential

Table 4. Overview of histopathological findings observed in different mutations causing DFNA9. Mouse mutations are indicated in grey.

Reference	**Species**	**Mutation**	**Histopathology vestibular system**	**Histopathology inner ear**
(Wang et al., 2016)	Human	L114P	Atrophy of neuroepithelium semi-circular canals, maculi sacculi and utriculi Amorphous deposit in mesenchymal tissue between cribose area and neuro-epithelium Loss of 50% of Scarpa ganglion cells, degeneration of distal vestibular dendritic fibres	Acellularity of spiral ligament, distal end of osseous spiral lamina and base of spiral limbus 50% loss of SGN, some loss of IHC and OHC Deposit of amorphous deposits in spiral ligament, spiral osseous and limbus
(Khetarpal et al., 1991) **(Burgess et al., 2016)**	Human	G88E	Loss of cellularity of the stroma of maculae and cristae and replacement by an eosinophilic, acidophilic homogeneous substance severe loss of sensory hair cells, 50% loss of Scarpa ganglions, severe loss of dendritic fibers Otolithic membranes show an irregular granular degenerative change with basophilic staining deposits	Widespread and diffuse degeneration of the spiral ligament and the spiral limbus and replacement by an eosinophilic, uniformly staining, acellular material Variable losses of IHC and OHC, severe loss of peripheral processes of SGN, loss of SGN.
(Robertson et al., 2008)	Mouse	*Coch*$^{G88E/G88E}$	At 21 months and 26 months old, no obvious deterioration of cochlear and vestibular sensory epithelia is observed	Loss of fibrocytes same as in wildtypes"' At 21 month and 26 months old, no obvious deterioration of cochlear and vestibular sensory epithelia is observed, no eosinophilic deposits
(Robertson et al., 2006)	Human	P51S	Abundant eosinophilic deposition present in ampullary stroma with reduction and atrophy of stromal fibrocytes degeneration of sensory epithelium of the crista, atrophy of ampullary nerve	Abundant extracellular eosinophilic aggregates throughout the spiral ligament, spiral limbus and osseous spiral lamina Loss of fibrocytes in spiral ligament and spiral limbus Degeneration of organ of Corti and neural processes in osseous spiral lamina
(Khetarpal et al., 1991) (Burgess et al., 2016) (Khetarpal, 2000)	Human	V66G	Acellularity of stromata of the maculae and cristae Eosinophilic acidophilic deposits in stroma of cristae and maculae. Also, in cribrose area of vestibular nerve Severe loss of hair cells in all cristae and maculae, 50% loss of vestibular neurons	Acidophilic homogeneous deposits were noted in the spiral ligaments, limbi, and the spiral laminae There is a severe loss of fibrocytes of the limbus and spiral ligament. Scattered loss of outer hair cells is apparent, the stria vascularisis normal. Loss of SGN, total atrophy of cochlear dendrites in the basal turn and about 60% in the middle and apical turns were noted A marked excess of a branched microfibrillar/filamentous structure that fills up the extracellular spaces of the spiral ligament was observed absence of major fibrillar type II collagen bundles.
(Burgess et al., 2016)	Human	W117R	Loss of cellularity of the stroma of maculae and cristae and replacement by an eosinophilic, acidophilic homogeneous substance loss of Scarpa ganglions, loss of hair cells in all vestibular end organs	Severe loss of cellularity of the spiral ligament and spiral limbus with replacement by an eosinophilic acellular material. Stria vascularis shows partial atrophy Loss of hair cells, severe loss of nerve fibres in the osseous spiral lamina
(Jones et al., 2011)	Mouse	*Coch*$^{-/-}$	N/A	At 21 months of age: No hair cell loss, no obvious changes in morphology of accessory structures of the cochlear duct, including spiral ligament, stria vascularis, limbus or tectorial membrane No fibrillar deposits characteristic of DFNA9

IHC, inner hair cells; OHC, outer hair cells; SGN, spiral ganglion neuron.

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