

ONLINE MUTATION REPORT

A novel *COCH* mutation, V104del, impairs folding of the LCCL domain of cochlin and causes progressive hearing loss

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Recently the causative gene of autosomal dominant sensorineural nonsyndromic late onset hearing loss (DFNA9) has been identified as the *COCH* gene, which lies in the DFNA9 region of human chromosome 14 (gene map locus 14q12–q13). Molecular analysis of cases of DFNA9 have identified several families with five different mutations in this gene.^{1–5}

The cochlin protein encoded by *COCH* is an extracellular matrix protein that contains an LCCL domain and two von Willebrand type A domains.¹ Interestingly, all mutations causing DFNA9 type deafness disorder affect the LCCL domain of cochlin. In view of the central role of this domain in the DFNA9 disorder, we initiated studies on it to explore the molecular basis of the disease.⁶ We found that most DFNA9 mutations affected conserved structural elements of the LCCL fold and disrupted the proper folding of this domain; recombinant mutant LCCL domains expressed in *Escherichia coli* failed to adopt the wild type fold and instead formed insoluble aggregates.⁷ It is noteworthy that insoluble deposits are detected in temporal bone sections of individuals affected by DFNA9.⁸ These results support the notion that the DFNA9 mutations may act through a gain of function mechanism; it is the presence of the abnormal protein that causes the disease. According to this view, accumulation of deposits in vestibular and cochlear nerve channels leads to strangulation and progressive degeneration of the dendrites, and loss of cochlear and vestibular neurones.

Our present investigation was aimed at identifying novel mutations affecting the LCCL domain of cochlin in order to gain more insight into the pathomechanism of DFNA9. Hungarian patients selected according to the diagnostic criteria of DFNA9 were screened for the presence of mutations affecting the LCCL domain. These studies identified one person heterozygous for a novel mutation in the coding region of *COCH*. The mutation has resulted in the deletion of Val104, a residue conserved in the human, mouse, and chicken cochlin sequences. It affects a critical β strand of the LCCL domain and was found to prevent refolding of recombinant LCCL domain expressed in *E. coli*.

MATERIALS AND METHODS

Selection of patients and gene analysis

Patients were selected from the cochlear implantation list of the Department of Otorhinolaryngology of Semmelweis University according to diagnostic criteria of the DFNA9 disorder (OMIM #603196): progressive hearing loss associated with vestibular dysfunction, the age of onset (20–56 years) and probable familial origin. In the selected patients, audiometrically documented hearing loss initially affected only higher frequencies with later progression to lower frequencies. Many patients reported episodes of vertigo.

DNA from 17 hearing impaired persons from 14 different families was used for genetic analysis, and compared with DNA from 50 unrelated controls. A DNA sample was also

Key points

- Autosomal dominant nonsyndromic sensorineural deafness 9 (DFNA9, OMIM #601369) is a rare, late onset hearing loss. In DFNA9 patients, hearing loss usually begins in the third decade of life as a high frequency loss with concomitant vestibular dysfunction.
- DFNA9 is caused by mutations in the *COCH* gene, which encodes cochlin, an extracellular matrix protein that contains an LCCL domain and two von Willebrand type A domains.
- Molecular analysis of cases of DFNA9 has identified five different mutations in this gene. Interestingly, all mutations causing DFNA9-type deafness disorder affect the LCCL domain of cochlin.
- Here we describe a novel *COCH* mutation in a Hungarian patient, which results in the deletion of Val104, a residue conserved in the human, mouse, and chicken cochlin sequences. The deletion affects a critical β strand of the LCCL domain and was found to prevent refolding of a recombinant LCCL domain expressed in *Escherichia coli*.

collected from a relative of the proband identified in the present study. Genomic DNA (20 ng) was isolated from peripheral blood cells using the Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN, USA).

Mutation screening was performed only for exons 4 and 5 of *COCH* (NM 004086), which encode the LCCL domain of the protein. Intronic PCR primers (sense: 5'-CCACTATGCCCC AAGAAGTCTAAGAATGC-3', antisense: 5'-GGGTGGGAGA ATATTACAGAATGAGAAC-3') were used to amplify the genomic region encompassing exon 4, intron 4, and exon 5 of *COCH*. PCR was performed with an initial denaturation step for 2 minutes at 95°C followed by 35 cycles of 94°C for 30 seconds, 65°C for 30 seconds and 68°C for 2 minutes. Amplification products were purified using the NucleoSpin Extract Kit (Macherey-Nagel Gmb, Düren, Germany).

Dideoxy sequencing of the PCR products for exons 4 and 5 was carried out using the primers 5'-CGTCTGCATT TCTCTCCACCCTGGTG-3', flanking exon 4, and 5'-TGA AAAAGTGTGGATAGCATCTCAGCTGC-3' flanking exon 5. To verify the sequence of the genomic DNA of the patient carrying a mutation in exon 5 of *COCH*, PCR primers (sense: 5'-CTGAAAAAGTGTGGATAGCATCTCAGCTGC-3', antisense: 5'-GGGTGGGAGAATATTACAGAATGAGAAC-3') were used to amplify only the affected exon 5. The PCR product was blunt end ligated into *Sma*I digested M13mp19 vector, and phages carrying the mutant or wild type alleles were sequenced. Restriction enzymes and the M13 sequencing kit used for dideoxy sequencing of cloned DNA were

Table 1 Comparison of the V104del mutation with other DFNA9 mutations

Mutation	Genotype	Age of onset	Refolding of LCCL domain
V104del*	Heterozygous	4th decade	Impaired*
I109N ⁵	Heterozygous	2nd–3rd decade	–
V66G ²	Heterozygous	2nd–3rd decade	Impaired ⁷
G88E ²	Heterozygous	5th decade	Impaired ⁷
W117R ²	Heterozygous	5th decade	Preserved ⁷
P51S ³	Heterozygous	4th decade	Impaired ⁷

*Present work.

problem and her genetic analysis did not identify a mutation in the LCCL domain of COCH. Although the possibility that the proband's father carried the mutation cannot be ruled out, it is also possible that the mutation arose de novo.

In order to examine the effect of the mutation on the structure of the LCCL domain we expressed the mutant domain using the same protocol as for the wild type protein.⁶ Whereas in the case of the wild type recombinant LCCL domain, this protocol yields a soluble protein that adopts a unique three dimensional structure,⁷ the same refolding protocol did not yield any soluble protein for the V104del mutant LCCL domain, and dialysis led to complete precipitation of the recombinant protein. As the deletion of Val104 affects a conserved residue of the β 6 strand structure of the LCCL domain (figs 1 and 2), it seems likely that this deletion affects this essential structural element of the LCCL fold, impairing its ability to form the native structure. In this respect, the V104del mutation is similar to most other DFNA9 causing mutations identified so far, which also impair refolding of recombinant LCCL domain (table 1).

In our opinion, this novel mutation provides additional support for the notion that the characteristic eosinophilic deposits in DFNA9 affected inner ear structures could be the result of accumulation and aggregation of aberrant, mutated cochlins. Recent studies have indeed shown that DFNA9 mutations do not impair secretion and post-translational processing of cochlin produced by mammalian cells.⁹ It seems likely that in vivo the mutations may lead to a slow aggregation of cochlin over a longer time course, consistent with the late onset and progressive nature of this disorder.

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