Sequencing of exons 4, 5, 12 of COCH gene in patients with postlingual sensorineural hearing loss accompanied by vestibular lesion

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Abstract

Introduction: Mutations at the DFNA9 locus on chromosome 14q12 are the third most common form of DFNA hearing loss, which is clinically characterized by late onset (in adulthood) progressive sensorineural hearing loss accompanied by vestibular dysfunction. The aim of the study was to search for COCH gene mutations (P51S, V66G, G87W, G88E, V104del, I109N, W117R, A119T, M512T, C542Y) in patients with severe or profound sensorineural hearing loss accompanied by a vestibular lesion.
Material and methods: The study was based on a group of 30 patients. Qualification criteria comprised the presence of progressive postlingual, severe to profound sensorineural hearing loss with tinnitus, early age of sensorineural hearing loss onset, before the 40th year of life, and a positive family history of early onset hearing loss. All patients were diagnosed with peripheral vestibular lesions.
Results: The authors did not find P51S, V66G, G87W, G88E, V104del, I109N, W117R, A119T, M512T, or C542Y mutations in the COCH gene in the tested group (no differences were found in the nucleotide sequences of exomes 4, 5 and 12 when compared to the published cDNA sequence of the COCH gene).
Conclusions: No cochlin mutations were found in the group of patients with severe to profound sensorineural hearing impairment accompanied by a vestibular lesion. The COCH gene needs further exploration and analysis of genotype-phenotype correlations.

Key words: postlingual sensorineural hearing loss, vestibular lesion, COCH gene.

Introduction

Hearing loss is the most common sensory deficit and human communication disorder, with around half of patients over 60 years of age experiencing hearing loss greater than 25 dB [1]. Age-related epidemiological research on the genetic contribution to hearing impairment is not available. If positive family history is present, the condition can be attributed to genetic causes. The condition is genetically heterogeneous, with the most common distinction in hereditary hearing loss being syndromic versus nonsyndromic, which in 80% of cases is inherited in an autosomal recessive mode (DFNB loci) and in 20% in an autosomal dominant mode (DFNA loci) [2].

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Mutations at the DFNA9 locus on chromosome 14q12 are the third most common form of DFNA hearing loss, which is clinically characterized by late onset (in adulthood) progressive sensorineural hearing loss accompanied by vestibular dysfunction [3]. The coagulation factor C homology gene (COCH) is probably the only human gene known to cause DFNA hearing loss associated with vestibular disorders. COCH mutation is rare, and only 21 examples of mutations have been reported so far [4]. Mutations at the DFNA9 locus result in alterations in the protein cochlin: the product of the COCH gene. Cochlin is a major non-collagen component of the inner ear extracellular matrix which constitutes up to 70% of the inner ear proteins [5]. Apart from the inner ear, cochlin mRNA has been found in the eye, cerebellum, spleen, lung, brain and thymus [6]. Mutated forms of cochlin have been found to interfere with disulfide bonds and correct protein folding, resulting in disturbances affecting substantial cochlear functions [7, 8]. Most reported mutations have been found within the LCCL domain (exons 4 and 5); however, recent reports describe mutations in exon 12 in the vWFA domain. Histopathological examinations of temporal bones from DFNA9 patients have revealed the presence of large amounts of acellular eosinophilic deposits (mucopolysaccharide) in the cochlear and vestibular nerve channels, the supporting structures of the organ of Corti, including the limbus, and the spiral structures: the lamina and ligament [9, 10]. Robertson *et al.* suggest that hearing loss in DFNA9 patients is due to obstruction of the nerve channels resulting in neuronal damage (gain-of-function mechanism) [11]. In addition, the accumulation of mutated cochlins over a long period is consistent with the late onset and progressive nature of DFNA9 hearing loss. There are some reports on histopathologically confirmed Ménière's disease (endolymphatic hydrops) in DFNA9 patients [12].

The aim of the study was to search for COCH gene mutations (P51S, V66G, G87W, G88E, V104del, I109N, W117R, A119T, M512T, C542Y) in patients with severe or profound sensorineural hearing loss accompanied by vestibular lesions; and furthermore, to identify phenotypes that would qualify for COCH gene screening.

Material and methods

The study was based on a group of 30 patients diagnosed in the Department of Otolaryngology, Laryngological Oncology, Audiology and Phoniatrics for severe or profound sensorineural hearing loss accompanied by tinnitus and vertigo (peripheral vestibular lesion). The group comprised 17 females and 13 males with a mean age of 53.2 years, ranging from 24 to 73. All subjects gave their informed consent to be included in the study. The qualification criteria comprised the presence of progressive postlingual sensorineural hearing loss with tinnitus, an early age of sensorineural hearing loss onset, before the 40th year of life, and a positive family history of early onset hearing loss. As well as a blood sample (3 ml) being collected from all patients, a bank of clinical data was collected: age of hearing loss onset, family history, previous medical history of noise or ototoxic drug exposure. Patients with a positive history of a risk factor for hearing loss (e.g. noise exposure, ototoxic drug intake, severe head traumas) were disqualified from the study.

Blood samples were processed at the Central Scientific Laboratory, Medical University of Lodz. The Gene Matrix Quick Blood DNA Purification Kit (Eurx) was used to process the blood samples according to the protocol provided by the manufacturer. The primers used for exon 4 were forward: 5′ATCTGGAATGGTATGGAAGGGTAT and reverse 5′-TAAAGCAGGAAAGGAAATAATCACG-3′, for exon 5 forward 5′-CTTTAGATGACTTCCCTGATGAGC-3′ and reverse 5′-ATCACAGGTTTTTCCATCAAGGTTA-3′, for exon 12 forward 5′-CACTCTTTTGCCACTCTCGTCAC-3′ and reverse 5′-GCTAAAGCATTTCAGTATTATGAGAA-3′. The cycling conditions were as follows: 1 min at 96°C, followed by 25 cycles of 10 s at 96°C, 50 s at 50°C, and 4 min at 60°C. Sequencing chemistry was conducted using Big Day Terminator V1.1 (Applied Biosystems Life Technologies).

The research was approved by the Institutional Review Board of the Medical University of Lodz (RNN/414/11/KB).

All patients gave their written, informed consent prior to inclusion in the study.

Results

Of the 30 patients, 26 had bilateral symmetrical severe to profound hearing loss and 4 demonstrated asymmetrical sensorineural hearing loss. All patients were diagnosed with peripheral vestibular lesions.

The authors did not find P51S, V66G, G87W, G88E, V104del, I109N, W117R, A119T, M512T, or C542Y mutations in the COCH gene in the tested group (no differences were found in the nucleotide sequences of exomes 4, 5 and 12 when compared to the published cDNA sequence of the COCH gene).

Discussion

Due to advances in molecular and genetic technology, more than 100 genes which lead to hearing loss when mutated have been identified [2]. Given the common ectodermal embryonic origin of the sensory inner ear structures (cochlea, semicircular

canals, the otolithic organs), it might be concluded that a gene mutation known to cause inherited hearing loss would also lead to vestibular dysfunction. The literature points to the heterogeneity of the DFNA9 patient group, which probably results from cochlin isoform heterogeneity.

Enzymatic processing, post-translational modifications and alternative splicing are possible explanations [13]. Saracyn *et al.* point to genetic and environmental factors which may play an additional role in gene expression regulatory processes [14]. However, in the present study, no tested COCH gene mutations were found in the patients with severe to profound hearing loss. None of the sequences analyzed in the patients differed from the published COCH cDNA sequences. Based on mutation analysis studies, Ligget and Stephen propose a group size of 30 individuals for the detection of a new polymorphism in a nucleotide sequence found in 5% of the general population [15]. In the group of DFNA9 patients, although the symptoms may vary, they do not seem to depend on the mutation. In the group of P51S mutation, the patients present sensorineural hearing loss and a number of additional symptoms, including recurrent episodes of vertigo, aural fullness and tinnitus, suggesting the presence of Ménière's disease. The authors, however, point to the differences in patterns of hearing loss in DFNA9 and Ménière's disease. The P51S mutation patient had a late onset progressive condition, initially manifesting as mild hearing loss at high frequencies, reaching severe to profound hearing loss across all frequencies, after around 20 years with total vestibular areflexia. Fransen *et al.* stated that in patients with Ménière's disease symptoms, a COCH mutation should be considered as one of the genetic contributing factors [12]. However, Sanchez *et al.* demonstrated that no such linkage exists in a group of 30 definite Ménière's disease patients [16]. Usami *et al.* obtained similar results in a group of 20 patients with Ménière's disease. The authors stated that for some mutations (P51S in Belgian and Dutch families, and I109N in an Australian family) the penetrance of vestibular symptoms is complete. However, they note that the American mutations V66G, G88E, and W117R may be associated with incomplete penetrance of vestibular symptoms. They suggest that penetrance of the vestibular symptoms may vary according to the mutation [17]. On the other hand, some authors point to compensatory mechanisms of the vestibular system, which may cause lack of vestibular symptoms in patients with a COCH mutation [18].

A novel mutation reported by Gallant *et al.* within the vWfA2 domain seems not to cause vertigo, as the patients did not suffer from any balance problems. Furthermore, some of the affected family members did not report tinnitus. The patients presented vertigo, resembling even Ménière's disease, when the LCCL domain was affected, but did not report balance disorders when the vWfA2 domain was involved [19].

The slow aggregation of aberrant, mutated cochlin causes progressive degeneration of neural structures of the inner ear and results in late onset, progressive hearing loss. According to the literature, the age of DFNA9 hearing loss onset ranges from the second (Manolis *et al.* 1996, approximately 20 years of age) to the fifth decade of life (Yuan *et al.*) [15]. Street *et al.* reported the youngest age of DFNA9 onset, in a 17-year-old male patient, due to mutation in the vWFA2 domain [20]. Fransen *et al.* report that symptoms of hearing loss were more severe and started earlier (at age 34) in homozygotes, due to a higher abundance of mutant protein, while heterozygotes typically had an older age of onset and less severe hearing loss. Despite complete penetrance of hearing loss across DFNA9 patients, the prevalence of vestibular symptoms seems to depend on the mutation [12]. According to Usami *et al.*, COCH mutation screening should be limited to autosomal dominant families with adult onset hearing loss combined with vestibular symptoms [17]. Gallant *et al.*, reporting a novel mutation within the vWFA2 domain in exon 11, supports the notion of COCH gene screening, but provided that it is expanded beyond exons 4, 5, and 12 [19].

The true world-wide incidence of COCH mutation is not known yet, as genetic screening for this gene is not routinely performed. Moreover, there are no established clinical features indicating a group of patients suspected of COCH gene mutations. Audiological manifestations of the deleterious function of the mutant cochlin may resemble those which appear as a result of hearing organ aging (presbyacusis). The earlier age of the hearing loss onset may then be a main distinctive factor. Further research is needed to obtain more insight into clinical presentation of DFNA9 patients. Early identification of COCH mutation cases would allow one to provide genetic counseling, as well as prompt audiological and vestibular rehabilitation. However, in a group of patients with severe or profound hearing loss, one possible management is cochlear implantation [21]. Eppsteiner *et al.* point to the need for genetic screening in cochlear implantation evaluation, in order to exclude mutations in genes expressed in the spiral ganglion (and poor performers after implantation) [22]. Furthermore, early environmental counseling (elimination of the risk factors for hearing loss) should be a main element of audiological prevention in this group of patients.

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In conclusion, no cochlin mutations were found in the group of patients with severe to profound sensorineural hearing impairment accompanied by a vestibular lesion. The COCH gene needs further exploration and analysis of genotype-phenotype correlations.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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