POSTLINGUAL SENSORINEURAL HEARING LOSS DUE TO A VERY RARE COCH PATHOGENIC VARIANT

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Abstract

Background: The COCH gene encoding cochlin is highly expressed in the inner ear but the exact physiological function of the protein still remains unknown. Pathogenic variants located in COCH cause autosomal dominant hearing loss with possible vestibular involvement.

Material and methods: A five-generation Polish family with autosomal dominant hearing loss and tinnitus was recruited for the study. Audiological and vestibular assessments were conducted and clinical exome sequencing was performed in the index patient. Next, co-segregation of the detected variant with hearing loss in the family was confirmed using Sanger sequencing.

Results: All affected individuals presented postlingual, progressive hearing loss mainly affecting high frequencies. No vestibular dysfunction was detected. In this study, we have identified a very rare COCH p.Ile374Thr pathogenic variant that segregated with the disease.

Conclusions: Our study provides an independent confirmation of the pathogenic role of COCH c.1115T>C in hearing loss. In addition to hearing loss, individuals with COCH pathogenic variants may also suffer from tinnitus and vertigo.

HIPOACUSIA NEUROSENSORIAL POSTLINGUAL CAUSADA POR UNA VARIANTE PATOGÉNICA RARA EN EL GEN COCH

Resumen

Introducción: El gen COCH que codifica la cochlina es altamente expresado en el oído interno, sin embargo, la función fisiológica exacta de la proteína codificada por este gen sigue permaneciendo desconocida. Las variantes patogénicas localizadas en el gen COCH causan hipoacusia heredada según un patrón autosómico dominante que puede afectar el sistema vestibular.

Materiales y métodos: Para el estudio se reclutó una familia polaca de cinco generaciones con una hipoacusia que sigue el patrón autosómico dominante y tinnitus. Se evaluó la función del sistema vestibular-cochlear y a partir de la muestra de ADN se llevó a cabo la secuenciación clínica del exoma. A continuación, en la familia estudiada se realizó un análisis de la segregación de las variantes genéticas detectadas con la hipoacusia.

Resultados: Todos los miembros de la familia examinada presentaron hipoacusia de tipo postlingual y progresivo, que sobre todo afectaba las altas frecuencias. No se detectaron trastornos del funcionamiento del sistema vestibular. En las pruebas genéticas, se identificó una variante patogénica muy rara p.Ile374Thr en el gen COCH, que segregaba completamente con la enfermedad.

Conclusiones: Nuestro estudio constituye una confirmación independiente del papel patogénico de la variante p.Ile374Thr en el gen COCH en el desarrollo de la hipoacusia. Aparte de la hipoacusia, los pacientes con variantes patogénicas en el gen COCH pueden también experimentar tinnitus y trastornos del equilibrio.

Palabras clave: COCH, DFNA9, hipoacusia autosómica dominante, secuenciación de segunda generación
Hearing loss (HL) is the most common birth defect affecting about 1–6/1000 newborns and the most common disability of human senses in developed countries [1]. It may be caused by a number of different factors, including genetic as well as environmental factors e.g. severe prematurity, congenital rubella, mumps or cytomegalovirus infection, severe neonatal hyperbilirubinemia, or exposure to ototoxic drugs or noise [2]. It has been estimated that genetic factors may account for almost 50% of all HL cases [3]. The genetic background of HL is complex and heterogeneous. So far, pathogenic variants in merely 100 different genes have been identified in patients with HL (www.heeditaryhearingloss.org; accessed 10/2017). The majority of cases with genetically determined HL is represented by those with an autosomal recessive (AR) mode of inheritance (80%), and this type of HL is usually prelingual and severe to profound [4].

Another important type of HL is that inherited in an autosomal dominant (AD) manner. AD HL accounts for almost 20% of HL cases, is usually progressive, and manifests itself later in life [4]. To date, 59 loci have been found to cause AD HL but only 36 genes have been described (www.heeditaryhearingloss.org; accessed 10/2017). A significant number of these genes have been identified in recent years with the use of massively parallel sequencing methods [5]. One of the genes commonly tested in patients with the dominant postlingual form of HL is COCH.
unknown [8]. The full-length COCH protein is made up of 550 amino acids and comprises 3 main domains: a LCCL domain highly homologous to factor C of Limulus, and two von Willebrand factor A (vWFA) domains [9, 10]. Pathogenic variants identified in the COCH gene are associated with AD HL (DFNA6; OMIM # 601369), with onset ranging from the 2nd to 6th decade of life [11]. At the beginning high frequencies are affected, with progression of HL slowing later in life [12]. Based on localization of the pathogenic variants to particular COCH domains, HL may be the only symptom in patients, or it may be accompanied by vestibular dysfunction and balance problems [11, 13].

In this study we have searched for the genetic cause of HL in a five-generation Polish family. We have applied a high throughput sequencing method and identified a very rare COCH pathogenic variant.

**Material and Methods**

**Patients and clinical diagnosis**

A five-generation Polish family with a history of HL over four generations was recruited for the study at the Department of Genetics, Institute of Physiology and Pathology of Hearing. The study included four patients with HL (III.2, III.4, IV.1, IV.7) and four unaffected individuals (III.3, IV.5, V.1, V.2) (Figure 1). All tested subjects gave informed consent for participation in the study, in accordance with the tenets of the Declaration of Helsinki.

Assessment of auditory function in the index patient (IV.1) was performed with pure-tone audiometry. Hearing thresholds for air and bone conduction were determined at frequencies of 125–8000 Hz and 500–4000 Hz with an AC40 clinical audiometer (Interacoustics, Middelfart, Denmark) and a 10/5 dB descending–ascending threshold estimation procedure [14].

In patient IV.7 neurootological clinical examination was performed. Objective vestibular function was measured using cervical and ocular evoked myogenic potentials (cVEMP, oVEMP) recorded with 500 Hz, 97 dBnHL air-conducted sound stimulation (EclipsVemp, Interacoustics, Assens, Denmark).

**Targeted next-generation sequencing**

Genomic DNA was isolated from whole blood samples with a standard salting out procedure. Concentration of the genomic DNA was determined with a Qubit HS Assay Kit using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). In the index patient (IV.1), clinical exome sequencing (TruSightOne, Illumina, Cambridge, UK) was performed according to the manufacturer's protocol. The sample was run on a MiSeq (Illumina) using 2 × 150 bp paired-end reads. Bioinformatics analysis was performed as described previously [15]. Selected variants were annotated with Annovar and converted to MS Access for further analyses. Integrative Genomics Viewer was used to inspect selected reads and validate candidate variants [16].

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**Figure 1.** Family pedigree. The proband is marked with an arrow. Affected individuals are indicated by black symbols, unaffected individuals are indicated by open symbols; presymptomatic carrier is indicated by an open symbol with vertical line, diagonal line denotes deceased individuals.
Our analysis pipeline included variant population frequencies from the database of the 1000 Genomes Project [17], the NHLBI GO Exome Sequencing Project (ESP) (https://esp.gs.washington.edu/drupal), and the Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org) (accessed 10/2017). Functional pathogenicity predictions for non-synonymous variants were performed using SIFT [18], PolyPhen-2 [19], and MutationTaster2 [20] computational algorithms.

Sanger sequencing

Presence of the candidate pathogenic variant was confirmed by Sanger sequencing. The forward primer 5’ – TGAAACATTCAGGATTTTCCAGT and reverse primer 5’ – ATGAGATGAGTAGGGCTGCTTTA were used for amplification of exon 11 of the COCH gene. Next, PCR products labeled with BigDye Termination cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA, USA) were sequenced with a 3500xL Genetic Analyzer (Applied Biosystems) and analyzed using Variant Reporter Software v1.1 (Applied Biosystems).

Results

A five-generation Polish family with postlingual, progressive HL and tinnitus was recruited for the study. The pedigree of this family showed a typical autosomal dominant pattern of inheritance, i.e. there was a male-to-offspring transition of HL and affected adult individuals were present in every generation (vertical pattern of inheritance).
Affected individuals had moderate to severe high frequency HL with a mean age of onset of 28.25 years (range, 15–47 years). In the index patient (IV.1), HL was diagnosed at the age of 22 and progressed gradually. Initially, the high frequency HL was followed by the involvement of mid frequencies. The latest audiological assessment at the age of 39 revealed mild to moderate HL at low frequencies, moderate HL at mid frequencies, and severe to profound HL at high frequencies (Figure 3). The patient suffered from tinnitus from the age of 15 years. He was fitted with binaural hearing aids at the age of 22 and since then he has reported improved speech intelligibility and reduced tinnitus.

Neither the index patient nor the other affected family members reported balance problems. This is consistent with the results of the vestibular function evaluation. Normal cVEMP and oVEMP responses were recorded in patient IV.7.

On the DNA sample from the index patient we have performed targeted next-generation sequencing of more than 4800 genes and identified 37,392 different genetic variants. After exclusion of variants in the non-coding regions (intronic, 3′, and 5′ UTR) and synonymous alterations we focused on variants with an allele frequency lower or equal to 0.01. Next, we selected variants located in genes involved in the development of HL (Table 1).

A detailed analysis of how the selected variants are inherited and their corresponding phenotype resulted in the prediction of one candidate disease-causing variant – a heterozygous transition c.1115T>C (NM_004086.2) located in the COCH gene (Figure 2 A, B). This change results in a substitution of isoleucine to threonine at position 372 (NP_004077.1:p.Ile372Thr) of the COCH protein. Segregation analysis confirmed that p.Ile372Thr was present in all affected subjects but also in the normal hearing son of the proband (8 y.o.). The genetic alteration was not present in other unaffected relatives.

The p.Ile372Thr pathogenic variant was not found in the analyzed population databases. To date, the p.Ile372Thr variant has been described in only two Japanese families with AD HL [13] and has been reported in the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php) with the accession number CM152070. In silico analysis predicted a deleterious effect of the detected variant with the following scores: PolyPhen-2 (score 0.978), SIFT (score 0.01), and MutationTaster2 (score 0.998).

Discussion

In this study we have identified the first pathogenic variant in the COCH gene in a Polish family. We have applied targeted next-generation sequencing to more than 4800 genes and detected a very rare COCH genetic variant that was not present in any of the analyzed population databases. These results, together with the data from computational approaches, showed that the genetic alteration has a deleterious effect on COCH protein structure and function. This strongly indicates that the identified variant represents a HL-causing change. This assumption is further confirmed by a segregation analysis of the genetic alteration with HL in the examined family. Testing a large number of genes in the index patient also resulted in the identification of five potentially pathogenic variants in genes, other than COCH, that were previously associated with HL. Based on the phenotype reported for these variants and their mode of inheritance, they have been excluded as causative for the nonsyndromic postlingual AD HL observed in the studied family.

The COCH c.1115T>C variant identified here is predicted to result in a p.Ile372Thr amino acid change. It localizes in the vWFA domain of the COCH protein and leads to substitution of highly conserved leucine to threonine. Pathogenic variants affecting this domain cause postlingual progressive HL without dysfunction of the vestibular system [21].
This COCH c.1115T>C genetic alteration was first identified in Japanese families that also had progressive HL without vertigo [13]. In contrast to our family, the Japanese patients had a higher age of HL onset (third vs fourth/fifth decade of life, respectively). In all families there was interfamilial variability in the age of HL onset (15–47 y.o. in the Polish vs 33–42 y.o. in the Japanese patients). One of the unaffected offspring of the index patient was also found to carry the COCH c.1115T>C variant. Currently, he is 8 y.o. and we presume he will most probably develop HL with age.

In the Polish index patient there was a strong association of exposure to noise with the occurrence and progression of his HL, although other affected family members did not report acoustic trauma. While this is an interesting observation, currently there are no data on the triggering role of noise in the development and/or progression of HL due to COCH pathogenic variants. Noise may be an independent factor causing HL and one may speculate that the earlier age of HL onset observed in our family may have been a consequence of exposure to noise. No information on the occurrence of tinnitus in the Japanese family was provided.

The index patient is currently using hearing aids, but considering the progressive nature of his HL in the next few years he may require a cochlear implant. Based on the partial deafness treatment strategy proposed by H. Skarżyński et al., the optimal treatment for this patient seems to be electric-acoustic stimulation (EAS) of the auditory system using a hearing aid combined with a cochlear implant [22, 23].

Our study provides an independent confirmation of the pathogenic role of COCH c.1115T>C in HL. It is important to underline that in addition to HL individuals with COCH HL-causing variants may also suffer from tinnitus and vertigo. On the other hand, information on the presence of tinnitus and/or vertigo may be beneficial for proper targeting of molecular genetic testing.

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References


