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SHORT REPORT

# Absence of *COCH* mutations in patients with Meniere disease

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**Missense mutations in the coagulation factor C homology (*COCH*) gene (14q12–q13) cause the autosomal dominant sensorineural hearing loss and vestibular disorder DFNA9 (OMIM 603196), and a high prevalence of symptoms of Meniere disease (MD) has been described in families with a mutation in the *COCH* gene. In this study, we search for mutations in the *COCH* gene in peripheral blood from patients with definite MD. DNA was extracted from peripheral blood cells of 30 individuals with MD and 30 controls. Exons 4 and 5 of the *COCH* gene were amplified by PCR reaction, using primer pairs flanking both exons. Sequences were analysed by a DNA sequencing system and compared with the published *COCH* cDNA sequence. No differences were found in the nucleotide sequences of exons 4 and 5 in the *COCH* gene in patients with definite sporadic MD when they were compared with the control group. Patients with definite MD have a low prevalence of mutations in exons 4 and 5 of the *COCH* gene.**
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## Introduction

Meniere disease (MD) is a clinical syndrome characterized by episodes of hearing loss associated with tinnitus or aural fullness followed by spells of spontaneous vertigo lasting at least 20 min. The estimated prevalence of MD in Caucasians is 1–2 cases per 10 000.[1] MD is multifactorial and the interaction of several genes with the environment is probably involved. Familial Meniere disease (FMD) represents 2.6–12% of MD.[2] FMD is heterogenetic and at least two possible genes have been suspected. Several mutations have been described in the coagulation factor C homology (*COCH*) gene (14q12–13), which cause the autosomal dominant sensorineural hearing loss DFNA9 (OMIM 603196),[3] and a high prevalence of symptoms of MD has been described in three European families with a P51S mutation in exon 4 of *COCH* gene.[4]

A group of British patients with FMD have shown an autosomal dominant inheritance with 60% penetrance, anticipation and an association with the class I HLA Cw7 antigen.[5] Linkage analysis has pointed to chromosome 14, but sequencing has excluded any mutation of the 12 coding exons of *COCH* gene in the British families.[6]

Autoimmunity appears to be associated with the pathogenesis of MD.[7] Early studies had found an association between MD and HLA antigens—the Cw7 antigen in British patients[5] and with the allele DRB1*1602 in a Japanese population[8]—but these results were not consistent. Later studies have not confirmed these associations between HLA antigens and sporadic MD.[9,10] The elevated levels of circulating immune complexes observed in

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**Table 1** Clinical features of patients with MD

| | |
|---|---|
| Uni/bilateral disease | *13/17* |
| Hearing stage (pure tone average 0.5–1–2–3 kHz) | |
| | *N* |
| 1 (⩽25 dB) | 1 |
| 2 (26–40 dB) | 4 |
| 3 (41–70 dB) | 21 |
| 4 (>75 dB) | 4 |
| Vertigo frequency (VF, average number of episodes per month for the previous 6 months). Class = FV × 100 | |
| | *N* |
| A (0) | 8 |
| B (1–40) | 16 |
| C (41–80) | 2 |
| D (81–120) | 3 |
| E (>120) | 1 |
| AOO-HNS functional level scale for MD (six levels, from one (= no limitation in daily activities) to six(= unable to work because of dizziness)) | |
| Level | *N* |
| 1 | 4 |
| 2 | 4 |
| 3 | 15 |
| 4 | 1 |
| 5 | 2 |
| 6 | 0 |
| No answer | 4 |

*N* is the number of individuals in each category.

patients with MD,[11,12] and the presence of nonspecific autoantibodies in 50–70% patients in MD, the most common being the antinuclear antibodies,[13,14] have also supported the hypothesis of autoimmunity or a response to exogenous antigen, such as microorganisms or proteins. Moreover, cochlin, a protein with a high expression in the cochlea and the vestibule,[15] is also a target antigen of autoantibodies found in the autoimmune inner ear disease.[16]

The aim of this study was to search for mutations in the *COCH* gene in peripheral blood cells from patients with definite MD.

## Subjects

The MD cases were part of a consecutive series of blood samples that had been collected after informed consent at the Hospital de Poniente at El Ejido and Virgen de las Nieves Hospital, Granada, Spain, during 1998–2002. In all, 30 Caucasian patients (12 males and 18 females) with definite MD according to the diagnostic scale for MD of the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) were included.[17] Median age at diagnosis was 54 years, range 31–70 years. Blood samples from probable and possible MD patients were excluded. An audiological, vestibular and functional evaluation was carried out in all patients following AAO-HNS guidelines to define stage, class and functional status in each patient.[17]

The control group was 30 Caucasian adults from the southeast of Spain. Individuals belonging to other racial populations were excluded from this study.

## Methods

### Polymerase chain reaction amplification

Peripheral blood was obtained from patients and controls, and genomic DNA was extracted by proteinase K digestion and salting out. A standard PCR protocol was used to amplify exons 4 and 5 using the primer pairs and reaction conditions described previously.[3] The primers used for exon 4 were forward 5′-CTTAAATCTCACACTGTAGTAC-3′ and reverse 5′-AAAGGAAATAATCACGTCTGC-3′, and for exon 5 forward TCTTTAGATGACTTCCCTGATGAG-3′ and reverse 5′-TCACAGGTTTTTCCATCAAGGTTA-3′. The cycling conditions were as follows: 5 min at 94°C, then 35 cycles at 94°C for 30 s, 58 and 62°C for 30 s for exons 4 and 5, respectively, and 72°C for 30 s, thereafter 72°C for 5 min. The presence of amplified product was confirmed by electrophoresing a 10 $\mu$l aliquot of PCR product in a 2% agarose gel.

### Sequencing

All sequencing reactions were performed using the ABI dye terminator cycle sequencing reaction kit (PE Biosystem, Foster City, CA, USA). Following PCR product purification with Microcon PCR columns (Millipore Corporation, Bedford, MA, USA), the product was subjected to cycle sequencing in both directions. Sequencing samples were resuspended in formamide and separated on an ABI 377 sequencer (PE Biosystems, Foster City, CA, USA) and analyzed with 377 DNA sequencing software.

## Results

A total of 17 and 13 patients had bilateral and unilateral MD, respectively. Only two women patients had a familial history of hearing loss with vestibular disorder. Table 1 shows the clinical features of the selected patients with MD.

No differences were found in the nucleotide sequences of exons 4 and 5 in the *COCH* gene in patients with definite MD when compared with the control group or the published cDNA sequence of the *COCH* gene.

## Discussion

In this study, we demonstrate that patients with definite MD do not carry any of the described mutations of the *COCH* gene. Mutation analysis studies have determined 30 individuals as the estimated sample size required to detect

a new polymorphism in the nucleotide sequence found in 5% of the general population.[18] So, we included 30 patients with MD to investigate exon 4 and 5 sequences of the *COCH* gene.

This gene was isolated by an organ-specific approach, and it was found to be expressed abundantly in the human cochlea and vestibule.[15] Combining genetic linkage analysis and positional candidate gene strategies, three different missense mutations were found in the *COCH* gene in three US families: V66G, G88E and W117R.[3] Subsequently, a P51S mutation was identified in one large Belgian and two small Dutch families at the DFNA9 locus,[4,19] and a fifth I109N mutation was identified in an Australian family with sensorineural hearing loss beginning in the second or third decade with concomitant vestibular dysfunction.[20] More than 25% of the patients affected with P51S mutation in the European kindreds showed episodes of vertigo, tinnitus and aural fullness with sensorineural hearing loss, which were consistent with the criteria for MD.[4] We have not found any of these mutations in exon 4 or 5 in patients with MD. All the sequences analyzed in patients or controls did not differ when compared with the published *COCH* cDNA sequence.

We support that there are significant clinical and pathological differences between DFNA9 and MD.[6,21] The predominant clinical features in MD are episodes of vertigo lasting minutes to hours associated with ipsilateral aural fullness, tinnitus and sensorineural hearing loss, and the presence of total deafness is unusual, whereas in DFNA9 the vestibular symptoms are more variable and most individuals do not describe vertigo attacks. The sensorineural hearing loss starts at high frequencies, with onset in the second to fourth decades of life and it progresses to anacusis.[22,23]

The pathological feature in MD is the endolymphatic hydrops. Immunohistochemical studies have demonstrated deposits of C3 and C1q in the membranous labyrinth located at the basal membrane, subepithelial connective tissue, vestibular ganglion and endolymphatic sac of patients with MD.[24,25] It appears that immune injury may be induced in the inner ear at sites where immune complexes are deposited in the vessels. Therefore, it has been suggested that deposits of immune complexes in the vessels of the endolymphatic sac may impair the resorptive function of it, resulting in endolymphatic hydrops. In DFNA9, the predominant feature is widespread acidophilic deposits observed in the lateral wall of the cochlear duct in the area of the spiral ligament and osseous spiral lamina, as well as in the stroma of the crista ampullaris and the macula in the vestibular system.[26] DFNA9 histopathological findings also include marked loss of fibrocytes in the spiral limbus, the spiral ligament and the stromal cells of the crista and macula. Neuroepithelial degenerations of the organ of Corti, crista and macula, and the dendrites and ganglia of the cochlear and vestibular nerves are also seen in some DFNA9 cases.[21,27] Moreover, endolymphatic hydrops is absent in most DNFA9 specimens.

Although genetic factors are probably involved to a significant extent in MD, the known mutations in exons 4 and 5 of the *COCH* gene have a low prevalence in MD.

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