High prevalence of symptoms of Menière’s disease in three families with a mutation in the COCH gene

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We report the genetic analysis of one large Belgian and two small Dutch families with autosomal dominant non-syndromic progressive sensorineural hearing loss associated with vestibular dysfunction. Linkage studies in the Belgian family mapped the disease to the DFNA9 locus on chromosome 14. Mutation analysis of the COCH gene, which is responsible for DFNA9, revealed a missense mutation changing a highly conserved residue. One of the patients, who had an earlier age of onset in comparison with most of the affected family members, was shown to be homozygous for the mutation. After the mutation was found in the Belgian family, we discovered that the same missense mutation was also present in two Dutch families with similar cochleo-vestibular symptoms. In all three families with hearing loss and imbalance problems, >25% of the patients showed additional symptoms, including episodes of vertigo, tinnitus, aural fullness and hearing loss. Clinically, these symptoms are consistent with the criteria for Menière’s disease. The importance of genetic factors in Menière’s disease has been suggested on many occasions, but this study is the first report of a mutation in a gene leading to the symptoms of Menière’s disease in a significant portion of the carriers. The COCH gene may be one of the genetic factors contributing to Menière’s disease and the possibility of a COCH mutation should be considered in patients with Menière’s disease symptoms.

INTRODUCTION

Hearing impairment is the most common human communication disorder and hereditary causes play an important role in its etiology. Although it has been known for several decades that hereditary non-syndromic hearing impairment (NSHI) is highly genetically heterogeneous, only three gene localizations were known prior to 1994. Since 1994, the localization of genes responsible for hearing impairment has greatly accelerated (1). To date, 21 loci for autosomal dominant NSHI (prefix DFNA) and 25 loci for autosomal recessive NSHI (prefix DFNB) are known. Considerably fewer disease genes have been identified: nine genes have been identified associated with DFNA and four with DFNB loci. A continuously updated table of all gene localizations and identifications is maintained on the Hereditary Hearing Loss Home page (http://dnalab-www.uia.ac.be/dnalab/hhh).

The inner ear consists of two organs that are evolutionarily related and have a similar physiology: the cochlea and the peripheral vestibular system (labyrinth). The cochlea is involved in the perception of sound, whereby sound waves are transformed into afferent nerve impulses that are sent to the hearing centers in the brain. The vestibular labyrinth consists of the saccus and utriculus, which register predominantly linear accelerations, including gravity, and the semicircular canals, which register rotatory motions. The sensory epithelia of the cochlea, semicircular canals, saccus and utriculus are very similar: in each case, afferent signals are generated by mechanical stimulation of hair cells. Central integration of the impulses generated by the vestibular system with signals from the proprioceptive and visual systems leads to the maintenance of balance as well as gaze stabilization during head movements.

Due to the remarkable resemblance between both parts of the inner ear, it seems logical that a considerable number of inner ear-specific genes will have both cochlear and vestibular functions and, consequently, mutations in these genes would be expected to lead to both auditory and vestibular dysfunction. Although several mouse mutants with both deafness and vestibular dysfunction have been described and some of the responsible genes have been identified (reviewed in ref. 2), DFNA9 is the only NSHI locus in...
DFNA9 is localized to chromosome 14q12–13 (3) and the responsible gene, named $COCH$, probably encodes a secreted molecule that may be an extracellular matrix protein (4). Very recently, de Kok et al. (5) confirmed the involvement of $COCH$ in DFNA9, by identifying a Pro→Ser mutation (P51S) in four Dutch families. Patients with a mutation in the $COCH$ gene suffer from progressive autosomal dominant sensorineural NSHI (6). The penetrance of vestibular symptoms was incomplete and the clinical nature of the vestibular dysfunction was not described in detail. In the original DFNA9 families, histopathological examination of the temporal bone revealed the presence of mucopolysaccharide depositions in cochlear and vestibular nerve channels and in the supporting structures of the organ of Corti, including the limbus, spiral lamina and spiral ligament. In the latter structures, severe loss of cellularity was observed (6,7). One patient also had histopathologically confirmed endolymphatic hydrops (7).

Endolymphatic hydrops, or hydropic distension of the endolymphatic system, is a histological hallmark of Menière’s disease, the clinical correlate of which is auditory and vestibular dysfunction. The diagnostic criteria for definitive Menière’s disease, as proposed by the American Academy of Otolaryngology and Head and Neck Surgery (AAO-HNS), include: (i) two or more episodes of vertigo of at least 20 min to 24 h duration; (ii) audiometrically documented hearing loss; (iii) tinnitus or aural fullness; and (iv) exclusion of other causes (8). Most patients with these symptoms do not report extensive positive family histories, although genetic involvement has been suggested (9,10). These data suggest that both environmental and genetic factors, including reduced penetrance and clinical var-

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**Figure 1.** Pedigree of family 1. This pedigree consists of a subset of the entire pedigree of family 1, comprising only family members from whom DNA was available. Spouses were omitted. Affected family members are denoted by solid symbols. Family members who were not clinically affected, many of whom are younger than the age of onset, are shown by open symbols. The affected status of the deceased family members was determined on an anamnestic basis. The age of the living family members is indicated. Family members that are heterozygous for the P51S mutation are marked by a + sign. In persons above the age of onset, the mutation co-segregates with the disease. In the younger generations, the presence of the mutation reveals whether or not these persons will get the disease at a later age, which can be used in genetic counseling. Three patients are marked by a bold italic number: the meaning of these is explained in the text.

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**Figure 2.** Confirmation of the P51S mutation by restriction analysis. The P51S mutation destroys a $BglI$ restriction site. The presence of the mutation can therefore be assayed by PCR of exon 4 followed by $BglI$ digestion. In controls, the 295 bp PCR product is cleaved into two fragments of 157 and 138 bp, respectively. In the case of the P51S mutation, the PCR product is not cleaved and the undigested 295 bp band is visible. Lane 1, 100 bp ladder; lane 2, patient homozygous for P51S; lanes 3 and 4, patients heterozygous for P51S; lane 5, control; lane 6, undigested PCR product; lane 7, blank (no DNA added).
iability, are involved in disease pathogenesis, although none of these factors has ever been identified.

Here we present three families linked to the DFNA9 locus with a mutation in COCH. Late onset functional degeneration of the inner ear occurs, leading to bilateral total deafness and vestibular areflexia. A significant fraction of the affected persons suffer from symptoms that include episodes of vertigo, hearing loss and tinnitus or aural fullness, fulfilling the clinical criteria for Menière’s disease.

RESULTS

Linkage analysis

For linkage analysis, a subset of the pedigree was used which included 23 family members. Because DFNA9 is the only reported locus for autosomal dominant non-syndromic hearing loss associated with vestibular pathology, we first analyzed whether the disease in our family was linked to this locus. Two-point LOD scores >4 were obtained for marker D14S262. Subsequently, haplotyping for the entire family (119 family members) was performed using markers D14S1060, D14S1071, D14S262 and D14S1021 (data not shown). The size of the candidate region was 6.1 cm, with no recombinations between markers D14S262 and D14S1071. One individual (Fig. 1, patient 4), who was born from a consanguineous marriage, was homozygous for the disease haplotype.

Mutation analysis

To investigate whether mutations in the COCH gene were responsible for the otovestibular symptoms in family 1, mutation analysis was performed. All coding exons of COCH, including the intron–exon boundaries, were sequenced in two heterozygotes, two controls and in the affected individual who is homozygous for the disease haplotype (Fig. 1, individual 4). In this latter individual, a homozygous C→T point mutation was found at bp 151 of the coding region in exon 4 [nucleotide numbering of COCH is based upon the sequence of Robertson et al. (4)]. The mutation was not found in the controls, whereas the heterozygotes carried both mutant and wild-type alleles. The mutation leads to a Pro→Ser substitution at residue 51 of the COCH protein (P51S). Like the three previously described COCH mutations, P51S occurs in the cysteine-rich factor C homologous (FCH) domain. This mutation is the same one that has recently been described in four Dutch DFNA9 families (5). No other DNA variations leading to amino acid changes were found.

The P51S mutation destroys a site for the restriction enzyme BglII, providing a rapid screening assay for the mutation (Fig. 2). Restriction digestion in all family members from whom DNA was available indicated that segregation of the mutation was completely concordant with the haplotype results. To exclude that the P51S variation is a common polymorphism, 100 independent controls of Belgian origin were analyzed using this assay, none of whom showed this mutation.

To investigate whether the P51S mutation was also present in other families with a similar phenotype, we analyzed three patients from family 2 and two patients from family 3 for the presence of this mutation. In both families, the identical mutation was detected.

DISCUSSION

In this study, we have performed linkage analysis on an extended Belgian pedigree with hereditary sensorineural hearing loss associated with vestibular problems. We found close linkage between the disease and markers surrounding the DFNA9 locus, on chromosome 14q12–13. Mutation analysis of the COCH gene, responsible for DFNA9, revealed a missense mutation changing Pro to Ser (P51S). The same mutation was also found in two small families with similar vestibulocochlear symptoms. This mutation is most likely disease-causing because: (i) no other variation was found in the COCH coding region; (ii) it was never found in the control population; (iii) the mutated Pro residue is conserved in homologous genes in mouse, chicken and horseshoe crab; and (iv) this mutation was found in three families with similar vestibulo-cochlear symptoms. The P51S mutation was also reported by de Kok et al. (5) in four Dutch DFNA9 families. Combined with the data presented here, this indicates that the P51S mutation has been found in seven families originating from Flanders or the southern part of The Netherlands. Further studies will have to be performed to determine whether all these families are related to a common founder.

The most consistent histopathological finding in DFNA9 patients is the presence of acid mucopolysaccharide deposits in cochlear and vestibular nerve channels and in the supporting tissues of the organ of Corti (6,7). In situ hybridization of the cochlea and vestibule in the chicken revealed that the sites where COCH is expressed in the chicken correspond with the sites where mucopolysaccharide deposits are found in human DFNA9 patients (4). Based upon these findings, Robertson et al. (4) hypothesized that COCH mutations act through a gain-of-function mechanism, whereby the formation of an insoluble deposit would be the direct consequence of a deleterious novel property of mutant COCH protein. Furthermore, they suggested that the accumulation of acidophilic deposits in vestibular and cochlear nerve channels leads to strandulation and degeneration of the dendrites and loss of cochlear and vestibular neurons.

The person in family 1 who is homozygous for the COCH mutation lends further support to the gain-of-function hypothesis. In this individual, born from a consanguineous marriage between two deaf persons, the age of onset of symptoms is lower than in most other persons (~25 years versus a median of 42 years in heterozygotes). While it cannot be excluded that this variation is due to intrafamilial clinical variability, as age of onset does vary between affected family members, at age 34 the homozygote is more severely affected than several older heterozygotes. Therefore, the earlier age of onset may reflect a higher abundance of deleterious protein, resulting in earlier impairment of cochlear and vestibular function.

In all three DFNA9 families we present here, >25% of the affected persons suffer from a number of additional symptoms, including recurrent episodes of vertigo, aural fullness, hearing loss and/or tinnitus. These symptoms are consistent with those observed in patients with Menière’s disease, where disturbances in inner ear fluid homeostasis lead to expansion of the endolympathic compartments at the expense of the perilymphatic compartments (endolympathic hydrops) (11,12). The
presence of endolymphatic hydrops can be proven only by post-mortem histopathological analysis of temporal bones or by electrocochleography (ECoG). Since Menière’s disease manifests as recurrent attacks of vertigo, hearing loss and tinnitus or aural fullness, in clinical practice the diagnosis is based on these criteria, combined with a full diagnostic work-up to exclude other causes. The fraction of Menière’s disease patients who truly have endolymphatic hydrops is unknown.

It is not clear whether DFNA9 patients who suffer from recurrent vertigo attacks represent true cases of Menière’s disease, as there are subtle clinical differences between the COCH-related episodic vertigo reported here and a typical Menière attack. First, in Menière’s disease, the hearing loss that accompanies the attack is generally considered to be fluctuating. Fluctuating hearing loss has been documented in some persons in family 1, but we were unable to demonstrate that the fluctuating hearing loss occurred during vertigo attacks. Second, Menière-related hearing loss is usually a flat or low tone hearing loss, whereas DFNA9 starts in the high tones.

Under the hypothesis previously formulated by Robertson et al. (4), one would expect the DFNA9 phenotype to be characterized by a steady deterioration in cochlear and vestibular function without sudden fluctuations. However, we have observed many DFNA9 patients with episodic vertigo and a fluctuating hearing impairment. As an alternative hypothesis, it is possible that the P51S mutation exerts its pathogenic effect by disturbing inner ear fluid homeostasis. In DFNA9 patients, insoluble deposits are present in the spiral ligament, the spiral lamina and the limbus and a severe loss of cellularity is observed (6). These tissues are very rich in gap junction proteins (13) and have been implicated in the recycling of K⁺ ions from the hair cells to the endolymph. Spicer and Schulte (14,15) found evidence for intercellular ion transport through gap junctions via both the medial pathway, returning K⁺ ions from the inner hair cells through the spiral limbus to the scala media, and the lateral pathway, returning K⁺ ions from the outer hair cells via the spiral ligament to the stria vascularis.

The importance of these recycling pathways is underlined by the observation that mutations in two cochlear gap junction proteins (connexin26 and connexin31) lead to non-syndromal deafness (16,17). The COCH-related defect in the supporting tissues of the organ of Corti may obstruct the K⁺ recycling pathways in the inner ear, resulting in a disturbance of ion homeostasis and perturbations in hearing and vestibular function, as seen in the persons we report.

Whether the Menière’s disease signs and symptoms in DFNA9 reflect a fluid imbalance, a slow deterioration of the afferent nerve channels or both, must await elucidation of the normal role of COCH in the inner ear. ECoG on DFNA9 patients, which will be carried out in the future, can possibly indicate disturbances in fluid homeostasis. It remains noteworthy that a considerable fraction of the DFNA9 patients show symptoms that are highly reminiscent of Menière’s disease. In family 1, a number of affected persons were diagnosed with Menière’s disease and it is conceivable that in the general population, a number of people diagnosed with Menière’s disease are in fact DFNA9 patients. Therefore, the possibility of a COCH mutation should be considered in sporadic Menière cases.

MATERIALS AND METHODS

Family pedigree

Family 1 is a large Belgian family with non-syndromic progressive sensorineural hearing impairment and progressive vestibular dysfunction. The pedigree of family 1 spans seven generations (Fig. 1) and demonstrates clear autosomal dominant inheritance with complete penetrance. Blood samples were obtained from 119 family members after informed consent. One documented consanguineous marriage occurred (Fig. 1) but since the family originates from an area that has remained relatively isolated and rural until two generations ago, it is not unlikely that other consanguineous marriages occurred in the past. This point is especially relevant when considering generation 3, which contains two affected siblings (1 and 2) whose children (8 and 14 children, respectively; not all sibs are shown in Fig. 1) are all affected (the ninth child of patient 1 died at the age of 7, well before the age of onset of the disease, and was not considered in the analysis). The most likely explanation for this observation is that both individuals 1 and 2 were homozygous for the disease allele, implying that their parents were both affected. Unfortunately, it has not been possible to determine the disease status of individual 3, the mother of the two putative homozygotes.

Family 2 is a Dutch family described previously (18,19) that includes four persons with vestibulo-cochlear dysfunction. Family 3 is another previously described Dutch family (20) in which five persons with vestibulo-cochlear dysfunction have been described.

Clinical findings

In family 1, clinical data with special relevance to hearing and vestibular complaints were collected using questionnaires and interviews. Clinical examination, otoscopy and pure tone audiometry were performed. These data revealed a progressive perceptive hearing impairment generally starting between ages 35 and 56 (median 42) and resulting in profound hearing loss. Individual 4 (Fig. 1, bold italic), who was born from the consanguineous marriage between individuals 5 and 6, was the youngest family member who could be diagnosed clinically as affected. All patients reported instability in darkness and the majority also reported periods in their life with gait instability or imbalance and positional vertigo. The vestibular symptoms started at approximately the same age as the hearing loss. In total, 34 family members had both hearing loss and vestibular dysfunction.

Nine affected family members reported recurrent vertigo attacks that lasted >20 min as well as hearing loss, tinnitus and/or aural fullness. These attacks fulfil the AAO-NHS clinical criteria for Menière’s disease. In five family members, a fluctuating hearing loss was audiometrically documented. No other relevant clinical abnormalities were reported.

Twenty-six affected family members had a complete audiological and vestibular test battery performed at the Antwerp University Hospital; when possible, previous audiometric data were also collected. Audiometric test results combined with anamnestic data indicated a late onset progressive hearing loss, initially manifesting as a mild hearing loss in the high frequencies, but progressing to a severe-to-profound loss across all frequencies after ~20 years. Vestibular testing indicated progressive deterioration of semicircular canal and otolith
function, with the same age of onset as for the hearing loss, ultimately leading to total vestibular areflexia.

Both families 2 and 3 have been described previously (18–20). In brief, in family 2, affected persons suffer progressive hearing loss and instability in the dark, both starting at ~40 years of age. Two of the four family members suffered from episodic vertigo. In family 3, progressive hearing loss and vestibular complaints also started around age 40 and two of five affected persons reported episodes of vertigo.

**Linkage analysis**

DNA was isolated from blood using standard procedures. Polymorphic markers were analyzed radioactively. One primer was end-labeled with 32P and a standard PCR reaction was performed. DNA was isolated from blood using standard procedures. Polyacrylamide gels were used in a cycle sequencing reaction using a Dye Terminator (Perkin-Elmer Applied Biosystems, Foster City, CA). Fragments were analyzed using the LINKAGE v.5.1 software package (21). The gene frequency for hearing loss due to mutations in this gene was set at 0.0001. Recombination frequencies were assumed to be equal for males and females. The allele frequencies were set at 1/n, where n is the number of observed alleles in the family. The disease was coded as a fully penetrant condition, without phenocopies.

**Mutation analysis**

All coding exons of the COCH gene were amplified in a standard non-radioactive PCR reaction, using the primers and reaction conditions described previously (4). PCR products were analyzed on an agarose gel, cut out of the gel and purified using a Sephaglass BandPrep kit (Amersham Pharmacia Biotech), according to the manufacturer’s instructions. Reaction products were analyzed on a 1% agarose gel and stained with ethidium bromide.

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