Biochimica et Biophysica Acta 1792 (2009) 27–32

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Are MYO1C and MYO1F associated with hearing loss?

Cristina Zadro [a], Maria Stella Alemanno [b], Emanuele Bellacchio [c], Romina Ficarella [d], Francesca Donaudy [d], Salvatore Melchionda [b], Leopoldo Zelante [b], Raquel Rabionet [e], Nele Hilgert [f], Xavier Estivill [e], Guy Van Camp [f], Paolo Gasparini [a,*], Massimo Carella [b]

[a] Unit of Medical Genetics, Department of Reproductive Science and Development, Institute for Maternal and Child Health – IRCCS "Burlo Garofolo" – Trieste, Italy
[b] Unit of Medical Genetics, Instituto di Ricovero e Cura a Carattere Scientifico, Casa Sollievo della Sofferenza, 71013 San Giovanni Rotondo, Italy
[c] CSS-Mendel Institute, Rome, Italy
[d] Telethon Institute of Genetics and Medicine, 80131 Naples, Italy
[e] Genes and Disease Program, Center for Genomic Regulation, Barcelona, Spain
[f] Department of Medical Genetics, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium

ARTICLE INFO

Article history:
Received 9 May 2008
Received in revised form 15 October 2008
Accepted 17 October 2008
Available online 5 November 2008

Keywords:
Hearing loss
MYO1C
MYO1F

ABSTRACT

The role of myosins in the pathogenesis of hearing loss is well established: five genes encoding unconventional myosins and two genes encoding nonmuscle conventional myosins have so far been described to be essential for normal auditory function and mutations in these genes associated with hearing impairment. To better understand the role of this gene family we performed a mutational screening on two candidate genes, *MYO1C* and *MYO1F*, analyzing hundreds of patients, affected by bilateral sensorineural hearing loss and coming from different European countries. This research activity led to the identification of 6 heterozygous missense mutations in MYO1C and additional 5 heterozygous missense mutations in *MYO1F*. Homology modelling suggests that some of these mutations could have a potential influence on the structure of the ATP binding site and could probably affect the ATPase activity or the actin binding process of both myosins. This study suggests a role of the above mentioned myosin genes in the pathogenesis of hearing loss.
© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Myosins are actin-based molecular motors, ubiquitously expressed as multiple isoforms in all eukaryotic cells. Almost all myosins known to date are composed of one or two conserved heavy chains, formed by the N-terminal globular motor domain, with ATP- and actin-binding sites, of a "neck" with one or more IQ motifs (that binds calmodulin or calmodulin-like chains) and of at least one variable light chain responsible for the specific function of the myosin [1–3]. Myosins are commonly classified into conventional myosins, which are similar in the head sequence and structure to muscle myosin, and unconventional myosins whose sequence is similar in the head but different in the tail. During the past years, considerable attention has been paid to the role of unconventional myosins in inner ear sensory hair cells. As a matter of fact, several myosins have been shown to play a relevant role in sound detection and signal transmission processes occurring in sensory hair cells of the cochlea [4,5]. Five genes encoding unconventional myosins (Ia, IIIa, VI, VIIa and XVa), and two genes encoding nonmuscle conventional myosins (*MYH9* and *MYH14*) are essential for normal auditory function and mutations in these genes have been associated with hearing impairment [6–10]. Myosin VIIA has been shown to be responsible for Usher syndrome type IB [11] and of a dominant form of nonsyndromic deafness DFNA11 [12,13], while its role in causing recessive forms of nonsyndromic deafness (DFNB2) is still controversial [14–16]. Mutations in *MYO6* have been described in human and mice, with a recessive inheritance in the Snell's waltzer mouse [17], and both autosomal dominant and recessive patterns in humans (DFNA22 and DFNB37) [18,19]. *MYO15A* is mutated in human DFNB3 and in mouse shaker-2 [20,21]. Recently, mutations in *MYO3A* and in *MYO1A* have been shown to cause non syndromic forms of recessive hearing loss, DFNB30 and DFNB48, respectively [8,9].

Recent data suggest that *MYO1C* and *MYO1F*, encoding unconventional myosins expressed in the inner ear, may represent good candidates for hereditary hearing loss in mammals. To evaluate *MYO1C* and *MYO1F* as candidate genes for deafness, we analyzed 450 subjects by mutational screening using denaturing high-performance liquid chromatography (DHPLC) and direct sequencing of electrophoretically abnormal fragments.

2. Materials and methods

2.1. Sample

Samples included 450 subjects showing bilateral sensorineuronal hearing loss, without any feature, clinical sign and/or symptoms of known syndromes (visual defects, facial appearance, skin

* Corresponding author.
E-mail address: gasparini@burlo.trieste.it (P. Gasparini).

0925-4439/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2008.10.017

Table 1
Primers and annealling temperature of *MYO1C*

Amplicon	Size bp	Primer sequence (5′–3′)	Annealing temperature
1	326	F: GTTCCCTCTCAGTCCCACAG R: GGTGCACCT TGGTGTCTCTT	60 °C
2	290	F: CGGATTTCTCCATGTTGGTC R: TGCGACATTCATCTCTGGTC	60 °C
3	332	F: TCGACTCCCACAAGAGGACT R: ACTGGCACTGGGCTTCTCT	60 °C
4	277	F: GGGCAGCAGTAGCTACAAGG R: GCTTTGTTTGACTGCTGGAA	58 °C
5	466	F: GTGGGCTGTTTTCCCAGTC R: AGATTTTGGGGGTGTGACAG	58 °C
6	418	F: TCTGCTAGTTGCCCTCTTGG R: AGGGGAGTGATGGGAGTAGG	60 °C
7	446	F: AGGCTCTGTGGCATTCATCT R: ATGAGAGGCTGGAGGACAGA	58 °C
8	468	F: TGTTTCTCACCTCCTGTGCTC R: ACGGAGAAGGGGGACATC	60 °C
9	230	F: CTCTGACCTGTCCCCCATAC R: GTGGCTGGTGTTTTGTGATG	58 °C
10	366	F: GCTGGTTCCTGCTCCTGA R: GTGCAGAGCTGTGGGCTATC	60 °C
11	235	F: TGTCCTGCCTGTCCCTGT R: GCTCACACGCGTTCACAC	62 °C
12	434	F: AGCAGCTTTAGCCCAGCTC R: GACCTAGGGGCTCCTACCC	60 °C
13	408	F: GTGCCCAGAGAAAGCTGATT R: GGAGTGACTTCCCTGCTTCC	60 °C
14	280	F: TTGGTTCCTTCAGCCACTCT R: TGTCGTGGAGACCACAGATG	58 °C
15	459	F: CCATGGTAAGAAGATAGAGTTCAGC R:CCGAAGAAATCAGACCCTTC	60 °C
16	184	F: CGATGTCTTCAGAGGGAAGGGAAGG R: TCCACTTCCTCATCCTCACC	60 °C
17	392	F: GGAGGCTGTCTCAGGTCCTA R: TCAGCTCTCAGGCAGTAGCA	60 °C
18	387	F: GAGAGCTGAAGGCAGGTCAG R: CCTCCCCATCCATGAAAAG	58 °C
19	447	F: GGGGGTTCCTCAAGTTCTG R: AGGAAGGCTTGGCTATCACA	60 °C
20	358	F: CGAGACCCCATTCTCCATAA R: CCTGGCTTTCCTATTGCTGT	58 °C

abnormalities, cardiac and renal defects, etc.). Inclusion criteria were: absence of the most common mutations within *GJB2*, bilateral sensorineural hearing loss and normal tympanometric evaluation. Hearing function was evaluated using standard audiometric instrumentations according to worldwide accepted guidelines and protocols. The sample set includes cases with a variable degree of hearing loss, ranging from mild to profound (according to ASHA classification) and with a variable age of onset, from congenital to late onset. In all cases, vestibular data were obtained by clinical examination and routine vestibular tests (one or more of the following: caloric, rotatory, optokinetic, swinging torsion, statokinesimetric, and vestibulo-vegetative). Familial records were also available. The majority of patients came from central and southern Italy (200), while 140 were from Spain, 60 from Belgium, and 50 from Israel. All patients have been recruited trough medical genetics diagnostic services. For Italian patients informed consent was obtained according to our national rules and laws on genetic tests and privacy [i) art.10 and 22 of the Italian law 196/03 on privacy and following updates published on the Gazzetta Ufficiale n.65 of 19 March 2007, ii) National authorization n.2-2004 to conduct research activities on genetics, iii) Government authorization to referral research centers (an IRCCS in our National Health Care System) to perform genetic tests 02-2007]. For samples coming from Spain, Belgium and Israel consent form was obtained according to respective national rules and laws. Peripheral blood was obtained from all subjects, and DNA was isolated from blood leukocytes using standard methods.

2.2. Genetic analysis

A set of 20 and 22 primer pairs were designed to amplify *MYO1C* and *MYO1F*, respectively (Tables 1 and 2). PCR fragments include the coding regions and the splice sites. All amplicons were screened by DHPLC, performed on a WAVE Nucleic Acid Fragment Analysis System HSM (Transgenomic), according to supplier protocols. DHPLC data analysis was based on a subjective comparison of sample and reference chromatograms. PCR products that showed an abnormal chromatographic profile on DHPLC analysis were sequenced directly on an automated sequencer (ABI 3130; Applied Biosystem, USA) using the ABI-PRISM big-dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystem, USA). The possible pathogenicity of variants was also assessed, evaluating the conservation of residues by multiple sequence alignments of homologous proteins obtained with the WU-BLAST algorithm. The presence of mutated alleles was tested on 200 normal chromosomes of individuals coming from the same geographical area of patients (100 healthy controls coming from Belgium, 100 from Italy and 100 from Spain).

2.3. Homology modelling

In order to understand the effects of the mutations on protein level, the structures of myosin-1c and myosin-1f proteins were built by

Table 2
Primers and annealling temperature of *MYO1F*

Amplicon	Size bp	Primer sequence (5′–3′)	Annealing temperature
1	387	F: GAGATGGGGAAGGAGGAATG R: TGTCACCACTTGGTGCTCTC	62 °C
2–3	385	F: CACACGACCCTTAACCATCC R: GATTGGGAGTTTCAGGCAAA	62 °C
4–5	399	F: CCACTGATCATTTATTCCTCCA R: TGGGTGTGTGTGTAGAAAGGTC	64 °C
6	247	F: TCATAAGACCCATCCCCAAC R: AGGTGGTGCTCCCTCTCC	62 °C
7	231	F: CCAGGCAAGCACCAGATG R: GTTTCTGGGGCTGAGGTCT	60 °C
8	236	F: GGATTCACGGCACAGTTTCT R: GCTAGGAGGACGCAGTTCAC	60 °C
9	291	F: ACCAACTGTCCCATTTTTGC R: AATGTTTGTGACCCCAGAGC	58 °C
10–11	389	F: ATGCCCACTGACTTCCTGTC R: AGGGATCCTCATGGTCCTTC	60 °C
12	240	F: GATGGAGAAGGGGAGAGGAC R: TGAGTCTGGAAGATGAGGGG	60 °C
13	298	F: CCTCCCTCCCCTAATCTCTG R: CTGTGCCTCCTCGCAGA	60 °C
14	223	F: CCAGGTTTCTCAGTCCCTCA R: CCATCTTACCCAGAACTGCC	60 °C
15	207	F: TGGAGGAAGAGTACCCGAAA R: GCTGAGTTCCACCCTACCTG	60 °C
16	247	F: CAGAGGGTCCCATCTCTGTG R: AGACACCTCCCTGACCTCCT	60 °C
17–18	425	F: GAGGGGACTCAGCCTACCC R: GGAGAGGGGAAGAGAGAGAAA	58 °C
19–20	398	F: GAGGGGACTCAGCCTACCC R: AGTGTGTTTGAGGGACAGGC	64 °C
21	241	F: GATGGAGAAGGGGAGAGGAC R: TGAGTCTGGAAGATGAGGGG	58 °C
22	388	F: GCTGACCCTTCCCTAAATCC R: ACCAGCGAGAGTTGGGTG	60 °C
23	246	F: CCCCAAGCTTTCTTCTCTCC R: TAAGAGTGGACGAAGGCCAG	58 °C
24	214	F: CGGAGCCTCAGAATGGACTA R: ATTCCCTTGTCTGTTGTGGG	58 °C
25	347	F: GAAGCCAAGTCCTCCTCAAA R: GTGACTCAAACCCCCTGTG	60 °C
26	244	F: CTGTGACTCAAACCCCCTGT R: GGTGAGGGGAGTGGTAAGAA	58 °C
27	246	F: AGGCATGAGAGCTTTCTCCA R: TTTACCTGGGCTGCAATAGC	60 °C

Table 3
MYO 1C mutations

MYO1C *Gene ID*: 4641				
Exon	Nucleotide variation	Amino acid variation	Amino acid conservation[a]	Effects of variation[b]
4	476 C>T	R156W	+ + +	+ + +
5	756G>A	V252A	+ + +	+ +
7	1136G>A	T380M	+ +	+ + +
15	2248C>A	Q750K	+ + +	n.d.
16	2469A>C	K823N	+	n.d.
16	2491G>A	E831K	+ + +	n.d.

[a] + + + residue well conserved; + + residue partially conserved; + residue very partially conserved.
[b] + + + very relevant; + + relevant; n.d. not determined.

homology modelling. In particular, the structures of myosin-1c (a.a. 12–852) and myosin-1f (a.a. 9–733) have been modelled with the program MODELLER (release 8v2) [22] using the crystallographic structure of myosin-2 heavy chain (nonmuscle) from Dictyostelium d. (PDB entry 1g8x, chain A) as a template. In the modelled region, myosin-1c and myosin-1f share respectively 36% and 39% amino acid identity with the sequence of the template myosin (sequence alignments available on request). The ADP molecule co-crystallized with the template myosin structure 1g8x has been retained in its corresponding position also in the myosin-1c and myosin-1f models to indicate the putative binding site of the ATP nucleotide phosphate cofactor. This computational method has allowed prediction of the effects of the mutations falling inside the modelled regions of myosin-1c and myosin-1f.

3. Results and discussion

Variations detected in *MYO1C* and *MYO1F* are summarized in Table 3 and Table 4, respectively.

3.1. Myosin-1c

Six missense mutations, at heterozygous state, have been detected in *MYO1C* gene. 5 of the affected residues are well conserved across species. Variations R156W, V252A and T380M are located in the myosin head domain, while Q750K is located in the third IQ domain; K823N, E831K are located between the third IQ domain and the TH1 domain.

R156W is due to a C>T nucleotide change at position 467. It was detected in a Spanish sporadic case (coming from Mallorca Isle) affected by bilateral sensorineural hearing loss. In this mutation, an arginine, a residue evolutionarily conserved and probably important in ATP binding, is substituted with a tryptophan. Homology modelling predicts that the R156W mutation would cause a strong change in the physical–chemical properties of an amino acid that makes direct contact with the ATP co-factor (Fig. 1A).

In an Italian patient with a moderate form of bilateral sensorineural hearing loss, the mutational screening highlighted the substitution 756G>A leading to the amino acid change V252A. The evolutionary conserved Val 252 interacts with a group of amino acids that includes Val 145, Ala 148 and Gln 207, contributing to stabilize the arrangement of α-helices nearby the binding site of ATP (Fig. 1B). Mutations involving Val 252 are therefore expected to interfere with the binding of ATP.

Mutation T380M is due to a G>A nucleotide change at position 1136; it was detected in Spanish subject with a family record of progressive bilateral sensorineural hearing loss and an autosomal dominant pattern of inheritance; the age of onset was 18. The T380M mutation implies the replacement of a polar with a non-polar amino acid in a protein site characterized by tight packing of secondary structure elements. The effects of this mutation are important since T380 interacts with amino acids that contribute to form α-helix directly involved in the binding of ATP (Fig. 1C). R156W, V252A and T380M mutations occur in the myosin head-like domain, and particularly in sites of the protein that are relevant in ATP binding and/or in the conversion of the chemical energy of this co-factor into mechanical energy, which is fundamental in the myosin–actin interaction.

The Q750K mutation is due to a nucleotide change C>A at position 2248, with a glutamine/lysine substitution and was detected in a Spanish patient affected by bilateral sensorineural hearing loss. K823N, with nucleotide change 2469A>C, was observed in two members of a family: a 10 years old girl with a bilateral mild hearing loss and a 7 years boy affected by moderate/severe bilateral sensorineural hearing loss. The age of onset was approximately 3 years old. Biological samples of their parents were not available.

Finally, E831K was detected in a Spanish patient affected by bilateral sensorineuronal hearing loss: this variation, characterized by a nucleotide change G>A at 2491 position, causes a glutamic acid to lysine substitution. Mutation Q750K lies in the third IQ domain and might affect the calmodulin binding function of the protein. Genetic studies have shown that the region of interaction between myosin-1c and PHR1 is located in the c-terminal region of myosin-1c (a.a. 762-1028): mutations K823N and E831K could affect myosin-1c and PHR1 interaction.

In conclusion, 5 of the six alleles identified in *MYO1C* affect residues highly conserved across species and for 3 of them bioinformatic analysis strongly suggest a relevant functional role.

3.2. Myosin-1f

For *MYO1F*, six missense variations have been detected, each one at heterozygous state. Five of these amino acid changes involve evolutionary highly conserved residues. The missense mutation P84L was detected in a patient coming from Belgium; it is due to a C>T nucleotide change at position 251, near the ATP-binding site (relative to ATG, designated +1), with a proline/glutamic acid substitution. Pro 84 makes direct contact with groups of surrounding residues (Asp 20 carboxylic group, Phe 47 amide carbonyl group, and Tyr 49 hydroxyl group). In addition, Pro 84 is also solvent-exposed, which is a common feature for proline residues. The P84L mutation implies replacement of Pro 84 with a leucine, a residue characterized by a high tendency to locate away from the solvent. This mutation is likely to cause a local structural destabilization and influencing the nearby binding site of ATP (Fig. 1D).

Mutation c.649A>T was detected in an Italian patient with a moderate form of sensorineural hearing loss. This nucleotide variation is located in exon 9 and causes the substitution of the alanine at position 217 with a threonine (A217T). Ala 217 is partially conserved in the evolutionary scale and is involved in hydrophobic interactions with the side chains of Leu 213 (one of the two δ-

Table 4
MYO 1F mutations

MYO1F *Gene ID*: 4542				
Exon	Nucleotide variation	Amino acid variation	Amino acid conservation[a]	Effects of variation[b]
2–3	251C>T	P84L	+ + +	+ + +
7	649A>T	A217T	+ +	+ + +
13	1504A>G	I502V	+ +	+ + +
15	1656G>C	K552N	+ +	n.d.
19–20	2296G>A	D766N	+ + +	n.d.
26	3080G>A	R1027Q	+	n.d.

[a] + + + residue well conserved; + + residue partially conserved; + residue very partially conserved.
[b] + + + very relevant; + + relevant; n.d. not determined.

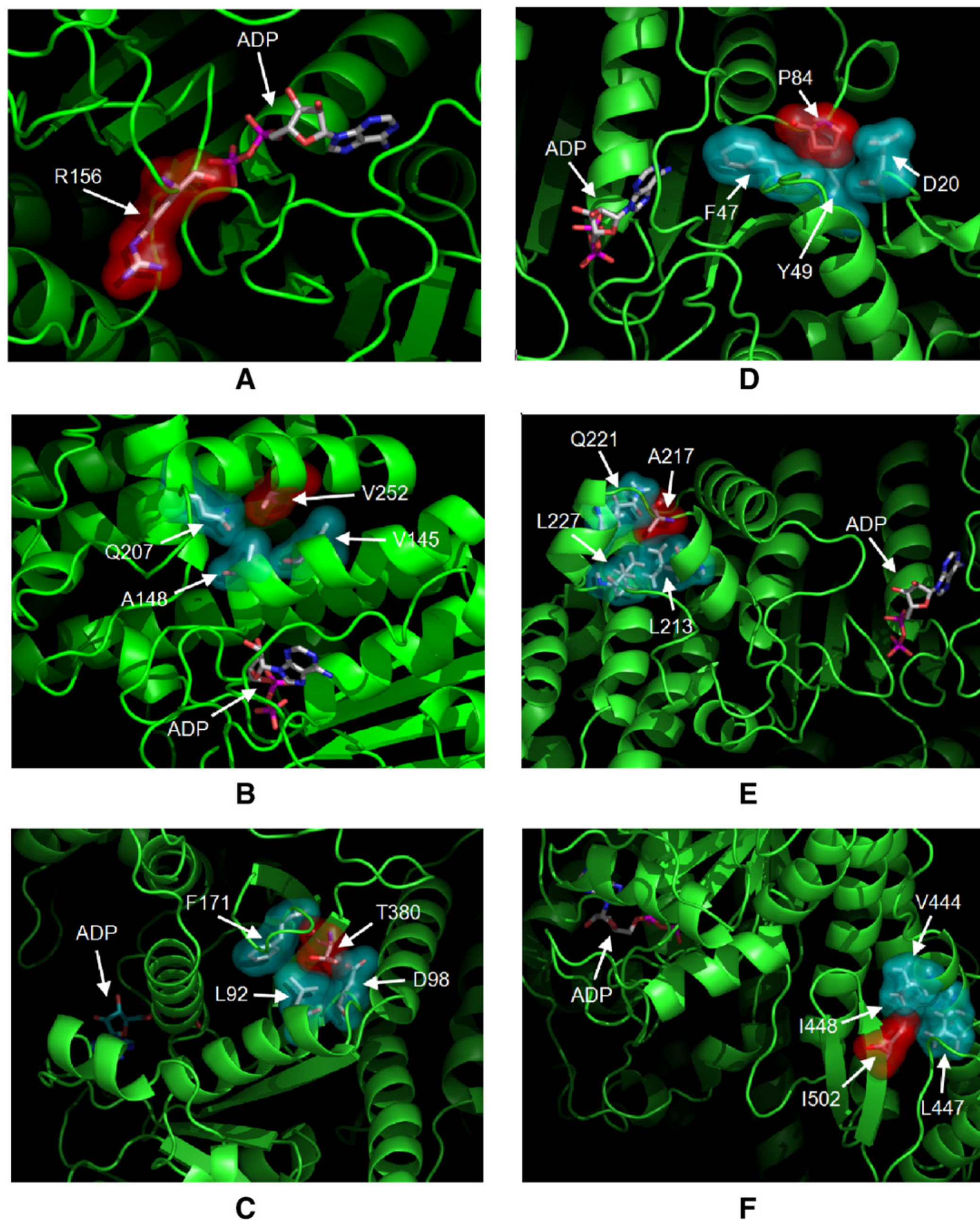

Fig. 1. Homology modelling of the sites of mutation falling within the head domain of myosin-1c and myosin-1f obtained using the crystallographic structure of myosin-2 heavy chain (nonmuscle) from Dictyostelium d. (PDB entry 1g8x, chain A) as a template. Represented are the protein backbone (green ribbon-like structure), the amino acid site of mutation (sticks with red surface), sets of selected amino acids (sticks with cyan surface) showing critical interactions with the mutated residues, and the ADP ligand (stick representation) used to evidence the putative site of binding of the nucleotide phosphate co-factor. Details of myosin-1c structure in proximity of the mutating residues R156, V252 and T380 are shown in panels A, B and C, respectively. Details of myosin-1f regions containing the mutated residues P84, A217 and I502 are shown in panels D, E and F, respectively.

carbon atoms), Gln 221 (the β-carbon atom) and Leu 227 (one of the two δ-carbon atoms). The A217T amino acid change introduces a threonine containing a polar side chain that cannot properly replicate the hydrophobic interactions of the smaller and apolar alanine residue. This mutation is expected to introduce an alteration in a region of the protein that is also characterized by tight interactions between secondary structure elements that are close to the ATP binding site (Fig. 1E).

I502V and K552N are two missense mutations detected in patients coming from Belgium and affected by mild to moderate bilateral sensorineuronal hearing loss; these variations are due to an A>G and C>G change, at positions 1504 and 1656, implying an isoleucine/valine and a lysine/asparagine substitution respectively. These amino acids are located near the actin-binding sites of myosin-1f. Ile 502 is part of β-sheet in the head of myosin-1f and contributes to stabilize the protein structure through its hydrophobic interactions with Val 444, Leu 447 and Ile 448 residues located on a neighbouring α-helix (Fig. 1F). The I502V mutation may disrupt this set of interactions interfering with the binding of ATP. The P84L, A217T and I502V mutations occur close to the ATP binding site in the myosin head-like domain and might interfere with the correct binding of the co-factor.

D776N is a missense mutation due to a G>A nucleotide change at position 2296, in the TH1 domain and leads to a substitution of an

aspartic acid with an asparagine. Aspartic acid is a residue highly conserved and probably functionally important. It was detected in a Spanish patient affected by bilateral sensorineural hearing loss. It segregates within the family since it was inherited from the affected father and also transmitted by the proband to the affected daughter but not to the healthy one (dominant patter of inheritance). It was also detected in a normal hearing member of the family; this information could exclude its pathogenic role, even if we cannot discard the hypothesis that this subject can manifest a hearing impairment in the future.

Finally, R1027Q was detected in a Spanish patient affected by early onset progressive sensorineuronal hearing loss. This variation is due to a G>A nucleotide change at position 3080; arginine 1027 is located between the TH1 and SH3 domains. For this variation a pathogenic role was excluded since it wasn't detected in the other affected members of the probands family.

Thus, summarizing, 5 alleles affect residues conserved across species, and prediction by homology modelling suggest a role for 3 of them.

4. Conclusions

The pathogenic role of more than 40 genes has already been described for hearing impairment. Within this heterogeneous group of genes, 7 belong to myosin superfamily and explain both syndromic and nonsyndromic forms. Expression, functional data and involvement in hearing physiology suggest that other members of the myosin superfamily could contribute to hearing loss. Since both *MYO1C* and *MYO1F* are known to be expressed in cochlea and since *MYO1F* is located in DFNB15, they can be considered good candidate genes for nonsyndromic hearing loss.

Here we report the identification of 11 heterozygous missense mutations in *MYO1F* and *MYO1C*, both clearly expressed in inner ear, in patients affected by bilateral sensorineuronal hearing loss.

MYO1C is a member of the family of unconventional type 1 myosins, characterized by the presence of the head domain (or motor domain), three or four IQ domains and the TH1 domain (or tail domain) at the C-terminus; the tail domain is rich in basic residues which allow interactions with negatively charged membrane phospholipids. Myosin-1c plays an essential role in adaptation of hair-cell mechano-electrical transduction [23]. Recent experiments confirmed that myosin-1c is a component of the hair cell's adaptation-motor complex, directly or indirectly interacting with other components of the transduction apparatus to mediate slow adaptation [24].

Furthermore, myosin-1c has been proposed as a motor protein that sets a resting tension on the tip link and the channels during fast adaptation processes [25–27]. Moreover, recent data indicate that myosin-1c interacts *in vitro* with three other molecules proposed to be important for transduction: cadherin 23 (CDH23), phosphatidylinositol 4,5-bisphosphate (PIP2) [28] and PHR1, an integral membrane protein [29].

The six missense mutations here reported suggest a pathogenic role of myosin-1c gene in hearing loss. The homology modelling proposes that mutations located in the head of myosin-1c have a strong influence on its functionality: they are predicted to affect sites of the protein relevant in ATP binding and/or in the conversion of the chemical energy of this co-factor into mechanical energy. Instead, the other missense mutations are located in IQ3, a domain, as suggested by Phillips et al. [28], involved in the regulation of myosin-1c activity by Ca^{2+} and in calmodulin binding, or in the region of interaction with PHR1. As hypothesized by Etournay et al. [29] PHR1 and myosin-1c form a complex acting as elastic molecular cross linker that contribute to and modulate the membrane tension in the slow adaptation process: the increasing of the stereociliar Ca^{2+} concentration through the open MET channels leads to the dissociation of myosin molecules from actin filaments, decreasing the MET channel open probability.

Summarizing, for *MYO1C*, all the mutated alleles described in this study can modify the adaptation process in which myosin-1c and PHR1 have an important role: a) mutations in the head of the protein could modify the conversion of the chemical energy of this co-factor into mechanical energy or the actin binding process; b) mutation in IQ motifs could change the regulatory function of Ca^{2+} or calmodulin on the myosin-1c activity; c) mutations in IQ4 or in the tail of the protein could interfere with the correct assembly and function of PHR1-myosin complexes. Other members of the transduction apparatus have been shown to be involved in hearing loss: mutations in *CDH23*, *Harmonin*, *PCDH15*, *MYOVIIA*, *PMCA2* have been shown to cause *Usher Syndrome or* autosomal forms of Hearing loss (see hereditary hearing loss homepage at http://webh01.ua.ac.be/hhh/); mutations in *MYO1C* suggest that proteins of the transduction apparatus have a key role in the sensorineural forms of hearing loss.

Regarding myosin-1f, very limited information is available. *MYO1F* is expressed in the cochlea and maps to chromosome 19p13.3–13.2 where is located the DFNB15, an autosomal recessive nonsyndromic deafness locus [30]. The amino acid sequence suggests the presence of a head domain, a single IQ domain, the TH1 domain and at the C-terminus an SH3 domain as mediator of protein–protein interactions [31]. Kim et al. [32] demonstrated that in mice, Myo1f is expressed predominantly in the mammalian immune system, and it was hypothesized that this protein could direct immune cell motility and innate host defence against infection. *MYO1F* was selectively expressed in neutrophils and was shown to be indispensable for both phagocytosis of bacteria and their destruction: it inhibits exocytosis of integrin containing granules in neutrophils, preventing excess of adhesion. Myosin-1f deficient neutrophils show a stronger adhesion to integrin ligands [33]. Since the inner ear immune response seems to be basically cellular [34], a possible affection of integrin mediated adhesion related to myosin-1f defects could increase the susceptibility to infections or could cause a neutrophil impaired response, leading to auditory tissues damage due to reactive oxygen species. Homology modelling showed that all mutations detected in the *MYO1F* head can seriously affect ATP binding, probably by reducing or modifying myosin-1f function. On the basis of the role of myosin-1f in the mammalian immune system, we can assume that hearing loss in these patients can be due to an increased susceptibility to infections or to an abnormal neutrophilic response with the production of reactive oxygen species (ROS) and the subsequent involvement of proteolysis enzymes and antimicrobial proteins [32].

For both myosin-1c and myosin-1f, mutations detected in the head domain could have a potential influence on the structure of the ATP binding site and could probably affect their ATPase activity, although it cannot be excluded that mutations in the head domain might also interfere with the actin binding process. Moreover, structural studies on myosins suggest that the interaction with actin requires large conformational changes that involve several residues, and no clear identification of all the amino acids composing the actin-binding regions is available yet. For *MYO1C* mutations located in other portions of the protein, we can hypothesize a modification of the regulatory function of Ca^{2+} or calmodulin on the myosin-1c activity or that of PHR1 interaction.

In conclusion, a) both genes are expressed in the inner ear and thus have been selected as good candidates; b) mutants have been detected at the heterozygous state in hearing impaired people but not in normal controls; c) the vast majority of the alleles here described affect residue highly conserved across species; d) modelling homology, whenever applied, strongly suggest a functional role of mutants; e) whenever possible a segregation within families was shown. We cannot rule out the possible presence of rare recessive alleles or the identification of carriers in which the cause of hearing loss is attributable to other genes, but all our findings suggest to exclude a mutational load and propose a possible causative role of the above

mentioned genes in hearing loss. Future studies will better define the role of *MYO1C* and *MYO1F* mutants in causing hearing loss.

Acknowledgment

We thank Professor Karen Avraham for her important contribution in providing DNA samples.

References

[1] J.S. Berg, B.C. Powell, R.E. Cheney, A millenial myosin census, Mol. Biol. Cell. 12 (2001) 780–794.
[2] V. Mermall, P.L. Post, M.S. Mooseker, Unconventional myosins in cell movement, membrane traffic, and signal transduction, Science 279 (1998) 527–533.
[3] J.R. Sellers, Myosin, Protein Profile, 2nd ed.Oxford University Press, Oxford, 1999.
[4] A. Forge, T. Wright, The molecular architecture of the inner ear, Br. Med. Bull. 63 (2002) 4–5.
[5] J.Y. Tinevez, F. Jülicher, P. Martin, Unifying the various incarnations of active hair-bundle motility by the vertebrate hair cell, Biophys. J. 93 (2007) 4053–4067.
[6] T.B. Friedman, Y. Liang, J.L. Weber, J.T. Hinnant, T.D. Barber, S. Winata, I.N. Arhya, J.H. Asher Jr, A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17, Nat. Genet. 9 (1995) 86–91.
[7] A.K. Lalwani, J.A. Goldstein, M.J. Kelley, W. Luxford, C.M. Castelein, A.N. Mhatre, Human nonsyndromic hereditary deafness DFNA17 is due to a mutation in nonmuscle myosin MYH9, Am. J. Hum. Genet. 67 (2000) 1121–1128.
[8] T. Walsh, V. Walsh, S. Vreugde, R. Hertzano, H. Shahin, S. Haika, M.K. Lee, M. Kanaan, M.C. King, K.B. Avraham, From flies' eyes to our ears: mutations in a human class III myosin cause progressive nonsyndromic hearing loss DFNB30, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 7518–7523.
[9] F. Donaudy, A. Ferrara, L. Esposito, R. Hertzano, O. Ben-David, R.E. Bell, S. Melchionda, L. Zelante, K.B. Avraham, P. Gasparini, Multiple mutations of MYO1A, a cochlear-expressed gene, in sensorineural hearing loss, Am. J. Hum. Genet. 72 (2003) 1571–1577.
[10] F. Donaudy, R. Snoeckx, M. Pfister, H.P. Zenner, N. Blin, M. Di Stazio, A. Ferrara, C. Lanzara, R. Ficarella, F. Declau, C.M. Pusch, P. Nürnberg, S. Melchionda, L. Zelante, E. Ballana, X. Estivill, G. Van Camp, P. Gasparini, A. Savoia, Nonmuscle myosin heavy-chain gene MYH14 is expressed in cochlea and mutated in patients affected by autosomal dominant hearing impairment (DFNA4), Am. J. Hum. Genet. 74 (2004) 770–776.
[11] D. Weil, S. Blanchard, J. Kaplan, P. Guilford, F. Gibson, J. Walsh, P. Mburu, A. Varela, J. Levilliers, M.D. Weston, P.M. Kelley, W.J. Kimberling, M. Wagenaar, F. Levi-Acobas, D. Larget-Piet, A. Munnich, K.P. Steel, S.D.M. Brown, C. Petit, Defective myosin VIIA gene responsible for Usher syndrome type 1B, Nature 374 (1995) 60–61.
[12] Y. Tamagawa, K. Kitamura, T. Ishida, K. Ishikawa, H. Tanaka, S. Tsuji, M. Nishizawa, A gene for a dominant form of non-syndromic sensorineural deafness (DFNA11) maps within the region containing the DFNB2 recessive deafness gene, Hum. Molec. Genet. 5 (1996) 849–852.
[13] X.Z. Liu, J. Walsh, Y. Tamagawa, K. Kitamura, M. Nishizawa, K.P. Steel, S.D.M. Brown, Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene, (Letter) Nature Genet. 17 (1997) 268–269.
[14] D. Weil, P. Kussel, S. Blanchard, G. Levy, F. Levi-Acobas, M. Drira, H. Ayadi, C. Petit, The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene, Nature Genet. 16 (1997) 191–193.
[15] L.M. Astuto, P.M. Kelley, J.W. Askew, M.D. Weston, R.J. Smith, A.F. Alswaid, M. Al-Rakaf, W.J. Kimberling, Searching for evidence of DFNB2, Am. J. Med. Genet. 109 (2002) 291–297.
[16] S. Riazuddin, S. Nazli, Z.M. Ahmed, Y. Yang, F. Zulfiqar, R.S. Shaikh, A.U. Zafar, S.N. Khan, F. Sabar, F.T. Javid, E.R. Wilcox, E. Tsilou, E.T. Boger, J.R. Sellers, I.A. Belyantseva, S. Riazuddin, T.B. Friedman, Mutation spectrum of MYO7A and evaluation of a novel nonsyndromic deafness DFNB2 allele with residual function, Hum. Mutat. 29 (2008) 502–511.
[17] K.B. Avraham, T. Hasson, T. Sobe, B. Balsara, J.R. Testa, A.B. Skvorak, C.C. Morton, N.G. Copeland, N.A. Jenkins, Characterization of unconventional MYO6, the human homologue of the gene responsible for deafness in Snell's waltzer mice, Hum. Mol. Genet. 6 (1997) 1225–1231.
[18] S. Melchionda, N. Ahituv, L. Bisceglia, T. Sobe, F. Glaser, R. Rabionet, M.L. Arbones, A. Notarangelo, E. Di Iorio, M. Carella, L. Zelante, X. Estivill, K.B. Avraham, P. Gasparini, MYO6, the human homologue of the gene responsible for deafness in Snell's waltzer mice, is mutated in autosomal dominant nonsyndromic hearing loss, Am. J. Hum. Genet. 69 (2001) 635–640.
[19] Z.M. Ahmed, R.J. Morell, S. Riazuddin, A. Gropman, S. Shaukat, M.M. Ahmad, S.A. Mohiddin, L. Fananapazir, R.C. Caruso, T. Husnain, S.N. Khan, S. Riazuddin, A.J. Griffith, T.B. Friedman, E.R. Wilcox, Mutations of MYO6 are associated with recessive deafness, DFNB37, Am. J. Hum. Genet. 72 (2003) 1315–1322.
[20] Y. Wakabayashi, Y. Takahashi, Y. Kikkawa, H. Okano, Y. Mishima, T. Ushiki, H. Yonekawa, R. Kominami, A novel type of myosin encoded by the mouse deafness gene shaker-2, Biochem. Biophys. Res. Commun. 248 (1998) 655–659.
[21] A. Wang, Y. Liang, R.A. Fridell, F.J. Probst, E.R. Wilcox, J.W. Touchman, C.C. Morton, R.J. Morell, K. Noben-Trauth, S.A. Camper, T.B. Friedman, Association of unconventional myosin MYO15 mutations with human nonsyndromic deafness DFNB3, Science 280 (1998) 1447–1451.
[22] A. Sali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815.
[23] C. Batters, C.P. Arthur, A. Lin, J. Porter, M.A. Geeves, R.A. Milligan, J.E. Molloy, L.M. Coluccio, Myo1C is designed for the adaptation response in the inner ear, EMBO J. 23 (2004) 1433–1440.
[24] K.R. Phillips, J.L. Cyr, In situ binding assay to detect Myosin-1c interactions with hair-cell proteins, Methods Mol. Biol. 392 (2007) 117–131.
[25] P.G. Gillespie, J.L. Cyr, Myosin-1c, the hair cell's adaptation motor, Annu. Rev. Physiol. 66 (2004) 521–545.
[26] P.G. Gillespie, Myosin I and adaptation of mechanical transduction by the inner ear, Philos. Trans. R. Soc. Lond. B. Biol. Sci. 359 (2004) 1945–1951.
[27] E.A. Stauffer, J.D. Scarborough, M. Hirono, E.D. Miller, K. Shah, J.A. Mercer, J.R. Holt, P.G. Gillespie, Fast adaptation in vestibular hair cells requires myosin-1c activity, Neuron 47 (2005) 541–553.
[28] K.R. Phillips, S. Tong, R. Goodyear, G.P. Richardson, J.L. Cyr, Stereociliary myosin-1c receptors are sensitive to calcium chelation and absent from cadherin 23 mutant mice, J. Neurosci. 26 (2006) 10777–10788.
[29] R. Etournay, A. El-Amraoui, A. Bahloul, S. Blanchard, I. Roux, G. Pézeron, N. Michalski, L. Daviet, J.P. Hardelin, P. Legrain, C. Petit, PHR1, an integral membrane protein of the inner ear sensory cells, directly interacts with myosin 1c and myosin VIIa, J. Cell. Sci. 118 (Pt 13) (2005) 2891–2899 Jul 1.
[30] A.H. Chen, D.A. Stephan, T. Hasson, K. Fukushima, C.M. Nelissen, A.F. Chen, A.I. Jun, A. Ramesh, G. Van Camp, R.J. Smith, MYO1F as a candidate gene for nonsyndromic deafness, DFNB15, Arch. Otolaryngol. Head Neck Surg. 127 (2001) 921–925.
[31] F. Crozet, A. El Amraoui, S. Blanchard, M. Lenoir, C. Ripoll, P. Vago, C. Hamel, C. Fizames, F. Levi-Acobas, D. Depétris, M.G. Mattei, D. Weil, R. Pujol, C. Petit, Cloning of the genes encoding two murine and human cochlear unconventional type I myosins, Genomics 40 (1997) 332–341.
[32] S.V. Kim, W.Z. Mehal, X. Dong, V. Heinrich, M. Pypaert, I. Mellman, M. Dembo, M.S. Mooseker, D. Wu, R.A. Flavell, Modulation of cell adhesion and motility in the immune system by Myo1f, Science 314 (2006) 136–139.
[33] R. Bovo, C. Aimoni, A. Martini, Immune-mediated inner ear disease, Acta Otolaryngol. 126 (10) (2006) 1012–1021.
[34] Y. Sibille, F.X. Marchandise, Pulmonary immune cells in health and disease: polymorphonuclear neutrophils, Eur. Respir. J. 6 (1993) 1529–1543.