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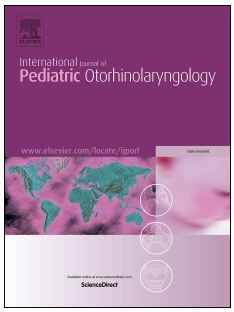

Homozygous mutations in PJVK and MYO15A genes associated with non-syndromic hearing loss in Moroccan families

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Abstract

Objectives

Autosomal recessive non-syndromic hearing loss is a heterogeneous disorder and the most prevalent human genetic sensorineural defect. In this study, we investigated the genetic cause of sensorineural hearing loss in Moroccan patients and presented the importance of whole exome sequencing (WES) to identify candidate genes in two Moroccan families with profound deafness

Methods

After excluding mutations previously reported in Moroccan deaf patients, whole exome sequencing was performed and Sanger sequencing was used to validate mutations in these genes.

Results

Our results disclosed the c.113_114insT (p.Lys41GlufsX8) and c.406C>T (p.Arg130X) homozygous mutations in PJVK and a homozygous c.5203C>T (p.Arg1735Trp) mutation in *MYO15A*, both genes responsible for non-syndromic recessive hearing loss DFNB59 and DFNB3, respectively.

Conclusion

We identified in Moroccan deaf patients two mutations in *PJVK* and one mutation in *MYO15A* described for the first time in association with non-syndromic recessive hearing loss. These results emphasize that whole exome sequencing is a powerful diagnostic strategy to identify pathogenic mutations in heterogeneous disorders with many various causative genes.

Keywords: *PJVK*; *MYO15A*; Hearing Loss; Whole Exome Sequencing; Morocco.

Introduction

Hearing loss is the most prevalent human sensorineural defect, due to genetic or environmental causes, or both [1,2]. It affects approximately 0.2% of newborns [3]. To date, a total of 159 loci have been mapped for non-syndromic deafness and 109 genes identified (http://hereditaryhearingloss.org). In most cases, the search for the gene responsible for the hereditary deafness by screening autosomal recessive non-syndromic hearing loss (ARNSHL) genes using standard molecular procedures is extensively long, expensive and time consuming [4-6]. In this study, we present the genetic and molecular characterization of three consanguineous Moroccan families affected by ARNSHL. Using whole exome sequencing (WES) on the index cases of two Moroccan families, we identified two homozygous mutations in *PJVK* and *MYO15A* genes. Using direct sequencing, a second mutation of PJVK gene was identified in one additional family.

The *PJVK* gene contains 7 exons along a 9.8 kb region of chromosomal locus 2q31.2. The protein encoded by *PJVK* gene, is involved in neural signal transmission [7]. This gene is expressed in the cell bodies of neurons, hair cells, supporting cells and spiral ganglion cells in the inner ear [7,8]. Mutations in *PJVK* cause autosomal recessive non syndromic hearing loss in humans at the DFNB59 locus [7–13]. Also, *PJVK* mutation has been reported to cause vestibular dysfunction along with deafness in one Moroccan family [14]. *MYO15A* gene consists of 66 exons spanning 71 kb of genomic sequence. It is located at chromosome 17p11.2 and encodes a protein myosin XVa which is an unconventional myosin, critical for the elongation and differentiation of the stereocilia [15]. It has an important role in actin organization in hair cells [16,17]. Mutations of *MYO15A* at the DFNB3 locus are the third most common cause of autosomal recessive non syndromic deafness in Pakistan and the second cause in Iranian population [18-19]. In this study, we report the implication of the *PJVK* and *MYO15A* genes in sensorineural hearing loss in Moroccan consanguineous patients.

Methods

In this study, we recruited 3 unrelated families (SF02, SF211, SF170), with more than one deaf patient to refine the genetic profile of deafness in Moroccan families. All patients presented profound congenital bilateral hearing loss and were from consanguineous families. They were clinically examined to exclude environmental causes for the hearing impairment. No other abnormality was encountered indicating the non-syndromic feature of the disease. Molecular analysis was performed after obtaining informed consent. The genetic study was approved by the committee on research ethics of the Pasteur Institute of Morocco.

Genomic DNA was extracted from the blood from all family members, using PureLinkTM Genomic DNA Minikit (Invitrogen, Made in USA). Patients were negative for mutations in *GJB2*, the m.1555A>G mitochondrial mutation in *MT-RNR1* and the c.242G>A mutation in *LRTOMT*. To identify the gene responsible for their condition, whole exome sequencing (WES) was performed on a single index case SF02.02 and SF211.04 in each of families SF02 and SF211, respectively, at Otogenetics Corporation (Norcross, GA, USA), as described elsewhere [20].

Using the 1000 Genomes Project database, the Exome Variant Server (EVS) and the ExAC (Exome Aggregation Consortium) browser, candidate pathogenic variants were defined as missense, nonsense, splice-site and frameshift mutations. Variants with a frequency of less than 1% were considered as rare. SIFT (Sorting Intolerant From Tolerant), PolyPhen-2 (Polymorphism Phenotyping) and Proven softwares predicted the impact of each variant on protein structure [21-23]. PCR amplification and Sanger sequencing were performed to verify that the identified mutations co-segregate with the phenotype in the studied families. In addition, direct sequencing of causative genes in SF02 and SF211 families were analyzed in 30 other ARNSHL Moroccan families. Direct sequencing of PCR products was performed

with the ABI prism Big Dye Terminator cycle sequencing Ready Reaction kit v. 3.1 (ABI Prism/Applied Biosystems, Foster City, CA) and run on an ABI Prism 3130 Genetic Analyzer (Applied Biosystem). Sequence analysis was carried out with the ABI SeqScape v. 2.5 Software. Specific primers were designed using Primer3 (http://primer3.ut.ee/) (Table 1).

Results

In the index case (SF02.02) from family SF02 (Fig1.A), a homozygous 1-bp insertion in exon 2 of the *PJVK* gene, c.113_114insT was identified by WES (Fig1.B). Sanger sequencing confirmed that the two affected offspring (SF02.03 and SF02.04) were homozygous for the mutation, whereas their unaffected father (SF02.01) and the healthy brother (SF02.05) were heterozygous for this mutation, and the healthy sister had a wild-type genotype. This mutation, already described in a Moroccan family, [24] induces a stop codon 28 bp downstream, truncating 47 residues (p.Lys41GlufsX8) of the protein. Direct sequencing of all *PJVK* exons in 30 other ARNSHL Moroccan families identified a novel c.406C>T mutation in exon 3 in the consanguineous SF170 family (Fig1.A). Segregation analysis showed that the two affected individuals (SF170.03 and SF170.04) are homozygous for this mutation, whereas their unaffected parents (SF170.01 and SF170.02) are heterozygous (Fig1.B). The *PJVK* c.406C>T mutation leads to a nonsense codon at position 136 and is reported (rs367688416) with an allelic frequency of 1.841e-05.

In the index case (SF211.04) from family SF211 analyzed by WES (Fig1.A), we disclosed a *MYO15A* homozygous mutation, c.5203C>T (p.Arg1735Trp) and confirmed it by Sanger sequencing (Fig1.B). This mutation was also detected in his two affected sisters (SF211.03 and SF211.05), whereas the heterozygous mutation was identified in both of their unaffected parents (SF211.01 and SF211.02) and brother (SF211.06). The *MYO15A* c.5203C>T mutation

is predicted to lead to a substitution of arginine at position 1735 to tryptophan, and is reported in ExAC database (rs778354646) with an allelic frequency of 8.25887e-06.

Discussion

There are multiple technologies available to undertake the identification of casual genetic variants associated with disease. Whole Exome Sequencing is the most efficient method if compared to other classical molecular methods. In this study, two mutations in the *PJVK* gene and one mutation in the *MYO15A* gene have been found. The protein encoded by PJVK (pejvakin), which is known to be responsible for DFNB59, is expressed in inner and outer hair cells, in a subset of supporting cells as well as in the spiral ganglion and in structures of the afferent auditory pathway[7]. The pejvakin protein contains a nuclear localization signal (residues 249-258) and a zinc-binding motif (residues 305-331) in the C-terminal domain. PJVK plays an important role in the activity of auditory pathway neurons [25-26] and also in the cell signaling of hair cells and sensory neurons [24]. To date, 17 pathogenic *PJVK* mutations have been identified (Table 2) (http://deafnessvariationdatabase.org/letter/d), all associated with non-syndromic hearing loss from which the homozygous c.113_114insT mutation that we found in SF02 family. While the father of this family is heterozygous and the mother's genotype could not be identified, we suggested that the mother had heterozygous c.113_114insT mutation, which explained the homozygosis of the affected offspring. The mutation c.113_114insT that we identified has been previously reported in a consanguineous Moroccan family with autosomal recessive congenital progressive hearing loss [24]. The second c.406C>T *PJVK* mutation found induced congenital bilateral profound hearing loss in this consanguineous family, that might result from a founder effect originating from a Israeli Arab village, including patients with prelingual non-syndromic hearing impairment and absent otoacoustic emissions, with the same *PJVK* mutation [11,12].

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Among the myosin superfamily, changes in the unconventional myosin XVA, which is encoded by *MYO15A*, are known to be responsible for DFNB3. MYO15A contains an N-terminal motor domain, 2 light-chain binding IQ motifs, and a tail region containing a MyTH4 and a talin like domain [27-28]. To date, 135 pathogenic *MYO15A* mutations have been identified (http://deafnessvariationdatabase.org/letter/m). Search for *MYO15A* mutations have been mainly performed in the Middle East, where consanguineous families are common [30-33]. Other mutations were also been identified in Tunisian, Brazilian and Korean families [32;34;35] .

The frequency of the *MYO15A* mutation, related with nonsyndromic autosomal recessive deafness is more important in the Middle East and Asia, than in North Africa [36-38]. In Turkey, 9.9% of patients are affected by this type of deafness [38] and only 2.1% were reported in Korea [39]. Therefore the high frequency of the *MYO15A* mutation in the Middle East might be due to the high rate of consanguineous families.

Conclusions

In two deaf Moroccan consanguineous families, mutations of *PJVK* gene were identified. In the third consanguineous family, the previously reported pathogenic variant p.Arg1735Trp in *MYO15A* was identified for the first time in association with ARNSHL. Compared to the classical genetic method (homozygosity mapping and Sanger sequencing), Whole Exome Sequencing is the most effective method to identify mutations in highly heterogeneous disease as hearing loss. Further analysis of these genes should facilitate diagnosis of congenital deafness of affected subjects in Morocco and other countries with high level of consanguineous families.

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Competing interests

The authors declare that they have no competing interest.

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Figures legends

Fig.1. Mutations in *PJVK and MYO15A* segregate with non-syndromic hearing loss. (A) Pedigrees of the three families are shown with the segregation of the mutations identified in *PJVK* (family SF170 and SF02) and *MYO15A* (family SF211). Index cases analyzed with whole exome sequencing (WES) are marked with a red arrow. NA: Not Available. (B) Electrophoregrams showing the 3 mutations identified in this work at the heterozygous (top) and homozygous (bottom) states.

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Table 1

Sequence of the primers used to validate the mutations by Sanger sequencing**.**

Gene and Exons	Sequence 5'→ 3'	SIZE (bp)
PEJVAKIN-E2F	TGAGCAGAGGCAGGGAATTA	496
PEJVAKIN-E2R	TTGCTTTAATCATTGAAAATGTG	
PEJVAKIN-E3F	TGGTGAGTCATGTTGCCTTT	572
PEJVAKIN-E3R	CAGCCTCTCTGCCTCTTCAT	
PEJVAKIN-E4F	CATTTCTTTTGGGTGTATTTTCTG	441
PEJVAKIN-E4R	GACATTGCTGTGCAAAATCC	
PEJVAKIN-E5F	AAAAATGGTTTAATTCAAGCAGAA	402
PEJVAKIN-E5R	GATTGACAGTGCTGAGAGCATC	
PEJVAKIN-E6F	GGTGGGCCAGAGACCTATTT	460
PEJVAKIN-E6R	CTCCCAAAGTGCTGGGATTA	
PEJVAKIN-E7F	CACATTTCTTTTCTGTTTTTGTCC	465
PEJVAKIN-E7R	GGGAATATGATACCCAGACAGG	
MYO15A-E1F	AGGATTACCCAGGCCATTGT	247
MYO15A-E1R	GCCACCCTTGTCTTTGTCTG	

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Table 2
Overview of all *PJVK* (NM_001042702) mutations

Origin of family	cDNA change	Protein change	References
Iranian	c. 161C>T	p.T54I	[7,9,24]
	c. 547C>T	p. R183W	
	c.726delT	p.F242LfsX7	
	c.988delG	p.V330LfsX7	
Turkish	c.499C>T	p.R167X	[25]
	c.548G>A	p.Arg183Gln	[8]
Dutch	c.509_512delCACT	p.S170CfsX35	[8]
	c.731T>G	p.L244R	
Chinese	c.930_931delAC	p.C312WfsX19	[13]
Moroccan	c.113_114insT	p.Lys41GlufsX8	[24] This study
Israeli Arab Moroccan	c.406C>T	p.R136X	[12] This study
	c.490C>T	p.Arg164Stop	
	c.970G>T	p.Gly324Trp	
Pakistani	c.121delA		
	c.211+1G>T		
	c.274C>T	p.Arg92Stop	
	c.1028G>C	p.C343S	[40]

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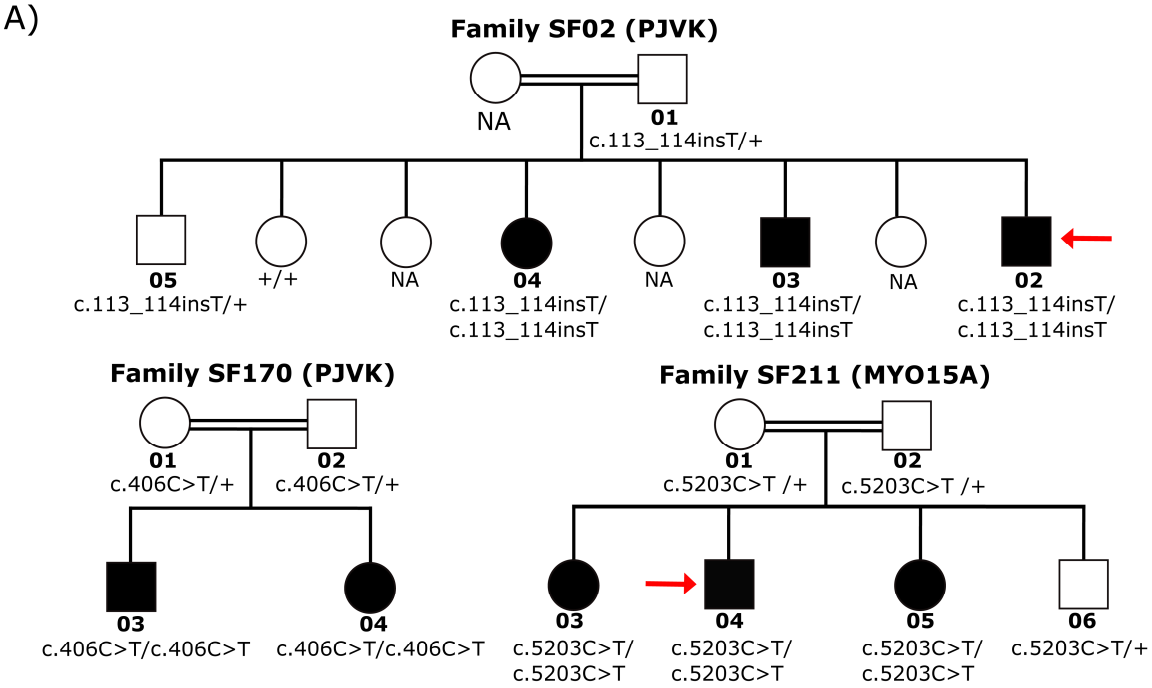
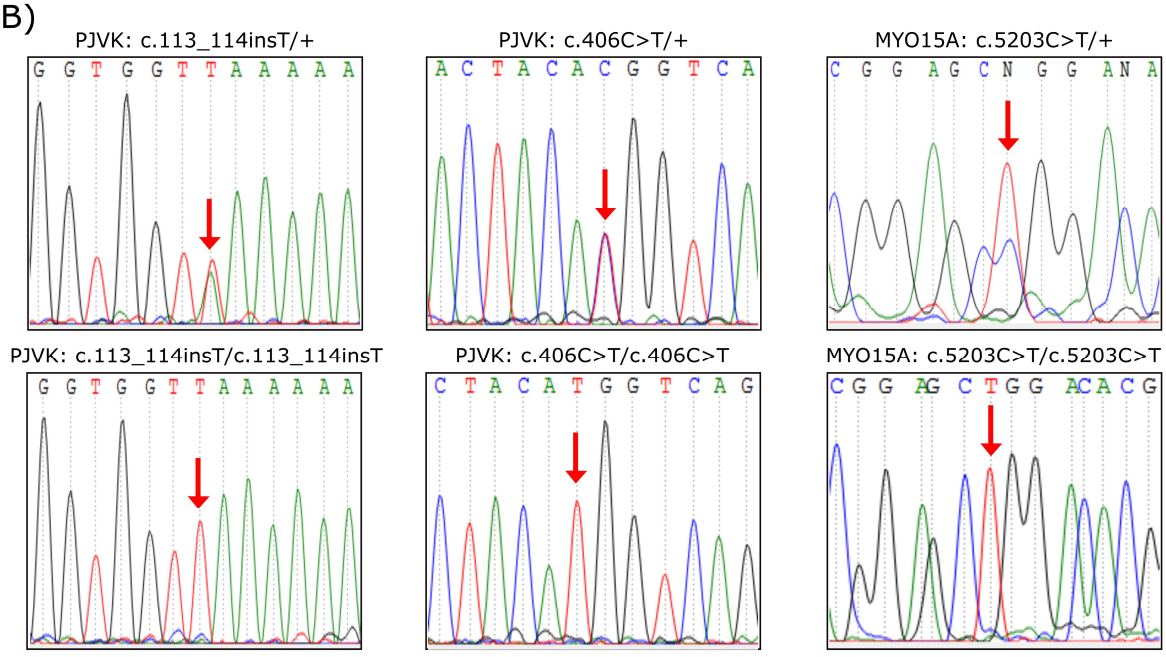
A)


Family SF02 (PJVK)
NA
01
c.113_114insT/+
05
c.113_114insT/+
+/+
NA
04
c.113_114insT/
c.113_114insT
NA
03
c.113_114insT/
c.113_114insT
NA
02
c.113_114insT/
c.113_114insT
Family SF170 (PJVK)
01
c.406C>T/+
02
c.406C>T/+
03
c.406C>T/c.406C>T
04
c.406C>T/c.406C>T
Family SF211 (MYO15A)
01
c.5203C>T /+
02
c.5203C>T /+
03
c.5203C>T/
c.5203C>T
04
c.5203C>T/
c.5203C>T
05
c.5203C>T/
c.5203C>T
06
c.5203C>T/+

B)


PJVK: c.113_114insT/+
PJVK: c.406C>T/+
MYO15A: c.5203C>T/+
PJVK: c.113_114insT/c.113_114insT
PJVK: c.406C>T/c.406C>T
MYO15A: c.5203C>T/c.5203C>T