Genetic heterogeneity in GJB2, COL4A3, ATP6V1B1 and EDNRB variants detected among hearing impaired families in Morocco

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Abstract

Background Deafness is the most prevalent human sensorineural defect. It may occur as a result of an external auditory canal involvement, or a deficiency in the sound conduction mechanism, or an impairment of the cochlea, the cochlear nerve or central auditory perception. The genetic causes are the most common, as approximately 70% of hearing disorders are of hereditary origin, divided into two groups, syndromic (associated with other symptoms) and no syndromic (isolated deafness).

Methods A whole exome sequencing was performed to identify the genetic cause of hearing loss in six Moroccan families and Sanger sequencing was used to validate mutations in these genes.

The results The results of four out of the six families revealed four genetic variants in the genes GJB2, COL4A3, ATP6V1B1 and EDNRB responsible for non-syndromic and syndromic hearing loss. Multiple Bioinformatics programs and molecular modelling predicted the pathogenic effect of these mutations.

Conclusions We identified in Moroccan deaf patients four homozygous mutations. These results show the importance of whole exome sequencing to identify pathogenic mutations in heterogeneous disorders with multiple genes responsible.

Keywords Whole exome sequencing · Mutation · Hearing loss · Moroccan patients

Introduction

The global population living with disabling hearing impairment is approximately 6.1% as estimated by the WHO recently[1]. Hearing loss is the most common human neurosensory defect in the world, occurring in approximately 1 in 1000 newborns, and 60% of these cases have a genetic etiology[2]. Hereditary hearing loss is transmitted by various modes: autosomal recessive, autosomal dominant, X- or Y-linked, and mitochondrial. It can be either isolated (non-syndromic) or associated with damage to other organs (syndromic)[3]. The GJB2 gene is the main cause of hereditary...
non-syndromic hearing loss (NSHL) in Morocco as several mutations in this gene have been described as causing autosomal recessive and more rarely dominant hereditary deafness. The most prominent variant in Morocco and the Mediterranean region is the deletion c.35delG[4]. The second cause of deafness in Morocco is the presence of the substitution c.242G>A located on LRTOMT gene [5]. Among the syndromic genes linked to deafness and which occur in the Moroccan population MYO7A[6], PEX1[7], ADGRV1[8] and many others. For non-syndromic genes, there is TMC1[9], CLDN14[10], PJVK[11] and MPZL2[12]. These genes play a very important role in maintaining the

| Genes | Forward Primers (5'-3') | Reverse Primers (5'-3') | Product size |
|-------|-------------------------|-------------------------|--------------|
| GJB2  | AGAGTTGGTGTTTGCT-       | GACTGAGCCTTG-           | 900pb        |
|       | CAGGA                  | GACAGCTGA               |              |
| COL4A3| AGAACCTTC-             | GTCTCCCCAGC-            | 383pb        |
|       | CAAGCTCCCTG            | CATGTAGAA               |              |
| EDNRB | CACCTCGGTTC-           | AAAATG-                 | 398pb        |
|       | CACTTACACA             | GTAGTCT-                |              |
|       | AAAATG-                 | GTCTTTCTGC              |              |
| ATP6V1B1| TTTAGGAATGTGTG-     | ACAATTGCGGA-            | 567pb        |
|       | GTGGGG                 | CAGGGG                  |              |

Fig. 1 The pedigree of all six families
normal physiology of the inner ear, each mutation of these can affect normal hearing physiology[13]. The genetic diagnosis of hearing loss plays a crucial role for the clinical evaluation of deaf persons and their families. The Next Generation Sequencing (NGS) provides an opportunity to explore the genetic structure of the disease, and can be further be used as a benchmark for medical genetic testing[14]. In this study, whole exome analysis was used to resolve the etiology of hearing loss in six Moroccan families.

**Patients and methods**

We recruited six families with syndromic and no syndromic hereditary deafness SF55, SF175, SF177, SF181, SF188 and SF193. Family members were informed of the purpose of the study and gave their informed consent. The genetic study was approved by the medical ethics committee of the Pasteur Institute of Morocco and carried out in accordance with the protocol of the Helsinki declaration.

First, the Whole Exome Sequencing (WES) was performed at IntegraGen (Evry, France). The Agilent Human exome V5 (50 Mb) capture kit allows the capture of libraries, followed by paired end sequencing on Illumina Hiseq 2000. Using Illumina Real-Time Analysis Pipeline version 1.14 image analysis and call up databases were carried out with default parameters. The short reads with paired ends were aligned against the human genome reference sequence hg19 (GRCH37). Then, the bioinformatics analysis of the sequencing data was based on the Illumina pipeline (CASAVA.1.8). We have filtered the variants based on dbSNP (build132) and 1000 genome project databases. Finally, the functional effects of the novel variants were predicted using SIFT and PolyPhen-2. To determine the segregation with the phenotype of the disease in these families, sanger sequencing was carried out to validate the mutation in the candidate gene. Specific primers have been designed using primer 3 (Table 1).

The 3D structures of the native and mutant EDNRB protein were predicted using SWISS Model, a fully automated

Fig. 2 (a) Alignment of EDNRB amino acid from different species (b) The potential structural impact of the p.Arg409Trp mutation of the EDNRB gene is revealed by molecular modeling and analysis of amino acid conservation. Yellow dotted lines represent hydrogen bonds, and green lines represent hydrophobic interaction
protein structure homology-modelling server [15]. The predicted models were generated based on the structure of the Endothelin type B receptor in complex with Endothelin-3 (PDB ID: 6IGK). We noticed that targets (native and mutated EDNRB proteins) and template proteins share more than 94% of sequence similarity. The obtained structures were minimized using the Yasara Energy Minimization Server [16].

Amino acids interactions analysis and visualization were performed using YASARA software [17]. We used the following bioinformatics tools to analyze the impact of amino acid substitutions on the stability of the EDNRB protein structure: mCSM [18], SDM [19], DUET [20] et DeepDDG [21].

Results

For the six families analyzed, results were obtained for the following four families SF55, SF175, SF181 and SF193. For the remaining two (SF177 and SF188), the analysis of the WES results did not identify any gene or variant. (Figure1).

SF177.04 patient was affected with profound prelingual congenital deafness with a trisomy 21 and cardiac disease; whereas the patient SF188.04 had a profound bilateral prelingual deafness with an operated cardiac malformation.

Syndromic genes

For syndromic families, SF181.5 patient suffered from Waardenburg syndrome characterized with profound congenital prelingual bilateral deafness, hypopigmented skin patches, scratching, bright blue irides whereas his older sister only exhibited a profound hearing loss and some white hairs. WES results revealed a novel homozygous mutation NM_001201397.1:c.1225 C>T;p.(Arg409Trp) (rs200363611) of EDNRB. Then, sanger sequencing confirmed that the parents and the brother were heterozygous.

The c.1225 C>T missense variant has been predicted to be possibly damaging to damaging using the SIFT, FATHMM-MKL and POLYPHEN programs, deleterious by LRT software and disease causing by MutationTaster.

Moreover, the alignment of multiple sequences of orthologous EDNRB proteins of different species showed that the missense mutation p.(Arg409Trp) affected a highly conserved residue (Fig. 2.a).

The amino acid interactions analyses showed that this variant did not affect hydrogen bounds. However, it may disturb the hydrophobic interactions between the residue in 409 position and its adjacent amino acids as we observed that the interaction with Leu405 residue was replaced by one with Glu410 (Fig. 2.b, c, d). A destabilizing effect was predicted based on the four different computational tools (Table 2).

The SF193.3 patient is from consanguineous parents, affected with congenital profound bilateral deafness, bilateral medullary nephrocalcinosis, distal renal tubular acidosis (dRTA), hypokalemia and growth retardation. WES analysis identified an insertion of ATP6V1B1 NM_001692.4: c.1155dupC; p.(Ile386HisTer56) (rs781969081) has been identified in the proband. Sanger sequencing confirmed that the affected patient (SF193.3) was homozygous for the mutation, while the unaffected father (SF193.1) and healthy mother (SF193.2) were heterozygous for this mutation.

Concerning the SF175.3, the proband is a girl with bilateral, prelingual deafness and early onset myopia. WES results showed homozygous variant, NM_000091.5:c.3829G>A:p. (Gly1277Ser) (rs190598500) in COL4A3, and had been confirmed in a heterozygous state in the parents and the proband’s sister.

Non-syndromic genes

The SF55 family has severe deafness. The results showed that a heterozygous variant was already described in GJB2, and was involved in a dominant form. Sanger sequencing confirmed that the affected members (SF55.1, SF55.3 and SF55.4) harboured the heterozygous missense mutation NM_004004.6:c.551G>A:p.(Arg184Gln) (rs80338950), while the mother (SF55.02) is homozygous wild-type.

Discussion

In this work, we describe two genes that had been described in the Moroccan population. Complete sequencing of the exome revealed the first characterization of a new mutation in Morocco in GJB2, identified as being the main cause of hereditary NSHL in Morocco affecting around 35% of cases [4]. The most prominent mutation in Morocco and around the Mediterranean is the c.35delG deletion, that is, in our laboratory, always initially sought by direct sequencing as the first step in molecular diagnosis. Other mutations in this gene have been described by Abidi et al. [22] as causing autosomal recessive hereditary deafness, while dominant inheritance rarely occurs in the Moroccan population. The Arg184Gln variant cited in our study was observed for the

Table 2 Mutation effects on EDNRB 3D structure stability

| Software | Prediction | Stability change (ΔΔG :Kcal/mol) |
|----------|------------|----------------------------------|
| mCSM     | Destabilizing | −0.017                          |
| SDM      | Destabilizing | −0.31                           |
| DUET     | Destabilizing | −0.31                           |
| DeepDDG  | Destabilizing | −0.369                          |
first time in the Moroccan population. According to Hamelmann et al. [23], this mutation was observed for the first time in Ghana in 2001 without any associated syndrome. It was then reported in the province of Jiangsu in China in 2016 [24] and in the Indian population in 2017 [25].

The second gene, ATP6V1B1, encodes the vacuolar H + ATPase B1 subunit located on the apical surface of alpha intercalated cells in the distal tubule, and is also expressed in epithelium of the human cochlea and the endolymphatic sac [26]. People with ATP6V1B1 mutations have sensorineural hearing loss, the homozygous variant c.1155dupC in ATP6V1B1 causes a change in the reading frame of isoleucine 386 introducing a premature stop codon with a frequency of 3.20e-5. This variant of ATP6V1B1 was found in a homozygous patient from a Mexican family [27], two Moroccan patients [26] as well as the patient SF193.3 of this study, and Tunisian children with recessive form of dRTA associated to precocious hearing loss [28].

Besides the common genes previously published in Morocco, we have described here the first mutation of the COL4A3 gene in the Moroccan population in a patient affected with Alport Syndrome. The p.(Gly1277Ser) has a frequency of 3.63e-4 on the gnomAD database. Mutations in the COL4A3 gene are linked to kidney problems, deafness, and eye damage known as Alport syndrome [29]. Fallerini et al. had already described the p.(Gly1277Ser) variant in COL4A3 in 3 families in Italy with Alport syndrome, and was found here in a Moroccan family for the first time with hearing loss and an onset of myopia and no kidney problem for the moment but probably because the patient is still very young. They also found this mutation in the heterozygous state in a patient with the autosomal dominant form [30].

The c.1225 C>T missense in EDNRB is reported in the gnomAD database (rs200363611) with a frequency of 1.78e-4. EDNRB mutations are linked to the Waardenburg type IV (WS4) [31]. The SF181 family presents an intrafamilial WS with a variable severity and no declared Hirschsprung disease. Other studies [32, 33] have suggested that EDNRB should be considered as another pathogenic gene prevalent in WS type 1 to the heterozygous state, whereas [31] (Issa 2017 et al.) estimated that the EDNRB mutations are responsible for 5–6% of WS type 2.

A molecular modeling study was performed to evaluate the structural impact of the p.Arg409Trp missense mutation. This mutation appears to cause a change in hydrophobic interactions, which resulted in a change in the 3D structure of the EDNRB protein between the native state and the mutated state. In addition, this mutation has a destabilizing effect on the stability of proteins.

In conclusion, this study describes the involvement of genetic variants of the GJB2, COL4A3, EDNRB and ATP6V1B1 genes in syndromic and non-syndromic deafness in Moroccan patients. These results can be considered very important because they allow to show even more the genetic diversity of hereditary deafness in Moroccan patients. In addition, they also make it possible to enrich national and international data concerning the Moroccan population.

Acknowledgements The authors are indebted to the families who contributed to this study. This project was supported by the Institut Pasteur du Maroc (IPM), we also thanks Dr. Snoussi Khaldi for the clinical auditory investigation.

Author contributions Conceptualization: [Houria Abdelghaffar], [Abdelhamid Barakat] and [Hicham Charoute]; Formal analysis: [Imane Ait Raise], [Ghita Amalou] and [Amale Bousfiha]; Funding acquisition: [Abdelhamid Barakat], [Hassan Rouba]; Investigation: [Imane Ait Raise], [Ghita Amalou]; Methodology: [Imane Ait Raise], [Amale Bousfiha] and [Crystel Bonnet]; Resources: [Christine Petit] and [Abdelhamid Barakat]; Software: [Imane Ait Raise], [Hicham Charoute]; Writing—original draft: [Imane Ait Raise], [Ghita Amalou]; Writing—review & editing: [Houria Abdelghaffar], [Crystel Bonnet], and [Abdelhamid Barakat].

Funding No funds, grants, or other support was received.

Availability of data and material Data will be provided by the authors upon request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The genetic study was approved by the medical ethics committee of the Morocco Pasteur Institute.

Consent to participate Informed consent was obtained from legal guardians.

Consent to publish Patients signed informed consent regarding publishing their data.

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