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## Biallelic variants in *TMIE* and *PDE6B* genes mimic Usher syndrome

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### ABSTRACT

In the case of consanguineous families, comorbidity cannot be ruled out. Here, we reported a child of eleven years-old who presented profound congenital hearing impairment and progressive visual defect who was suspected to have Usher syndrome. We searched for mutations in Usher syndrome genes but we failed to detect any. We then performed whole exome sequencing to identify the causality of their phenotype. Interestingly, we found two homozygous missense variants, p.(Arg84Trp) in *TMIE*, responsible for deafness and p.(His337Arg) in *PDE6B*, responsible for retinitis pigmentosa. The combination of both variants mimics Usher syndrome. This article highlights the importance of genetics in avoiding clinical misdiagnosis, which is important for genetic counseling and in the perspective of gene therapy.

### 1. Introduction

Deafness is the most prevalence sensorineural disorder (World Health Organization, 2018) and retinitis pigmentosa (RP) is the most common inherited retinal dystrophies (Cross et al., 2022). The prevalence of hearing impairment is estimated to 2.21 per 1000 neonates (Bussé et al., 2020) whereas the prevalence of RP is about 1 per 5000 worldwide (Fahim et al., 2000). To date, 148 genes have been reported to be responsible for isolated deafness (<https://hereditaryhearingloss.org/>; last update January 2024) and 86 genes have been implicated in RP (Fahim et al., 2000). Causal deafness genes have been identified for 83 recessive genes (DFNB), 63 dominant genes (DFNA), 6 X-linked genes (DFNX), 1 Y-linked gene (DFNY) and 9 mitochondrial genes. Of note, 14 of which are responsible for both DFNA and DFNB forms. To date, 57 genes have been reported as responsible for recessive RP, 26 for dominant RP and three for X-linked RP (Fahim et al., 2000). Moreover, hundreds of syndromic forms of deafness have been described, and about 300 causal genes for these forms have been identified (Bolz, 2016). In many cases, deafness is associated with other symptoms such

as retinal disorders. Indeed, there are >70 causes of deaf-blindness identified (Causes of Deaf-Blindness | National Center on Deaf-Blindness [Internet], n.d.). Among them, Usher syndrome (USH, MIM 276900, MIM 276905, MIM 605472), an autosomal recessive disorder, characterized by deafness and retinitis pigmentosa (RP) and in some cases vestibular dysfunction, accounts for 50 % of all deaf-blind patients (Yan and Liu, 2010). The prevalence of USH is about 1 per 10,000 (Delmaghani and El-Amraoui, 2022). This syndrome is clinically and genetically heterogeneous. There are three clinical subtypes of Usher syndrome (USH1, USH2, USH3). USH1 combines by profound congenital hearing impairment, prepubertal onset of RP, and vestibular areflexia. USH2 is characterized by congenital moderate to severe hearing impairment, onset of RP in the first or second decade of life, and absence of vestibular dysfunction. USH3 patients present with congenital or early onset progressive hearing impairment, variable age of onset and severity of retinitis pigmentosa, and variable vestibular dysfunction. Nine causal genes have been reported to be responsible for USH; *MYO7A*, *USH1C*, *CDH23*, *PCDH15* and *USH1G* are responsible for USH1, *USH2A*, *ADGRV1* and *WHRN* for USH2 and *CLRN1* for USH3. An earlier

**Abbreviations:** cGMP, Cyclic guanosine monophosphate; DFNA, autosomal dominant gene; DFNB, autosomal recessive gene; DFNX, X-linked gene; gnomAD, Genome Aggregation Database; PDE6B, Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta; PHARC, Polyneuropathy, hearing loss, ataxia, RP, and cataract; RP, retinitis pigmentosa; TMIE, Transmembrane inner ear; USH, Usher syndrome; WES, Whole exome sequencing.

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molecular diagnosis of deaf-blindness is of utmost importance because these patients should be given priority for cochlear implant in early childhood, due to the visual loss. The development of next-generation sequencing enables rapid and efficient diagnosis, but the use of these techniques is still limited in developing countries. In Algeria, very few studies have reported cases of deaf-blindness. In fact, only three studies on USH reveal the genetic diversity of this syndrome in the Algerian population (Ammar-Khodja et al., 2009, 2015; Abdi et al., 2016). At the present time, diagnosis is based mainly on clinical data (severity of deafness and visual disorder). The aim of this study was to genetically characterize a young patient who was clinically diagnosed as USH2. We searched for the causal variant responsible for the phenotype of this patient by both targeted-exome sequencing screening USH genes and whole exome sequencing (WES).

2. Materials and methods

2.1. Ethics statement

This study was approved by the local ethics committee of the CHU Bab El-Oued, in accordance with the "Loi sanitaire n° 85-05 du 16 février 1985" available in Algeria. This study was conducted according

to the principles of the declaration of Helsinki. We proceeded to the patients' anonymization. Written consent for genetic testing was obtained for all family members (parents and children).

2.2. Patients

The patient was recruited in the ENT department of the University hospital center in Algiers (Algeria). The patient was born to consanguineous parents (see Fig. 1A). The patient underwent audiogram, visual field, ocular fundus autofluorescence imaging, and electroretinogram. The patient has severe-to-profound bilateral deafness which required cochlear implantation. An elevation of hearing thresholds between 70 and 90 dB was found for both ears, for pure tone at all frequencies tested (250 to 8000 Hz). During the speech therapy sessions following any implantation, questioning the mother for other signs associated with deafness enabled us to gather information related to the presence of RP. Notably, the mother noted that her daughter suffered from photophobia. The patient was very clumsy at dusk and was afraid of the dark (linked to the reduction in night vision) which prompted an ophthalmological consultation that revealed pronounced signs of RP, such as a reduced visual field and papillary pallor at the fundus. The electroretinogram was abnormal, typical of RP. Moreover, the mother mentioned no signs

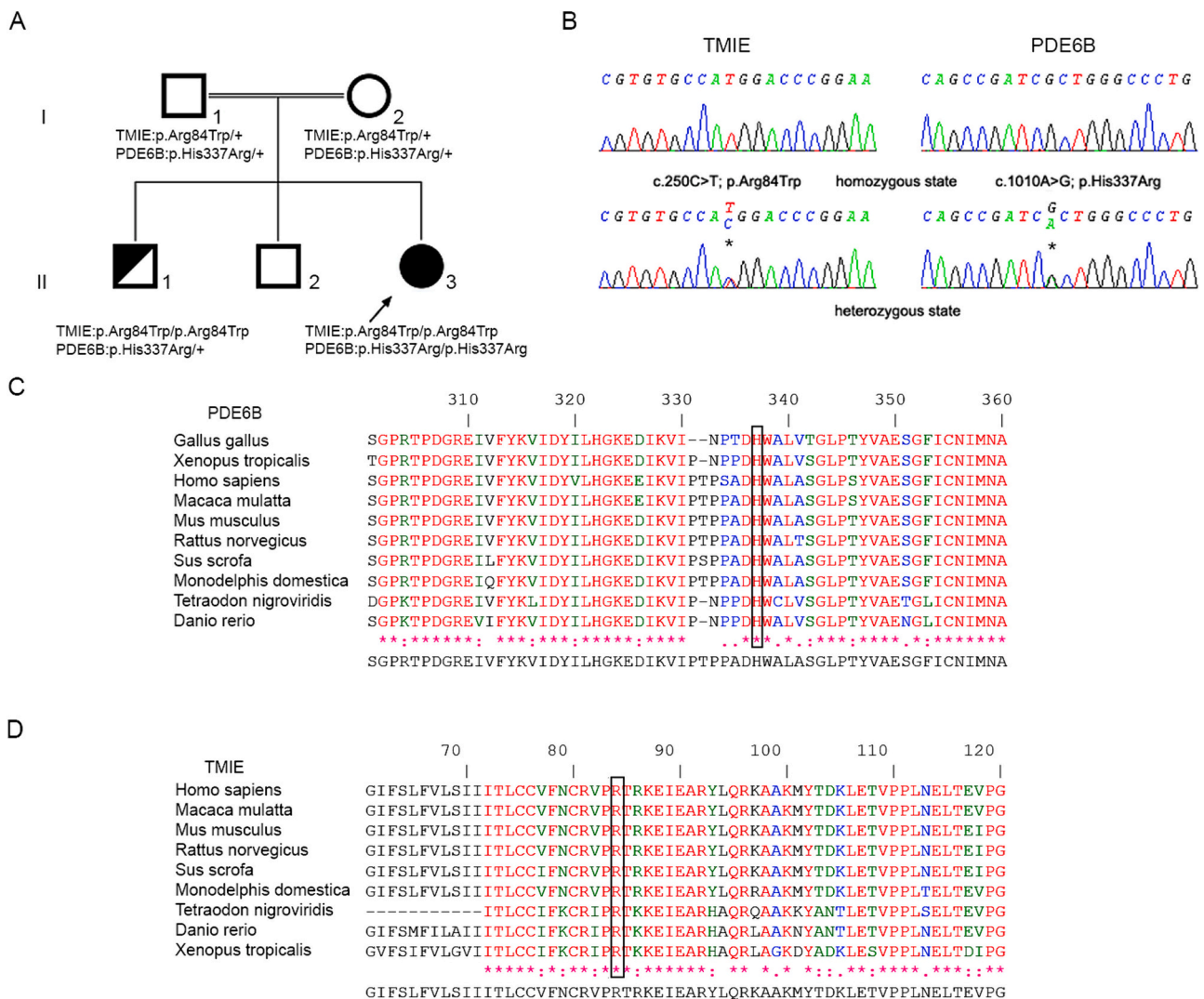


Fig. 1. Family pedigree, DNA chromatogram sequences, and sequence alignment of both variants reported. A. Pedigree of the family. Index case is indicated by an arrow. B. Partial DNA sequencing chromatograms showing both variations. \*indicates the position of the variations. C. Interspecies alignment of PDE6B showing the conservation of the histidine residue. D. Interspecies alignment of TMIE showing the conservation of the arginine residue. Corresponding residues are boxed.

of vestibular dysfunction, in fact, her daughter acquired a sitting position at 6 months, and was walking by 18 months of age. Her older brother also has severe-to-profound deafness without any other visual signs. The parents had normal hearing and no retinal degeneration on fundus examination.

### 2.3. Targeted-exome sequencing and whole exome sequencing

Genomic DNA was extracted from peripheral blood using the Promega Wizard Genomic DNA Purification Kit (Promega, Madison, MI, USA, Cat. # A1120) according to the manufacturer's instructions. Targeted-exome sequencing of USH genes was used to check the presence of pathogenic variants as previously reported (Bonnet et al., 2016). To summarize, we used a multiplex PCR method with an Access Array microfluidic support (Fluidigm). Primer pairs were designed with Intergen internal pipeline based on Primer3 (<http://frodo.wi.mit.edu/primer3/>) PCR and a tiling approach. This pipeline included specificity checking based on In-Silico PCR. The nine USH genes (*MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G*, *USH2A*, *ADGRV1*, *WHRN*, *CLRN1*), the *CIB2* gene, initially reported as USH1 and more recently refuted, and the USH2 modifier gene *PDZD7* were thereby covered with 1268 primer pairs generating an equal number of PCR products, 165 bp in length on average (primer sequences available upon request). All PCR products were sequenced on a HiSeq Illumina Sequencer (Illumina, San Diego, CA, USA). Whole-exome sequencing was performed as previously described (Delmaghani et al., 2012) with minor modifications, using the SureSelect V5 capture kit (Agilent) and HiSeq 2000 sequencer (Illumina). Bioinformatics analysis of sequence data was based on the pipeline provided by Illumina (CASAVA 1.8), and the algorithm ELANDv2e (Malony alignment and multi-seed mismatch reducing artifact) was used for sequence alignment. Sanger sequencing was performed to validate each presumably pathogenic variant identified by whole exome sequencing in the index case and their relatives. Sequences were run on ABI 3730 DNA analyzer and compared with Genbank reference sequences (<http://www.ncbi.nlm.nih.gov/genbank/>) using ABI Prism Seqscape 2.1. The primers used for PCR amplification of the DNA fragments were designed using Primer3. PDE6B-7F forward primer: 5'-CATCCAGTCCCTCTGACTCC-3' and PDE6B-7R reverse primer: 5'-TCGCTGAATCAAATC-TCCTC-3', TMIE-3F forward primer 5'-ACATGTGGGTGGATGGGG-3' and TMIE-3R reverse primer 5'-TTGGTTGGAGCAGAGGAACA-3' and GUCY2F-4F forward primer 5'-GGCTTGGAAATATACTCTGAGATCG-3' and GUCY2F-4R reverse primer: 5'-TCCCCTGTAAACCAAGCTG-3' were used to amplify and sequence PDE6B exon 7, TMIE exon 3, GUCY2F exon 4, respectively. Sorting Intolerant from Tolerant (SIFT) (Sim et al., 2012), Mutation Taster (Schwarz et al., 2014), CADD (Rentzsch et al., 2019) and PolyPhen-2 (Adzhubei et al., 2013) software programs were used to predict the impact of missense variants on the protein.

### 2.4. Protein 3D-structure modeling

Structure modeling was performed with UCSF-Chimera (Pettersen et al., 2004), based on the cryo-electron microscopy (cryo-EM) structure of the bovine Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta (PDE6B) (3.4 Å resolution) (Gulati et al., 2019).

## 3. Results

The patient was suspected to have a USH when the patient was three years old (see Materials and methods). A moderate-to-severe deafness associated with visual defect as RP led to a diagnosis of USH2. The first step was obviously to check the presence of pathogenic variants in USH genes by using targeted-exome sequencing. Surprisingly, except polymorphisms, we did not detect any pathogenic variant in USH genes. The second step was then to perform WES to identify a possible novel USH gene and the associated variants. After performing bioinformatic

analyses, we identified a total of 280 homozygous and 3247 heterozygous variants. For the analysis, we first excluded variants with a prevalence >1 % in the Genome Aggregation Database (gnomAD) database (<https://gnomad.broadinstitute.org/>). Second, we focused on variants present in the coding exons and flanking splice sites (nonsense, frameshifting, missense, or splice site mutations). After filtering, 60 homozygous and 682 heterozygous variants remained. As the parents were consanguineous, we expected the causative mutations to be present in the affected child at the homozygous state. This is why we retained only the 60 homozygous variants and proceeded to an extended analysis of these variants. All of them are missense variants. We excluded all the variants predicted to be benign or likely benign by *in silico* pathogenicity predictor programs: SIFT, PolyPhen-2, MutationTaster and CADD. Regarding these criteria, we found six homozygous predicted to be pathogenic missense variants listed in Table 1. Interestingly, we found two homozygous missense variants which can answer to the phenotype and classified as pathogenic variants in ClinVar database. Indeed, we identified both homozygous missense variants (Fig. 1B), c.250C>T; p.(Arg84Trp) in *TMIE*, a deafness gene and c.1010A>G; p.(His337Arg) in *PDE6B*, a RP gene. We also found a homozygous missense variant in *GUCY2F* gene, and variants in this gene may be a cause of X-linked RP (Yang et al., 1996). However, the father carried this variant at heterozygous state without any signs of RP. So, we excluded this variant as responsible for the RP in this family. Moreover, the brother was deaf without sign of RP and carried the c.250C>T; p.(Arg84Trp) in *TMIE* gene at homozygous state. This variant was first reported in one Southern Indian family (Naz et al., 2002). This missense variant is predicted damaging by SIFT, probably damaging by PolyPhen-2 and disease causing by MutationTaster, all with the highest pathogenicity score. In the gnomAD database, the p.(Arg84Trp) had an allele frequency of 0.0022 % (26/1,180,048) in the non-Finnish European population and 0.003 % (3/91,090) in the South Asian population. This missense variant was also reported as a common cause of hearing loss in Turkish population with a high prevalence (2.4 %) (Sirmaci et al., 2009). The second missense variant (c.1010A>G; p.(His337Arg)) in *PDE6B* gene was previously reported in one Tunisian family (Habibi et al., 2016). It is predicted to be pathogenic by all prediction algorithms. In the gnomAD database, the p.(His337Arg) had an allele frequency of 6.857e-7. Both residues (Arginine in *TMIE* and Histidine in *PDE6B*) were highly conserved through the evolution (Fig. 1C–D). To highlight the pathogenicity of the missense variant (p.(His337Arg) in *PDE6B*, we have modeled using UCSF-Chimera the substitution of a histidine residue by an arginine residue, based on the 3D structure of the bovine *PDE6B* (PDB ID code 6MZZB) (Fig. 2A–C). There is >90 % of identity between bovine and human *PDE6B* proteins. Residues implicated in the interactions were identical in the two species. The p.(His337Arg) variant leading to the replacement of the histidine residue by an arginine residue in position 337 results in the replacement of the imidazole side chain of histidine by the long charged arginine side chain. The presence of the arginine residue in position 337 introduces steric clashes with the valine and isoleucine residues in positions 316 and 330. Both residues (histidine and arginine) have a different polarity and the mutated residue (arginine) is predicted to alter the hydrogen bond network. These changes suggest that this could lead to protein disorganization (Fig. 2B–D).

## 4. Discussion

In this study, we identified two missense variants (c.250C>T; p.(Arg84Trp) in *TMIE* and c.1010A>G; p.(His337Arg) in *PDE6B*), which were reported for the first time in Algeria, in a patient initially diagnosed as affected by Usher syndrome. The combination of these both variants, one in RP gene (*PDE6B*) and one in deafness gene (*TMIE*), mimics Usher syndrome and explains the patient's phenotype. *TMIE* (Transmembrane inner ear) protein is a key component of the mechanoelectrical transduction machinery which contributes to the maintenance and function

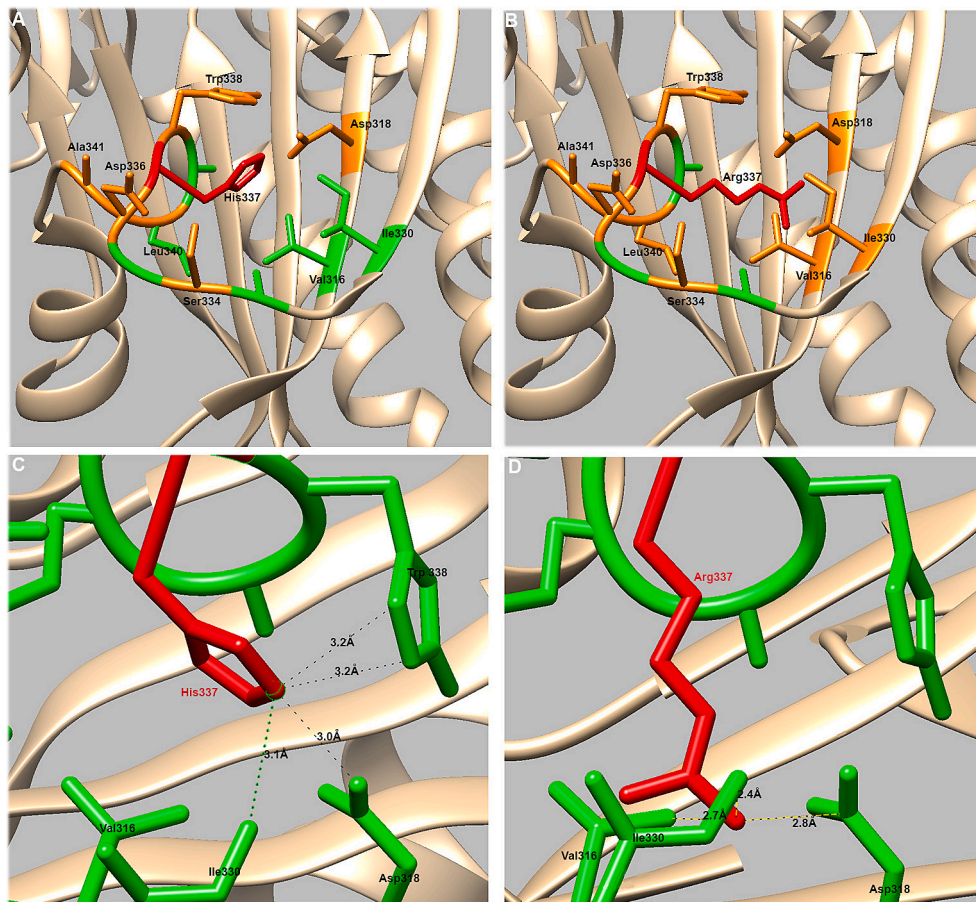


**Table 1**

List of predicted pathogenic homozygous variants carried by the index case.

Gene	Nucleotide change	Protein name	Protein change	Allele frequency (gnomAD)	ClinVar
<i>TMIE</i>	c.250C>T	Transmembrane inner ear expressed protein	p.(Arg84Trp)	2.4e <sup>-5</sup>	Pathogenic
<i>PDE6B</i>	c.1010A>G	Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta	p.(His337Arg)	NR	Pathogenic
<i>GUCY2F</i>	c.1033G>T	Retinal guanylyl cyclase 2	p.(Val345Phe)	NR	NR
<i>OR2L8</i>	c.719G>C	Olfactory receptor 2 L8	p.(Cys240Ser)	1.1e <sup>-3</sup>	NR
<i>TGM6</i>	c.688G>A	Protein-glutamine gamma-glutamyltransferase 6	p.(Asp230Asn)	6.3e <sup>-5</sup>	Likely benign
<i>FLNB</i>	c.7627G>A	Filamin-B	p.(Ala2543Thr)	5.9e <sup>-5</sup>	VUS

NR: not reported; VUS: variant of unknown significance.

*OR2L8*, *TGM6* and *FLNB* genes were excluded gene because variations in these genes were not associated with hearing impairment and/or retinitis pigmentosa.First, *OR2L8* gene is member of a large multigene family encoding transmembrane signaling proteins required for odorant discrimination. Second, variations in *TGM6* are responsible for autosomal dominant spinocerebellar ataxia 35. Finally, *FLNB* is an actin binding protein. Variations in this gene are responsible for either autosomal recessive spondylocarpotarsal synostosis syndrome or autosomal dominant atelosteogenesis-Larsen syndrome (early death).**Fig. 2.** Predicted structural change in the p.(His337Arg) variant of PDE6B.

A. Cryo-electron microscopy (cryo-EM) structure of the bovine PDE6B (3.4 Å resolution, PDB: 6MZY). The residues in green have distances between 3 and 5 Å with the histidine residue in position 337, while the residues in orange have only distances between 2 and 3 Å. B. Cryo-EM structure of the bovine PDE6B (3.4 Å resolution, PDB: 6MZY). The arginine residue in position 337 is shown in red. C. Magnification of the region around the histidine residue in position 337 showing the distances between the neighboring residues. D. Magnification of the predicted structure of the same region showed in C but with the histidine residue in position 337 replaced with an arginine. The most likely rotamer is represented (probability of 90 %): long charged side chain of arginine is predicted to interact with the valine and isoleucine residues in positions 316 and 330 located in β-strands, respectively leading to potential protein disorganization. The arginine residue is also predicted to interact with the aspartate residue in position 318 located in a β-strand. By establishing these interactions with the two neighboring β-strands of PDE6B (carrying Val316, Asp318 and Ile330), this variant presumably affects the overall fold and stability of the β-strands in the GAF2 (cGMP-specific phosphodiesterases, adenylyl cyclases and Fh1A) domain. The predicted steric hindrances are represented in yellow.

of the hair cells (Zhao et al., 2014; Farhadi et al., 2021). *TMIE* is located on the 3p21 chromosomal region. Variations in this gene are responsible for autosomal recessive deafness (DFNB6) (Naz et al., 2002). The patient presented a severe-to profound hearing impairment that corresponds to the phenotype previously reported in other studies (Sirmaci et al., 2009; Naz et al., 2002). As also reported in Naz et al. (Naz et al., 2002), no vestibular dysfunction was observed in the index case as well as her

older brother. The *PDE6B* gene is located on chromosome 4p16.3 and encodes a protein which is part (the beta subunit) of a protein complex called cGMP-PDE. This complex is found in specialized photoreceptor cells called rods. These cells transmit visual signals from the eye to the brain, particularly in low-light conditions. When light enters the eye, a series of rod cell proteins are activated, including GMP-PDE. GMP molecules are then broken down, triggering the closure of channels in

the cell membrane, resulting in the transmission of signals to the brain, which are interpreted as vision. In response to a light stimulus, PDE6B (Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta) plays an important role in visual function by hydrolyzing cGMP (Kameni Tchoudji et al., 2001). Variations in this gene are responsible for autosomal-recessive RP or autosomal-dominant stationary night blindness in humans (Kuehlewein et al., 2021) with a prevalence of 2.4 % in France (Khateb et al., 2019). A gene therapy trial in patients with biallelic variants of the *PDE6B* gene is currently ongoing (ClinicalTrials.gov Identifier: NCT03328130). The patient in our study has also visual impairment, mainly characterized by reduced visual field and photophobia. The concomitance of these two signs led to the suspicion of Usher syndrome. Given the patient's young age, the signs of RP are not highly developed and establishing a genotype/phenotype correlation is difficult.

In countries with high rates of consanguinity, comorbidity is also common, accounting for up to 50 % of the cases (Romdhane et al., 2016). As there are a large number of genes responsible for deafness as well as retinitis pigmentosa, comorbidity is possible, especially when the index case is born of consanguineous parents. Other patients who had been diagnosed as Usher syndrome had been reported with a combination of two different genes, two Slovenian patients with variants in *GJB2* and in *PRPH2* (Fakin et al., 2012) and one patient with variants in *SLC26A4* and *BBS1* (DeLuca et al., 2015). In the first study, the two Slovenian patients carried the common homozygous frameshift variant in *GJB2* gene (c.35delG; (p.Gly12Valfs\*)) responsible for the hearing impairment and one heterozygous missense variant (c.389 T > C; (p. Leu130Pro)) in *PRPH2* gene responsible for the RP. This second is dominantly inherited. The presence of isolated RP in both father and daughter facilitated the diagnosis. Clinicians could suspect the existence of another gene responsible for deafness (Fakin et al., 2012). In the second study, the patient carried one homozygous missense variant (c.1169T>G; (p.Met390Arg)) in *BBS1* gene responsible for RP and two compound heterozygous variants, one missense (c.707T>C; (p. Leu236Pro)) and one splice variant (c.-3-2A>G) in *SLC26A4* gene responsible for deafness. Usually, variants in *BBS1* gene are responsible for Bardet-Biedl syndrome. This syndrome is a syndromic form of RP associated with other symptoms such as obesity, cognitive impairment, postaxial polydactyly, and/or hypogonadism. Interestingly, variants in *BBS1* gene can also cause nonsyndromic RP (Estrada-Cuzcano et al., 2012). However, in the case of this patient, the presence of inner ear malformation, more specifically Mondini dysplasia, was an indicator for finding variants in *SLC26A4* gene. Indeed, this gene when mutated is responsible for Pendred syndrome and/or DFNB4 with inner ear malformations such as enlarged vestibular aqueduct or Mondini dysplasia (Forli et al., 2021).

Moreover, several genes have been reported as responsible for USH-like phenotype (Igelman et al., 2021), among them, *HARS* (Abbott et al., 2017), *ABHD12* (Eisenberger et al., 2012), *CEP78* (Fu et al., 2017), *CEP250* (Fuster-García et al., 2018), *ARSG* (Khateb et al., 2018) and more recently *SGSH* (De Falco et al., 2024). In fact, for each of these genes, at least one patient had a hearing impairment associated with RP without vestibular trouble. Centrosomal protein 78 (CEP78) and Centrosomal protein 250 (CEP250), both ciliary proteins, play an important role in retinal photoreceptor cells (Igelman et al., 2021). However, patients with variants in *CEP78* gene presented with cone-rod dystrophy instead of RP with the onset of deafness is variable (Fu et al., 2017) whereas patients with *CEP250* variants had a progressive hearing impairment combined with a mild retinal defect (Fuster-García et al., 2018). The *ARSG* gene encodes the arylsulfatase G (ARSG), a sulfatase lysosomal enzyme. Variants in this gene were reported as responsible for USH4 (Abad-Morales et al., 2020). USH4 is defined by both late-onset of hearing impairment and RP without vestibular defect. The Polyneuropathy, hearing loss, ataxia, RP, and cataract (PHARC) syndrome was caused by homozygous or compound heterozygous variants in *ABHD12* ( $\alpha/\beta$ -hydrolase domain containing 12) gene (Fiskerstrand

et al., 2010). In some patients, penetrance is incomplete and only deafness and RP are present, leading to a diagnosis of USH. The onset of hearing impairment and RP are variable. More recently, one patient initially diagnosed with USH due to the coexistence of hearing impairment and RP carried biallelic missense variants in *SGSH* (N-sulfoglucosamine sulfohydrolase) gene (De Falco et al., 2024). Variants in this gene are currently responsible for Mucopolysaccharidosis type IIIA (MPS IIIA or Sanfilippo syndrome type A). Additional tests have shown other clinical symptoms such as complete deficiency of sulfamidase activity. In MPS IIIA, there is great phenotypic variability correlated with the genotype. This is why the late-onset of other clinical signs led to the diagnosis of USH.

## 5. Conclusions

Taken together, these cases illustrate the importance of molecular genetics in validating a clinical diagnosis for the purposes of genetic counseling and gene therapy. Although Usher syndrome is the most common form of deaf-blindness, the possibility of a combination between two genes, one responsible for deafness and the other for retinitis pigmentosa, cannot be ruled out, in particular in the case of families with a high rate of consanguinity.

## CRedit authorship contribution statement

**Samia Abdi:** Writing – original draft, Investigation, Formal analysis, Data curation. **Mohamed Makrelouf:** Writing – review & editing. **Issa Nazim Rous:** Resources. **Kheireddine Ounnoughi:** Resources. **Akila Zenati:** Writing – review & editing. **Christine Petit:** Writing – review & editing. **Crystal Bonnet:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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