Identification a novel pathogenic LRTOMT mutation in Mauritanian families with nonsyndromic deafness

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IDENTIFICATION OF A NOVEL PATHOGENIC LRTOMT VARIANT IN MAURITANIAN FAMILIES WITH NONSYNDROMIC DEAFNESS

Running title: Novel *LRTOMT* mutation in Mauritanian patients with hearing impairment.

Abstract

Purpose: Although recessive mutations in *GJB2* are the common genetic etiology of sensorineural hearing impairment (SNHI), variants in *LRTOMT* gene were also identified, mostly in Middle East and North African populations. ***Methods:*** Using Sanger sequencing we screened the exon 7 of *LRTOMT* in a cohort of 128 unrelated Mauritanian children with congenital deafness. ***Results:*** One biallelic missense mutation, predicted as pathogenic (c.179T>C;p.Leu60Pro) was found at homozygous state in four independent families. This variant, not reported before, was predicted to have a deleterious effect by SIFT (score: 0.01) and a disease-causing effect by Mutation Taster (prob: 1). Exploration of the encoded protein 3D structure revealed a disruption of an organized α helix (in the normal protein structure) to a random conformation. Early fitting of a cochlear implant seemed to improve the auditory ability of the patient carrying the biallelic variant. ***Conclusion:*** Further screening using a panel of deafness genes should provide a more comprehensive view of the heterogeneity of deafness genes underlying hearing impairment in our population.

Keywords: SNHI, Deafness, LRTOMT, Mutation, Mauritania

1. Introduction

Deafness is the most common sensorineural disability in developing countries as it affects approximately 5% of the population [1,2]. In children, 1 to 6 per 1,000 newborns are suffering from a form of hearing impairment [3]. Despite the lack of reliable statistics on the affected populations in developing countries, the proportion of hearing loss was estimated to be greater than in states with higher income [4, 5]. In a random WHO survey, the prevalence of deafness was indeed found to be closely affected by widespread issues such as deprived life environment, lack of proper care services but also genetic background including the effect of consanguinity [6, 7]. SNHI were related to about 134 identified causal genes with most cases passed down through variations in *GJB2* gene with autosomal inheritance (Hereditary Hearing loss Home page: Last update August 2021). Several variations of *LRTOMT* gene, mostly in exon 7, have also been associated with deafness in different populations including cohorts from North African populations making of this gene the second most frequent, after *GJB2*, involved in congenital severe to profound hearing impairment [8, 9, 10, 11, 12, 13]. In a previous work using Sanger sequencing of *GJB2* gene, we have detected two pathogenic variants c.35delG (p.Gly12Valfs*2) and c.94C>T (p.Arg32Cys) in only 5 out of the 53 families with sporadic or hereditary deafness investigated [8]. This finding left thus about 95% of the families with other putative genetic cause to be assigned. The aim of this study was to identify the molecular etiology of non-syndromic hearing loss not linked to *GJB2* mutations in a large cohort of Mauritanian patients using a targeted mutation screening of exon 7 in *LRTOMT* gene.

2. Materials and methods

2.1. Patients

The cohort was composed of 128 unrelated Mauritanian children (1 child per family) respectively from 83 families with multiple affected siblings and 45 families with no history of deafness (sporadic cases), recruited from two deaf schools in Nouakchott, Mauritania. Forty-one children of the cohort were identified as negative for *GJB2* variants in a previous study we carried out on non-syndromic hearing impairment associated *GJB2* variants in Mauritania [8] and 87 children were newly recruited. All patients were diagnosed with severe-to-profound non-syndromic congenital hearing impairment following medical examination. This study was approved by the ethics committee of the University of

Nouakchott, Mauritania as stated by the ethics clearance letter N°002/2020/CE/UNA. Written informed consent was obtained from all parents or legal guardians of the children. A questionnaire was used to collect demographic data and medical history of the subjects.

2.2. Molecular Analysis

Blood samples were collected in EDTA tubes and used to extract genomic DNA by Qiagen DNA Blood Minikit procedure (QIAGEN Genomic DNA Handbook.2015). PCR reactions (20 µl) contained 1 µl (20-30 ng) of genomic DNA, 1 µl (10µM) of each specific primer (5'-AGGATAATAATTGCTACTGGCAAAA-3'and5' ATCCCAAATATTCCTTCACTGTCTT-3'), 10µl of AmpliTaqGold 360 Master Mix (Life Technologies) and 7 µl of distilled water. The PCR program presented a DNA denaturation step at 95°C for 10 min followed by 35 amplification cycles (denaturation at 94°C for 30s, annealing at 53°C for 35s and extension at 72°C for 40s) tailed by a 5 min final extension at 72°C. PCR products were sequenced on capillary ABI3730 Genetic Analyzer (Applied Biosystems, California, USA). Data obtained were then matched with reference sequences of the *LRTOMT* gene (NM_001145309.4) using Seqscape3.0 software program package (Gene Codes, MI, and USA). Amino acid change was considered as potentially deleterious if predicted by (PolyPhen2, http://genetics.bwh.harvard.edu/pph2), Sorting Intolerant from Tolerant (SIFT, http://sift.jcvi.org/) and Mutation Taster (http://www.mutationtaster.org/). In families with multiple cases of deafness, DNA of one proband was sequenced. Sanger sequencing was then extended to available parents and all affected siblings carrying the mutation in homozygous state.

2.3. *LRTOMT* protein 3-D structure modeling

The predicted tertiary structure of *LRTOMT* protein (AF-Q8WZ04-F1) was obtained using AlphaFold protein structure database (https://alphafold.ebi.ac.uk/). Similarity between wild-type and mutated 3D structures of *LRTOMT* protein was explored by using UCSF Chimera version 1.15.

3. Results

3.1. Screening of exon7 in *LRTOMT*

Analysis of sequencing data from exon 7 of *LRTOMT* gene of the 128 selected patients showed only one biallelic missense variant (c.179T>C;p.(Leu60Pro) in four (3.1%) unrelated patients. The genomic coordinates of the newly identified variant were

Chr11(GRCh37):g.71817077T>C; NM_001145309.4:c.179T>C. Sequence alignment revealed that this variant was not reported before. Notably, the arginine residue was highly conserved through evolution among multiple-species (from Human to opossum considering 6 species) (Fig. 1). The variant was predicted to have deleterious effect on gene function by SIFT (score: 0.01) and a disease-causing effect by Mutation Taster (prob: 1). Exploration of effect of p.Leu60Pro on the predicted three-dimensional (3D) structure of the encoded protein showed a disruption from an organized α helix in the wild protein, to a random conformation in the mutant form (Fig.1). The healthy parents of the four patients in families (F7-DFN, F28-DFN, F31-DFN, F81-DFN) carried the mutation in heterozygous state and all affected children were homozygous for the variant. Three of the four patients had other affected relatives and one presented no family history of HI. The four affected families originated from the Trarza region in southern Mauritania (Fig.2 and 3).

3.2. Cochlear implant outcome

Based on clinical assessment and audiological data, the proband of family F28-DFN, a 7-year-old boy, diagnosed with profound bilateral congenital deafness, received a cochlear implant fitted in the right ear. Now, aged 9 years, signs of auditory recovery are largely perceptible (Fig.3) on the basis of his score on APCEI scale used to assess his communication skill progress.

4. Discussion

In this study, we identified a novel biallelic missense pathogenic variant in exon 7 of *LRTOMT* gene in 3 Mauritanian children with family history of congenital deafness and one child with no other relative being affected. The variant was found in both girls and boys and only carriers at homozygous state showed the phenotype which supported an autosomal recessive segregation of the variant. Because of their higher number of inter-residue contacts, helices are known to tolerate sequence amino-acid changes without affecting their secondary structure [14]. As a result of this relative robustness, compared to strands and coils conformations, any variation in the helix organization was implied to be enough significant to cause alteration of the protein structure and therefore likely to affect its function. For instance, the wild residue Arg81 formed a salt bridge and hydrogen bonds between the helix and following loop, central for the protein stability. Its substitution with glutamine c.242G>A in exon 7: (p.Arg81Gln) was found to disrupt the alpha helix resulting in local destabilization of the protein tertiary structure [12]. Although involving a radical

structurally distinct, our variant (p.Leu60Pro), located in the same exon 7, appeared to be inducing a comparable disorganizing effect on the protein alpha helix which likely resulted in the hearing impairment observed in the four patients of our cohort and also predicted for the (p.Arg81Gln) mutation [11]. Since the first reported case of direct involvement of proline in human diseases [15], mutant proteins for this amino acid have been implicated in the etiology of number of genetic disorders including non-syndromic recessive hearing impairment [16]. For instance, different studies showed that p.Ser50Pro and p.Thr56Pro variants, both consisting of replacement of wild amino acid by proline residue in connexin 50 (Cx50, *GJA8*), affected the protein contribution to the lens normal survival through angular restriction of α helix or β sheet secondary structure [17, 18]. Proline is indeed a distinctive amino acid with a nitrogen atom covalently locked within a ring inducing a unique constrained φ angle of approximately −65° [19]. This irregular geometry disrupts protein secondary structure by steric hindrance i.e. in inhibiting the backbone to conform into a normal alpha-helix turn conformation. In addition, the lack of hydrogen on proline's nitrogen (proline cannot donate protons) prevents it from participating in hydrogen bonding essential for the helix structure. The apparent deleterious consequence we observed (SIFT score (0.01) and disease-causing effect (prob: 1) by Mutation Taster) for this novel mutation was therefore likely due to a change of the protein secondary structure induced by the substitution to proline residue consisting in an alpha helix breaking. The wild type leucine residue was also located in an evolutionary highly conserved region [12] suggesting an important functional position of the residue (Fig 1B). Modeling, using WHATIF server (http://swift.cmbi.ru.nl) on rat COMT crystal structure as template for catechol-Omethyltransferase domain of the human *LRTOMT2* (NM_001145309.4) showed that common *LRTOMT* variants associated with non-syndromic recessive hearing impairment led to hair cell degeneration process [9, 11, 20]. Close to p.Leu60Pro variant, various changes in the same exon 7 were also associated with cases of moderate to profound congenital deafness making of this segment a mutation hot spot in the *LRTOMT* gene [10]. Besides the above homozygous c.242G>A (p.Arg81Gln) variant found in Tunisian, Libyan and Egyptian families [9,20] we may also cite missense variants c.122G>A (p.Arg41Gln), c.193T>C (p.Trp65Arg) in Morocco and Tunisia [11, 12] and a nonsense mutation c.208C>T (p.Arg70*), in Tunisia [11, 12, 13] (Table 1). In this ethnic context, we noticed that all four affected families carrying the *LRTOMT* mutation (c.179T>C;p.(Leu60Pro) identified in this report belonged to the Moors (Maures), a predominant racial group of the Mauritanian

population. This group, of Berber-Arab origin, ethnically and culturally self identifies with the neighboring North Africa populations [21].

In a previous study, we detected *GJB2* (MIM 121 011) variants in only 5 out of the 53 (9.4%) investigated families with congenital hearing impairment [8]. The present work showed that *LRTOMT* was also involved in hearing impairment in our population (3.1%; 4/128). However, a limitation of this study was that we have only screened exon 7 of *LRTOMT*. This screening only in exon 7 was mostly guided by very scarce findings of deafness associated variants in other exons reported by various studies in the region [9-13]. Indeed, a total of 14 pathogenic variants in exon 7 out of 18 causal variants have presently been reported. Search in these sequences along multi-gene hear panel is underway to locate other deafness related variants in our families.

Conclusions

This study identified a novel biallelic predicted pathogenic variant (c.179T>C;p.(Leu60Pro) in exon 7 of *LRTOMT* gene likely causing a localized alpha helix breaking in the LRTOMT protein. Being the single exon 7 variant identified in our patients underlined the significance of a genetic screening for this variant in the context of an accurate molecular diagnosis of hearing impairment or in assessing the transmission of the disease in our population and, broadly, among Northern African populations.

Further hearing tests are still required to clearly establish the effect of early cochlear implant fitting on audition rehabilitation in patients carrying mutations in the *LRTOMT* gene. Screening for other parts of this gene or using multigene panel in a larger population study is also recommended.

Declarations

Ethics approval and consent to participate

Research in this study has been performed in accordance with the Declaration of Helsinki and was approved by an appropriate ethics committee: the Ethics Committee of the University of Nouakchott, Mauritania (ref. n°002/2020/CE/UNA). We confirm that all methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Informed consent was obtained from all study participants. The informed consent of all patients was also obtained for the data publication.

Availability of data and materials

The datasets on variants generated during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors'contributions

MS, ECM, SMB, CTH and VT collected and organized patients' files. AD and LAV examined patients and analyzed clinical data. CB, CP and AH contributed in paper conception and writing of the manuscript. All authors read and approved the final manuscript.

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References

1 Wilson BS, Tucci DL, Merson MH, O'Donoghue GM (2017) Global hearing health care: New findings and perspectives. Lancet 390: 2503–2. https://doi.org/10.1016/S0140-6736(17)31073-5

2 Sheffield AM, Smith RJ (2019) The Epidemiology of Deafness. Cold Spring HarbPerspect Med 9(9):a033258. https://doi: 10.1101/cshperspect.a033258

3 Kimberly A, Gifford MD, Michael G, Holmes, Henry H, Bernstein, DO (2009) Hearing Loss in Children. Pediatrics in Review 30(6):207-15. https://doi.org/10.1542/pir.30-6-207

4 Prasansuk S (2000) Incidence/prevalence of sensorineural hearing impairment in Thailand and Southeast Asia. Audiology 39 (4): 207–211. https://doi.org/10.3109/00206090009073080

5 Elahi MM, Elahi F, Elahi A, Elahi SB (1998) Paediatric hearing loss in rural Pakistan. J Otolaryngol 27(6):348–353.

6 Smith AW (2001) WHO activities for prevention of deafness and hearing impairment in children. Scan Audiol Suppl (53):93-100. https://doi.org/10.1080/010503901750166808

7 Zakzouk S (2002) Consanguinity and hearing impairment in developing countries: a custom to be discouraged. J LaryngolOtol 116:811–816. https://doi.org/10.1258/00222150260293628

8 E.C Moctar, Z. Riahi, H. El Hachmi, F. Veten, G. Meiloud, C. Bonnet, S. Abdelhak, M. Errami, A. Houmeida (2016) Etiology and associated GJB2 mutations in Mauritanian children with non-syndromic hearing loss. Eur Arch Otorhinolaryngol 273 pp. 3693-3698.https://doi.org/10.1007/s00405-016-4036-z

9 MosratiMA, Fadhlaoui-Zid K, Elgaaied AB et al (2021) Deep analysis of the LRTOMT c.242G>A variant in non-syndromic hearing loss North African patients and the Berber population: Implications for genetic diagnosis and genealogical studies. Mol Genet Genomic Med 9:e1810. https://doi.org/10.1002/mgg3.1810

10 Ichinose A, Moteki H, Hattori M ,Nishio SY, Usami SI (2015) Novel Mutations in *LRTOMT* Associated With Moderate Progressive Hearing Loss in Autosomal Recessive Inheritance. Annals of Otology, Rhinology & Laryngology 124 Suppl 1:142S-7S. https://doi.org/10.1177/0003489415575043

11 Charif M, Bounaceur S, Abidi O et al (2012) The c.242G>A mutation in LRTOMT gene is responsible for a high prevalence of deafness in the Moroccan population. Mol Biol Rep 39(12):11011-11016. https://doi.org/10.1007/s11033-012-2003-3

12 Ahmed ZM, Masmoudi S, Kalay E et al (2008) Mutations of LRTOMT a fusion gene with alternative reading frames cause non syndromic deafness in humans. Nat Genet 40 1335–1340. https://doi.org/10.1038/ng.245

13 Riahi Z, Bonnet C, Zainine R, Louha M, Bouyacoub Y, Laroussi N, Chargui M, Kefi R, Jonard L, Dorboz I, Hardelin JP, Salah SB, Levilliers J, Weil D, McElreavey K, Boespflug OT, Besbes G, Abdelhak S, Petit C (2014) Whole exome sequencing identifies new causative mutations in Tunisian families with non-syndromic deafness. PLoS One 9:e99797.https://doi:10.1371/journal.pone.0099797

14 Abrusán G, Joseph A (2016) Marsh Alpha Helices Are More Robust to Mutations than Beta Strands. Plos computational biology. https://doi.org/10.1371

15 Schafer IA, Scriver CR, Efron ML (1962) Familial hyperprolinemia, cerebral dysfunction and renal anomalies occuring in a family with hereditary nephropathy and deafness. N Engl J Med; 267:51–60. https://doi.org/10.1056/NEJM196207122670201

16 Batissoco AC, Auricchio M.T.B.M, Kimura L, Tabith-Junior A, Mingroni-Netto RC (2009) A novel missense mutation p.L76P in the GJB2 gene causing non-syndromic recessive deafness in a Brazilian family Brazilian Journal of Medical and Biological Research 42: 168-171. https://doi.org/10.1590/S0100-879X2009000200004

17 Adam M, De RA, Meşe G, Li L, Sellitto C, Brink PR, Gong X, White TW (2009) The cataract causing Cx50-S50P mutant inhibits Cx43 and intercellular communication in the lens epithelium. Exp Cell Res 315(6):1063-75. https://doi.org/10.1016/j.yexcr.2009.01.017

18 Hadrami M, Bonnet C, Veten F, Zeitz C et al (2018) A novel missense mutation of GJA8 causes congenital cataract in a large Mauritanian family. European Journal of Ophthalmology 29(6):621-628.https://doi.org/10.1177/1120672118804757

19 Alexander AM, Edward R, Dulce EC (2013) Proline: The Distribution, Frequency, Positioning, and Common Functional Roles of Proline and Polyproline Sequences in the Human Proteome. PLoS One 8(1): e53785. https://doi.org/10.1371/journal.pone.0053785

20 Gibriel AA, Abou-Elew MH, Masmoudi S (2019) Analysis of p.Gly12Valfs*2, p.Trp24* and p.Trp77Arg mutations in GJB2and p.Arg81Gln variant in LRTOMT among non-syndromic hearing loss Egyptian patients: implications for genetic diagnosis. Molecular Biology Reports 46, 2139–2145. https://doi.org/10.1007/s11033-019-04667-0

21 Vernet R. (1986) La Mauritanie, des Origines au Début de l'Histoire. Centre Cultural Francais Nouakchott.

22 Crowgey EL, Washburn MC, Kolb EA, Puffenberger EG (2019) Development of a Novel Next-Generation Sequencing Assay for Carrier Screening in Old Order Amish

and Mennonite Populations of Pennsylvania. The journal of Molecular Diagnostics vol 21(4): 687–694.https://doi.org/10.1016/j.jmoldx.2019.03.004

23 Babanejad M, Fattahi Z, Bazazzadegan N et al (2012) A Comprehensive Study to Determine Heterogeneity of Autosomal Recessive Non-Syndromic Hearing Loss in Iran.Medical genetics (10):2485-92. https://doi.org/10.1002/ajmg.a.35572

24 Vanwesemael M, Schrauwen I, Ceuppens R et al (2011) A 1 bp Deletion in the Dual Reading Frame Deafness Gene LRTOMT Causes a Frameshift from the First In to the Second Reading Frame. Medical genetics (8):2021 3. https://doi.org/10.1002/ajmg.a.34096

25 Wang R, Han S, Khan A, Zhang X (2017) Molecular Analysis of Twelve Pakistani Families with Non-syndromic or Syndromic Hearing Loss. GENETIC TESTING AND MOLECULAR BIOMARKERS 21(5):316-321. https://doi.org/10.1089/gtmb.2016.0328

26 Marková S, Brožková DS, Mészárosová A et al (2016) Mutations in eight small DFNB genes are not a frequent cause of non-syndromic hereditary hearing loss in Czech patients. International Journal of Pediatric Otorhinolaryngology (86): 27–33. https://doi.org/10.1016/j.ijporl.2016.04.005

27 Du X, Schwander M, Marie Y E et al (2008) A catechol-O-methyltransferase that is essential for auditory function in mice and humans. PNAS 105 (38): 14609–14614. https://doi.org/10.1073/pnas.0807219105

28 Wu CC, Lin YH, Liu TC, Lin KN, Yang WS, Hsu CJ, et al (2015) Identifying children with poor cochlear implantation outcomes using massively parallel sequencing. Medicine 94: e1073.https://doi.org/10.1097/MD.0000000000001073

Table 1: Overview of known pathogenic variants in the *LRTOMT* Gene and associated phenotypes. Report from Deafness variation Database (**https://deafnessvariationdatabase.org/gene/LRTOMT**)

Exon number	Variants	Amino acid change	Phenotype	Population	Reference
7	c.96_109del	p.Thr33HisfsTer57	Non syndromic-HL	USA	[22]

7	c.102G>A	p.Met34Ile	Non syndromic-HL	Iran	[23]
7	c.107del	p.Pro36LeufsTer12	Sensorineural-HL	Iran	[24]
7	c.122G>A	p.Arg41Gln	HL-severe	Tunisia	[12]
7	c.154C>T	p.Arg52Trp	Sensorineural-HL	Pakistani	[25]
7	c.161G>A	p.Arg54Gln	HL- moderate, Progressive	Japan	[10]
7	c.193T>C	p.Trp65Arg	NA	Tunisia	[12]
7	c.208C>T	p.Arg70Ter	Non syndromic-HL	Tunisia	[13]
7	c.240G>T	p.Glu80Asp	Non syndromic-HL	Iran	[23]
7	c.241C>T	p.Arg81Trp	Non syndromic-HL	Iran	[23]
7	c.242G>A	p.Arg81Gln	Non syndromic-HL	Maroco	[11]
7	c.249C>G	p.Phe83Leu	Non syndromic-HL	Czech	[26]
7	c.333C>G	p.Tyr111Ter	Non syndromic-HL	China	[27]
7	**C.179 T>C**	**p.leu60Pro**	**Non syndromic-HL**	**MauritaniaThis study**	
8	c.473G>A	p.Arg158His	Non syndromic-HL	China	[27]
9	c.566del	p.Ile189ThrfsTer8	HL- moderate, Progressive	Japan	[10]
9	c.655C>T	p.Arg219Ter	HL- severe to Profound	China	[28]

Abbreviation: NA, not applicable; HL, Hearing loss

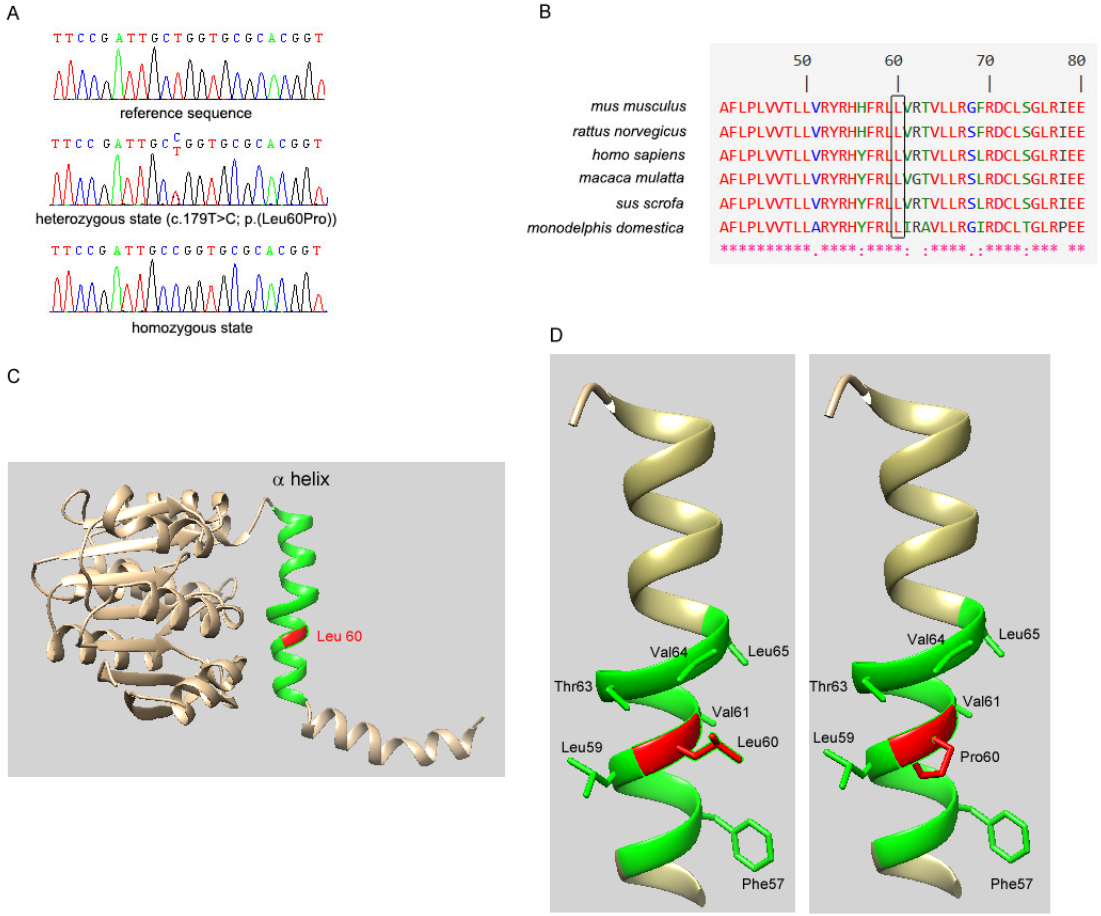

Figure 1: Sequencing data, alignment and prediction of protein secondary structure of the LRTOMT protein. (A) *Electropherogram* plots showing the reference sequence and the mutation in heterozygous and homozygous states. (B) Sequence alignment presenting conservation of the region of *LRTOMT* protein containing (Leu60) among various species. (C) *LRTOMT* protein tertiary structure modeling performed with Chimera, based on the AlphaFold prediction of the transmembrane O-methyltransferase (AF-Q8WZ0F1). (D) Localized structural change in the wild type alpha helix (Phe57, Leu59, **Leu60**, Val61, Thr63, Val61, Val64 and Leu65) (left) showing the breaking of the alpha helix induced by substitution of Leu60 by Prolin (right).

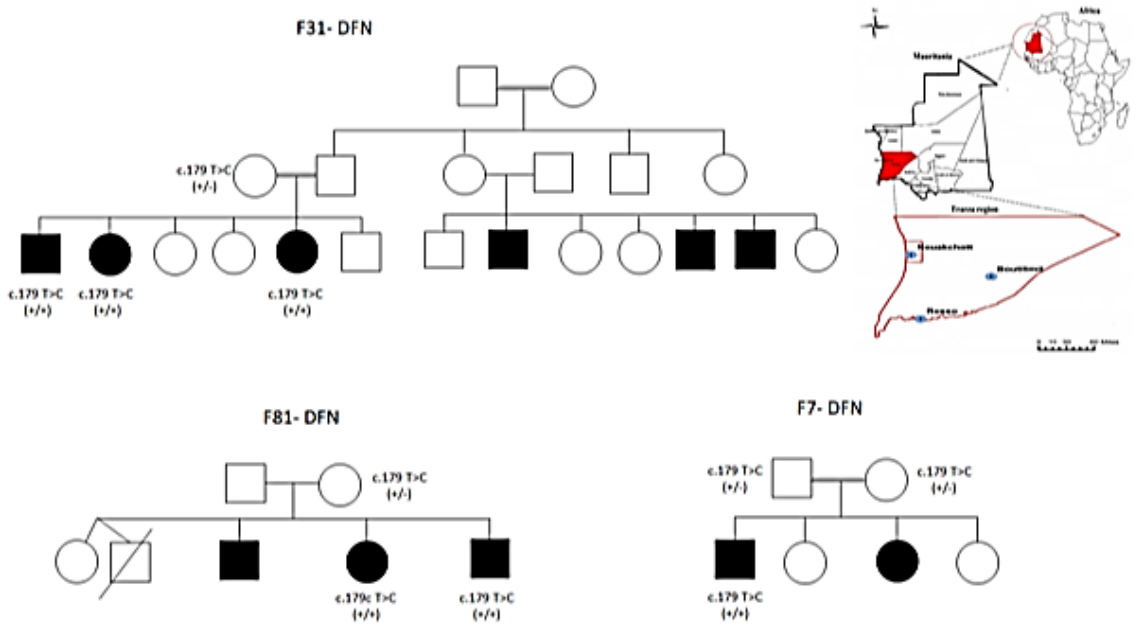

Figure 2: Pedigrees of affected children with family history. Segregation of the mutation pattern was compatible with autosomal recessive inheritance. All cases originated from southern Mauritania (map top right).

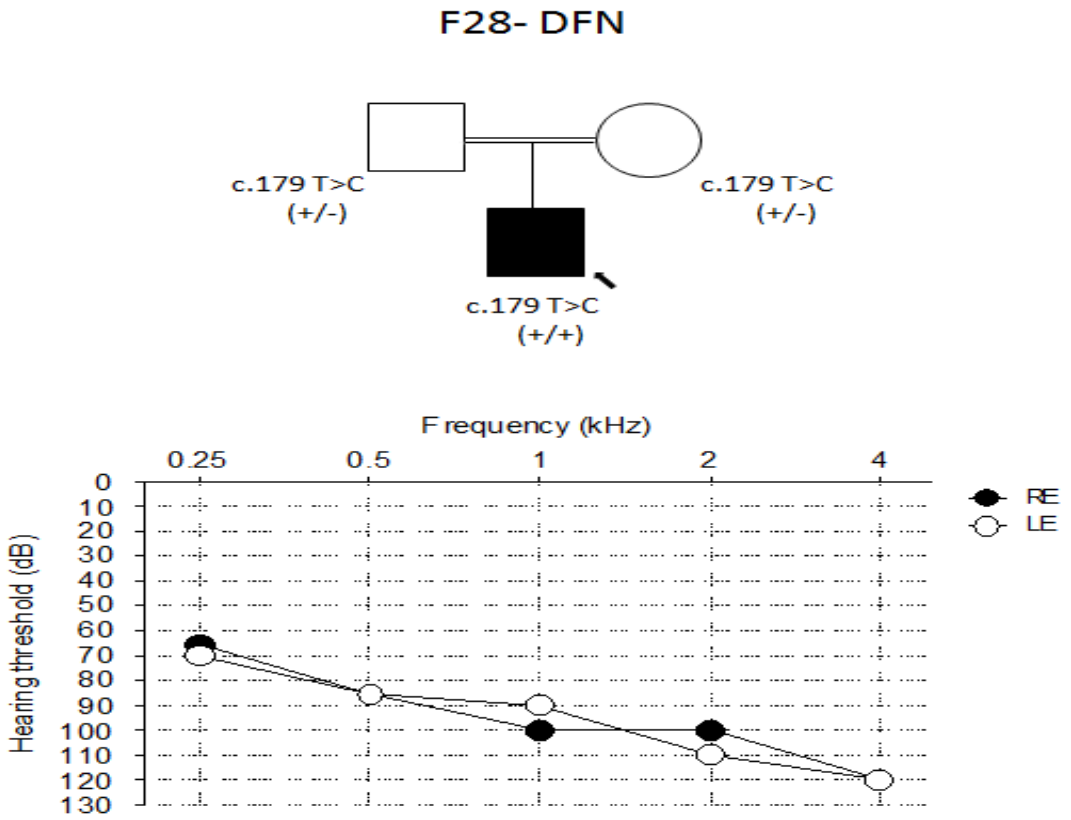

Figure 3: Pure-tone audiogram of a patient from family F28-DFN who received cochlear implant. Proband, indicated by an arrow, had profound hearing loss in both ears.