Nonsense variant in MYO7A underlies non-syndromic hearing loss in an Indian family

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Abstract

Myosin VIIA (also known as MYO7A) is a protein that is encoded by the *MYO7A*. Several mutations in the *MYO7A* have been linked to non-syndromic hearing loss, in which the deafness occurs independently of any other symptoms. DFNA11 and DFNB2 are two types of non-syndromic hearing loss that may be caused by mutations in this gene. After considering the significant impact of *MYO7A* mutations, we tried to identify the same from some Indian families suffering from non-syndromic hearing loss. According to the results of the present investigation, four members of a Maharashtrian family were found to have inherited hearing loss. The results of this family's investigation point to the chr11:77,192,243C>T [hg 38]; c.4117C>T; p.Arg1373Stop homozygous stop gain variant in *MYO7A*. This family has a high probability of having autosomal recessive non-syndromic hearing loss due to this premature stop codon (p.Arg1373Stop) in *MYO7A*. This is the first report documenting MYO7A-related inherited hearing loss in India, and it is caused by the p.Arg1373Stop variant. As a result of this study, more people in our community will become aware of genetic illnesses, which might improve future patient diagnosis and counseling. In future, treatment therapies for these illnesses may also develop as a result of information disseminated by this research work.

Keywords: Myosin VIIA; MYO7A; Mutation; Hearing loss; Genetic; Usher syndrome

Introduction

Myosin VIIA (also known as MYO7A) is a protein that is encoded by the *MYO7A*. It is a member of the family of proteins known as unconventional myosins. Similar in structure, these proteins function in intracellular transport. Actin, a protein crucial to cell motility and shape, interacts with myosins. Long actin filaments are thought to serve as rails used by myosins to move other molecules (Zhang & Yu, 2020).

Both the inner ear and the retina (the light-sensitive tissue at the back of the eye) produce myosin VIIA. Myosin VIIA aids in the formation and maintenance of stereocilia, which are hair-like projections in the inner ear. Actin-rich stereocilia line the inner ear and deflect in response to vibrations in the air. Sound waves must undergo this bending motion in order to be transformed into nerve impulses and sent to the brain. The vestibular system, located in the inner ear, is responsible for helping the body maintain its balance and sense of spatial direction (Terziev & Vasileva, 2022). In order for the vestibular system to send signals to the brain, these stereocilia must bend. Retinal pigment epithelium (RPE) cells are where myosin VIIA is most concentrated in the eye. The growth and maintenance of this tissue, which is essential for the health of the retina, likely involves myosin VIIA. It has been hypothesised that myosin VIIA transports melanosomes, tiny sacs of pigment, around the RPE. This pigment is essential for healthy eyesight. There are other regions of the retina where myosin VIIA is present, suggesting that it transports other proteins and chemicals crucial to eye function (MedlinePlus, 2022).

Several mutations in the *MYO7A* have been linked to non-syndromic hearing loss, in which the deafness occurs independently of any other symptoms. DFNA11 and DFNB2 are two types of non-syndromic hearing loss that may be caused by mutations in this gene. Because of its autosomal dominant inheritance pattern, DFNA11 may be caused by the presence of even a single mutation *MYO7A* copy in any given cell. This kind of hearing loss manifests itself in early infancy, when a kid has the ability to communicate verbally (post-lingual), and worsens with time (Hildebrand et al., 2010).

Usher syndrome type I is characterized by hearing loss, visual loss, and issues with balance and coordination, and more than 200 mutations in the *MYO7A* have been found in

persons with this condition. More than half of all instances of Usher syndrome type I are due to mutations in the *MYO7A*, which causes Usher syndrome type IB (USH1B). Many of these mutations modify only one amino acid in key spots in the myosin VIIA protein. Other mutations alter the instructions for synthesising myosin VIIA by adding a premature stop codon. Still other changes to the *MYO7A* include deletions or insertions of very small fragments of DNA. All of these modifications either cause the development of a myosin VIIA variant that is dysfunctional or completely eliminate its production. Myosin VIIA deficiency causes hearing loss, imbalance, and coordination issues because it prevents stereocilia from developing and functioning normally in the inner ear. Progressive vision loss is the outcome of retinitis pigmentosa, a disorder caused by a deficiency of myosin VIIA in the retina (Lenassi et al., 2014; Liu et al., 1997; Riazuddin et al., 2008; Rong et al., 2014). After considering the significant impact of *MYO7A* mutation, we tried to identify the same from some Indian families suffering from non-syndromic hearing loss.

Materials and Methods

Ethical approval

All the samples were collected after receiving duly filled and signed consent forms from each individual. Identity of each individual was concealed using appropriate identifiers to comply with confidentiality norms. Photographs were taken with patients' or their guardians' permission wherever required.

Subjects

Blood samples were obtained from affected children, parents and unaffected family members with informed consent. DNA was extracted by standard procedures. Blood samples were taken from all the affected and unaffected individuals from family. All samples were obtained with approved informed consent. All affected individuals underwent a detailed physical examination.

DNA extraction

DNA was extracted from the blood samples obtained from the family members of the affected individuals using ReliaPrep Blood gDNA Miniprep Sytem.

Whole Exome Sequencing

The SureSelect Target Enrichment method is a solution-based approach that captures areas of interest using ultra-long - 120 merbiotinylatedcRNA baits - and enriches them from an NGS genomic fragment pool.

Captured Library Construction

We employ the Agilent SureSelectXT Low Input Target Enrichment procedure for Illumina paired-end sequencing libraries with 1ug of input gDNA to build standard exome capture libraries. PicoGreen and agarose gel electrophoresis are used to determine the amount and quality of DNA. We utilise 1 g of genomic DNA from each cell line diluted in EB Buffer and sheared to a desired peak size of 150–200 bp using the Covaris LE220 focused-ultrasonicator (Covaris, Woburn, MA) according to the manufacturer's instructions. End-repair and the addition of a 'A' tail follow fragmentation. The fragments are subsequently ligated to Agilent adapters.

The adapter ligated product is PCR amplified after the ligation efficiency is assessed. TapeStation DNA screentape D1000 is used to measure the final purified product (Agilent). According to the Agilent SureSelect Target Enrichment technique, 250 ng of DNA library is combined with hybridization buffers, blocking mixes, RNase block, and 5l of SureSelect all exon capture library for exome capture. The DNA is extracted, washed, and amplified. The resulting purified product is then quantified using qPCR (KAPA Library Quantification kits for Illumina Sequecing platforms) and validated using the TapeStation DNA screentape D1000 according to the qPCR Quantification Protocol Guide (Agilent).

Clustering & Sequencing

Illumina employs a one-of-a-kind amplification process that takes place on the flow cell's surface. The Illumina platform is filled with a flow cell holding millions of distinct clusters for automatic extension and imaging cycles. Sequencing-by-four proprietary nucleotides with reversible fluorophore and termination features are used in the synthesis. Each sequencing cycle happens with all four nucleotides present, resulting in better accuracy than approaches in which only one nucleotide is present in the reaction mix at a time. This cycle is repeated one base at a

time, resulting in a sequence of pictures that each represent a single base expansion at a particular cluster.

Generation of Raw data

RTA, the Illumina platform's integrated primary analysis programme, generates raw pictures and base calling (Real Time Analysis). The binary base calling files are translated into FASTQ using the Illumina programme bcl2fastq v2.20.0. The value of the demultiplexing option (— barcode-mismatches) is 0 (Fu et al., 2012; Logan et al., 2022; Witten, 2011; Yang & Xu, 2020).

Results and Discussion

MYO7A information is tabulated in Table 1. Family pedigree indicating affected and unaffected individuals are depicted in Fig. 1. The clinical details of affected individuals from the family are exemplified in Table 2. The Sanger sequencing results are illustrated in Table 3.

Table 1. *MYO7A* gene information.

Gene name	MYO7A
Code protein	Myosin protein VIIA
Site of expression	Expressed in epithelial tissue of retina and inner ear

Table 2. The clinical details of affected individuals from the family.

Clinical Features	**Individual**			
	II:1	**II:3**	**II:4**	**II:5**
Hearing	No	No	No	No
Vision	Normal	Normal	Normal	Normal
Birth/Birth related issues, if any	Normal birth	Normal birth	Normal birth	Normal birth
Spoken communication	No word, use sign language	No word, use sign language	No word, use sign language	No word, use sign language
Weight at the time of examination	60 Kg	62 Kg	65 Kg	64 Kg
Age at the time of	45 years	43 years	40 years	38 years

examination				
Head circumference	Normal	Normal	Normal	Normal

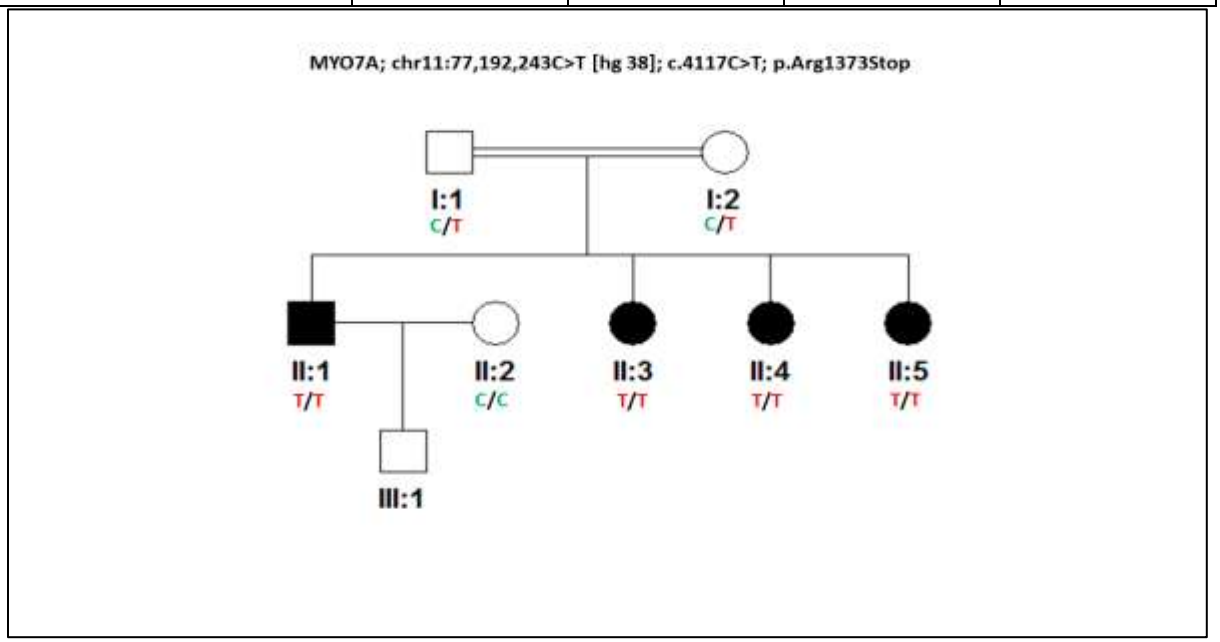

Fig. 1. The family pedigree indicating affected and unaffected individuals from studied cohort. Empty squares and circles indicate unaffected male and females from this family, respectively. Whereas filled in squares and circles indicate affected individuals suffering from autosomal recessive non-syndromic hearing loss.

Table 3. The Sanger sequencing results of the study

Individual	**Genotype**	**Zygosity**	**Affectation Status**	**Gene**	**Variant**
I:1	C/T	Heterozygous	Unaffected	MYO7A	c.4117C>T; p.Arg1373*
I:2	C/T	Heterozygous	Unaffected	MYO7A	c.4117C>T; p.Arg1373*
II:1	T/T	Homozygous	**Affected**	MYO7A	c.4117C>T; p.Arg1373*
II:3	T/T	Homozygous	**Affected**	MYO7A	c.4117C>T; p.Arg1373*
II:4	T/T	Homozygous	**Affected**	MYO7A	c.4117C>T; p.Arg1373*
II:5	T/T	Homozygous	**Affected**	MYO7A	c.4117C>T; p.Arg1373*
II:2	C/C	Wild type	Unaffected	MYO7A	c.4117C>T; p.Arg1373*

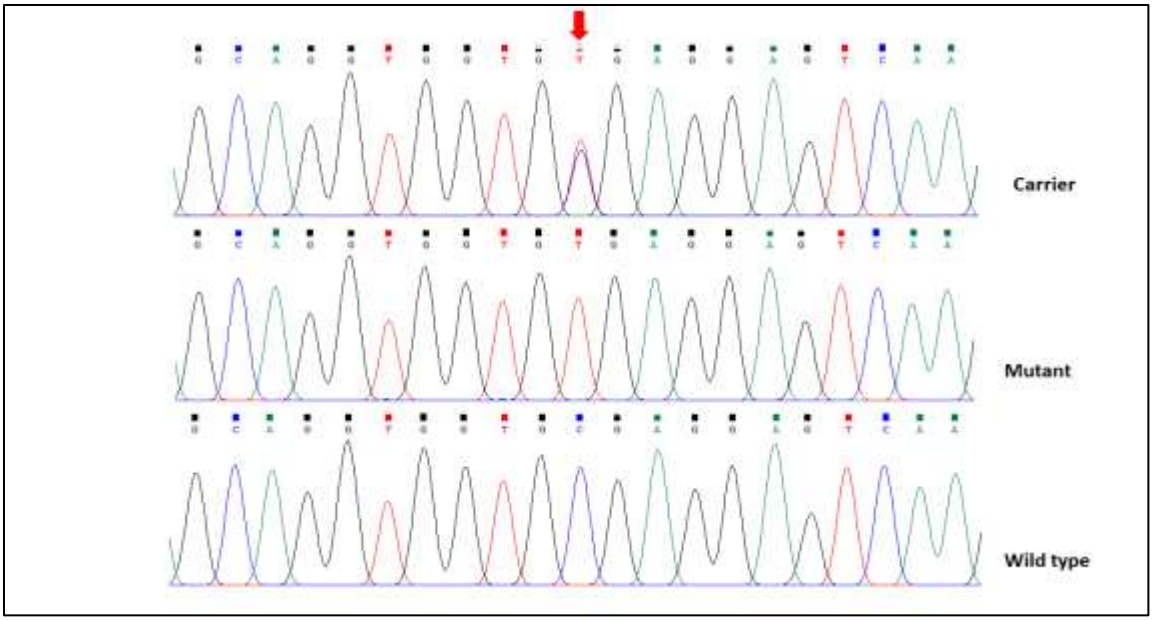

Fig. 2. Sequence chromatograms for carrier, mutant and WT

The *MYO7A* is found on chromosome 11q13.5 and encodes for an unusual myosin that is found in the inner ear, lung, kidney, testis, and retina. p.Arg1373Stop variant identified in this family has been reported in the French and Spanish families in the past. Sanger sequencing of all the affected and unaffected individuals from this family was performed for this specific variant to corroborate the fact this variation, p.Arg1373Stop in MYO7A is the underlying cause of this disease (autosomal recessive non-syndromic hearing loss) in this family. p.Arg1373Stop homozygous variant in MYO7A co-segregates completely with disease phenotype in this family.

During the course of the present research, a family from Maharashtra was uncovered that had four afflicted individuals who suffered from inherited hearing loss. Results of this study confirm that MYO7A homozygous stop gain variant chr11:77,192,243C>T [hg 38]; c.4117C>T; p.Arg1373Stop is the underlying cause of inherited non-syndromic hearing loss in this family.. This is the first study that describes this form of inherited hearing loss in India that is caused by this variant (p.Arg1373Stop) in MYO7A. In conclusion, this study helps to improve understanding about inherited illnesses in our community, which may in future contribute towards accurate diagnosis and counselling of similarly affected individuals as well as their

family members. This research could also improve the prospects of developing a potential treatment therapy for inherited hearing loss in future.

Conclusion

In the current study, a family from Maharashtra with four affected individuals suffering from inherited hearing loss was identified. Investigation carried out in this family suggests that MYO7A homozygous stop gain variant chr11:77,192,243C>T [hg 38]; c.4117C>T; p.Arg1373Stop in MYO7A is likely to be the underlying cause of autosomal recessive non-syndromic hearing loss in this family. This is the first report describing this form of inherited hearing loss due to this variation (p.Arg1373Stop) in MYO7A in India. In conclusion, this research raises awareness regarding inherited disorders in our community and may aid in the diagnosis and counselling of similarly affected patients and their families in future. This study may also increase chances of development of treatment for these type of disorders at some point in future. This study may prove to be beneficial to the community, health care professional and researchers.

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Conflicts of Interests

Declared none

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