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ORIGINAL ARTICLE

Novel Homozygous missense variant in calcium and integrin-binding protein 2 (CIB2) underlies autosomal recessive non-syndromic hearing loss in an Indian family

Sandip Hurpade[1*], Neelam Khan[2]
[1*]Research Scholar, Oriental University, Department of Pharmacy, Indore (M.P.), India
[2]Research Guide, Oriental University, Department of Pharmacy, Indore (M.P.) India
*Corresponding author Email: hurpadess@gmail.com

ABSTRACT
The etiology of hearing impairment (HI) can be attributed to genetic factors, environmental factors, or a combination thereof. Genetic factors account for a majority, specifically 50-60%, of cases involving congenital hearing loss. Currently, there exists a body of knowledge indicating that around 300 genes have been identified as having a connection to the auditory process. The majority of these genes are responsible for encoding proteins that play a role in the structure and function of the inner ear. The inclusion of the CIB2 (calcium- and integrin-binding protein 2) gene in the comprehensive catalogue of genes linked to hearing loss has occurred in recent times. After considering the significant impact of CIB2 mutation, we tried to identify the same from some Indian families suffering from non-syndromic hearing loss. The present investigation involved the identification of a family hailing from Maharashtra, India, comprising three individuals who exhibited symptoms of inherited hearing loss. The findings of this investigation provide confirmation that the presence of a homozygous missense variant, specifically the chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly mutation in the CIB2 gene, serves as the primary etiological factor for the autosomal recessive non-syndromic hearing loss observed within this particular familial cohort. This study presents the initial investigation of a specific type of hereditary hearing impairment in the Indian population, which is attributed to a particular genetic variant (chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly) in the CIB2 gene. In conclusion, this study contributes to a deeper understanding of genetic disorders in our population, potentially resulting in improved accuracy in identifying and providing support for affected individuals and their family members. The present study holds promise in expanding the prospects for the advancement of a prospective therapeutic intervention for hereditary hearing impairment.
Keywords: *CIB2; Mutation; Hearing loss; Genetic; Usher syndrome; Gene analysis*

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INTRODUCTION

Usher syndrome (USH) is the prevailing type of hereditary sensory deaf-blindness. The condition is distinguished by auditory impairment and visual abnormalities, which may or may not be accompanied by issues related to equilibrium. USH is categorized into three types, namely USH1, USH2, and USH3, based on the clinical symptoms[1,2]. USH1 is considered the most severe form of the disorder, characterized by the presence of profound congenital deafness, retinitis pigmentosa, and vestibular areflexia. USH2 is characterized by a moderate congenital hearing impairment, along with retinitis pigmentosa and mild balance issues. USH3, the mildest manifestation, is characterized by a gradual decline in auditory function accompanied by retinitis pigmentosa and minor vestibular impairments[2,3]. Currently, a total of ten genes have been identified as being associated with Usher syndrome (USH). These genes include MYO7A, USH1C, CDH23, PCDH15, USH1G, CIB2, USH2A, ADGRV1, WHRN, and CLRN-1. Moreover, PDZD7 has been proposed as a modifier of USH and as a potential contributor to digenicUSH. Research has demonstrated that mutations occurring in the USH genes can result in the development of nonsyndromic hearing loss[2].

The acquisition and interpretation of auditory stimuli play a crucial role in the optimal development of communication abilities in young children. Hearing impairment is a prevalent sensory disability that commonly impacts individuals. Based on data provided by the World Health Organization, it is estimated that approximately 5% of the global population, which amounts to approximately 360 million individuals, including 32 million children, experience hearing impairment (HI). The term "sensorineural hearing loss" pertains to a condition characterized by a degree of auditory impairment exceeding 40 decibels (dB) in adults and 30 dB in children, specifically in the ear with better hearing ability[4]. The estimated prevalence of newborns with HI is approximately 2-4 per 1000 in developed countries and 6 per 1000 in developing countries. In addition to environmental factors, such as noise exposure, infections, and the administration of ototoxic drugs. The aetiology of HI can be attributed to genetic factors, environmental factors, or a combination thereof. Genetic factors account for a majority, specifically 50-60%, of cases involving congenital hearing loss. Currently, there exists a body of knowledge indicating that around 300 genes have been identified as having a connection to the auditory process. The majority of these genes are responsible for encoding proteins that play a role in the structure and function of the inner ear. The inclusion of the *CIB2* (calcium- and integrin-binding protein 2) gene in the comprehensive catalogue of genes linked to hearing loss has occurred in recent times[5,6].

Hearing impairment is a prevalent sensory disability that commonly impacts individuals. The etiology of this phenomenon can be attributed to a combination of genetic and environmental factors. *CIB2* is a gene that has been recently discovered and is known to play a role in the pathogenesis of hearing impairment. *CIB2* exhibits broad expression patterns across diverse human and animal tissues, with prominent localization in skeletal muscle, nervous tissue, inner ear, and retina. The CIB2 protein plays a crucial role in the regulation of intracellular calcium levels and its interaction with integrins, which are integral membrane receptors that are vital for various cellular processes such as adhesion, migration, and activation of signalling cascades. The calcium signaling pathway plays a pivotal role in facilitating signal transduction within the inner ear, while integrins are responsible for governing the process of hair cell differentiation and the maturation of stereocilia. Currently, the identified mutations in *CIB2* have been determined to be responsible for non-syndromic hearing loss (DFNB48) or Usher syndrome type 1 J. Individuals with biallelic mutations in the *CIB2* gene experience bilateral, moderate to profound hearing impairment that begins at an early age[2,7–10].After considering the significant impact of *CIB2* mutation, we tried to identify the same in some Indian families suffering from non-syndromic hearing loss.

MATERIAL 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 this 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 gDNAMiniprepSytem.

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 a NGS genomic fragment pool.

Captured Library Construction

We employed 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 were used to determine the amount and quality of DNA. We utilised 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 were subsequently ligated to Agilent adapters.

The adapter ligated product was PCR amplified after the ligation efficiency was assessed. TapeStation DNA screentape D1000 was used to measure the final purified product (Agilent). According to the Agilent Sure Select Target Enrichment technique, 250 ng of DNA library was combined with hybridization

buffers, blocking mixes, RNase block, and 5l of SureSelect all exon capture library for exome capture. The DNA was extracted, washed, and amplified. The resulting purified product was then quantified using qPCR (KAPA Library Quantification kits for Illumina Sequencing 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 [11–14].

RESULTS AND DISCUSSION

CIB2 gene information is tabulated in Table 1. Family pedigree indicating affected and unaffected individuals are depicted in Fig. 1. The phenotypic features of the affected individuals from family group are depicted in Table 2.The clinical details of affected individuals from the family are exemplified in Table 3. The Sanger sequencing results are illustrated in Table 4. This family (family G; figure 1) was originally from Parbhani District in Maharashtra and had three affected individuals (I:1, II:1 and II:3; figure 1). The overall clinical presentation in all affected individuals from this family was mutism and deafness. Father and his two daughters (I:1, II:1 and II:3; figure 1) from this family were found to be suffering from hearing loss. Exome sequencing was performed in one affected individual (II:1; figure 1) from this family to understand underlying cause of inherited deafness in this family. All the affected individuals from family G were mute and deaf by birth and used sign, simple words and sounds to be able to communicate.

Table 1.*CIB2* gene information[15]

Gene name	*CIB2*
Function of gene/protein	○ Protein: Calcium and integrin binding family member 2 ○ Calcium- and integrin-binding protein ○ Required for the normal development and function of the inner ear hair cell stereocilia
Clinical phenotype	○ Usher syndrome type 1J ○ Autosomal recessive hearing loss
Inheritance	○ Autosomal recessive
Ocular features	○ Retinitis pigmentosa (Usher syndrome only)
Visual function	**Usher syndrome** ○ Nyctalopia with pre-adolescent onset ○ Peripheral visual field loss ○ Loss of central and colour vision in later life
Systemic features	○ Congenital, bilateral, severe to profound sensorineural hearing loss ○ Failed newborn hearing screen or hearing difficulties suspected in infancy ○ Vestibular dysfunction affecting balance from birth

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

Question	**Individual I:1**	**Individual II:1**	**Individual II:3**
What is their hearing like?	No	No	No
What is their vision like?	Normal	Normal	Normal
Were they born at the right time, were there any problems when they are born?	Normal birth	Normal birth	Normal birth
What did they weight at birth?	NA	3.5 kg	2.5Kg
What is their speech / communication like? Are they able to use any words or if not do they make any sound to communicate, or use any signs?	No word, using sign language	No word, using sign language	No word, using sign language
Height, weight and head circumference and state the age (year or month) that these were obtained	Wt-55 kg Ht- 5.5.age -65 year, head circumference measurement- normal	Wt-45 kg Ht- 5.5.age -26 year, head circumference measurement- normal	Wt-45 kg Ht- 4.8.age -21 year, head circumference measurement- normal

Table 3. Phenotypic features of the affected individuals from family G.

Family and individual	Age	Sex	Age at onset	Severity	Progressive HL	Laterality
Family G Individual 1 (I:1)	55 yr	Male	Congenital	Yes	No	Bilateral
Family G Individual 2 (II:1)	26 yr	Female	Congenital	Yes	No	Bilateral
Family G Individual 3 (II:3)	21 yr	Female	Congenital	Yes	No	Bilateral

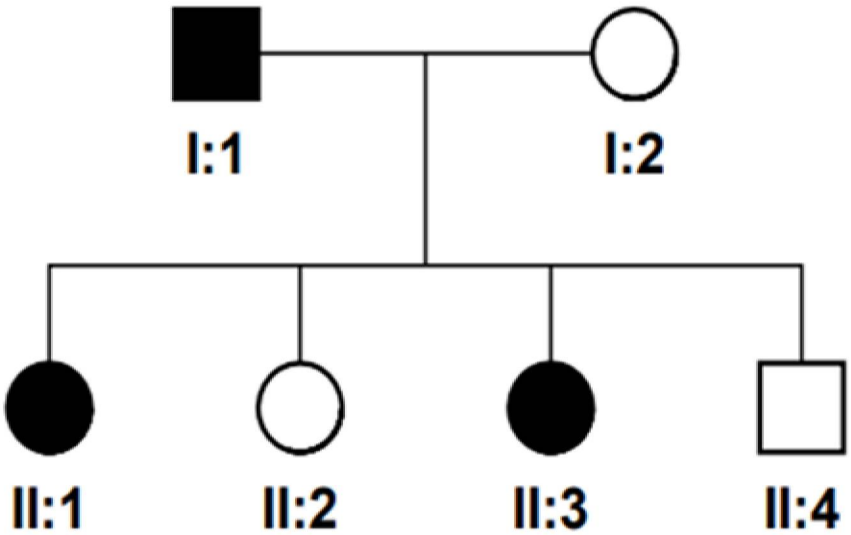

Fig. 1. The family pedigree indicating affected and unaffected individuals from studied cohort. Empty squares and circles indicate unaffected male and females from this family, respectively. Whereas filled in squares and circles indicate affected individuals suffering from autosomal recessive non-syndromic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

Table 4. The Sanger sequencing results of the study

Individual	Genotype	Zygosity	Affectation Status	Gene	Variant
I:1	**G/G**	**Homozygous**	**Affected**	**CIB2**	**chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly**
I:2	A/G	Heterozygous	Unaffected	CIB2	chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly
II:1	**G/G**	**Homozygous**	**Affected**	**CIB2**	**chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly**
II:2	A/G	Heterozygous	Unaffected	CIB2	chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly
II:4	A/A	Wild type	Unaffected	CIB2	chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly
II:3	**G/G**	**Homozygous**	**Affected**	**CIB2**	**chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly**

To date, seven mutations in the CIB2 gene have been discovered: c.97C>T (p.Arg33*), c.192G>C (p.Glu64Asp), c.196C>T (p.Arg66Trp), c.272T>C (p.Phe91Ser), c.297C>G (p.Cys99Trp), c.368T>C (p.Ile123Thr), and c.556C>T (p.Arg186Trp). All of them except c.97C>T and c.556C>T affect the three of four alternatively spliced isoforms, i.e., A, B, and C, of the CIB2 protein. Mutation c.97C>T affects isoforms B and C, but not the isoforms A and CIB2-006, while c.556C>T affects presumably, isoforms A, B, and CIB2-006. The assignment of all identified CIB2 mutations exclusively to isoform B suggests that this specific isoform plays a crucial role in the sound transduction process. However, additional research is required to validate this assumption.

Sanger sequencing of all the affected and unaffected individuals from this family was performed for chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly specific variant to corroborate the fact this variation, p.Asp45Gly in *CIB2* is the underlying cause of this disease (autosomal recessive non-syndromic hearing loss) in this family.

During the course of the present research, a family from Maharashtra was uncovered that had three afflicted individuals who suffered from inherited hearing loss. Results of this study confirm that *CIB2* homozygous missense variant chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly 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 (chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly) in CIB2. In summary, this research enhances comprehension regarding hereditary diseases within our society, potentially leading to more precise identification and guidance for individuals and their relatives who are similarly impacted. This research has the potential to enhance the possibilities of developing a future treatment therapy for inherited hearing loss.

CONCLUSION

In the current study, a family from Maharashtra with three affected individuals suffering from inherited hearing loss was identified. Results of this study confirm that *CIB2* homozygous missense variant chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly 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 (chr15:78,111,229A>G [hg38]; c.134A>G; p.Asp45Gly) in *CIB2*. In summary, this research enhances comprehension regarding hereditary diseases within our society, potentially leading to more precise identification and guidance for individuals and their relatives who are similarly impacted. This research has the potential to enhance the possibilities of developing a future treatment therapy for inherited hearing loss.

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CONFLICTS OF INTERESTS

Declared none

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