Novel splice-site variation in SLC26A4 causes autosomal recessive nonsyndromic hearing loss in a consanguineous Indian family

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Abstract

Background: Variants in the SLC26A4 gene are correlated with autosomal recessive nonsyndromic hearing loss. This study aimed to identify the genetic causes in a in a consanguineous Indian family and cause pathogenicity of the detected variants.

Methods: We collected blood samples and clinical data of affected and unaffected individuals from consanguineous Indian family. The DNA was isolated using standard methods. Whole-exome sequencing (WES)of two affected individuals from this family was performed. The purity of DNA was confirmed using a Nanophotometer. Variant analysis software (GATK) was used to extract the potential sites of SNP and InDel in the whole genome and compared to the reference database Human_GRCh38_dbSNP141. SLC26A4 gene variants identified by WES in the pedigree were confirmed by Sanger sequencing for confirmation of genotype in affected individuals.

Results: In current study, we identified a novel homozygous splice-site variation NM_000441.1 (SLC26A4) GRCh37:Chr7:107323987; GRCh38:Chr7: 107683542; c.1001+5G>A in exome sequencing data of two affected individuals As per splice site prediction tool, Berkeley Drosophila Genome Project (https://www.fruitfly.org/seq_tools/splice.html), c.1001+5G>A mutation in SLC26A4 leads to abolition of donor splice site of coding exon 7 in SLC26A4.

Conclusions: On the SLC26A4 gene, c.1001+5G>A is a pathogenic mutation. This finding enriches the mutational spectrum of the SLC26A4 gene and provides a basis for the genetic diagnosis ARNS Hearing loss

Keywords: SLC26A4 gene, c.1614+5G>A, ARNSHL.
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Introduction

Hearing impairment is the most prevalent sensory impairment. Around the world, 1 in 1000 infants are affected by it, and 4% of adults under 45 have some kind of hearing loss.[1]all syndromic hearing loss and non-syndromic hearing loss are classifications for inherited hearing loss. Conductive hearing loss and sensorineural hearing loss are the two causes of hearing loss (SNHL).[2] Many proteins are needed for the function of the inner ear. The inner ear consists of the cochlea (responsible for hearing), the saccule, the utricle, and three semicircular canals that modify balance and spatial direction. This system requires genes for its development, differentiation, and maintenance. These mutations cause sensorineural hearing impairment. In cases of autosomal recessive hearing loss, the GJB2, SLC26A4, MYO15A, OTOF, CDH23, and TMC1 genes occur most frequently.[3]About 75% to 80% of instances of non syndromic hearing loss (NSHL) are inherited in an autosomal recessive pattern, while another 20% to 25% are inherited in an autosomal dominant pattern, and the remaining 1% to 2% are inherited in an X-linked or mitochondrial pattern.[4]

For autosomal recessive nonsyndromic hearing loss, Biallelic mutations in 42 unique genes have been found (ARNSHL). More than eighty loci have been mapped to various chromosomal sites, which are denoted by DFNB followed by a number indicating when a locus was first published.[5] The bulk of currently documented deafness genes have been identified in India, where paternal consanguinity and high family size permit the use of genetic linkage with autozygosity mapping.[6]SLC26A4 mutations are the second most common cause of inherited hearing loss, after GJB2 mutations.[7]

The extremely hydrophobic membrane protein pendrin is encoded by the SLC26A4 gene, which is located on chromosome 7q22.3 and contains 20 coding exons. Pendrin is a member of the SLC26 family of anion transporters. Most of the pendrin is located in the inner ear and thyroid.[8]

Pendrin, a transmembrane anion exchanger and a member of the solute carrier 26 family, is encoded by the SLC26A4 gene. It transports chloride, iodide, bicarbonate, and format. It is expressed in a number of tissues, including the inner ear, kidney, and thyroid. It can be found in the cochlea's apical membranes of the spiral prominence and outer sulcus epithelial cells that border the endolymph, the spiral ganglion, and supporting cells.[9]

All exons as well as the surrounding fan-in regions are included in the broad range of SLC26A4 mutations. The majority of SLC26A4 mutations are missense, although the remainder can be classified as either frameshift, splice site, stop gain or deletion.[10]In current study, we identified a novel homozygous splice-site variation NM_000441.1 (SLC26A4) GRCh37:Chr7:107323987; GRCh38:Chr7: 107683542; c.1001+5G>Ain exome sequencing data of two affected individuals from a consanguineous family with four affected individuals from Maharashtra, India. c.1001+5G>A variant in was found to be the underlying cause of autosomal recessive nonsyndromic hearing SLC26A4loss in this family as this was confirmed by co-segregation analysis using Sanger sequencing of all the affected and unaffected individuals from this family (fig.). Co-segregation analysis revealed that this variation c.1001+5G>A in SLC26A4 cosegregates completely with disease phenotype in this family. Two different disease mutations, with HGMD IDs viz. CS083275 and CS084673, are reported in the past as per HGMD database at this specific genomic position. This splice-site variation c.1001+5G>A in SLC26A4 is also predicted to be disease causing by Mutation Taster, a mutation prediction tool. As per gnomAD browser database, no variation or nucleotide change is reported at this position i.e. NM_000441.1 (SLC26A4) GRCh37:Chr7:107323987; GRCh38:Chr7:107683542; c.1001+5G. These findings add up to the list of mutations known to cause autosomal recessive nonsyndromic hearing loss due to mutations in SLC26A4. This will prolongate the scope of knowledge of the spectrum of SLC26A4 mutations in the Indian population, and it would be helpful to explore the relationship between phenotype and genotype in the SLC26A4 mutations.

Methods

Subjects involved in this study that were investigated for identifying the underlying cause of autosomal recessive non-syndromic hearing loss are indicated in the pedigree drawn using HaploPainter1.043 software. This study was approved by the Institutional Review Board, Oriental School of Pharmacy, Oriental University, Indore, M.P. (India). All participants (or their parents if they were minors) gave their written consent for participation in the study. After obtaining properly completed and signed consent forms from all participants, blood samples were collected. To ensure confidentiality, proper identifiers were implemented to conceal people's identities. The DNA was isolated using standard methods. A specific medical history was acquired, including instances of family history of hearing loss and consanguineous marriages, history of ototoxic drugs and noise exposure, years of onset of deafness, age and progression.Normal hearing was defined as PTA 25 dB HL (Hearing Level), mild hearing loss as 25 PTA 40 dB HL

moderate hearing loss as 40 PTA, 60 dB HL severe hearing loss as 60 PTA, 80 dB HL as profound hearing loss as PTA > 80 dB HL.

SLC26A4 mutation screening

Whole-exome sequencing (WES)

WES of two affected individuals from this family was performed. The SureSelect Target Enrichment workflow is solution-based system utilizing ultra-long - 120 mer biotinylated cRNA baits - to capture regions of interest, enriching them out of a NGS genomic fragment library.To generate standard exome capture libraries, we used the Agilent SureSelect Low Input Target Enrichment protocol for Illumina paired-end sequencing library with 1ug of input gDNA. The DNA quantity and quality is measured by PicoGreen and agarose gel electrophoresis, respectively. We used 1µg of each cell line's genomic DNA diluted with EB Buffer and sheared to a target peak size of 150–200 bp with the Covaris LE220 focused-ultrasonicator (Covaris, Woburn, MA) according to the manufacturer's recommendations. Fragmentation was followed by end-repair and the addition of an 'A' tail. Agilent adapters are then ligated to the fragments. After assessing the efficiency of ligation, the adapter ligated product was PCR amplified. The final purified product is quantified by TapeStation DNA screen tape D1000 (Agilent). For exome capture, 250 ng of DNA library was mixed with hybridization buffers, blocking mixes, RNase block and 5 µl of SureSelect all exon capture library, according to the standard Agilent SureSelect Target Enrichment protocol. The captured DNA was washed and amplified. Then final purified product was quantified by qPCR according to the qPCR Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platforms) and qualified by the TapeStation DNA screentape D1000 (Agilent). Illumina utilizes a unique amplification reaction that occurs on the surface of the flow cell. A flow cell containing millions of unique clusters was loaded into the Illumina platform for automated cycles of extension and imaging. Sequencing-by-Synthesis utilized four proprietary nucleotides possessing reversible fluorophore and termination properties. Each sequencing cycle occurs in the presence of all four nucleotides leading to higher accuracy than methods where only one nucleotide is present in the reaction mix at a time. This cycle was repeated, one base at a time, generating a series of images each representing a single base extension at a specific cluster. The Illumina platform generated raw images and base calling through integrated primary analysis software called RTA (Real Time Analysis). The base calling files which were expressed in binary were converted into FASTQ by Illumina package bcl2fastq v2.20.0. The demultiplexing option (--barcode-mismatches) was set to as value: 0.

Sanger sequencing

SLC26A4 gene variants identifed by WES in the pedigree were confrmed by Sanger sequencingfor confirmation of genotype in all affected as well as unaffected individuals in family was performed and Sanger sequencing data was analysed using ChromasLite software. Likely pathogenic homozygous variant c.1001+5G>A in SLC26A4 was identified in the DNA of the other affected subjects (III;1; IV-1,IV-2,IV-5).(Figure 1) DNA was extracted from each individual sample, and the main DNA quality testing procedures were as follows: (1) the DNA concentration was tested by Nanodrop (Thermo scientifc, CA, USA);(2) the purity of DNA was confirmed using a Nanophotometer (IMPLEN, CA, USA); and (3) the integrity was detected by 1% agarose gel electrophoresis. Libraries were tested for: (1) exon enrichment using a Sure Select XT Target Enrichment System (G7530–90000); (2) for size distribution using an Agilent 2100 Bioanalyzer system; and (3) for concentration using a BioRad CFX 96 quantitative fuorescence PCR instrument and Bio-Rad KIT iQ SYBR GRN. Samples were then sequenced on a NovaSeq 6000 platform. Variant analysis software (GATK) was used to extract the potential sites of SNP and InDel in the whole genome and compared to the reference database Human_GRCh38_dbSNP141.

Genomic DNA of all family members was prepared from 3 mL of peripheral blood with a QIAGEN Universal DNA Purification kit.

Primers were designed using Primer 3 software. PCR was carried out according to these steps: an initial denaturation at 96 °C for 1 min, 96 °C for 10 s (denaturation), 50 °C for 5 s (annealing), 60 °C for 4 min (extension) and a final 25 cycles. In the end, the PCR products were sequenced with an ABI 3730 Sequencer (Applied Biosystems).

Results

Phenotypic evaluation (Family investigation)

Here we identified a three-generational consanguineous Indian family with 11 members. (Figure 1) Affected individual (III-3) 52 years age had hearing loss by birth and with no other clinical symptoms like behavioural abnormalities as well as anxiety, aggression or any seizure. They born right time with normal weight and head circumference was normal. This individual had learning difficulties and only spoke some words using sign language. They do not have any other unusual physical characteristics that appear different from normal members.

Another affected individual (IV-1) 12 years old had hearing loss by birth and with no other clinical symptoms like behavioural abnormalities as well as anxiety, aggression or any seizure. They born right time with normal weight and head circumference was normal. This individual had learning difficulties and only spoke some words using sign language. They did not have any other unusual physical characteristics that looked different from normal members.

Next affected individual (IV-2) 6 years old had hearing loss by birth and with no other clinical symptoms like behavioural abnormalities as well as anxiety, aggression or any seizure. They born right time with normal weight and head circumference was normal. This individual had learning difficulties and only spoke some words using sign language. They did not have any other unusual physical characteristics that looked different from normal members.

Last affected individual (IV-5) 4 years old had hearing loss by birth and with no other clinical symptoms like behavioural abnormalities as well as anxiety, aggression or seizure. They were born at right time with normal weight and their head circumference was normal. This individual had learning difficulties and only spoke some words using sign language. They did not have any other unusual physical characteristics looked different from normal members.

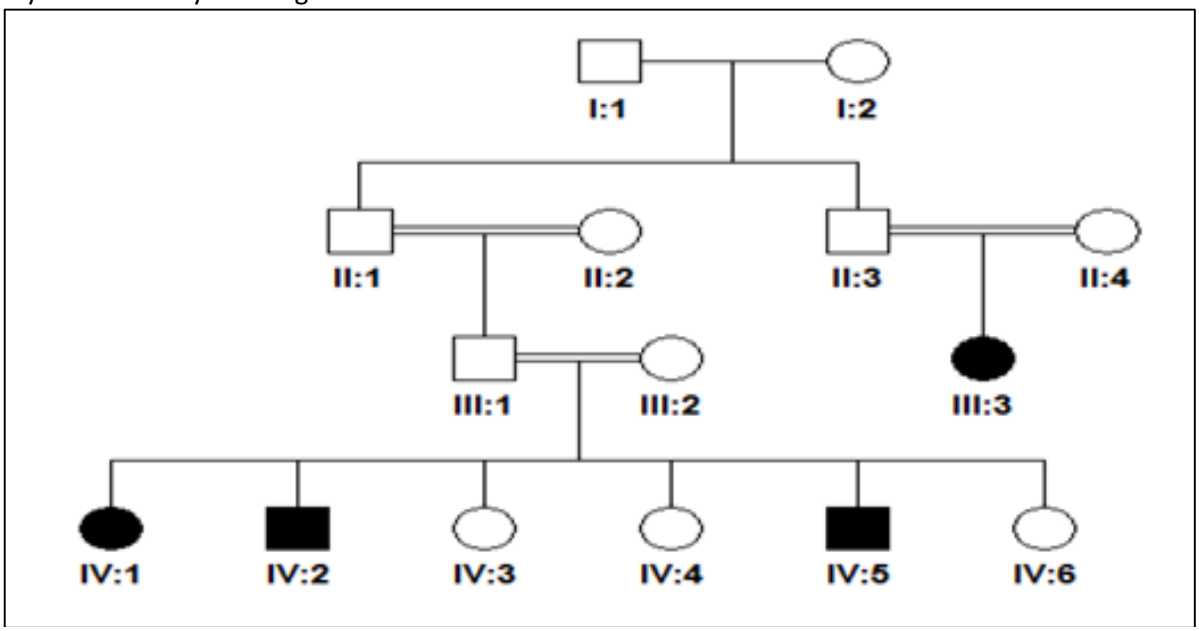

Figure 1: Pedigree map mutations in the four-generation, Squares denote males; circles denote females; black denotes affected individuals of hearing loss wile white denotes unaffected individuals and double line denotes consanguineous marriage.

Genotypic evaluation

Identification of SLC26A4 gene mutation

Homozygous mutation in SLC26A4 Chr7:107683542G>A(hg38) (c.1001+5G>A)

was detected in all four affected individuals as a result of Sanger sequencing and showed that all four affected individuals were homozygous for c.1001+5G>A mutation. Of note, we were able to determine details of the mutations and the pedigree map. Genotypes of the family members are illustrated in (Figure 2) As per splice site prediction tool, Berkeley Drosophila Genome Project (https://www.fruitfly.org/seq_tools/splice.html), c.1001+5G>A mutation in SLC26A4 leads to abolition of donor splice site of coding exon 7 in SLC26A4.

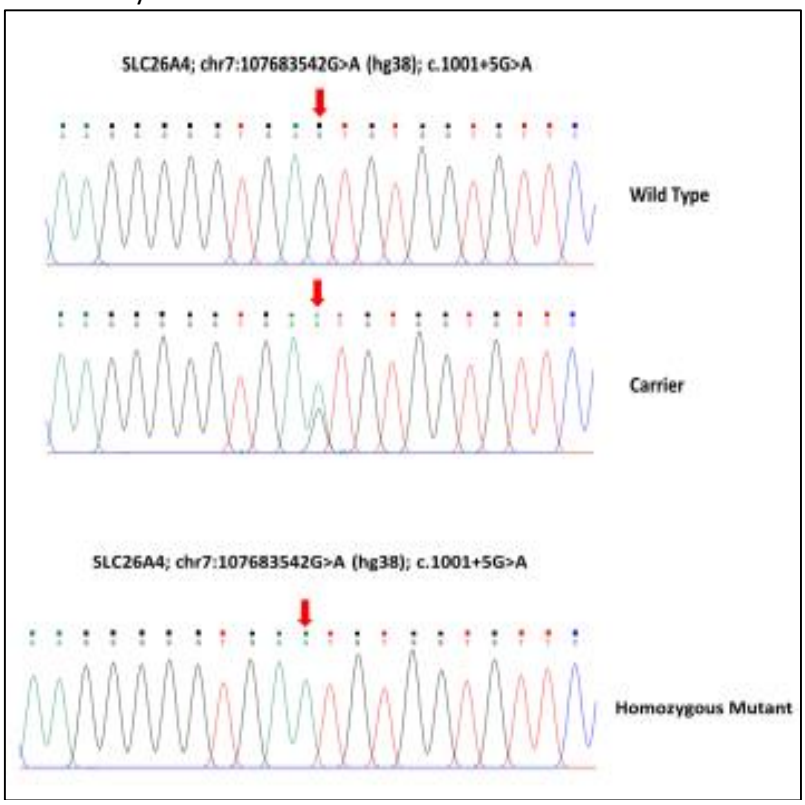

Figure 2: Partial sequence chromatograms of SLC26A4.Homozygous mutation in SLC26A4 Chr7:107683542G>Ahg38) (c.1001+5G>A) with wild type in unaffected, carrier, and homozygous in affected individuals as per splice site prediction tool, Berkeley Drosophila Genome Project (https://www.fruitfly.org/seq_tools/splice.html)

Discussion

SLC26A4 mutation was the main cause of ARNSHL, and no other mutations associated with these clinical phenotypes have been found. To incidence of SLC26A4 gene mutations is 20.35%, ranking second only to GJB2 mutations (25.65%) [13] some studies believe that the phenotype of EVA has a strong correlation with number of mutant alleles of SLC26A4 in Western populations.[14] In this study, we discovered that the proband carried a novel homozygous splice-site mutation (c.1001+5G>A) in the SLC26A4 gene and his clinical presentation was profound high frequency hearing loss. In general, SLC26A4 mutations give rise to hearing loss, which is more pronounced at high frequency.ARNSHLpatients with bi-allelic mutations had more severe deafness, earlier age of onset, and more fuctuating hearing levels compared to patients with non pathogenic mutations. As splicing site is very important for gene transcription, it may be a possible explanation on the lower mRNA/protein expression levels.

Conclusions

In summary, using whole-exome sequencing we identified a new homozygous mutation (c.1001+5G>A) in a four affected individuals from a consanguineous Indian family which will further strengthen the association between SLC26A4 mutation and ARNSHL. We also demonstrated that the novel mutation (c.1001+5G>A) is pathogenic. Further mRNA

and cDNA level functional/sequence analysis could assist in clarifying the relationship between the pathogenic mechanism and the novel mutation. In addition, there is still much room to explore the precise molecular mechanism of each mutation and how they affect phenotypic expression. Deciphering this disease mechanism and progression related information would pave the way for development of further treatment therapies for SLC26A4 associated ARNSHL.

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Conflict of Interest

The authors declare that they have no competing interests.

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