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RESEARCH NOTE

MCM2 mutation causes autosomal dominant nonsyndromic hearing loss (DFNA70): novel variant in the second family

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Abstract. Pathogenic variants in *MCM2* could result in mild to severe sensorineural hearing loss in the affected individuals (deafness, autosomal dominant 70; DFNA70; OMIM: 616968), an extremely rare autosomal dominant progressive disorder. Here, we report a novel missense variant (NM_004526:c.388C>T, p.R130C; Clinvar: SCV002072508) in *MCM2* in an Iranian family identified by whole-exome sequencing and confirmed by Sanger sequencing. The heterozygous variant (NM_004526:c.388C>T, p.R130C) in *MCM2* was identified in the proband and his mother. The proband is a nine-year-old male born to nonconsanguineous parents. The proband was characterized by nonsyndromic hearing loss, while his mother showed a mild form of the disorder. This study reports the second disease-causing variant in *MCM2* in the world and confirms that hearing loss arising from variants in *MCM2* is nonsyndromic. Nevertheless, as was reported in the previous family, phenotype could vary among the patients with the same variant.

Keywords. nonsyndromic hearing loss; deafness; autosomal dominant 70; DFNA70; *MCM2*; whole-exome sequencing; hearing loss; genetic hearing loss.

Introduction

The most common cause of hearing loss in humans is sensory impairment. Roughly, half of the cases with hearing loss are caused by genetic underlying disorders. According to the clinical presentations, hereditary hearing loss can be classified as non-syndromic hearing loss (NSHL) and syndromic hearing loss (SHL) (Gürtler and Lalwani 2002). SHL is a form of inherited hearing impairment that is accompanied by abnormalities of other systems of the body. It accounts for around 30% of all hereditary hearing impairments. NSHL, which accounts for ∼70% of inherited hearing loss, on the other hand, has no pertinent involvement of other organs attributable to the specific genetic mutation (Bayazit and Yilmaz 2006).

Our knowledge about hereditary hearing loss has considerably increased since the identification of the first human hearing loss gene. Hitherto, more than 152 genes have been identified that are involved in both NSHL and SHL. In the aforementioned genes, over 800,000 variants have been classified as likely pathogenic or pathogenic, about 96% of coding variants being rare or an extremely rare.

Hearing loss is a common disorder caused by many genes. Some of which are associated with syndromic deafness and some of them result in nonsyndromic deafness. It is determined that mutation in genes such as *KCNQ4*, *WFS1*, *DFNA5*, *MIR96*, *CRYM*, *COCH*, *TJP2*, *MYH9*, *ACTG1*, *DSPP*, *CCDC50* and *MCM2* leads to nonsyndromic autosomal dominant deafness (Raviv *et al.* 2010).

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The *MCM2*, also called *CDC19*, is located on human chromosome 3q21.3 and encodes minichromosome maintenance complex component 2 (MCM2) (Mincheva *et al.* 1994). MCM2 is a member of the MCM2-7 complex (MCM complex), a putative replicative helicase that is needed for DNA replication initiation and elongation during cell cycling in eukaryotic cells (Todorov *et al.* 1994; Lei *et al.* 1997).

The origin recognition complex (ORC) and Cdc6 place MCM2-7 double hexamer on the DNA strand, to initiate DNA replication (Ramer *et al*. 2013; Yuan *et al.* 2017).

Further, MCM2 is implicated in the transition of parental histone H3/H4 to newly replicated DNA in the eukaryotic cells. The amino-terminal region of MCM2 is necessary for the reassembly of the parental nucleosomal histones onto replicated DNA during cellular DNA replication. MCM2 is also involved in the assembly of the newly synthesized histones on the replicated DNA. As a result, different MCM2-7 proteins in the CMG (Cdc45-MCM-GINS) complex can have different functions in chromosomal DNA replication (Ishimi 2018). Moreover, decreasing levels of MCM2 protein in ageing cells have shown its crucial role in cellular senescence (Suzuki *et al*. 2019).

In former studies, it has been shown that mutations in *MCM2* induce apoptosis without any change in proliferation or cell cycle, in HL60 and HEK293 cells. Moreover, studies regarding the alterations in *MCM2* expression in the cochlea of rats and guinea pigs have demonstrated that a subtle increase in the apoptosis of inner ear hair cells can lead to progressive hearing loss (Suzuki *et al*. 2012; Gao *et al*. 2015).

Formerly, an autosomal dominant hearing impairment disease in four generations of a Chinese family with a novel mutation (NM_004526:c.388C>T, p.R44C; Clinvar: SCV002072508) in *MCM2* was described for the first time. Eight family members carrying the variation were diagnosed with sensorineural hearing loss ranging from mild to profound (Gao *et al*. 2015). Here, we report an Iranian family with two affected individuals that present a different severity of hearing loss caused by a novel pathogenic variant (c.388C>T, p.R130C) in the *MCM2* gene.

Materials and methods

Subject

The proband is a nine-year-old male presenting with the chief complaint of total hearing loss. In addition, his mother was a known case of hearing impairments, and needed hearing aids to achieve normal hearing functioning. The proband and his mother were examined thoroughly, and the father was observed for any problems related to this study's concerns. Further, peripheral blood samples were collected from the subjects for further investigations. The parents granted written informed consent (on behalf of themselves and their child) for participation in this study and publication of the results.

Exome sequencing

Using a QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany), genomic DNA was extracted from peripheral white blood cells for WES, Sanger sequencing, and further investigations. Genomic DNA was captured using the SureSelect XT Human All Exon V6 reagent kit (cat. no. 5190-8863; Agilent Technologies), which was applied for the enrichment of coding exons and flanking intronic sequences in accordance with the manufacturer's instructions. Captured coding DNA samples were sequenced using an Illumina NovaSeq6000 (Illumina San Diego, CA) with 100-bp paired-end sequencing.

The raw data were aligned against the human reference genome (hg19) using the Burrows-Wheeler Aligner (Li and Durbin 2010). Single-nucleotide polymorphisms (SNPs) were called by the software program Genome Analysis Toolkit (GATK). Variants were annotated using ANNOVAR (Wang *et al*. 2010). Each variant was classified into one of five categories, namely pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign, and benign, based on the American College of Medical Genetics and Genomics standards for the interpretation of sequence variations (Richards *et al*. 2015). The phenotypic features associated with the candidate genes were compared with the patient's phenotype. The core phenotype associated with the identified mutations was obtained from the OMIM database (OMIM: 616968).

Sanger sequencing

We confirmed the presence of the variant detected by WES using Sanger sequencing (figure 1b). To amplify the mutated sites of the genome, PCR was conducted. The primers were designed using Oligo Primer Designer (Rychlik 2007). To perform the PCR, we utilized the Hot start mix Ampliqon (25 μL), 70 ng of DNA, 1 μL of forward primer (5'-ATG GGGCTCCTGAACCTT), 1 μL of reverse primer (5'-GTT TCTCTGACAGACCTCT), and 21 μL deionized water. The primers were designed using Oligo Primer Designer (Rychlik 2007). The DNA was amplified using the following thermocycling steps: 95°C for 15 min; 35 cycles of 95°C for 30 s, 59°C for 30 s and 72°C for 15 s; 72°C for 5 min. Chromas v2.01 was utilized to illustrate the results of the Sanger sequencing.

Protein structure

The structure of DNA replication licensing factor MCM2 was retrieved from RCSB Protein Data Bank (https://www.rcsb.org/) (PDB ID: 6XTX) (Berman *et al*. 2000).

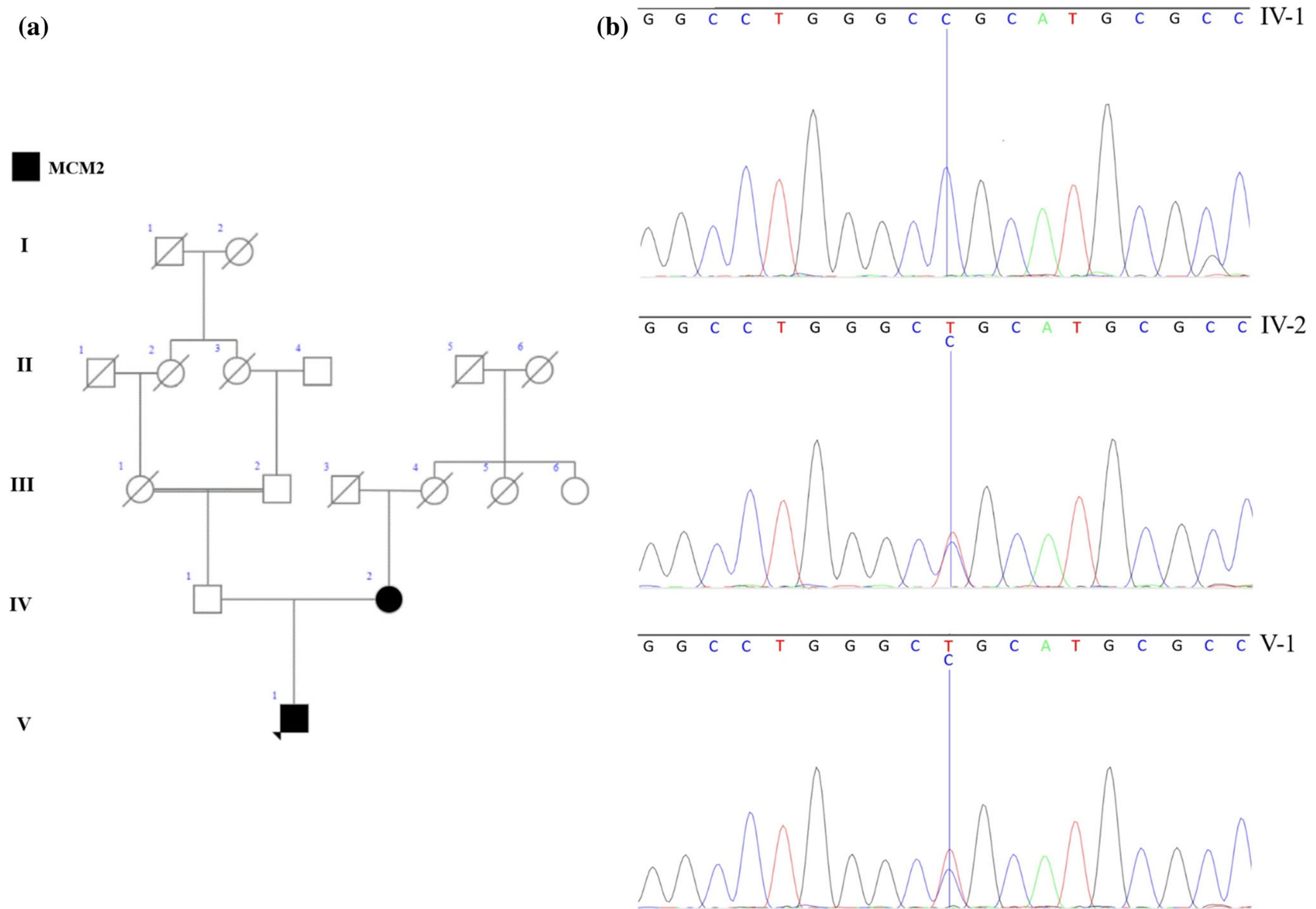

Figure 1. Pedigree and electropherograms of the family. (a) Pedigree illustrating the dominant inheritance of the pathogenic variant in the family where V-1 is proband. (b) Electropherogram of the proband and his parents. Heterozygous substitution of C to T results in alteration of arginine to cysteine in position 130 of the MCM2 protein in the proband (individual V-1) and his mother (individual IV-2).

Domains were collected from InterPro database (https://www.ebi.ac.uk/interpro/) (Hunter *et al*. 2009). The structure was analysed using DynaMut and represented by DOG 1.0 (Ren *et al*. 2009; Rodrigues *et al*. 2018).

Written informed consent was obtained from the patients or, in the case of minors, from their parents. This study was ethically approved by the Ethics Committee of Shiraz University of Medical Sciences.

Results

A nine-year-old Iranian boy with total hearing loss was referred to our centre along with his mother who was a known case of hearing disorder. Whole-exome sequencing conducted on the proband blood sample identified a heterozygous variant in the *MCM2* (NM_004526:c.388C>T, p.R130C). This variant was confirmed by Sanger sequencing in the proband and his mother and determined as the causative agent of the phenotype in this family (figure 1).

The pedigree demonstrates autosomal dominant inheritance pattern in the family. The mother has a *de novo* mutation while the proband inherited the pathogenic variant from his mother. There is no evidence in the examinations or medical record pointing to hearing loss in individuals III-3 and III-4 (figure 1a). Electropherogram of the proband and his parents illustrated heterozygous substitution of C to T in the proband and his mother (figure 1b).

The proband manifests total hearing loss. His mother has been identified with the same pathogenic variant, but despite her postlingual hearing impairments, she could hear by using hearing aids that presented a mild form of the disease. The proband has both hearing loss and speech disorder, which indicates a more severe form of the disease. There were no other symptoms similar to their hearing impairment in other members of the extended family.

The audiogram illustrates the audible frequency tests performed through the standard audiometric test inside a soundproof room according to the clinical standards. Threshold data were obtained from the worse ear of the proband. To diagnose the sensorineural hearing loss in the proband, we used pure tone audiometry. The audiometry result was symmetric and prelingual. Although the pure tone

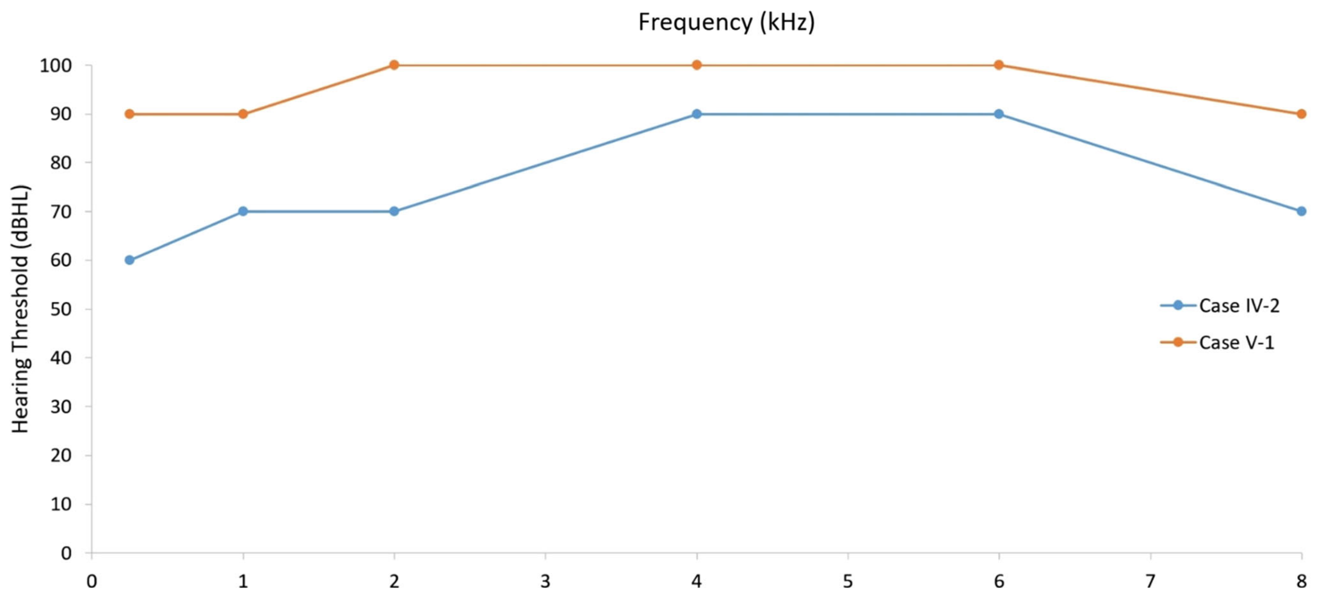

Figure 2. Audiogram of the proband and his mother. Based on the audiogram test it has been illustrated that case IV-2 (blue line) has hearing difficulties without using hearing aids. As it has been illustrated, the case V-1 (red line) has a total hearing disability.

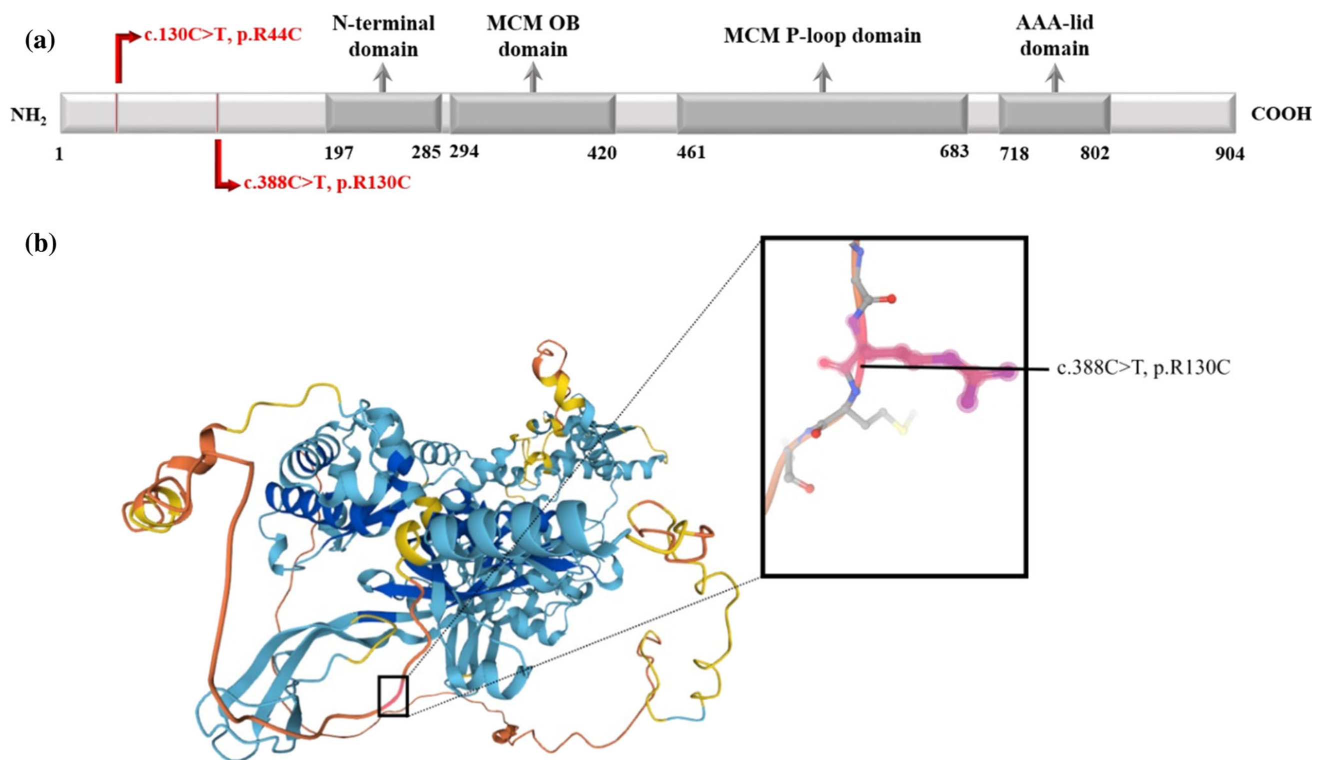

Figure 3. The schematic depiction and three-dimensional structure of MCM2 protein. (a) A schematic depiction of the human MCM2 protein. Arrows indicate pathogenic variants. (b) The three-dimensional structure of MCM2 and the position of the *de novo* missense mutation in this study. The p.R130C variation (pink) is shown on the protein structure. The mutation causes amino acid substitution that converts arginine to cysteine at the 130th position of the MCM2 protein.

audiometric result was abnormal in case IV-2, a mild form of hearing disorder was observed in this case (figure 2).

WES identified a heterozygous missense variant (NM_004526.4:c.388C>T) of *MCM2* in the proband; this variant was also found in his mother in a heterozygous state. Pathogenic variants of *MCM2* are associated with autosomal dominant NSHL. The variant detected by WES is a missense variant and is classified as 'variant of uncertain significance'

based on the PS3, PM2, PP3, PP1 and BP1 criteria of the ACMG/AMP guidelines (Richards *et al*. 2015). This variant was not listed in the gnomAD browser beta (https://gnomad.broadinstitute.org/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases.

The variant (c.388C>T) presented in the current study leads to the substitution of a conserved amino acid (arginine to cysteine) at the 130th position of the MCM2 protein. Both variants presented thus far in *MCM2* are missense and results in arginine to cysteine substitution but in the different positions of the MCM2 protein. In addition, both arginine 44 and arginine 130 are conserved and located at the beginning of the protein before the functional domains (figure 3).

Discussion

Based on the data presented here and the former study, we concluded that pathogenic mutations in *MCM2* present a variable range of mild to severe degree of NSHL in affected individuals. The disease-causing mutation in this family (c.388C>T, p.R130C) like the previously reported family (c.130C>T, p.R44C) is missense.

Hearing loss affects 5% of the global population. Genetic alterations causing hereditary hearing loss are mostly comprised of missense and rare mutations (Azaiez *et al*. 2018). Some of the genes associated with nonsyndromic hearing loss includes *KCNQ4*, *WFS1*, *DFNA5*, *MIR96*, *CRYM*, *COCH*, *TJP2*, *MYH9*, *ACTG1*, *DSPP*, and *CCDC5* (4). One of the rarest among them is *MCM2*.

In 2015, a disease-causing variant in *MCM2* was reported for the first time in a Chinese family with nonsyndromic hearing loss. Eight affected members in this family carrying the pathogenic variant (c.130C>T, p.R44C) were diagnosed with progressive hearing loss from mild to profound hearing loss and the onset of the disease varied among the individuals.

The present study reports the second pathogenic variant (c.388C>T, p.R130C) in this gene (Gao *et al*. 2015). Two affected individuals in this family, same as the former study, manifest various degrees of severity of hearing impairment. By introducing the second family affected by a pathogenic variation in *MCM2*, we could confirm that *MCM2* is one of the extremely rare genes causing the nonsyndromic form of hearing loss, albeit with varying severity.

Considering the notable prevalence of hearing loss and genetic variation in this disorder, it is important to identify the genetic factors that associate with this condition to help diagnose, determine the clinical presentations, and development of effective treatment for these patients. Further studies are recommended to determine the specific clinical presentation related to DFNA70.

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Authors' contributions

ZZ, conceptualization, methodology. MD, conceptualization, supervision. ASS, writing - original draft. NK, conceptualization, methodology, writing - original draft. HJK, methodology, project administration. SZ, investigation. SM, methodology. SST, writing and editing. SAD, physical examination and clinical characterization. SMBT, physical examination and clinical characterization.

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