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Bi-allelic Pro291Leu variant in *KCNQ4* leads to early onset non-syndromic hearing loss

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Abstract

Variants of *KCNQ4* are one of the most common causes of dominantly inherited nonsyndromic hearing loss. We investigated a consanguineous family in which two individuals had prelignual hearing loss, apparently inherited in a recessive mode. Whole-exome sequencing analyses demonstrated genetic heterogeneity as variants in two different genes segregated with the phenotype in two branches of the family. Members in one branch were homozygous for a pathogenic variant of *TMC1*. The other two affected individuals were homozygous for a missense pathogenic variant in *KCNQ4* c.872C > T; p.(Pro291Leu). These two individuals had prelingual, progressive moderate to severe hearing loss, while a heterozygous carrier had late onset mild hearing loss. Our work demonstrates that p.Pro291L variant is semi-dominantly inherited. This is the first report of semi-dominance of a *KCNQ4* variant.

Keywords

Hearing loss; Deafness; ADNSHL; ARNSHL; DFNA2A; *KCNQ4*; Pakistan; Whole exome sequencing; WES; Baylor-Hopkins Center for Mendelian; Genomics; BHCMG

1. Introduction

Hereditary hearing loss is a heterogeneous sensory disorder. To date, 45 genes have been reported for autosomal dominant non-syndromic hearing loss (ADNSHL) while 73 genes have been identified for autosomal recessive non-syndromic hearing loss (ARNSHL) (https://hereditaryhearingloss.org, accessed February 2019). Further, some genes cause both dominant and recessive forms of hearing loss, including *CEACAM16*, *COCH*, *GJB2*, *MYO6*, *MYO7A*, *PTPRQ*, *TECTA* and *TMC1*. Up till now, only one splice-site variant of *MYO6* (OMIM 600970) has been unambiguously shown to cause semi-dominant inheritance of nonsyndromic hearing loss (Brownstein et al., 2014). Although a splice-site

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variant in *SLC26A5* (OMIM 604943) was reported to be semi-dominant (Liu et al., 2003), subsequent studies have suggested that heterozygosity for variants in this gene does not cause hearing loss (Tang et al., 2005; Teek et al., 2009).

Autosomal dominant deafness-2A (DFNA2A-OMIM 600101) is caused by heterozygous variants in *KCNQ4* (Kubisch et al., 1999) (OMIM 603537). It is considered to be one of the most common disease causing genes for non-syndromic dominant deafness accounting for about 9% of ADNSHL (Sloan-Heggen et al., 2016). KCNQ4 is a voltage gated potassium channel that plays an important role in potassium recycling in the inner ear (Kharkovets et al., 2000). More than 30 *KCNQ4* variants have been identified that are involved in dominantly inherited progressive hearing loss (http://www.hgmd.cf.ac.uk/ac/all.php, accessed February 2019). Majority of these are missense variants and affect the pore region of KCNQ4.

Missense variants affecting the pore region are predicted to cause severe hearing loss in a dominant-negative manner (Huang et al., 2017). Heterozygous frameshift variants of this gene cause a less severe phenotype, possibly due to haploinsufficiency (Wang et al., 2014). However, *KCNQ4* variant c.1044_1051del8; p.(Ala349ProfsTer19) has been reported to cause autosomal recessive non-syndromic hearing loss which seems to contradict this hypothesis (Wasano et al., 2015).

In the present study we describe a *KCNQ4* variant associated with semi-dominant inheritance of hearing loss.

2. Materials and methods

This study was approved by Institutional Review Board of School of Biological Sciences, University of the Punjab, Lahore Pakistan. All the family members signed a written informed consent to participate in the research.

We enrolled a consanguineous family HLGM10 (Fig. 1A) in which some members had prelingual hearing loss, which was presumed to be recessively inherited. Medical history was obtained from the family members by asking relevant questions to exclude the involvement of environmental factors or syndromes related to hearing loss. Peripheral blood was drawn to extract DNA from the participants. Assessment of hearing for consenting participants was carried out in ambient noise conditions in 2009 and 2018. Romberg and Tandem Gait tests were performed to find any gross vestibular defects in the affected individuals.

Genes known to cause hearing loss were excluded by homozygosity mapping, utilizing microsatellite markers in close proximity to the respective genes. *GJB2* was analyzed by Sanger sequencing. Whole-exome sequencing (WES) was performed for individuals III:7 and VI:3 at Baylor-Hopkins Center for Mendelian Genomics (BHCMG). Variant filtering was completed using wANNOVAR and PhenoDB Variant Analysis Tool (Sobreira et al., 2015). We selected for rare variants (gnomAD, TOPMed, Exome Variant Server) and focused on exonic non-synonymous, splice-site and insertion and/or deletion (indel) variants. All targeted genes, including known deafness genes, were examined for variants.

Based on the prediction scores by multiple online programs, the shortlisted variants were tested for segregation by Sanger sequencing in all participants. The allele frequency of the selected variants was also checked in 200 ethnically matched controls.

3. Results

Four individuals in a consanguineous family, HLGM10 were affected with hearing loss. Individuals III:7 (65 years old) and IV:5 (20 years old) displayed bilateral, severe to profound or moderate to severe hearing loss, respectively (Fig. 1B and data not shown) when first evaluated in 2009. No vestibular defect was detected after testing for any affected individual.

Linkage to known deafness genes was not identified after homozygosity mapping. Whole exome sequencing did not reveal a pathogenic variant common to both individuals, III:7 and VI:3. When data from each individual were analyzed separately, homozygous variants in two different genes were identified. This included a known pathogenic variant c.236+1G > A in *TMC1* (NM_138691.2), which was detected in the sample from individual VI:3. Sanger sequencing confirmed that the same homozygous variant was present in affected individual IV:5 and was heterozygous in obligate carriers (Fig. 1A). In individual III:7, a homozygous pathogenic variant c.872C > T; p.(Pro291Leu) was identified in *KCNQ4* (NM_004700.3), which has been previously reported for dominantly inherited hearing loss (Naito et al., 2013). Sanger sequencing revealed that affected individual V:5 was also homozygous for the same variant of *KCNQ4* while others were either heterozygous for the variant or had the reference allele (Fig. 1A, C). The variant was not detected in 200 chromosomes from ethnically matched controls.

Audiometry was performed for individuals III:7, IV:7, V:3, and V:5 in 2018 while other members refused testing. The hearing loss of the affected individual III:7 evaluated first in 2009 (Fig. 1B) had progressed to profound degree in 2018 as no response was detected at any frequency (data not shown). The age of individual IV:7 who is heterozygous for the *KCNQ4* variant was 45 years in 2018. Audiometric data revealed mild to moderate hearing loss in this individual (Fig. 1B), of which she was not aware at the time of testing. Though audiometric evaluation was not possible for additional carriers of the variant, interviews from the family indicated that none of them had reported the presence of hearing loss.

Individual V:3, homozygous for the reference allele was 15 years old and had normal hearing (Fig. 1B). The two individuals III:7 (at 65 years) and V:5 (at 8 years) were homozygous for the *KCNQ4* variant and had an early onset moderate to profound or moderate to severe hearing loss, respectively (Fig. 1B) which progressed to profound degree for individual III:7 in a period of nine years.

4. Discussion

Affected individuals presented here show a classic case of semi-dominant inheritance due to a missense variant. This variant affects the pore region of KCNQ4 channel. The pore region of KCNQ4 maintains ion selectivity of the channel and regulates hair cell membrane potential in the inner ear. The Pro291amino acid residue is conserved from mammals to

teleost fish (Fig. 1D). Moreover, proline at this position is conserved in other KCNQ homologues and more broadly in other K^+ channel family members (Talebizadeh et al., 1999).

In a previous report of the p.(Pro291Leu) variant, two individuals of one family (one parent and a child) were affected with dominantly inherited hearing loss. The age of onset was 17 years for both affected individuals (Naito et al., 2013). In light of the hearing loss present in one individual heterozygous for the *KCNQ4* variant presented here, and the two reported before (Naito et al., 2013), it is possible that all carriers of the variant in family HLGM10 may also have a mild hearing loss of which they are as yet unaware. In addition, the progression of hearing loss detected for the oldest affected participant in this research, suggests that hearing loss is also progressive in individuals who are homozygous for the KCNQ4 p.(Pro291Leu) variant.

Semi-dominant inherited traits have been reported before for many other disorders. For example, a more severe phenotype in individuals homozygous for the same variant has been described for Olmsted syndrome, Charcot-Marie-Tooth disease, Epidermolytic ichthyosis and Optic atrophy (Pesch et al., 2001; Nicholson et al., 2008; Nousbeck et al., 2013; Cao et al., 2016).

5. Conclusion

In summary, our results together with a previous report (Naito et al., 2013), indicate that the KCNQ4 variant p.(Pro291Leu) causes early onset, progressive hearing loss in homozygous individuals and late onset, progressive mild to moderate hearing loss when inherited in a heterozygous state. It suggests that the same variant may affect the severity of the phenotype as well as the age of onset in mono-allelic or bi-allelic forms.

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Declaration of interests and funding

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Abbreviations:

ADNSHL	autosomal dominant non-syndromic hearing loss
ARNSHL	autosomal recessive non-syndromic hearing loss
DFNA2A	autosomal dominant deafness-2A
WES	whole exome sequencing
BHCMG	Baylor-Hopkins Center for Mendelian Genomics

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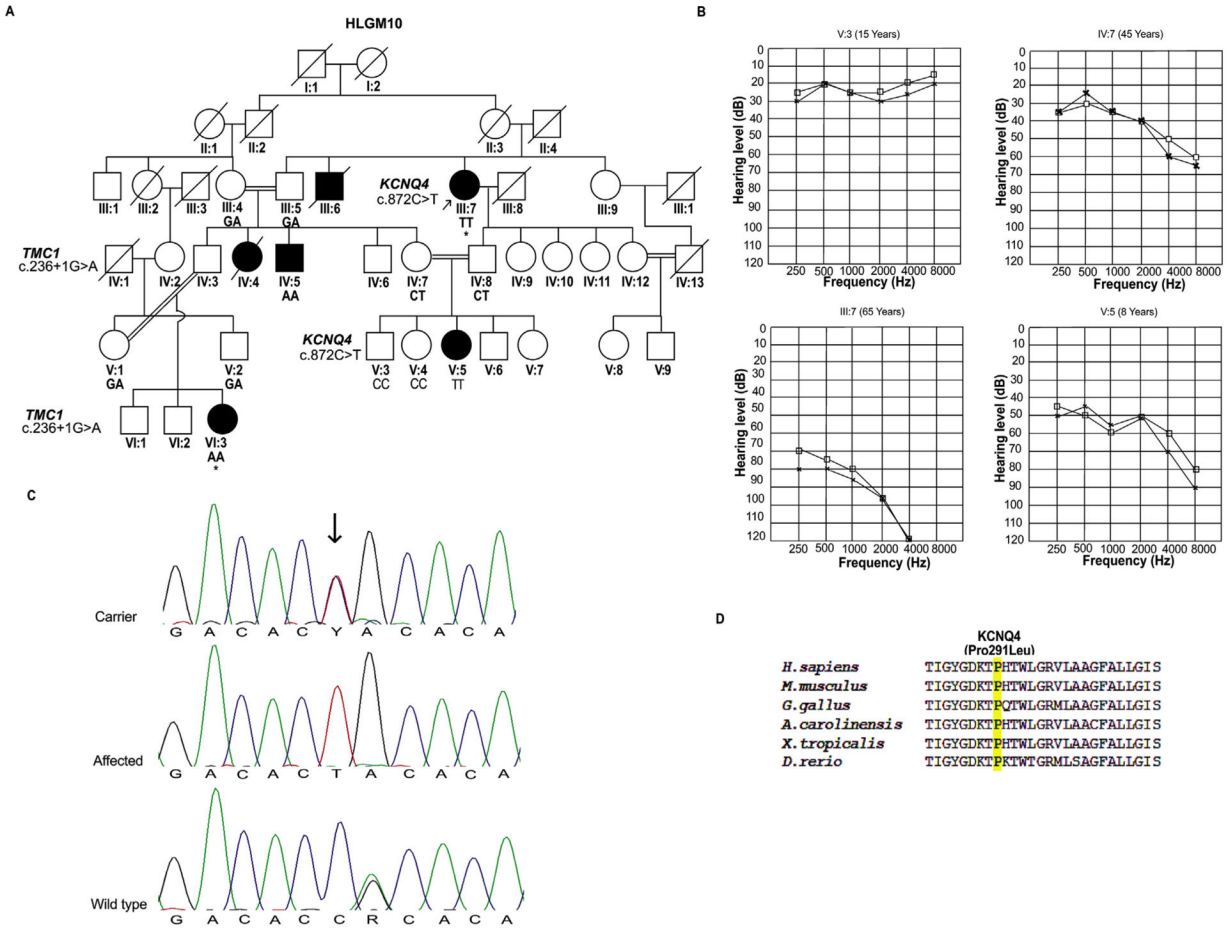

Fig. 1.

Pedigree of HLGM10, audiograms, electropherograms and multiple alignment of partial protein sequence for KCNQ4. (A) Family HLGM10. Arrow shows the proband. Solid circles and squares denote affected individuals. Asterisks mark the individuals whose DNA samples were subjected to whole-exome sequencing. The genotypes for *TMC1* c.236+1G > A, and *KCNQ4* c.872C > T nucleotide variants are indicated below the symbols of the participants. (B) Pure tone audiograms of individuals of the participating families including wild type, carrier and affected individuals for the branch segregating the *KCNQ4* variant. Squares and crosses represent the hearing thresholds for the left and right ears, respectively. The audiograms reveal the presence of mild to moderate hearing loss in the heterozygous carrier, moderate to severe or severe to profound deafness in individuals homozygous for the variant and normal hearing in the individual homozygous for the reference sequence. (C) Electropherograms of *KCNQ4* sequence analyses showing the missense variant c.872C > T; p.(Pro291Leu) in a carrier, homozygous affected and an individual homozygous for the reference wild-type allele, respectively. The arrow indicates the location of the variant. Note that the individual homozygous for the reference allele is heterozygous for an adjacent common SNP, *rs12117176*. (D) Clustal omega alignment of a segment of KCNQ4 from diverse vertebrate species. Alignment shows absolute evolutionary conservation of Pro291.