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A novel frameshift mutation in *KCNQ4* in a family with autosomal recessive non-syndromic hearing loss

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ABSTRACT

Mutation of *KCNQ4* has been reported to cause autosomal dominant non-syndromic hearing loss (DFNA2A) that usually presents as progressive hearing loss starting from mild to moderate hearing loss during childhood. Here, we identified a novel *KCNQ4* mutation, c.1044_1051del8, in a family with autosomal recessive non-syndromic hearing loss. The proband was homozygous for the mutation and was born to consanguineous parents; she showed severe hearing loss that was either congenital or of early childhood onset. The proband had a sister who was heterozygous for the mutation but showed normal hearing. The mutation caused a frameshift that eliminated most of the cytoplasmic C-terminus, including the A-domain, which has an important role for protein tetramerization, and the B-segment, which is a binding site for calmodulin (CaM) that regulates channel function via Ca ions. The fact that the heterozygote had normal hearing indicates that sufficient tetramerization and CaM binding sites were present to preserve a normal phenotype even when only half the proteins contained an A-domain and B-segment. On the other hand, the severe hearing loss in the homozygote suggests that complete loss of the A-domain and B-segment in the protein caused loss of function due to the failure of tetramer formation and CaM binding. This family suggests that some *KCNQ4* mutations can cause autosomal recessive hearing loss with more severe phenotype in addition to autosomal dominant hearing loss with milder phenotype. This genotype–phenotype correlation is analogous to that in *KCNQ1* which causes autosomal dominant hereditary long QT syndrome 1 with milder phenotype and the autosomal recessive Jervell and Lange-Nielsen syndrome 1 with more severe phenotype due to deletion of the cytoplasmic C-terminus of the potassium channel.

1. Introduction

The *KCNQ* (Kv7) gene family contains five members that encode proteins for voltage-gated potassium channels (Kv7.1 – Kv7.5) that play important roles in the brain, heart, kidney and inner ear [1,2]. Although the function of the pore domain of the channel is considered important, recently the function of the cytoplasmic C-terminus, where the A-domain and B-segment are located, has also attracted attention. The A-domain has an important role in the tetramerization of KCNQ proteins to form the potassium channel. The B-segment binds calmodulin (CaM), which regulates the function of the potassium channel with Ca ions [3–5].

Mutation of *KCNQ* genes cause various syndromic diseases: mutation of *KCNQ1*, which is expressed in the heart, causes hereditary long QT syndrome 1(LQT1), also known as Ward-Romano syndrome (OMIM:192500) and Jervell and Lange-Nielsen syndrome 1 (JLNS1) (OMIM:220400); mutations of *KCNQ2* or *KCNQ3*,

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which are expressed in the brain, result in benign familial neonatal seizures (OMIM:121200, 121201); mutation of *KCNQ4*, which is expressed in the inner ear, causes hereditary autosomal dominant hearing loss (DFNA2A) (OMIM:600101) [1,2]. To date, no diseases have been ascribed to mutation of *KCNQ5*.

In 1999, Kubisch et al. [6] reported that DFNA2A was the result of mutation of *KCNQ4*. This gene is expressed in outer hair cells and participates in the potassium recycling mechanisms of the inner ear [7]. The reported mutations in *KCNQ4* are mostly missense or non-truncating deletion mutations in the pore domain and cause progressive severe hearing loss as a dominant-negative effect [1,8]. A few mutations are associated with mild to moderate hearing loss due to haplo-insufficiency [1,8,9].

In this study, we report a novel *KCNQ4* mutation c.1044_1051del8 in a patient with sporadic severe hearing loss of congenital origin or of early childhood onset. The patient was born to consanguineous parents and was homozygous for the mutation. One of the patient's sisters was found to be heterozygous for the mutation but showed normal hearing. This analysis suggests that some *KCNQ4* mutations cause autosomal recessive hearing loss with more severe phenotypes in addition to autosomal dominant hearing loss with milder phenotypes, as is known for *KCNQ1* mutations that cause autosomal dominant LQT1 that has a milder phenotype and the autosomal recessive JLNS1 that has a more severe phenotype [1].

2. Materials and methods

The proband (IV-6) was a 33-year-old woman who had progressive hearing loss with congenital origin or of early childhood onset. There was no family history of childhood hearing loss. The parents of the proband were cousins (Fig. 1). We carried out interviews, and performed physical, otolaryngological, audiological and imaging examinations on the proband, and we also examined her two older sisters (IV-2, IV-5).

We carried out genetic tests on the proband and her two sisters and examined various candidate genes: *GJB2*, as it the most prevalent gene for hereditary hearing loss [10]; mitochondrial *12S rRNA*, $tRNA^{Ser(UCN)}$, $tRNA^{Glu}$, $tRNA^{Leu(UUR)}$, $tRNA^{Lys}$, $tRNA^{His}$, and $tRNA^{Ser(AGY)}$ [11]; *CDH23*, which causes autosomal recessive sensorineural hearing loss with congenital or early childhood onset, high frequency dominance and progression [12,13]; and *KCNQ4*, which is associated with early childhood onset, high frequency dominance and progression. Although *KCNQ4* is better known as a cause of autosomal dominant hearing loss, we considered the possibility of a *de novo* mutation in our patient. The results of the *KCNQ4* analysis are detailed here; details of the other sequencing results are available upon request.

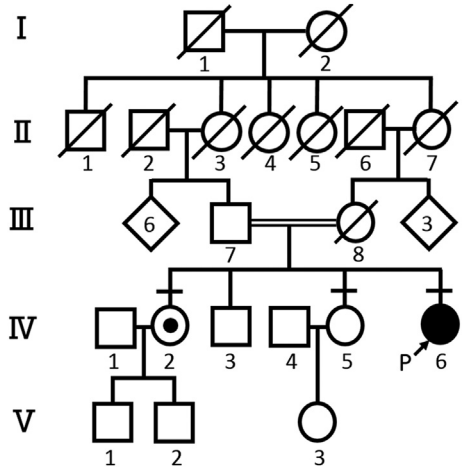

Fig. 1. Pedigree of the family described here. The parents of the proband were cousins. "P" indicates the proband.

Genomic DNAs were extracted from peripheral bloods using the Gentra Puregene Blood kit (QIAGEN, Hamburg, Germany). PCR primers specific for *KCNQ4* (GenBank NG_008139, hg19/GRCh Build37.1) were selected from the resequencing amplicon probe sets (NCBI). All of the exons, together with flanking intronic regions, of *KCNQ4* were analyzed by bidirectional sequencing using an ABI 3730 Genetic Analyzer (Applied Biosystems, CA, USA) and the ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems). The sequences were characterized using SeqScape software v.2.6 (Applied Biosystems).

All procedures were approved by the Ethics Review Committee of the National Hospital Organization Tokyo Medical Center and other participating institutions, and were conducted only after written informed consent had been obtained from each subject.

3. Results

3.1. Clinical features

Hearing loss in the proband was not suspected by the parents during her early childhood although she showed significant delay in speech development and only started to talk at around 6-years-old. She was first seen by a medical doctor at 10-years-old and then started to use hearing aids. Her average hearing threshold was reported as 70 dB at age 13 and hearing loss gradually progressed until her 20 s. At 29-years-old, she visited the International University of Health and Welfare Clinic, and severe sensorineural hearing loss with high frequency dominance was found in both ears (Fig. 2A). DPOAE showed no response in both ears. CT and MRI did not reveal any abnormal findings in the inner ear or in the central auditory pathway. The audiograms of her two older sisters were normal (subject IV-2 is shown in Fig. 2B).

3.2. Genetic analysis

Sequencing analysis of *KCNQ4* in the proband revealed a homozygous deletion of eight nucleotides at position 1044_1051. The deletion caused a frameshift at amino acid 349 (alanine) resulting in early termination after 19 abnormal amino acid residues (c.1044_1051del8, RefSeq NM_004700; p.A349Pfs*19, RefSeq NP_004691) (Fig. 3A), and eliminating the cytoplasmic C-terminal region (Fig. 4A and B). The sequencing analyses did not reveal any mutations of *GJB2*, the mitochondrial genes or *CDH23*. The eldest sister (IV-2) was heterozygous for the *KCNQ4* deletion (Fig. 3B) and her other sister (IV-5) did not carry the mutation (Fig. 3C). The mutation identified here is not listed in the Exome Variant Server (http://evs.gs.washington.edu/EVS/, last accessed December 16, 2014) or the Human Genetic Variation Database, which contain variations determined by exome sequencing of 1208 Japanese individuals (http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html, last accessed December 16, 2014). Similarly, the mutation was not present in a control group of 96 unrelated Japanese individuals with normal hearing. The alanine at position 349 was conserved in all 61 mammalian screened and in 35/36 non-mammalian vertebrate species (UCSC Genome Browser; http://genome.ucsc.edu/cgi-bin/hgGateway, accessed December 16, 2014).

4. Discussion

4.1. The normal and mutated structures of Kv7.4

The *KCNQ4* gene is located on chromosome 1p34 and encodes a protein (Kv7.4) composed of 695 amino acids that has six

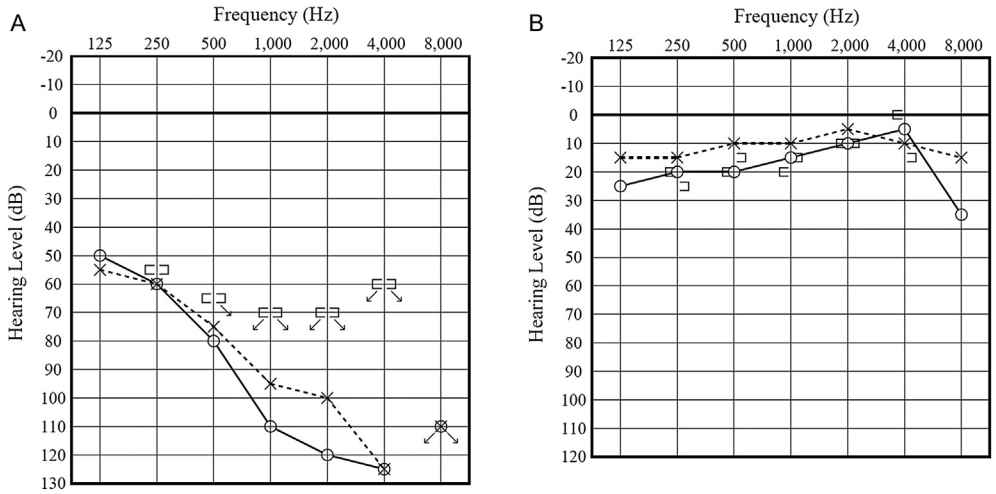

Fig. 2. Audiogram of the proband and her heterozygous sister. (A) The proband (IV-6). (B) The proband's elder sister (IV-2).

transmembrane domains called S1 − 6. Domains S1 − 4 are voltage sensors, while S5 and S6, and the pore helix between S5 and S6, are pore domains. Residues 1–97 of the N-terminal region and residues 318–695 of the C-terminal region of the transmembrane protein are located in the cytoplasm. The Kv7.4 potassium channel has a quaternary structure formed by a homotetramer of Kv7.4 proteins mediated by the A-domain (Assembly domain) in the C-terminus (Fig. 4C) [3,4]. Located between the S6 and A-domains is the B-segment that is a binding site for calmodulin (CaM) which regulates the channel function in conjunction with Ca ions [5]. The c.1044_1051del8 mutation was located next to the sequence encoding S6 and caused a frameshift that eliminated almost all of the cytoplasmic C-terminus (Fig. 4A and B). NNSPLICE (0.9 version) predicted that the mutation might cause a splice site mutation that would also eliminate most of the cytoplasmic C-terminus since it was located near to 5′ end of exon 8 (Fig. 3).

4.2. Nonsense mediated decay (NMD)

Eukaryotic cells contain a surveillance system (NMD) that acts as an mRNA quality control by eliminating sequences with a premature stop codon. An mRNA transcribed from a gene with a truncating mutation is liable to be destroyed via NMD, which therefore blocks formation of abnormal polypeptides [14]. Mutations with premature stop codons within the final exon or within 55 nucleotides upstream from the last exon–exon junction are not destroyed but those outside of these regions are destroyed by this monitoring system. However, escape from NMD is known to occur in some human genes. For example, the β globin gene, mutation of which causes β-thalassemia, is one such gene [15]. We suggest that *KCNQ4* is likely to escape from NMD.

Two frameshift mutations have been reported in *KCNQ4*, c.211_223del13 [16,17] and c.211delC [18] that affect the cytoplasmic N-terminus, and also a nonsense mutation, c.725G>A [19] in the S5 domain (Fig. 4A and B). The latter mutation causes severe hearing loss by a dominant-negative effect, which indicates escape from NMD. The mutation c.1044_1051del8 identified in the present study is located in exon 8, which is 6 exons upstream of the final exon, but closer to the final exon than the c.725G>A mutation. Thus, the mRNA of c.1044_1051del8 is also likely to escape NMD and to be translated to a polypeptide that lacks the cytoplasmic C-terminus.

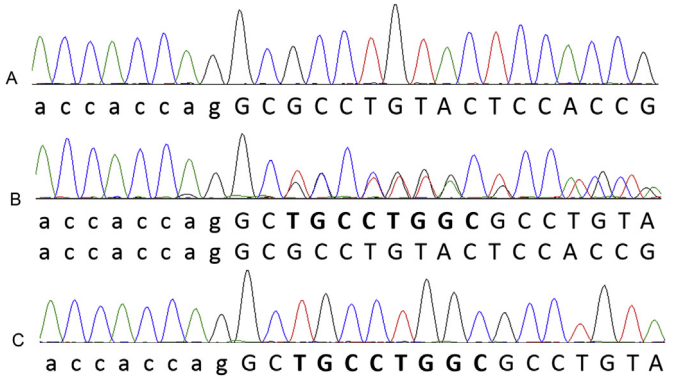

Fig. 3. Electropherograms of the part of *KCNQ4* sequence. (A) Proband (del8/del8). (B) Proband's heterozygous sister (del8/+). (C) Proband's homozygous wild type sister (+/+). (Lower case, intron 7; upper case, exon 8; boldface: the 8 bases deleted in this mutation).

4.3. Similarity of KCNQ4 mutations causing hearing loss to KCNQ1 mutations causing long QT (LQT) syndrome

LQT syndrome, an inherited heart condition, is caused by mutation of the *KCNQ1* gene, which has a similar structure to *KCNQ4*. The autosomal dominant LQT1 and the autosomal recessive JLNS1 are variants of the LQT syndrome [1]. LQT1 is caused by monoallelic mutations and JLNS1 is caused by biallelic truncating mutations in the cytoplasmic C-terminus. Clinically, JLNS1 presents with a more severe phenotype than the LQT1 [20]. The genotype–phenotype correlation for *KCNQ1* mutations is similar to that found for *KCNQ4* mutations. The previously reported *KCNQ4* cases had monoallelic mutations and presented with milder phenotypes compared to the phenotype in the present case. The present case had biallelic truncating mutations of the cytoplasmic C-terminus, and presented with a more severe phenotype.

4.4. Lack of cytoplasmic C-terminus in KCNQ4 and KCNQ1

A case with a nonsense mutation in the S5 domain of *KCNQ4* showed severe hearing loss as a result of a dominant-negative effect [19], indicating that abnormal polypeptides that lack the C-terminus can assemble with normal proteins to form a tetramer. We suggest that a similar phenomenon, except for the dominant-negative effect, occurred here in the heterozygous carrier of the c.1044_1051del8 mutation who did not show any hearing loss. Her

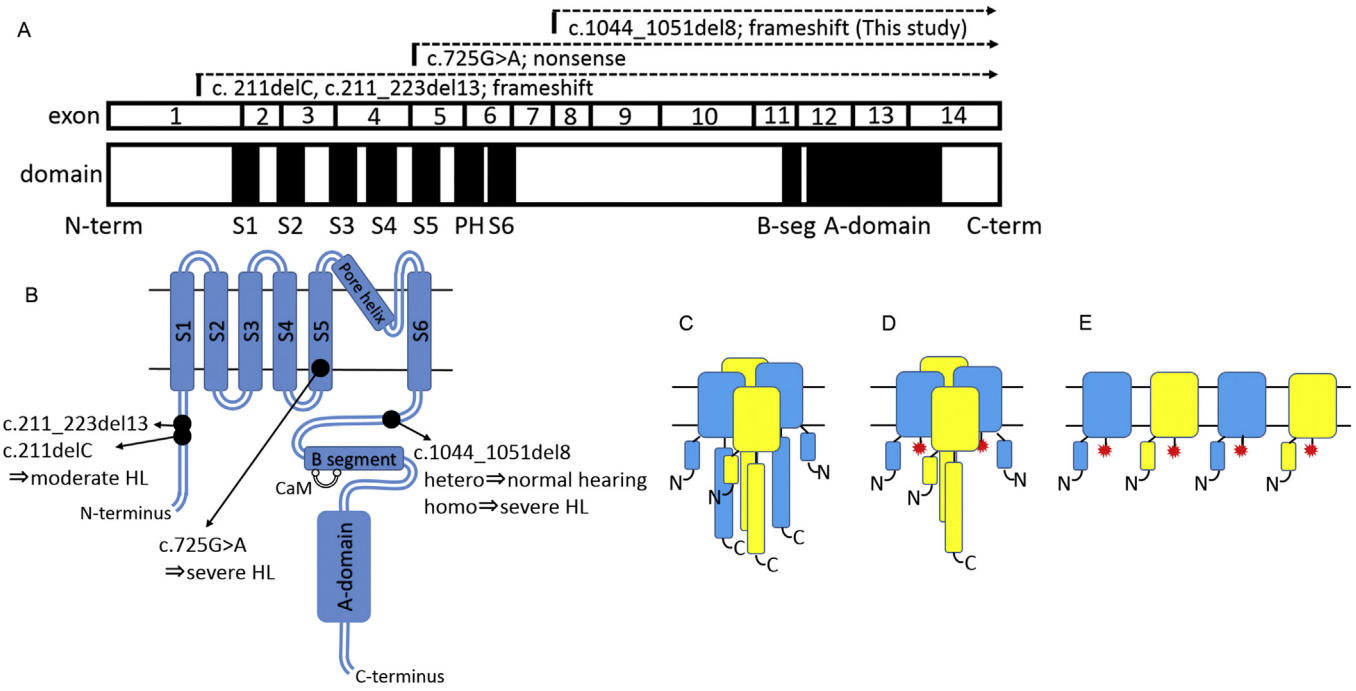

Fig. 4. Schematic representation of the structure of *KCNQ4* and Kv7.4 with annotation of all known truncating mutations and the structures formed by assembly of Kv.7 polypeptides. (A) Locations of *KCNQ4* truncating mutations. (B) Schematic drawing of the Kv7.4 protein, annotation of all known truncating mutations and genotype–phenotype correlations. (C) Normal tetramer structure produced by tetramerization of normal proteins. (D) Abnormal tetramer structures produced by the association of normal proteins and polypeptides lacking the cytoplasmic C-terminus. These structures are expected to function normally. (E) Abnormal polypeptides that lack the cytoplasmic C-terminus cannot assemble to form tetrameric structures. The potassium channel is not functional in this case. Blue square, polypeptide from one allele; yellow square, polypeptide from the other allele. Red star, mutation causing truncation of the cytoplasmic C-terminus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

retention of normal hearing also indicated that tetramer formation and potassium channel regulation by Ca ions functioned sufficiently to achieve a normal phenotype despite the absence of A-domains and B segments in half of the polypeptides (Fig. 4D). The absence of a dominant-negative effect in the carrier of the c.1044_1051del8 mutation might be due the presence of the transmembrane region, including the pore domains that retained the normal amino acid sequences. By contrast to her sister, the proband was homozygous for the mutation and showed a severe phenotype suggesting that the function of the potassium channel was severely impaired by the lack of A-domains and B-segments in all the polypeptides (Fig. 4E).

In a cell culture system designed to model the deletion of the cytoplasmic C-terminus domain in *KCNQ1* that leads to JLNS1, it was shown that mutated and normal proteins could assemble [21]. The function of the potassium channel was normal in heterozygotes for the JLNS1 mutation, but was lost in the homozygous state because the abnormal proteins did not form a tetramer [22]. We postulate that a similar mechanism is present in the proband here, i.e., because of homozygosity for the frameshift mutation of *KCNQ4* that deletes the C-terminus, the function of the potassium channels is lost and a more severe hearing loss occurs. On the other hand, because of heterozygosity for the mutation in the proband's sister, the function of the potassium channels is normal and allows normal hearing. This suggested mechanism needs to be verified in future experiments using cultured cells carrying these types of *KCNQ4* mutations.

Conflict of interest

The authors declare no conflict of interest.

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Transparency document

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