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Moderate hearing loss associated with a novel KCNQ4 non-truncating mutation located near the N-terminus of the pore helix

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ABSTRACT

Genetic mutation is one of the causative factors for idiopathic progressive hearing loss. A patient with late-onset, moderate, and high-frequency hearing loss was found to have a novel, heterozygous *KCNQ4* mutation, c.806_808delCCT, which led to a p.Ser260del located between S5 and the pore helix (PH). Molecular modeling analysis suggested that the p.Ser269del mutation could cause structural distortion and change in the electrostatic surface potential of the *KCNQ4* channel protein, which may impede K+ transport. The present study supports the idea that a non-truncating mutation around the N-terminus of PH may be related to moderate hearing loss.

1. Introduction

Currently, 50 loci and 27 responsible genes for autosomal dominant non-syndromic hearing loss (DFNA) have been identified [1]. *KCNQ4* is one gene that can cause DFNA, type 2 (DFNA2, OMIM: 600101) [2,3]. Patients with mutations in this gene present progressive sensorineural hearing loss starting in the high frequency range. *KCNQ4* (OMIM: 603537) is a voltage-gated KQT-like potassium channel. It modulates the resting membrane potential of the outer hair cells, a type of auditory sensory cell. A functional *KCNQ4* channel consists of four subunits. Each subunit contains six putative domains that span the cellular membrane (S1–S6), a K+-selective pore region consisting of S5, S6, a pore helix (PH), and a pore-loop (P-loop) domain, and N- and C-terminal regions [3].

So far, 11 missense mutations, one nonsense mutation, and three small deletion mutations in *KCNQ4* have been reported to be associated with hearing loss. Understanding the molecular pathology resulting from each *KCNQ4* mutation would be beneficial in predicting the clinical course of KCNQ-related hearing loss. *KCNQ4* mutations can be divided into non-truncating and truncating mutations (Table 1). Most of the *KCNQ4* non-truncating mutations in the pore region are associated with severe hearing loss, except for a non-truncating mutation at the N-terminus of PH, p.Tyr270His, which has been associated with moderate hearing loss [13]. In an electrophysiological study, co-expression of wild-type *KCNQ4* with each non-truncating mutation associated with severe hearing loss, including p.Leu274His, p.Trp276Ser, p.Leu281-Ser, p.Gly285Cys, p.Gly285Ser, p.Gly296Ser, p.Gly321Ser, and p.Gly322_Leu327del, has been shown to result in significantly reduced or non-detectable current [14]. These results indicate that the severe hearing loss in patients carrying these heterozygous mutations is due to a dominant negative effect. On the other hand, the protein products of two *KCNQ4*-truncating mutations, p.Gln71SerfsX138 and p.Gln71fs, lack structural motifs, such as transmembrane domains, and are probably not synthesized from these alleles. Moderate hearing loss in patients carrying these mutations in the heterozygous allele has been considered to be due to haploinsufficiency [3,11].

We identified a novel heterozygous *KCNQ4* non-truncating mutation, c.806_808delCCT, that leads to deletion of a serine residue at position 269 (p.Ser269del), located in the region between S5 and the PH of the protein. Unlike other patients with *KCNQ4* non-truncating mutations, the patient who carried this mutation presented moderate hearing loss. Previously, we reported that a patient having *KCNQ4* with p.Try270His, which is located next to Ser269, showed moderate hearing loss [13], raising the possibility that mutation at or proximal to the N-terminus of PH is associated

Abbreviations: DFNA2, nonsyndromic autosomal dominant sensorineural deafness type 2; *KCNQ4*, potassium voltage-gated channel; KQT-like subfamily, member 4; ABR, auditory brainstem response.

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Table 1
KCNQ4 mutations affecting the pore region of the channel protein in DFNA2 families.

	Exon		Nucleotide	Amino acid	Protein domain	Onset (y)	Progression	Severity	Mechanism	Refs.
Non-truncating mutation	5	Missense	c.778G>A	p.Glu260Lys	S5	1–20	Yes	SV	Unknown	[9]
	5		c.785A>T	p.Asp262Val	S5-PH	1–20	Yes	SV	Unknown	[9]
	5		c.808T>C	p.Tyr270His	N-terminus of PH	0	Yes	MD	Unknown	[13]
	5		c.821T>A	p.Leu274His	PH	1–20	Yes	SV	D.N.E.	[12]
	5		c.827G>C	p.Trp276Ser	PH	1–20	Yes	SV	D.N.E.	[3–5]
	6		c.842T>C	p.Leu281Ser	PH	1–20	Yes	SV	D.N.E.	[6]
	6		c.853G>T	p.Gly285Cys	P-loop	1–20	Yes	SV	D.N.E.	[3]
	6		c.853G>A	p.Gly285Ser	P-loop	1–20	Yes	SV	D.N.E.	[2]
	6		c.859G>C	p.Gly287Arg	P-loop	1–20	Yes	SV	D.N.E.	[7]
	6		c.886G>A	p.Gly296Ser	S6	1–20	Yes	SV	D.N.E.	[8]
	7		c.961G>A	p.Gly321Ser	S6	1–20	Yes	SV	D.N.E.	[3]
	4	Deletion	c.664_681del18	p.Gly322_Leu327del	S5	1–20	Yes	SV	D.N.E.	[10]
	5		c.806_808del3	p.Ser269del	S5-PH	1–20	Yes	MD	See discussion	This study
Truncating mutation	1	Deletion	c.211del1	p.Gln71SerfsX138	N-terminal cytoplasmic	Unknown	Yes	MD	H.I.?	[11]
	1		c.212_224del13	p.Gln71fs	N-terminal cytoplasmic	1–20	Yes	MD	H.I.?	[3]
	5	Nonsense	c.725G>A	p.Trp242X	S5	1–20	Unknown	SV	Unknown	[9]

SV: severe, MD: moderate D.N.E.: dominant negative effect, H.I.: haploinsufficiency, PH: pore helix.

with moderate hearing loss. In this study, we used molecular modeling to elucidate the molecular mechanism underlying moderate hearing loss associated with *KCNQ4* harboring the p.Ser269del mutation.

2. Materials and methods

2.1. Subjects

All procedures were approved by the Ethics Review Committee of National Mie Hospital and National Tokyo Medical Center, and were conducted after written informed consent had been obtained from each individual.

2.2. Clinical analysis

Hearing level was measured by pure tone audiometry and evaluated by averaging four frequencies, 500, 1000, 2000, and 4000 Hz in the better hearing ear and was classified according to the criteria of GENDEAF (moderate, 41–70 dB; severe, 71–95 dB) [1]. Clinical information, such as age of onset and presence of progression, was gathered from the medical records. Computed tomography (CT) and magnetic resonance imaging (MRI) were done to check whether the patient had an inner ear anomaly and/or retrocochlear disease. Auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) were also examined to evaluate inner ear function.

2.3. Genetic analysis

KCNQ4 was selected as the candidate gene on the basis of clinical features, including onset of hearing loss, audiogram patterns, imaging studies, and hereditary pattern [15]. Prior to this study, the patient was confirmed to have neither GJB2 mutations, the most common causative gene of hereditary hearing loss, nor mitochondrial m.1555A>G and m.3243A>G mutations. Genomic DNA was extracted from blood samples using the Gentra Puregene Blood kit (QIAGEN, Hamburg, Germany). PCR primers specific for *KCNQ4* (GenBank NG_008139, NCBI Build37.1) were selected from the resequencing amplicon probe sets (NCBI). All of the exons, together with the 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) and DNASIS Pro (Hitachisoft, Tokyo, Japan). Control DNA was obtained from 96 Japanese subjects with normal hearing.

2.4. Molecular model analysis

To predict the effects of the mutation on the KCNQ4 channel, molecular modeling of KCNQ4 was performed as previously described [13]. The crystal structure of Kv1.2 (PDB ID: 3LUT, chain B) [16] was used as the structural template for modeling of the *KCNQ4* sequence based on sequence homology as determined through Gapped BLAST [17] and PDBsum [18]. The pore regions of wild-type KCNQ4 and the p.Ser269del mutation were modeled using SWISS-MODEL Workspace [19] and validated using the Verify 3D Structure Evaluation server [20,21]. The models were each superimposed onto Kv1.2 using Chimera [22] to visualize ribbon models with electrostatic surface potentials and the hydrogen bonds of either wild-type KCNQ4 or KCNQ4 with the p.Ser269del mutation.

3. Results

3.1. Clinical features

The proband was a 25-years-old female in a pedigree of autosomal dominant progressive hearing loss (Fig. 1A). She has become conscious of progressive bilateral hearing loss, since she has become 20 years-old. At 24 years-old, severe mixed hearing loss with high frequency dominance was found in the right ear by pure tone audiometry. An air-bone gap was considered to have resulted from an operation for a right cholesteatoma at 8 years of age. Moderate sensorineural hearing loss with high frequency dominance was found in the left ear (Fig. 1B). No other symptoms accompanying the hearing loss were identified. ABR showed a threshold of 90 dB in the left ear, and no response at 90 dB in the right ear. DPOAE showed a response only at 1000 Hz in the left ear and no response in the right ear. CT and MRI failed to reveal deformity of the inner ear or structural abnormality in the central auditory pathway.

3.2. Novel mutation of KCNQ4

Sequencing analysis of *KCNQ4* from the patient identified a heterozygous deletion of three nucleotides, CCT, at position 806–808 (c.806_808delCCT). The deletion mutation causes a change of amino acid residues from Ser268-Ser269-Tyr270 to Ser268-Tyr269 (p.Ser269del) without a frameshift (Fig. 2A). Ser269 was located

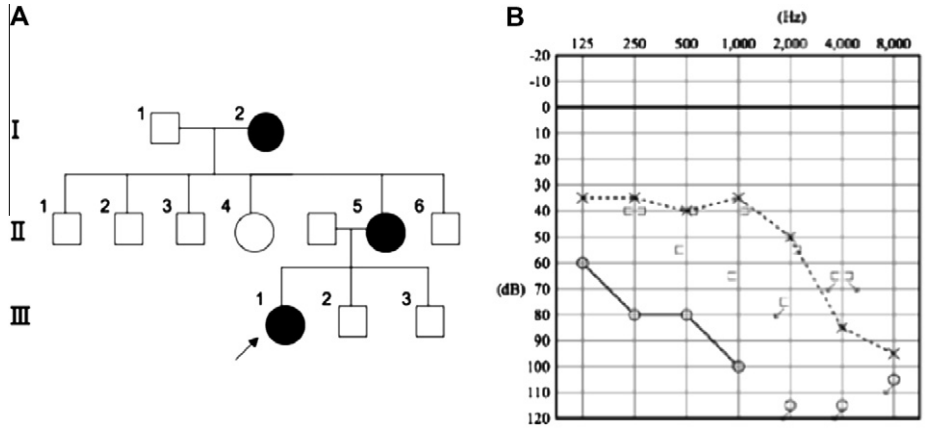

Fig. 1. Clinical information. (A) Pedigree of a family carrying heterozygous *KCNQ4* with the c.806_808delCCT (p.Ser269del) mutation. Individuals with hearing loss are indicated by filled symbols. The arrow indicates the proband. (B) Pure tone audiogram from the proband at 25 years old. Open circles with line: air conduction thresholds of the right ear; x with dotted line: air conduction thresholds of the left ear; left bracket: bone conduction thresholds of the right ear; right bracket: bone conduction thresholds of the left ear. Arrows indicate the non-detectable hearing level by profound hearing loss.

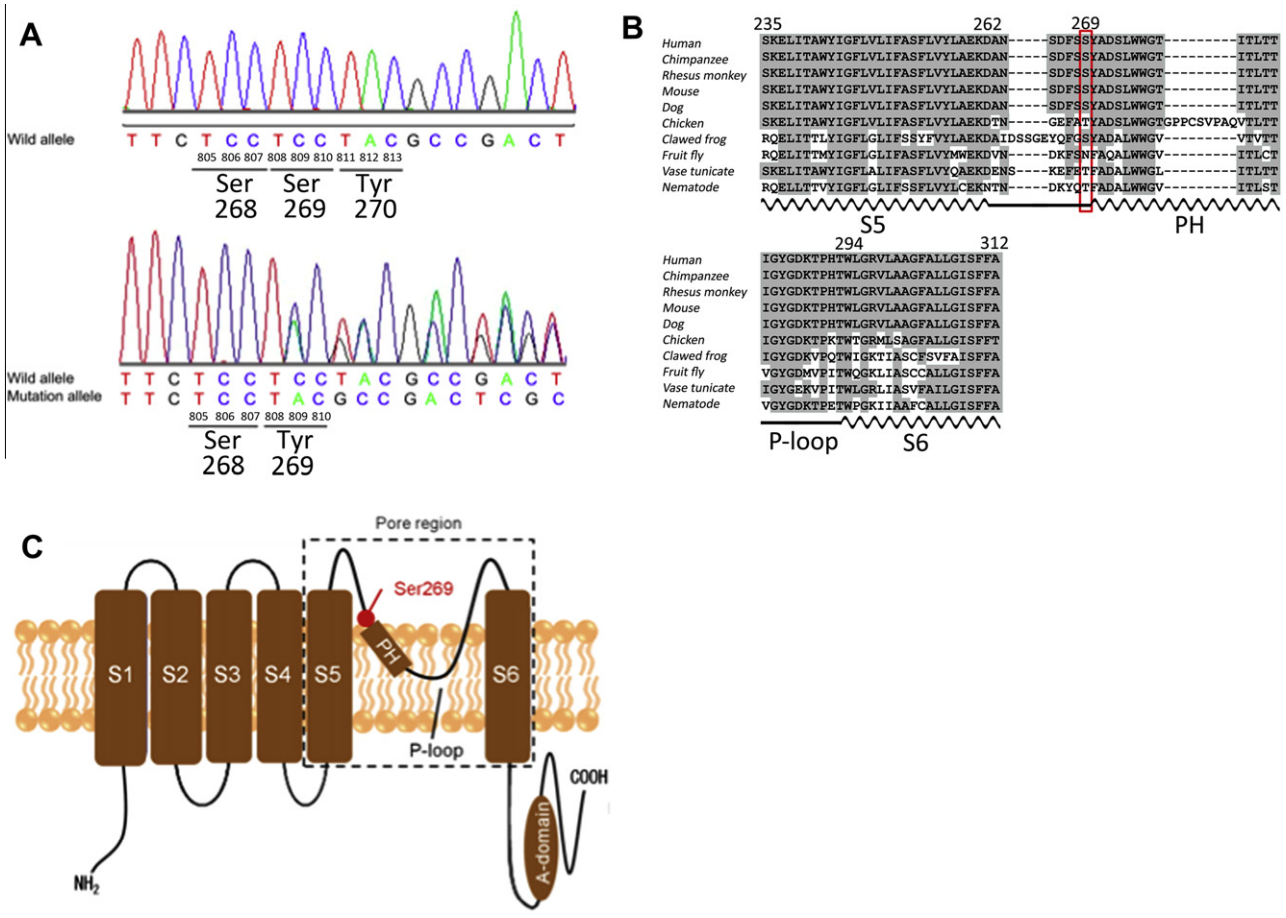

Fig. 2. Partial electrophoretogram of exon 5 of *KCNQ4* with the partial protein sequence for *KCNQ4*. (A) A partial electrophoretogram of exon 5 of *KCNQ4* from an individual with normal hearing (above) and the proband with the heterozygous c.806–808delCCT mutation (below). The positions of the heterozygotic deletion of CCT at 806–808 and the resulting amino acid deletion (p.Ser269del) are indicated. (B) Sequences of the orthologous *KCNQ4* pore region are aligned. Positions highlighted in gray indicate the residues identical to human *KCNQ4*. The position of Ser269 is enclosed by a red square. The positions of S5, pore helix (PH), S6 (wavy lines) and the P-loop (straight line) are shown below the sequences. (C) Schematic topology of *KCNQ4*. Putative domains, including transmembrane regions (S1-S6), channel pore region, PH, P-loop, and A-domain are indicated. Position of Ser269 is indicated by a red circle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in the region between the putative S5 and PH, a highly conserved region among animal species (Fig. 2B and C). This mutation was found neither on the Exome Variant Server [23] nor in the control group of 96 unrelated Japanese individuals with normal hearing.

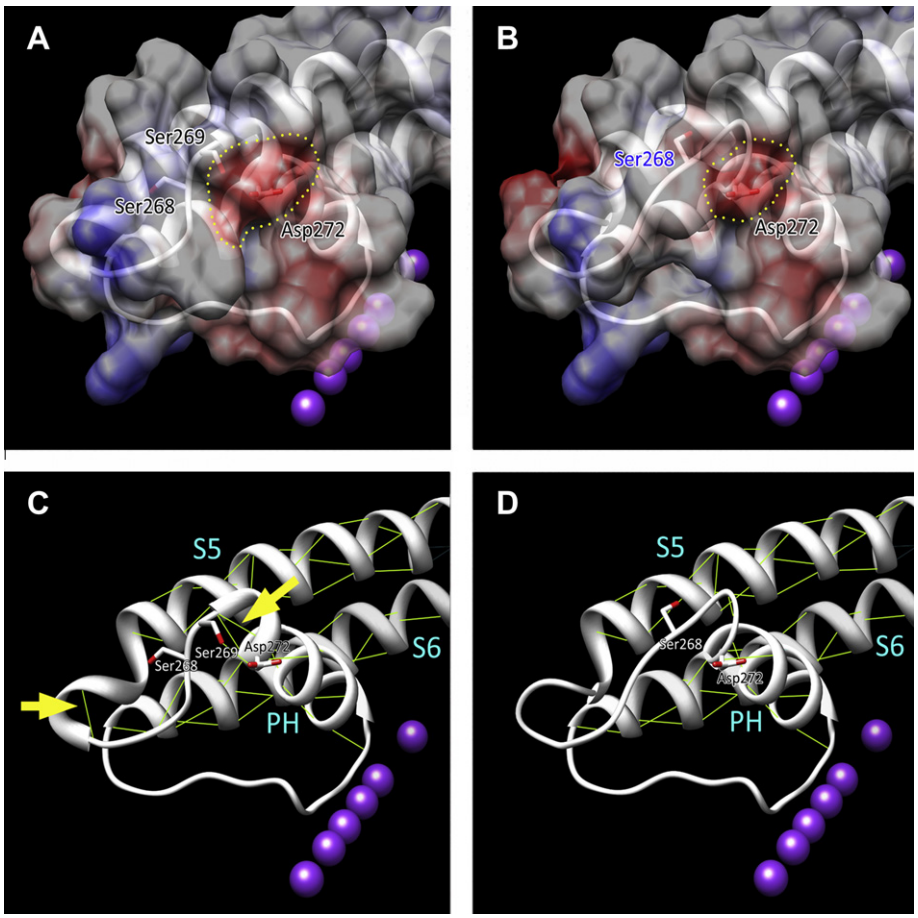

Fig. 3. Partial structural model of KCNQ4 and the p.Ser269del mutation. (A and B) The ribbon models of (A) wild-type KCNQ4 subunit and (B) KCNQ4 subunit with the p.Ser269del mutation overlaid with their corresponding electrostatic surface potential. Red or blue area: negatively or positively charged residues, yellow dot circle: negatively charged surface potential on the N-terminal region of the pore helix (PH). (C and D) Ribbon models of (C) wild-type KCNQ4 and (D) KCNQ4 with the p.Ser269del mutation. Green lines: putative hydrogen bonds; yellow arrows: hydrogen bonds within S5 and PH; purple spheres: potassium ions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Predicted structural change in KCNQ4 caused by the p.Ser269del mutation

The ribbon model of the wild-type KCNQ4 subunit overlaid with the corresponding electrostatic surface potential demonstrated that the surface of the N-terminal region of PH is negatively charged because of the negatively charged side chains of Ser269 and Asp272 (Fig. 3A). The model of KCNQ4 with the p.Ser269del mutation demonstrated reduction of the negatively charged surface area in this region (Fig. 3B). Reduction of the electrostatic surface potential in this area has been predicted to impede K+ transport because of the long range electrostatic attractive force between PH and K+ [13]. In addition, hydrogen bonds on the C-terminus of S5 and the N-terminus of PH of wild-type KCNQ4 (Fig. 3C, yellow arrows) were absent in KCNQ4 with the Ser269del mutation (Fig. 3D). Loss of the hydrogen bonds around the N-terminus of PH resulted in shortening of the PH and was attributed to destabilization of α-helix formation [24]. The disrupted helices would affect the structural stability of the pore region and lead to abnormal channel function.

4. Discussion

Most of the *KCNQ4* non-truncating mutations affecting the pore region are associated with severe hearing loss. However, we found that the non-truncating p.Tyr270His [14] and p.Ser269del mutations were associated with moderate hearing loss. *KCNQ4* mutations at or proximal to the N-terminus of PH are suggested to be associated with moderate hearing loss, because this site is predicted to have relatively smaller influence than other pore regions, such as S5, S6, the central region of PH, and the P-loop, on KCNQ4 channel function.

The molecular pathology associated with the p.Ser269del mutation, demonstrated in silico, indicates a reduction in the negatively charged electrostatic surface potential and structural distortion of the pore region by the mutated KCNQ4, which may explain the associated moderate hearing loss. The molecular mechanism in this case is likely to be a mild dominant negative effect resulting from the relatively small influence of *KCNQ4* with the p.Ser269del mutation on the normal channel subunit. However, another possibility is haploinsufficiency resulting from the loss of function of *KCNQ4* with the p.Ser269del mutation. This scenario, which would not affect the functioning of the other channel subunits, cannot be excluded.

5. Conclusion

We found a novel heterozygous *KCNQ4* mutation, c.806_808delCCT (p.Ser269del), in a pedigree with progressive and moderate hearing loss. Molecular modeling analysis of this mutation demonstrated that changes in electrostatic surface potential and structural distortion could be relevant to the pathology underlying

auditory dysfunction. Mutations at or proximal to the N-terminus of the PH of the KCNQ4 channel might cause mild molecular dysfunction and be associated with moderate hearing loss.

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