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A novel *KCNQ4* one-base deletion in a large pedigree with hearing loss: implication for the genotype–phenotype correlation

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Abstract Autosomal-dominant, nonsyndromic hearing impairment is clinically and genetically heterogeneous. We encountered a large Japanese pedigree in which nonsyndromic hearing loss was inherited in an autosomal-dominant fashion. A genome-wide linkage study indicated linkage to the DFNA2 locus on chromosome 1p34. Mutational analysis of *KCNQ4* encoding a potassium channel revealed a novel one-base deletion in exon 1, c.211delC, which generated a profoundly truncated protein without transmembrane domains (p.Q71fsX138). Previously, six missense mutations and one 13-base deletion, c.211_223del, had been reported in *KCNQ4*. Patients with the *KCNQ4* missense mutations had younger-onset and more profound hearing loss than patients with the 211_223del mutation. In our current study, 12 individuals with the c.211delC mutation manifested late-onset and pure high-frequency hearing loss. Our results support the genotype–phenotype correlation that the *KCNQ4* deletions are associated with later-onset and milder hearing impairment than the missense mutations. The phenotypic difference may be caused by the difference in pathogenic mechanisms:

haploinsufficiency in deletions and dominant-negative effect in missense mutations.

Keywords DFNA2 · *KCNQ4* · Linkage · Mutation · Haploinsufficiency

Introduction

Hearing impairment is one of the most common communication disorders in humans and is both clinically and genetically heterogeneous. Approximately 1 in 1,000 children is affected by hearing impairment (Morton 1991), and in half of the cases genetic factors are involved (Marazita et al. 1993). Nonsyndromic hearing impairment is classified according to its mode of inheritance as DFNA, DFNB, and DFN (autosomal dominant, autosomal recessive, and X-linked, respectively). Currently, 54 autosomal dominant, 59 autosomal recessive, and 8 X-linked loci associated with nonsyndromic hearing impairment have been mapped (Hereditary Hearing Loss Homepage, <http://webhost.ua.ac.be/hhh/>). A total of 21 DFNA genes have been reported to date. Several of the genes are involved in both dominant and recessive deafness (*GJB2*, *GJB6*, *MYO6*, *MYO7A*, *TECTA* and *TMCI*). For example, a null *GJB2* mutation, 35delG in Caucasians and 235delC in Asians, is responsible for the majority of autosomal recessive sensorineural deafness in the respective populations (Kenneson et al. 2002; Kudo et al. 2000; Usami et al. 2002). In contrast, some *GJB2* mutations, including R75W, segregate with deafness in an autosomal-dominant fashion (Richard et al. 1998). The dominant-negative effect of the R75W mutation was suggested by the transgenic expression in mice (Kudo et al. 2003).

DFNA2 is a locus responsible for autosomal-dominant, nonsyndromic hearing impairment in chromosome 1p34 (Coucke et al. 1994; Van Camp et al. 1997). Two

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hearing impairment genes, *GJB3* and *KCNQ4*, have been identified in the DFNA2 locus (Kubisch et al. 1999; Xia et al. 1998). The *KCNQ4* gene consists of 14 exons that encode a protein of 695 amino acids (MIM*603537). The *KCNQ4* protein contains six transmembrane domains and a P-loop region that forms the potassium-selective channel pore (Kubisch et al. 1999). Six missense mutations have been reported in the *KCNQ4* gene to date (Akita et al. 2001; Coucke et al. 1999; Kubisch et al. 1999; Talebizadeh et al. 1999; Topsakal et al. 2005; Van Camp et al. 2002; Van Hauwe et al. 2000). Co-expression studies in *Xenopus* oocytes revealed that the mutant channel protein with a missense mutation exerted a strong dominant-negative effect, which may explain the autosomal-dominant inheritance of *KCNQ4* deafness (Kubisch et al. 1999). Besides the missense mutations, one small deletion has also been reported (Coucke et al. 1999). Because the mutation generated a channel protein that is truncated before the first transmembrane domain, it is unlikely that the mutant protein had a dominant-negative effect. The pathogenic mechanism of this deletion in *KCNQ4*, therefore, remains elusive (Coucke et al. 1999). Recently, Topsakal et al. (2005) noted a phenotypic difference among eight pedigrees with six missense mutations and a single pedigree with the c.211_223del mutation. Based on these data, a hypothesis for the genotype–phenotype correlation is suggested in which younger-onset and all-frequency hearing loss is associated with missense mutations, and later-onset and pure high-frequency hearing loss with null mutations. Although it is an attractive hypothesis, more phenotypic information should be accumulated from individuals with other null *KCNQ4* mutations in order to evaluate the genotype–phenotype correlation.

We identified a novel one-base deletion in *KCNQ4* exon 1 in a large Japanese pedigree with hearing loss using genome-wide linkage analysis and candidate gene analysis. Individuals with the deletion manifested later-onset and pure high-frequency hearing loss, compared with reported patients with *KCNQ4* missense mutations. Our observations support the phenotype–genotype correlation in *KCNQ4* deafness, and suggest that haploinsufficiency is the most likely mechanism for development of hearing loss caused by the null *KCNQ4* mutations.

Materials and methods

Family data

A Japanese family affected with autosomal-dominant, nonsyndromic hearing impairment was identified (Fig. 1). All affected family members had an affected and an unaffected parent, and four male-to-male transmissions were noted. Twenty-four members of the family participated in this study after giving informed consent. They were asked to complete a questionnaire to exclude other causes of hearing impairment. Special attention was paid to features that might have caused

syndromic hearing impairment. All participants underwent otoscopic and audiological examinations. Pure-tone audiograms were obtained in a sound-treated room. Air conduction thresholds were measured in dB hearing level (HL) at 500, 1,000, 2,000, 4,000, and 8,000 Hz. Diagnosis of progressive sensorineural hearing impairment was based on pure-tone audiogram, questionnaire information, and medical records. Family members were considered to be affected if they had sensorineural hearing impairment of more than 25 dB at more than one frequency. Syndromic hearing impairment and environmental causes of deafness were excluded from this study. The ethics committee of Tohoku University School of Medicine approved this study.

Genetic analysis

Blood samples were obtained from 24 family members, 13 affected and 11 unaffected. Control samples were obtained from 100 Japanese subjects with normal hearing. DNA was extracted from peripheral blood leukocytes using the Genomic DNA purification kit (Promega, Madison, WI, USA). In the genome-wide linkage analysis, microsatellite markers of the Human MAPPAIRS (Invitrogen, Carlsbad, CA, USA) were used for genotyping on the ABI 373A DNA Sequencer. Detailed information for additional genetic markers used in this study can be found in the NCBI Human Map Viewer. We performed pedigree and haplotype constructions using the Cyrillic version 2.1 software. Two-point LOD scores were calculated by the MLINK program of the LINKAGE version 5.1 software package (Lathrop et al. 1984). The affected allele frequency and penetrance were set at 0.0001 and 1.0, respectively. Multipoint linkage analyses were conducted using the GENEHUNTER 2 (Kruglyak et al. 1996).

Mutation analysis was performed by genomic exon sequencing. PCR was carried out using primers flanking 1 *GJB3* exon and 14 *KCNQ4* exons. Reaction conditions were optimized for different primer sets. Primer sequences were based on the genomic sequence available in the Genbank (*GJB3*, AF099730; *KCNQ4*, AH007377). The amplification products from both genes were sequenced with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) on the ABI 310 Genetic Analyzer and analyzed using the ABI DNA Sequencing Analysis version 5.1 software.

Results

Genetic analysis

In the genome-wide screening, a maximum two-point LOD score of 5.42 at $\theta=0$ was obtained for GATA129H04 (penetrance=1.0, Table 1). In addition, multipoint linkage analysis with GATA129H04 revealed

Fig. 1 Pedigree of the Japanese family with nonsyndromic, autosomal-dominant hearing impairment. The asterisks indicate those individuals in whom a *KCNQ4* mutation was identified. The haplotype enclosed in the box shows the disease haplotype

Table 1 Two-point LOD scores for linkage between hearing impairment and microsatellite markers in a Japanese family

Marker	Marshfield map distance (cM)	LOD score at θ					
		0	0.05	0.1	0.2	0.3	0.4
D1S234	55.10	2.99	2.75	2.49	1.93	1.28	0.59
D1S496	64.38	−999.99	3.09	3.00	2.44	1.64	0.71
D1S186	67.22	−999.99	3.09	2.97	2.35	1.50	0.55
D1S432	69.86	−999.99	0.81	1.13	1.15	0.86	0.44
D1S2892	70.41	−999.99	−1.59	−0.69	−0.10	0.02	0.02
D1S2706	71.13	1.68	1.53	1.38	1.04	0.66	0.25
GATA129H04	72.59	5.42	4.97	4.50	3.48	2.32	1.01
D1S2134	75.66	−999.99	0.73	0.83	0.70	0.45	0.20
D1S200	82.41	−999.99	3.16	3.02	2.36	1.47	0.50

a maximum nonparametric linkage (NPL) score of 10.95 (data not shown). This marker is known to flank the *DFNA2* locus on chromosome 1p34. As shown in Fig. 1, the most likely haplotypes were constructed to determine the borders of the critical region. All affected family members shared the same disease haplotype. The recombination between D1S2134 and the gene for hearing impairment was found in family member II-5. In family member II-9, recombination occurred between the gene for hearing impairment and marker D1S2892. These events localized the gene for hearing impairment between D1S2892 and D1S2134. This region is at 1p34, where the *DFNA2* locus resides. These data clearly indicated linkage of the hearing impairment to the *DFNA2* locus in this family.

As linkage to the *DFNA2* locus was proven in this family, both *GJB3* and *KCNQ4* genes were examined for

mutations. First, we sequenced the coding region of *GJB3* in this family, but did not find any mutations. Subsequently, we analyzed all *KCNQ4* exons. In exon 1 we identified a deletion of C at nucleotide position 211 of the *KCNQ4* cDNA sequence (Fig. 2). All affected family members were heterozygous for this mutation (Fig. 1). The mutation was not detected in the 100 normal controls.

Clinical features

We performed a clinical study on the Japanese family members (13 affected) with a null *KCNQ4* mutation to delineate its phenotypic features (Table 2). Patients showed a nonsyndromic, postlingual, symmetric and sensorineural hearing impairment. The hearing impair-

Fig. 2 Sequence analysis of the *KCNQ4* in **a** an unaffected (WT/WT) and **b** an affected member (WT/c.211delC) of the pedigree. The *arrow* indicates the c.211delC mutation, shown in **c**. The *arrow* shows the deletion of C at nucleotide position 211 of the *KCNQ4* cDNA sequence. The deletion results in a frameshift after Gly70 (FS71), followed by 67 novel amino acids and a premature stop codon at amino acid position 138 (p.Q71fsX138)

Table 2 Clinical features of family members with c.211delC

Family members	Age	Onset (years)	Sex	Hearing threshold (dB)										Age at the test
				500 Hz		1,000 Hz		2,000 Hz		4,000 Hz		8,000 Hz		
				Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	
II-5	78	22	Male	50	40	75	65	85	>95	>95	>95	>95	>95	71
II-7	71	8	Male	85	85	>95	>95	>95	>95	>95	>95	>95	>95	64
II-9	69	50	Female	30	40	40	35	>95	>95	>95	>95	>95	>95	62
II-11	64	N/A	Female	25	20	30	35	85	75	>90	>90	>90	>90	58
III-3	48	20	Male	10	20	30	25	75	80	>95	>95	>95	>95	41
III-5	52	30	Male	30	20	30	40	80	80	>95	90	>95	>95	45
III-8	51	N/A	Male	20	10	10	10	80	>90	>90	>90	>90	>90	45
III-12	34	N/A	Female	10	15	15	10	15	20	60	60	80	70	34
III-13	46	N/A	Female	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IV-2	23	17	Female	15	15	5	10	10	15	45	45	65	65	18
IV-3	21	14	Male	10	5	10	10	40	45	70	60	85	70	20
IV-4	18	15	Male	15	15	10	10	40	30	50	45	65	65	18
IV-5	20	15	Male	10	10	10	10	10	10	10	10	30	20	15

N/A No information was available

ment in this family was progressive, starting with high frequencies and including middle and low frequencies later in life. According to the anamnestic data, the age at onset varied from 8–50 years.

Discussion

We have identified a novel *KCNQ4* mutation in a large Japanese pedigree with hearing loss by a genome-wide linkage analysis followed by the mutational analysis of candidate genes. The identified mutation was a one-base deletion in exon 1 of the *KCNQ4* gene, c.211delC, which caused a profoundly truncated peptide without the transmembrane portion of the potassium channel (p.Q71fsX138). Individuals with the apparently null mutation c.211delC had late-onset and pure high-frequency hearing loss, which did not resemble the phenotypes of the patients with the *KCNQ4* missense mutations. The individuals with c.211delC had phenotypes similar to those of patients with the c.211_223del (Topsakal et al. 2005). Dominant-negative mechanism has been suggested to underlie the pathogenesis of hearing loss caused by the missense *KCNQ4* mutations. Concordance of the mild phenotypes in two different null mutations, c.211_223del and c.211delC, suggests the genotype–phenotype correlation in which *KCNQ4* deletions are associated with later-onset and milder hearing loss than the missense mutations.

The phenotypic difference may be caused by the difference in the pathogenic mechanism. Two null mutations (c.211_223del and c.211delC) are expected to yield a *KCNQ4* protein that is truncated before the first transmembrane region. Although deleterious mutations result in haploinsufficiency, the function of normal *KCNQ4* protein produced from the intact allele may be spared. Fifty-percent decrease in normal *KCNQ4* channels may lead to mild hearing impairment. Haploinsufficiency has been suggested to underlie the pathogenesis of both long QT syndrome and benign familial neonatal convulsions caused by *KCNQ1* and *KCNQ2* or *KCNQ3* mutations, respectively (Gouas et al. 2004; Rogawski 2000). In contrast, the dominant-negative effect due to missense mutations significantly interferes with the normal channel subunit. Six missense mutations have been identified in the *KCNQ4* gene to date. Five mutations are located in the *KCNQ4* P-loop domain, and one mutation is located in the sixth transmembrane domain. Because these missense mutations are located in the critical domain of the protein, they may cause the dominant-negative effect.

The LOD score estimated at the early stage of this study was 3.0, which was far lower than the final estimation. The NPL score at the early stage was estimated to be 8.0, suggesting the possibility of incomplete penetrance. The identified *KCNQ4* mutation, c.211delC, was also present in a family member III-12, who had been classified as an unaffected individual. She did not complain of any difficulty in hearing. After the identifi-

cation of the causative mutation we performed the audiological examination on her. It turned out that she had mild hearing impairment only in high-frequency, in concordance with her genotype. Re-analysis using this information showed an LOD score of 5.42. Thus, the deafness caused by the c.211delC appeared to be transmitted at a level of complete penetrance. The current study illustrates the potential difficulty of linkage analysis in slowly progressive hearing loss in a limited frequency range. Thorough otological examinations may be required even in individuals who are seemingly not affected upon interview.

Although more than 30 deafness genes have been so far identified, the genotype–phenotype relation in each allelic mutation remains largely unidentified. Elucidation of the genotype–phenotype correlations would facilitate informative genetic counseling.

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