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Mutation analysis of *COL9A3*, a gene highly expressed in the cochlea, in hearing loss patients

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Abstract

cDNA microarray analysis indicated that *COL9A3* is one of the highly expressed genes in the cochlea. This suggests that collagen type IX has a crucial functional role in the inner ear and may be a candidate gene for hearing loss. Mutation analysis was carried out to find possible disease-causing mutations in this gene. The direct-sequencing method was applied to the *COL9A3* gene in 159 non-syndromic sensorineural deafness patients and 150 normal controls. Two possible disease-causing mutations were identified: an in-frame deletion of three amino acid residues (G181–P183 del) and a missense mutation (D617E). The patients with the mutations showed a moderate progressive bilateral sensorineural hearing impairment in all frequencies. The present data indicate that mutations of *COL9A3* may cause non-syndromic hearing impairment.

Keywords: *COL9A3*; Hearing loss; Mutation screening

1. Introduction

Genes that are expressed specifically in auditory tissues are likely to be good candidates to screen for genetic alterations in patients with deafness, and several genes associated with deafness have been efficiently identified by way of organ-specific and subtractive approaches [1,2]. cDNA microarray analysis indicated that *COL9A3* is one of the highly expressed genes in the inner ear [1], suggesting that collagen type IX has a crucial functional role in the inner ear. Collagen IX is known to be one of the important components together with types II and V collagens in the tectorial membrane of the organ of Corti [3,4]. The tectorial membrane, composed of collagens and non-collagenous glycoproteins, lies over the surface of the organ of Corti and plays a crucial role in the first step of sound transduction. Type IX collagen belongs to the FACIT (fibril-associated collagen with interrupted triplet helices) group of collagens, which is known to bind to the surface of fibril-forming type II collagen [5–7]. The alpha chain of collagen IX contains three triple helical domains (COL1, COL2, and COL3) separated by four non-triple helical (NC1-4) domains [5–7] (Fig. 1). In the present study, we performed screening for mutations in *COL9A3*, a gene reported to be one of those highly expressed in the cochlear tissues.

2. Materials and methods

2.1. Subjects

We screened 147 Japanese and 12 Korean probands with non-syndromic sensorineural hearing impairment. The composition of the subjects was as follows: 95 subjects from autosomal dominant or mitochondrial families (two or more generations affected); 15 subjects from autosomal recessive families (normal hearing parents and two or more affected

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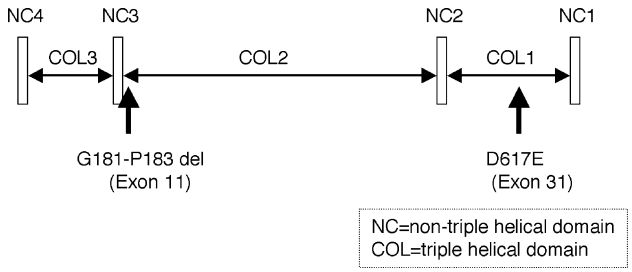

Fig. 1. Scheme showing gene structure of *COL9A3*, which contains three triple helical domains (COL1, COL2, and COL3), separated by four non-triple helical (NC1-4) domains. Arrows indicate the mutations that have been found in this study.

siblings); 49 subjects with sporadic deafness (also compatible with recessive inheritance or non-genetic hearing loss). None of the patients had any other associated neurological signs. The control group consisted of 150 unrelated Japanese individuals with normal hearing evaluated by auditory testing. All subjects gave prior informed consent for participation in the project and the study was approved by the Ethical Committee of Shinshu University.

2.2. Mutation analysis

All 32 exons and flanking intronic sequences except for exon 1 of the *COL9A3* gene [8] were amplified by polymerase chain reaction PCR. Primers were designed to flank all of the exon–intron boundaries through use of the Primer3 web-based server (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). Each genomic DNA sample (40 ng) was amplified, using Ex-Taq polymerase (Takara), for 5 min at 95 °C, followed by 37 three-step cycles of 95 °C for 30 s, 53–63 °C for 30 s, and 72 °C for 1.5 min, with a final extension at 72 °C for 10 min, ending with a holding period at 4 °C in a Perkin-Elmer thermal cycler. The PCR products varied in size at about 230–760 bp, and they were treated with 0.1 μl exonuclease I (Amersham) and 1 μl shrimp alkaline phosphatase (Amersham) by incubation at 37 °C for 30 min, and inactivation at 80 °C for 15 min. After the products were purified, we performed standard cycle-sequencing reactions with ABI Big Dye terminators in an ABI 377 autosequencer (Applied Biosystems).

3. Results

Direct DNA-sequencing identified two possible disease-causing mutations as well as five polymorphisms (Table 1). One mutation was a homozygous nine-base-pair (in-frame) deletion at position 541–549 in exon 11, removing a Gly–Pro–Hypro triplet in the 5′-end of the COL2 domain (Fig. 2). This mutation was detected in a 38-year-old female, who was affected by a moderate progressive bilateral sensorineural hearing impairment in all frequencies. Her parents were consanguineous, but not available for testing. Anamnestically, the onset of hearing impairment was at about the age of 5 and gradually progressed. She had a history of vertigo at the age of 35, but had no abnormal findings in a temporal bone CT scan. Aside from hearing loss, she was phenotypically normal. Consanguinity is a strong indication of true homozygosity and it is unlikely that the paired allele is a different large deletion leading to hemizygosity of the nine-base-pair deletion. Even if it were, the argument that this person's hearing loss is due to a defect in both *COL9A3* alleles would remain strong.

There is weaker evidence of possible dominant inheritance. This was seen in two families with a missense mutation, D617E (1851 C > A) in exon 31 (Fig. 3), localized in the COL1 domain. It was found in two (one Japanese and one Korean) independent autosomal dominant families (Fig. 3). The affected individuals with this missense mutation had moderate bilateral all frequency involved progressive sensorineural hearing loss unassociated with other symptoms. The age at onset of hearing impairment was 30 and 41 years, respectively. The probands did not have mutations in any of the other genes known to cause deafness at an appreciable frequency (*GJB2*, *SLC26A4*, or the 1555A > G mutation in the mitochondrial 12S rRNA) (data not shown).

4. Discussion

The present mutation analysis in the *COL9A3* gene detected two possible disease-causing mutations; an in-frame deletion of three amino acid residues (G181–P183 del) in the COL2 domain and a missense mutation (D617E)

Table 1
Nucleotide changes detected by mutation analysis of the *COL9A3* gene

CHANGE	EXON	AMINO ACID	ALLELE FREQUENCY/CHROMOSOME	
			In Patients	In Control Subjects
308 G>A	5	R103Q	6/318	4/300
541-549 9bp del	11	G181-P183 del	2/318	0/300
1304 C>A	25	A435E	6/318	4/300
1649 C>T	30	P550L	2/318	0/300
1740 T>C	30	P580P	79/318	45/300
1851 C>A	31	D617E	2/318	0/300
2044 C>A	32	R2044R	16/318	Not done