Genes Genom (2016) 38:961–966
DOI 10.1007/s13258-016-0440-4

Online ISSN 2092-9293
Print ISSN 1976-9571

RESEARCH ARTICLE

Genetic analysis of *COL11A2* in Korean patients with autosomal dominant non-syndromic hearing loss

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Received: 14 May 2016 / Accepted: 11 June 2016 / Published online: 6 July 2016

Abstract The collagen type XI alpha 2 gene (*COL11A2*) is associated with autosomal dominant non-syndromic hearing loss (ADNSHL), and all mutations of this gene in ADNSHL are missense mutations. To evaluate its potential as a major causative gene of ADNSHL in the Korean population, we performed genetic analysis of *COL11A2* in 75 unrelated Korean patients with ADNSHL. Consequently, 5 non-synonymous variants, 7 synonymous variants, and 6 intronic variants were identified in *COL11A2*. Among them, a novel variant, p.G829R (c.2485G>C) was found in a patient as a heterozygote. However, pedigree analysis showed this variation was not co-segregated with hearing loss. Previously reported variants p.G230W (c.688G>T) and p.P1422L (c.4265C>T) were discovered in Korean patients. However, these variants were also detected in normal individuals. These results suggest that *COL11A2* is not a major causative gene of ADNSHL in the Korean population.

Keywords Autosomal dominant non-syndromic hearing loss · *COL11A2* · DFNA13 · Mutation · Korean

Introduction

Collagen, the most abundant structural protein in mammals, is the major component of the extracellular matrix in various tissues including cartilage, skin, bone, tendon, and ligament (Fukunaga et al. 2003). So far, a total of 28 collagen types have been identified and categorized based on their structure (Ricard-Blum 2011). It is known that collagen proteins contribute to tissue integrity maintenance through interactions with other extracellular matrix proteins (Di Lullo et al. 2002). Many previous studies have reported that collagen deficiency or loss of collagen function through genetic mutation is associated with syndromic diseases such as osteogenesis imperfecta, Alport syndrome, Weissenbacher–Zweymüller syndrome (WZS), and otospondylomegaepiphyseal dysplasia (OSMED) (Barker et al. 1990; Gajko-Galicka 2002; Melkoniemi et al. 2000; Pihlajamaa et al. 1998).

Collagen type XI alpha 2 (COL11A2) also has been reported to cause several syndromic diseases such as WZS and OSMED (Melkoniemi et al. 2000; Pihlajamaa et al. 1998). However, McGuirt et al. discovered *COL11A2* mutations that were associated with autosomal dominant

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non-syndromic hearing loss (ADNSHL) (McGuirt et al. 1999). To date, 6 different pathogenic missense mutations have been reported in patients with autosomal dominant (DFNA13) or recessive (DFNB53) non-syndromic hearing loss (Chakchouk et al. 2015; Chen et al. 2005; Choi et al. 2013; McGuirt et al. 1999). Previous studies have reported that missense mutations in *COL11A2* cause non-syndromic disease, whereas nonsense or frameshift mutations lead to severe phenotypic effects, such as OSMED syndrome (Chakchouk et al. 2015; Tokgoz-Yilmaz et al. 2011).

COL11A2, located on the human chromosome 6p21.3, has four transcript isoforms due to alternative splicing, with the longest isoform (isoform 1, NM_080680.2) consisting of 66 exons (http://www.ncbi.nlm.nih.gov/). Because of large exon numbers, previous genetic studies of DFNA13 and DFNB53 have not performed mutation analysis in this gene (Ensink et al. 2001). More recently, however, a causative mutation of *COL11A2* was reported for the first time in the Korean ADNSHL population through targeted sequencing (Choi et al. 2013). This suggested the potential that *COL11A2* might be a main causative gene of ADNSHL in the Korean population. Nevertheless, there is no published genetic study of *COL11A2* in the Korean population, yet. Additional genetic studies targeting *COL11A2* are therefore required to investigate the prevalence of ADNSHL associated *COL11A2* mutations in the Korean population.

In this study, we performed genetic analysis of *COL11A2* in 75 unrelated Korean ADNSHL patients to evaluate its potential as a major causative gene of ADNSHL in the Korean population.

Materials and methods

Subjects

A total of 75 unrelated Korean patients with ADNSHL were recruited from the Department of Otorhinolaryngology-Head and Neck Surgery, Kyungpook National University Hospital, Daegu; Yonsei University Health System Hospital, Seoul; and Soree Ear Clinic, Seoul, Korea. We have used, clinically evaluated and reported on these subjects as part of a previous study (Ryu et al. 2016). An unrelated control group composed of 180 individuals with normal hearing was also evaluated. Written informed consent was obtained from all individuals, and the study was approved by the local ethics committee.

Genetic analysis

We designed 57 pairs of primers covering all exon and exon–intron boundaries using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) to amplify the coding region of *COL11A2*. Genomic DNA was extracted from blood using a FlexiGene DNA extraction kit (QIAGEN, Hilden, Germany). Coding regions were amplified by polymerase chain reaction (PCR) using H-Taq DNA polymerase (Solgent, Daejeon, Korea). To confirm the PCR products, gel electrophoresis was carried out using a 1.5 % agarose gel containing ethidium bromide (EtBr). The amplified products were subsequently purified using shrimp alkaline phosphatase (USB, Cleveland, OH, USA) and exonuclease I (USB), and then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). After ethanol precipitation, PCR products were finally sequenced with a 3130xl Genetic Analyzer (Applied Biosystems, Forster City, CA, USA).

The nucleotide sequences were analyzed using ChromasPro v1.6 (Technelysium, Brisbane, QLD, Australia) and SeqScape software v2.5 (Applied Biosystems). These data were compared with reference sequences (NG_011589.1, NM_080680.2) registered in the NCBI database. The dbSNP (http://www.ncbi.nlm.nih.gov/snp/) and the 1000 genomes (http://www.1000genomes.org/) databases were used as references to investigate the novelty and probable pathogenicity of detected variations. Comparison with conserved protein residues among different species was performed in CLC sequence viewer v6.9 (CLC bio, Aarhus, Denmark). To predict functional pathogenic effects of the variations, 3 types of bioinformatics tools were used: Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT and PROVEAN (http://provean.jcvi.org), and Mutation Taster (http://www.mutationtaster.org/).

Results and discussion

COL11A2 mutations have been reported in American, Dutch, Finnish, Northern European, and Korean populations, among others. Currently, 5 missense mutations (p.P779L, p.G808E, p.R1043C, p.G1220D, and G1441E), 3 nonsense mutations (p.R546X, p.R1331X, and G1584X), and 5 frameshift mutations (c.1918-2A>G, c.3032-3033insC, c.3906+5G>A, c.4392+G>A, and c.2775-2801del) have been identified in patients with autosomal dominant diseases (Choi et al. 2013; McGuirt et al. 1999; Melkoniemi et al. 2000; Pihlajamaa et al. 1998; Sirko-Osadsa et al. 1998; van Beelen et al. 2012; Vikkula et al. 1995). To investigate the prevalence of hereditary hearing loss caused by *COL11A2* mutations in the Korean population, we performed genetic analysis of *COL11A2* in 75 Korean patients with ADNSHL. As a result, we identified 18 variants: 5 non-synonymous variants, 7 synonymous variants, and 6 intronic variants (Table 1).

Table 1 Genetic variants of *COL11A2* in Korean patients with autosomal dominant non-syndromic hearing loss

Location[a]	Nucleotide change	Amino acid change	Protein domain	Heterozygote (n)	Homozygote (n)	Allele frequency[c]	dbSNP
Exon 5	c.688G>T	p.G230W	Non-helical region	2/75	0/75	0.0018	rs141430703[b]
Exon 6	c.826G>A	p.E276K	Non-helical region	26/75	5/75	0.3219	rs9277934
Intron 6	c.877-4T>A	–	–	28/75	5/75	0.3221	rs1799907
Intron 9	c.1179+10G>A	–	–	3/75	0/75	0.1026	rs2744507
Intron 12	c.1360-7A>C	–	–	0/75	75/75	1	rs3129201
Intron 15	c.1557+5C>T	–	–	1/75	0/75	–	–
Exon 20	c.1782C>T	p.D594D	Triple helical region	1/75	0/75	0.0042	rs41266697
Exon 24	c.1980C>T	p.P660P	Triple helical region	1/75	0/75	0.0004	rs2229786
Exon 27	c.2136A>T	p.G712G	Triple helical region	26/75	40/75	0.4529	rs1799908
Intron 32	c.2484+8delG	–	–	38/75	27/75	N/A	rs373127772
Exon 33	c.2485G>C	p.G829R	Triple helical region	1/75	0/75	–	–
Exon 33	c.2520G>A	p.R840R	Triple helical region	1/75	0/75	0.0164	rs117237998
Intron 35	c.2628+3G>A	–	–	10/75	65/75	0.3568	rs970901
Exon 36	c.2681C>T	p.P894L	Triple helical region	1/75	0/75	0.1022	rs2855430
Exon 37	c.2700T>C	p.D900D	Triple helical region	4/75	71/75	0.1218	rs2229785
Exon 43	c.3174G>A	p.P1058P	Triple helical region	3/75	3/75	0.4876	rs1799910
Exon 48	c.3576C>T	p.G1192G	Triple helical region	1/75	0/75	0.0006	rs138380958
Exon 59	c.4265C>T	p.P1422L	Triple helical region	1/75	0/75	–	–

N/A not available

[a] Isoform 1 (NM_080680.2 → NP_542411.2): this variant is the longest transcript and encodes the longest isoform

[b] Registered variations in the dbSNP database

[c] Allele frequency based on 1000 genomes database

The novel non-synonymous variant p.G829R (c.2485G>C) was found heterozygously in a patient with ADNSHL (Fig. 1a). His average air-conduction level showed moderately severe hearing loss, with a calculated threshold average of 60 and 76.25 dB in the right and left ears, respectively (Fig. 1b). This variant, a nucleotide substitution of guanine with cytosine at nucleotide position 2485 in exon 33, lead to an amino acid change from glycine to arginine at amino acid position 829 in the triple-helical region domain (Fig. 1c). Interestingly, most *COL11A2* mutations, including three mutations associated with ADNSHL, were located in the triple-helical region (Fig. 2). This region is mainly composed of the conserved 'Gly–X–Y' triple repeat sequences that are a structurally unique feature of the collagen family. Non-glycine positions, X and Y, can be any amino acids, but are often occupied by proline and hydroxyprolin, allowing this region to form a right-handed triple helix consisting of three individual collagen α-chains (Gelse et al. 2003). In this region, the glycine sites are highly conserved. Mutations in glycine sites are considered more likely to cause severe effects than mutations in X or Y positions. For example, in type I collagens (α1 and α2 chains), substitution of a cysteine residue at the X or Y position caused mild phenotypic effects, whereas substitution of glycine with cysteine led to perinatal lethality in a patient with osteogenesis imperfecta. Presumably, decreased structural stability of the triple helix and delayed secretion caused by this substitution results in excessive lysyl hydroxylation and hydroxylysyl glycosylation, leading to collagen degradation (Steinmann et al. 1986). The p.G829R also located in the triple-helical region and the glycine site was

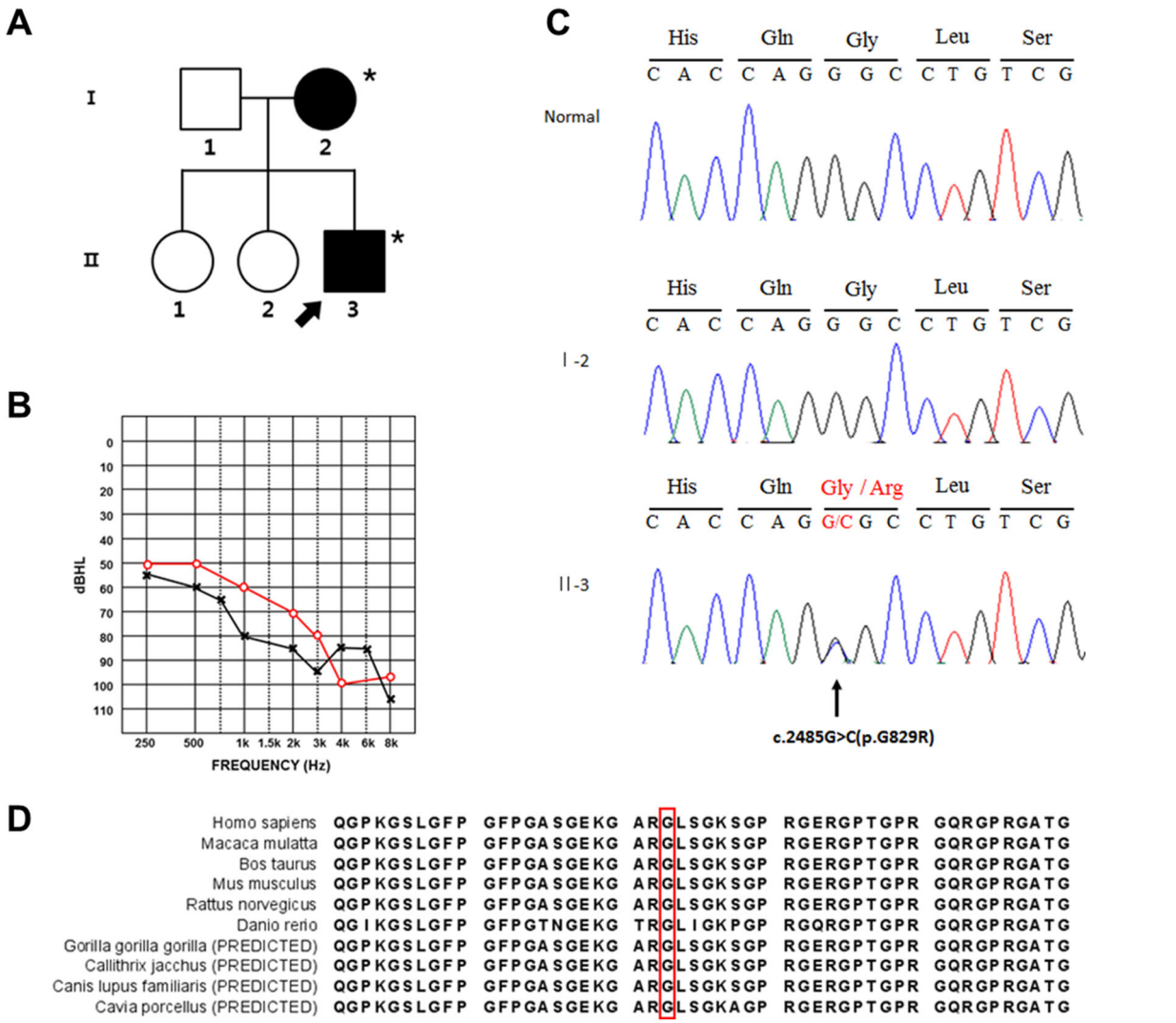

Fig. 1 The missense variant, p.G829R (c.2485G>C), was discovered in a Korean family with ADNSHL. **a** Pedigree of the patient with p.G829R. The *arrow* indicates the proband of the family, and the *filled symbols* represent affected individuals. Patients marked with *asterisks* were analyzed by Sanger sequencing. **b** The audiograms of the proband (II-3). *Red* (*circle*) and *black* (*cross*) *lines* represent the threshold of the right and left ear, respectively. **c** Chromatograms of nucleotide sequences showing the p.G829R variant in the proband (II-3), his mother (I-2), and a normal control. A *black arrow* indicates the location of p.G829R. **d** The evolutionary conservation of amino acids in the vertebrata species. The locations of p.G829R are *boxed*

highly evolutionary conserved sequence (Fig. 1d). Additionally, it has been predicted to be probably damaging with a score of 1.000 by Polyphen-2, damaging with a score of zero by SIFT & PROVEAN, and disease causing by Mutation Taster. Nevertheless, the p.G829R variant was not co-segregated in this family. This means that the substitution from glycine to arginine at amino acid position 829 may not affect normal collagen type XI functions, despite its location in the triple-helical region and high conservation.

Another non-synonymous variant, p.G230W (c.688G>T), was discovered heterozygously in two patients. In this variant, a change from guanine to thymine at nucleotide position 688 in exon 5 lead to substitution of a glycine with a tryptophan at amino acid position 230. Although this variant was already found in Japanese deafness patients (Miyagawa et al. 2013), there was no prediction or investigation about the pathogenicity of this variant. Additionally, this amino acid site was not conserved between other species, and this variant was detected in one of this study's normal control. This suggests that the p.G230W variant found in Korean and Japanese patients with ADNSHL is likely not a pathogenic mutation. A third non-synonymous variant p.P1422L (c.4265C>T) found heterozygously in one patient was also a single amino acid substitution, from proline to leucine at amino acid position

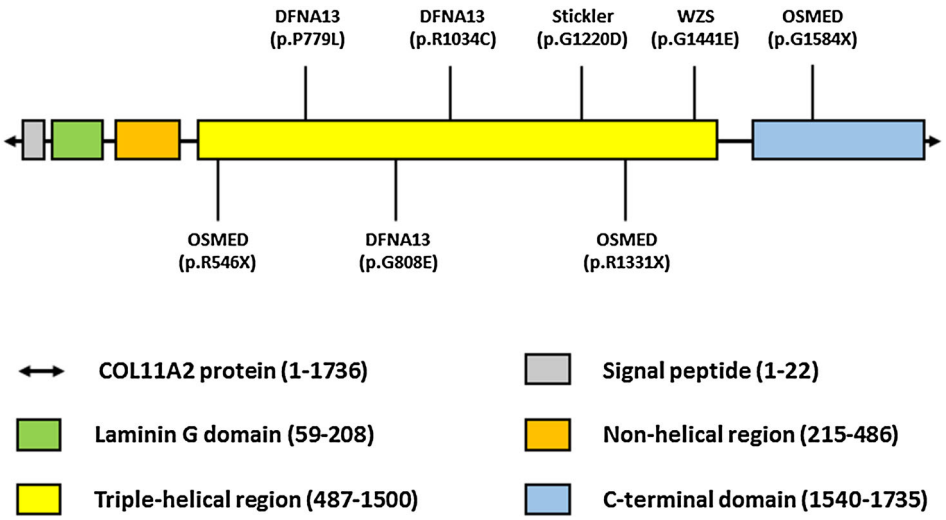

Fig. 2 Schematic representation of *COL11A2* protein with signal peptide, followed by laminin G domain, non-helical region, triple-helical region, and C-terminal domain. A total of 5 missense mutations and 3 nonsense mutations were previously reported showing autosomal dominant inheritance

1422. This non-synonymous variant was previously identified in Korean and Japanese populations (Baek et al. 2012; Miyagawa et al. 2013). However, it was reported that the proline at position 1422 was not conserved in several species and found in individuals with normal hearing (Baek et al. 2012). This suggests that the p.P1422L variant may be a probable polymorphism, and not a pathogenic mutation. The other two non-synonymous variants, p.E276K and p.P894L, were also reported in the 1000 genomes database with high minor allele frequencies (MAFs).

Autosomal dominant hearing loss is a highly heterogeneous genetic disease, with no prominent causative gene of ADNSHL existing in Korean or other East Asian populations. Although *COL11A2* was analyzed in more than 100 Korean patients with non-syndromic hearing loss in this study and in previous genetic research by Choi et al., only one missense variant (p.P693L) has been identified as a probable pathogenic mutation (Choi et al. 2013). However, the dbSNP database suggests that the p.P693L variant (rs150877886) is likely to be a benign allele. The MAF of this variant is 0.004, which is higher than the general prevalence of genetic hearing loss (1 in 1000) as a whole, with 9 of 489 individuals possessing this variant heterozygously in the South Asian population (1000 genomes database). In summary, these results indicate that no certain pathogenic mutations of *COL11A2* in Korean ADNSHL are known, and *COL11A2* may not be a major causative gene of ADNSHL among the Korean population.

Acknowledgments This work was supported by the National Research Foundation (NRF) of Korea, funded by the Ministry of Science, Information and Communications Technology (ICT) and Future Planning (Grant Number: 2015R1A2A2A01003438). This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (Grant Number: HI14C0384).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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