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MAIN ARTICLE

Novel connexin 30 and connexin 26 mutational spectrum in patients with progressive sensorineural hearing loss

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Abstract

Objective: Mutations in the gap junction protein beta-2 gene ('*GJB2*') are known to be responsible for mild to profound congenital and late-onset hearing loss. This study aimed to investigate the molecular basis of progressive hearing loss compared with non-progressive hearing loss.

Methods: Following clinical otorhinolaryngological evaluation, a genetic analysis was performed in a cohort of 72 patients with progressive sensorineural hearing loss.

Results: Pathological genotypes were established in 16 patients (22.2 per cent). Six different gap junction protein beta-2 gene mutations were detected in 15 patients, with the c.35delG mutation responsible for 56 per cent of the mutated alleles. A novel gap junction protein beta-6 gene ('*GJB6*') mutation (p.Met203Val) was observed in one patient with mild progressive hearing loss.

Conclusion: Analyses of gap junction protein beta-2 and -6 genes revealed that similar pathological genotypes, occurring with similar frequencies, were responsible for progressive hearing loss, compared with reported genotypes for non-progressive hearing loss patients. Thus, genotype cannot be used to differentiate non-progressive from progressive hearing loss cases; in this study, patients both with and without an established pathological genotype had a similar clinical course.

Key words: Hearing Loss, Sensorineural; Genetics; Connexin 26; Connexin 30

Introduction

Sensorineural hearing loss (SNHL) is the most common congenital sensory impairment. Approximately half of such cases are expected to have a genetic origin, and 70 per cent of these are non-syndromic cases. The inheritance pattern is autosomal recessive in 80 per cent of these non-syndromic cases, while it is autosomal dominant in 17 per cent, X-linked in 2–3 per cent and mitochondrial in less than 1 per cent.[1]

Mutations in the gap junction protein beta-2 gene, known as *GJB2* and encoding the connexin 26 protein, represent a major cause of prelingual non-syndromic hearing loss and account for 50 per cent of non-syndromic autosomal recessive hearing loss cases.[1,2] More than 100 *GJB2* mutations are listed in the human gene mutation database (HGMD); they are responsible for mild to profound hearing loss, with cases of incomplete penetrance and delayed disease onset. In most cases, *GJB2* mutations are inherited in an autosomal recessive manner, but some mutations are associated with dominantly inherited hearing loss.[3] More than 56 per cent of *GJB2*-related SNHL cases have hearing loss progression.[4]

Another connexin gene which has been characterised as a genetic cause of non-syndromic deafness is the gap junction protein beta-6 gene (known as *GJB6*), which encodes the connexin 30 protein and is located adjacent to the *GJB2* gene on chromosome 13q12. Two large deletions, including most of the *GJB6* gene coding region, are associated with hearing impairment in some populations.[5,6] Only seven *GJB6* mutations responsible for non-syndromic hearing loss have been reported (and listed in the human gene mutation database (HGMD)); four are inherited in an autosomal recessive manner and two in an autosomal dominant manner.

Mitochondrial hearing loss is believed to be responsible for less than 1 per cent of non-syndromic SNHL.[1] Nevertheless, the A1555G mutation in the 12S ribosomal RNA gene of the mitochondrial genome related to aminoglycoside ototoxicity has been reported to be important in the aetiology of SNHL in some populations,[7] but its importance in progressive hearing loss is unknown.

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This study aimed to investigate the molecular basis of progressive hearing loss and to compare it with that of non-progressive hearing loss.

Materials and methods

Patients

Seventy-two patients with progressive hearing loss, from 69 non-consanguineous Caucasian families, were recruited from the cochlear implant programme and from otorhinolaryngology and cervicofacial surgical out-patient clinics, between February 2003 and December 2010. All hearing tests and other evaluations were performed within the department.

The study protocol was approved by the Slovenian national medical ethics committee, and written, informed consent was obtained from all participants (or parents of minors) prior to the study.

The protocol for the evaluation of hearing-impaired patients comprised a detailed family history, a medical history focusing on potential causes of acquired hearing loss (i.e. acoustic trauma, intrauterine infections, perinatal complications,[8] meningitis, mumps, and pre- and postnatal ototoxic drug exposure), and a complete audiological history (including the age of hearing loss onset, rate of progression, and other audiological symptoms). Patients with a history of daily occupational or recreational noise exposure were excluded.

All patients underwent a clinical otorhinolaryngological examination, including ear microscopy, with a systematic search for signs of syndromic hearing loss.

Each patient received a hearing test, prior to which a tympanogram was performed and middle-ear causes of hearing loss excluded. The air–bone gap was never greater than 5 dB.

Infants underwent ophthalmological and paediatric neurological examinations. Babies underwent transient otoacoustic emission testing, behavioural pure tone audiometry and auditory brainstem response testing.

Younger children underwent age-appropriate audiometry tests (i.e. visual reinforced audiometry and conditioned play audiometry) administered by speech and language pathologists experienced in paediatric audiometric testing.

Adults and children older than two to three years underwent conventional pure tone 'hand-raising' audiometry performed using a diagnostic audiometer in a sound-proof room (0.25–8 kHz for air conduction and 0.25–4 kHz for bone conduction).

Pure tone averages (PTA) for air conduction in the conversational frequencies (0.5, 1, 2 and 4 kHz) were calculated for each ear, and the severity of hearing loss was determined by using pure tone audiometry data. The severity of hearing loss was categorised as mild (21–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–90 dB) or profound (>90 dB), for each ear separately.[4] Progressive hearing loss was diagnosed when thresholds worsened by 10 dB or more at two or more frequencies, or by 15 dB or more at one or more frequencies, in the same ear, on retest audiograms. The severity of hearing loss was followed for each ear separately.[4] Hearing loss was considered asymmetrical when the difference between the ears was 15 dB or more in at least two frequencies, as per the definition of the European Workgroup on Genetics of Hearing Impairment (The European Concerted Action Project on Genetic Hearing Impairment; see http://hear.unife.it).

To establish the level and onset of hearing impairment in children under the age of two years, the results of notched noise brainstem audiometry were used.[9] In children over two to three years of age, only conventional pure tone 'hand-raising' audiometry was used to calculate PTAs.

Morphological causes of deafness were excluded with high resolution computed tomography or, in selected cases, with magnetic resonance imaging of the temporal bone.

Adult patients with progressive hearing loss were also referred to a neurologist, an infectious diseases specialist and a rheumatologist, to exclude other, secondary causes of hearing loss.

Data were analysed for statistical significance by applying the *t* test, using the Statistical Package for the Social Sciences version 16 software program (IBM, Chicago, Illinois, USA). Differences were considered significant at a p value of less than 0.05.

Genetic analyses

The *GJB2* and *GJB6* gene coding regions were amplified using polymerase chain reaction with primers, under conditions shown in Table I. Amplicons were sequenced using a BigDye Terminator version 1.1 sequencing kit and an ABI Prism 310 automated sequencer (Applied Biosystems, Norwalk, Connecticut, USA).

Any novel mutations we detected were also sought in 100 unrelated, healthy control subjects.

Screening for the 309 kb GJB6-D12S1830 deletion was performed by sequence-specific duplex polymerase chain reaction, with one fragment amplifying the normal sequence and the other only the deletion-specific sequence. Sample DNA with the GJB6-D12S1830 deletion (kindly provided by Dr Ignacio del Castillo from the Unidad de Genetica Molecular, Hospital Ramon y Cajal, Madrid, Spain) was used as a positive control for the amplification. The primers and conditions used are presented in Table I.

Screening for the mitochondrial A1555G mutation was performed using the Custom TaqMan SNP Genotyping Assay and an ABI Prism 7000 HT sequence detection system (Applied Biosystems, Foster City, California, USA). Sample DNA with the A1555G mutation (kindly provided by Dr Chen-Chi Wu from the Department of Otolaryngology, National Taiwan University Hospital, Taiwan) was used as a positive control.

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TABLE I
PCR PRIMERS AND CYCLING CONDITIONS FOR *GJB2**, *GJB6** AND GJB6-D12S1830 DELETION† AMPLIFICATION

Protein or gene sequence	Primer name	Primer sequence‡	Amplicon length (bp)	Cycling conditions**
Connexin 26 (*GJB2*)	Cx26F1	tgtgtgcattcgtcttctcc	287	Ta 57°C, 1 mM $MgCl_2$, 5% DMSO
	Cx26R1§	cacacgaagatcagctgcag	287	Ta 57°C, 1 mM $MgCl_2$, 5% DMSO
	Cx26F2§	ccaggctgcaagaacgtgtg	520	Ta 58°C, 1.5 mM $MgCl_2$
	Cx26R2	cctcatccctctcatgctgt	520	Ta 58°C, 1.5 mM $MgCl_2$
Connexin 30 (*GJB6*)	Cx30 KR.F	agactagcagggcagggagt	1010	Ta 63°C, 1.5 mM $MgCl_2$
	Cx30 KR.R	ggttggtattgccttctgga	1010	Ta 63°C, 1.5 mM $MgCl_2$
GJB6-D12S1830 deletion	Con30 del F	ctcctttagggcatgattgg	462	Ta 56°C, 2 mM $MgCl_2$, elongation 40 s
	Con30 del R	ccatgcgtagccttaaccat	462	Ta 56°C, 2 mM $MgCl_2$, elongation 40 s
Normal sequence	KONT Con30del R	gtctttgggggtgttgctt	333	Ta 56°C, 2 mM $MgCl_2$, elongation 40 s
	KONT Con30del F	gcatgaagagggcgtacaa	333	Ta 56°C, 2 mM $MgCl_2$, elongation 40 s

*Coding region amplification; †sequence-specification amplification. ‡5′–3′. **Ta-annealing temperature. §Previously described primers.[10] bp = base pairs; PCR = polymerase chain reaction; *GJB2* = gap junction protein beta-2 gene; *GJB6* = gap junction protein beta-6 gene; T = thymine; G = guanine; C = cytosine; A = adenine; DMSO = dimethylsulphoxide

Results

Patient characteristics

The study included 72 patients with progressive hearing loss, aged between one and 65 years (mean age, 28 years). Pure tone audiometry documented the onset of hearing loss as occurring between two and 65 years of age (mean age, 18 years). Three patients were older than 60 years. The duration of documented hearing loss progression ranged from one to 39 years (mean duration, 10.5 years). The hearing loss (expressed as PTA loss) was between 15 and 100 dB (mean, 29.2 dB).

In the 56 patients without identified mutations, the documented mean age of hearing loss onset was 19 years, and the mean duration of documented hearing loss progression was 11.3 years.

In the 16 patients with pathological *GJB2* or *GJB6* variations, the documented mean age of hearing loss onset was 14 years, and the mean duration of documented progression was 10 years.

The clinical characteristics of individual patients with an established genotypic variation are summarised in Table II. No clinical differences were observed between the progressive hearing loss patients with and without an established pathological genotype. The mean documented duration of hearing loss progression was not significantly different ($p = 0.67$). Additionally, there was no significant difference in PTA loss between the two groups ($p = 0.71$); in patients with an established pathological genotype, PTA loss was 15–75 dB (mean, 27.6 dB), while in those without a pathological genotype it was 15–100 dB (mean, 29.7 dB).

Genetic analyses

A pathological genotype was established in 16 of the 18 patients with identified *GJB2* or *GJB6* variations (Table II). Six different mutations and two possibly pathological variations of the *GJB2* gene were detected: five patients had the common c.35delG mutation in a homozygous state, one patient had the c.313_326del14bp mutation in a homozygous state, two patients were compound heterozygotes for two different *GJB2* mutations, and seven patients had a *GJB2* mutation in a heterozygous state. Two controversial *GJB2* variations, namely p.Arg127His[11] and p.Val153Ile,[12] which are believed to be potentially pathogenic, were detected in family 11 and 12 patients, respectively. Two patients, a mother (family 14, born 1965) and a daughter (family 14, born 1988), had two probably non-pathological variations[12,13] inherited on the same allele (Table II).

Additionally, one novel mutation in the *GJB6* gene, changing methionine to valine in amino acid position 203 (p.Met203Val), was detected in a heterozygous state in the one patient from family 15 (Figure 1). This mutation was named starting the numbering from the AUG codon (GenBank Accession Number NM_001110219), as recommended by den Dunnen and Antonarakis.[14] This novel mutation was not detected in 100 healthy control subjects.

In total, eight (11.1 per cent) patients had biallelic mutations and eight (11.1 per cent) had only one heterozygous mutation. The common c.del35G mutation was detected in 56 per cent of the mutated alleles (Table II). None of the patients studied had the mitochondrial A1555G mutation or the GJB6-D12S1830 deletion.

The audiological phenotypes of our patients (all with progressive hearing loss) roughly correlated with the severity of their mutations (Tables II and III). The starting and final PTA values were highest in patients with a homozygous truncating c.35delG mutation (group I in Table II), who thus had the lowest rate of average hearing loss progression. Hearing loss progression in patients with a homozygous truncating mutation started from a higher PTA level, compared with patients with heterozygous truncating mutations ($p = 0.039$), compound heterozygous mutations ($p = 0.01$) or single heterozygous non-truncating mutations ($p = 0.03$). There was a significant difference in the final PTA of patients with homozygous truncating mutations versus compound heterozygous mutations ($p = 0.009$).

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TABLE II
DATA FOR PATIENTS WITH ESTABLISHED *GJB2* OR *GJB6* VARIATION

Patient			Genotype		Hearing loss progression				Mutn type
Fam no	YOB	G	*GJB2*	*GJB6*	Start to end severity (start–end PTA)	Onset (y)	Durn (y)	Mean rate (PTA change/y)	
1	1961	F	p.Gly12fx c.35delG	N	Sev to prof (85–105 dB)	39	10	20 dB/10 y	I
2	2000	M	p.Gly12fx c.35delG	ND	L: mod to deaf (55–100 dB) R: mod to sev (55–75 dB)	L: 2 R: 2	L: 2 R: 8	L: 45 dB/2 y R: 20 dB/8 y	I
2	1977	M	p.Gly12fx c.35delG	N	Mod sev to sev (65–80 dB)	3	14	15 dB/14 y	I
2	1974	F	p.Gly12fx c.35delG	N	Sev to prof (80–105 dB)	3	32	25 dB/32 y	I
3	1994	F	p.Gly12fx c.35delG	N	Sev to prof (90–105 dB)	3	11	15 dB/11 y	I
4	1968	F	p.Arg104fsX109 c.313_326del14	N	Sev to prof (85–100 dB)	3	2	15 dB/2 y	I
5	2006	F	p.[Gly12fx] + [L90P] c.[35delG]+ [269T > C]	N	Mod to mod sev (50–65 dB)	2	2	15 dB/2 y	III
6	1988	M	p.[L90P] + [Met34Thr] c.[269T > C] + [101T > C]	N	Mod to mod sev (50–68 dB)	10	12	18 dB/12 y	III
7	1961	F	p.[Gly12fx] + N c.[35delG] + N	ND	Mod to prof (50–105 dB)	21	16	55 dB/16 y	II
8	1951	F	p.[Gly12fx] + N c.[35delG] + N	N	Sev to prof (80–105 dB)	3	2	25 dB/2 y	II
9	1973	F	p.[Gly12fx] + N c.[35delG] + N	N	L: mild to deaf (10 dB to deaf) R: mild to mod (15–43 dB)	L: 25 R: 28	L: 2 R: 9	L: 100 dB/2 y R: 28 dB/9 y	II
10	1966	F	p.[Gly12fx] + N c.[35delG] + N	N	Mild to mod (35–50 dB)	37	6	15 dB/6 y	II
11	2003	M	p.[Arg127His] + N c.[380G >A] + N	N	Mod sev to sev (65–80 dB)	3	4	15 dB/4 y	IV
12	1971	M	p.[Val153Ile] + N c.[457G >A] + N	N	Mild to prof (30–105 dB)	13	25	75 dB/25 y	IV
13	2006	M	p.[Val37Ile] + N c.[109G > A] + N	N	Mod to mod sev (50–65 dB)	2	1	15 dB/1 y	IV
14	1965	F	p.[Val27Ile; Glu114Gly] + N c.[79G > A; c.341A > G] + N	N	Mild to sev (21–80 dB)	32	4	59 dB/4 y	IV
14	1988	F	p.[Val27Ile; Glu114Gly] + N c.[79G > A; c.341A > G] + N	N	L: congenitally deaf R: sev to prof (85–105 dB)	L: birth R: 5	R: 13	R: 20 dB/13 y	IV
15	1977	F	N	p.[Met203Val] + N* c.[607A > G] + N	Normal to mild (5–25 dB)	26	7	15 dB/7 y	New

Mutations are described as copy DNA change of the open reading frame, and as affecting the protein coding sequence. Mutation (mutn) types: I = homozygous truncating; II = heterozygous truncating; III = compound heterozygous; IV = heterozygous non-truncating. *Met203Val was a novel mutation. *GJB2*=gap junction protein beta-2 gene; *GJB6* = gap junction protein beta-6 gene; Fam no = family number; YOB = year of birth; G = gender; PTA = pure tone average; y = years; durn = duration; F = female; M = male; N = normal; ND = not done; sev = severe; prof = profound; mod = moderate; L = left; R = right

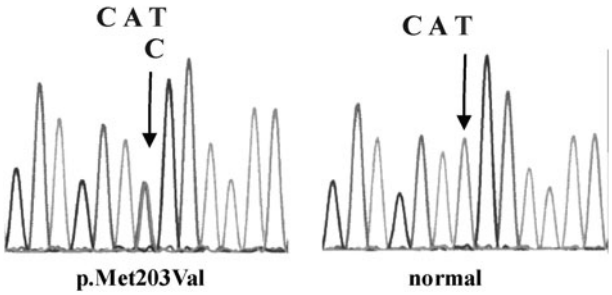

FIG. 1

Sequencing output for the novel connexin 30 gene mutation detected in patient 15, showing the sequence for the heterozygous p.Met203Val mutation compared with a normal sequence (sequences are shown in reverse). C = cytosine; A = adenine; T = thymine

Discussion

Mutations of the *GJB2* gene are known to be a cause of hearing loss which can range from mild to profound. They can be congenital or late-onset, and may be inherited in an autosomal recessive or dominant manner. As many as 56 per cent of patients with *GJB2* gene mutations show some degree of progression of their hearing loss.[4] Our study performed genetic analyses for 72 patients with progressive hearing loss, in whom causes of acquired hearing loss had been excluded. In 16 (22.2 per cent) of them, pathological *GJB2* or *GJB6* genotypes were established.

In several recent studies involving large cohorts of Caucasian patients with SNHL, a pathological *GJB2* or *GJB6* genotype was detected in 24.3 to 36.6 per cent of cases (mean, 29.5 per cent), and the common c.35delG mutation was present in 55.1 to 68.6 per cent of mutated alleles (mean, 60.3 per cent) (Table IV). In our group of patients with progressive hearing loss, a pathological *GJB2* or *GJB6* genotype was detected in 22.2 per cent, and the c.35delG mutation was present in 56 per cent of mutated alleles. Our results corroborate the finding that *GJB2*-related hearing loss is often progressive.[4,17] The c.35delG mutation accounted for a similar proportion of mutated alleles in all the above studies (Table IV). However, this is in contrast with the observation that progressive hearing loss is commonly related to non-truncating mutations.[18] In our study, the same *GJB2* mutations, occurring with similar frequencies, were responsible for non-progressive hearing loss as are known to be responsible for progressive hearing loss; however, further studies on larger cohorts are needed to confirm this observation.

None of our patients carried the GJB6-D12S1830 deletion. This is in accordance with several study findings,[9] including two performed in neighbouring populations, in Croatia[19] and Austria.[20]

It is generally believed that there is some degree of genotype–phenotype correlation in *GJB2*-related non-syndromic hearing loss. Indeed, in our study patients with hearing loss progression and a homozygous truncating mutation had a higher PTA level to begin with, compared with patients with hearing loss due to a heterozygous truncating mutation. However, there was no marked difference in the final hearing loss classifications of patients with different mutation types (Table III). Interestingly, an increasing number of exceptions show that the same mutation can be responsible for different hearing-loss phenotypes and, conversely, that different mutations might be responsible for the same phenotype. In family 2, our patient, born in 2000 and with a homozygous c.35delG mutation in the *GJB2* gene and documented long-term progression of hearing loss, had parents with progressive hearing loss (born in 1977 and 1974) and a congenitally deaf sister, all with the same mutation.

TABLE III
AUDIOLOGICAL PHENOTYPES, BY *GJB2* MUTATION TYPE

Mutn type	PTA (dB)		HL progression		Final HL class (ears; *n*)
	Initial	Final	Mean rate (PTA (dB)/y)	Onset (y)	
I	76.6	97.0	1.7	8.8	3 sev, 9 prof
II	44.3	82.5	5.5	22.0	3 mod, 5 prof
III	50.0	66.5	2.2	6.0	4 mod sev
IV	48.3	83.3	3.4	6.0	2 mod sev, 2 sev, 2 prof

Mutation (mutn) types: I = homozygous truncating; II = heterozygous truncating; III = compound heterozygous; IV = heterozygous non-truncating. *GJB2* = gap junction protein beta-2 gene; PTA = pure tone average; HL = hearing loss; y = years; sev = severe; prof = profound, mod = moderate

- **This study genotyped 72 progressive hearing loss patients and healthy controls**
- **Gap junction protein beta-2 and beta-6 gene mutation types were similar, and similarly frequent, compared with reported non-progressive hearing loss data**
- **A new gap junction protein beta-6 gene mutation (p.Met203Val) was found in a mild progressive hearing loss patient**

In general, progression of hearing loss can be influenced by variations in modifying genes and/or environmental factors. An interesting proposed mechanism of connexin mutation related hearing loss comprises a defect of gap junction mediated intracellular transport of metabolically important molecules (e.g. glucose) as the central cochlear cell defect.[21,22] It is therefore possible that any concomitant condition compromising glucose and/or oxygen supply to cochlear cells may additionally impede intracellular metabolism, causing cell death and thus promoting hearing loss

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TABLE IV
PREVIOUSLY REPORTED *GJB2* GENOTYPES

Population	Pts (*n*)	Path *GJB2* or *GJB6** (pts; %)	Biallelic mutn (pts; %)	Monoallelic mutn (pts; %)	c.35delG (mutd alls; %)	delGJB6-D12S1830 (mutd alls; %)	HL progression
Mainly European[9]	335	29.9	21.2	8.7	57.3	1.1	35.5% of 62 pts with biallelic mutn
North American[17]	7401	24.4	11.3	13.1	55.1	0.3	No data
Italian[15]	376	34	27.7	6.3	No data	2.1	10% of pts with pathological genotype
Italian[16]	734	36.6	27.5	9.1	68.6	1.1	No data
Average	–	29.5	21.9	9.3	60.3		–
Slovenian†	72	22.2	11.1	11.1	56	0	100%

*Pathological gap junction protein beta-2 gene (*GJB2*) or gap junction protein beta-6 gene (*GJB6*) genotype. †Current study.
Pts = patients; mutn = mutation; mutd alls = mutated alleles; HL = hearing loss

progression. Such conditions may include environmental factors such as pollution (with noise or specific neurotoxic chemicals), behavioural factors (e.g. smoking) and other inherent defects of intracellular energy metabolism. Theories proposing novel mechanisms of connexin (or other) mutation related deafness may bring new insights to the understanding of the genotype–phenotype correlation. Larger studies are warranted to expand our knowledge in this field.

A novel *GJB6* gene mutation, p.Met203Val, was detected in a heterozygous state in the one patient from family 15. This mutation was not detected in control subjects and was probably responsible for the progression of hearing loss in this patient. The p.Met203Val mutation is located in the fourth transmembrane helix of connexin 30, and causes a change from non-polar methionine to non-polar small aliphatic valine, resulting in an increase in Kyte–Doolittle hydrophobicity from 1.9 to 4.2.[23] As it changes the hydrophobic methionine to the smaller but also hydrophobic valine, this mutation probably does not significantly destabilise the helix. Thus far, there have been no reported *GJB6* mutations in amino acids 193 to 215, which form the fourth helix. Connexin 26 is similar in structure to connexin 30, and its fourth helix is also formed by amino acids 193 to 215. There is one known *GJB2* mutation changing methionine to valine in this region (p.Met195Val), reported in a heterozygous state in a hearing-loss patient of Japanese origin.[13] Therefore, a methionine to valine type mutation in the fourth helix of connexin 30 may also be pathogenic. Our family 15 patient had mild hearing loss with progression detected over a period of seven years; thus, we speculate that this mutation may have milder pathogenicity. A milder phenotypic effect is also supported by the fact that connexin 30 protein null mice have dramatically milder cochlear cell degeneration compared with connexin 26 protein null mice.[24] Our family 15 patient had no history of hearing loss in her family. An additional, undetected mutation or a dominant effect of p.Met195Val in *GJB6* might also be speculated. One mis-sense *GJB6* gene mutation (p.Thr5Met) has been reported to be responsible for non-syndromic, autosomal dominant, progressive hearing loss.[25] It has also been reported that several *GJB2* mutations with a dominant, negative effect, namely p.Thr44Cys,[26,27] R143Q[28] and p.Cys202Phe,[29] are associated with progressive hearing loss.

Conclusion

In this study, genetic analyses of the *GJB2* and *GJB6* genes indicated that similar pathological genotypes, occurring with similar frequencies, may be responsible for both progressive and non-progressive hearing loss; therefore, genotyping cannot differentiate between these two types of hearing loss. A novel *GJB6* gene mutation (p.Met203Val) was detected in a heterozygous state in a patient with mild progressive hearing loss. Patients both with and without an established pathological genotype had similar clinical courses.

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