遺伝子変異が同定されている症例は数少ない。現状では一般的な遺伝カウンセリングになる場合が多い。

遺伝カウンセリングの目的とは

遺伝子に関わる疾患（遺伝病）を正しく理解し、患者への診療とその家族への支援を行う事である。カウンセリングを行う医師カウンセラーには、正しい診断を行い、情報提供および疾患リスクの計算を行うだけではなく心理的問題を明らかにする役割が求められる。

優性遺伝形式をとる難聴に関する遺伝カウンセリングの留意点

・難聴の受容を促す（難聴者である自分を受け入れられるか)。
・責任論にならないように配慮する（自分を
責めない、親を責めない)。
・クライエントの希望、不安、理解度、家庭環境などに応じて説明していく
(家庭内で責任問題が根底にある場合が多い)
・出来れば配偶者にも来てもらい共通理解を図る。
・再発危険率は 1/2（出生前診断は無理、発症前遺伝子診断は慎重に行うべき。）
再発を畏れて生まない生き方を選択するケースも想定されるが、両親の生殖に関する意思決定は尊重しなければならない。

まとめ

・遺伝子(DNA)が完全である人間などいない。
・難聴を受け入れて前向きに生きて行く為には、クライエントを支える家族や周囲（社会）と医療側のサポートが必要。

D. 結論

自験例を基に遺伝カウンセリングを行う際のポイントと配慮すべき内容について検討を行い、遺伝カウンセリングの際に配慮すべき情報を明らかにした。

E. 研究発表

なし

F. 知的財産権の出願・登録状況

なし

当科に通院中の優性遺伝形式が疑われる遺伝性難聴症例

研究協力者：石川浩太郎（自治医科大学）
共同研究者：市村恵一（自治医科大学）

A. 研究目的

実際の臨床現場における優性遺伝形式遺伝性難聴症例の受診実態を把握することを目的とした。

B. 研究方法

自治医科大学附属病院耳鼻咽喉科外来に2009年度に複数回受診した優性遺伝形式が疑われる遺伝性難聴症例を受診録から検索した。

（倫理面への配慮）

個人名が特定できないよう匿名化を図った。

C. 研究結果

優性遺伝形式が疑われる遺伝性難聴症例を9家系同定できた。この中には家系図から常染色体優性遺伝形式が間違いないと考えられるものが3家系、母系遺伝の可能性が否定できないものが2家系、親子で難聴が認められるものの家系情報が不足しているものが4家系確認された。

D. 考察

遺伝性難聴外来と銘打っていない大学病院の外来においても、慎重に病歴をとりながら診療にあたれば、遺伝性難聴家系を同定できることが明らかになった。今回の発表には含まれていない劣性遺伝形式が疑われる遺伝性難聴家系も合わせて、どの症例も自分たちの難聴の遺伝について少なからず関心を持っている。遺伝カウンセリングも含めて、遺伝性難聴診療の重要性が改めて示唆された。

E. 結論

優性遺伝形式遺伝性難聴は日常の耳鼻咽喉科外来で遭遇する可能性のある疾患であり、その確実な診断法の研究が急務であることが明らかとなった。

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F. 研究発表

なし

G. 知的財産権の出願・登録状況

なし

Waardenburg症候群の一家系

研究協力者：武市紀人（北海道大学）
共同研究者：福田諭（北海道大学）

A. 研究目的

近年、先天性難聴を含む若年発症の難聴において診断目的に遺伝学的検査を施行する機会が増えている。遺伝性難聴の約７割を占めるとされる非症候群性難聴に対し、症候群性難聴や優性遺伝性難聴は症例数は少ないものの、随伴症状や遺伝様式より診断は比較的容易である。診断に当たり医師の側が十分な知識を有していることが必要なのはもちろんであるが、診断の際、患者や家族に病気の概要のみならず、再発率、浸透率を含む遺伝情報、将来の合併症の可能性など疾患の背景についても十分に説明を行い、必要に応じて複数回の面談を行うなどのいわゆる遺伝カウンセリングを行うことが重要である。

本報告では現在難聴に関して当科で診療を行っているWaardenburg症候群疑いの一家系についての経過を報告した上で、遺伝カウンセリングの重要性と今後の課題について検討した。

B. 研究方法

当院では平成17年より遺伝子診療部が難聴遺伝子を含む全ての遺伝子学的検査の窓口となっている。難聴遺伝子診断までの流れとして、まず始めに本人または医師からの依頼を受け、毎月開催される定例カンファレンスにて検査の妥当性について検討される。適応ありと判断された場合は検査前カウンセリングとして耳鼻科医と臨床遺伝専門医で本人との面談を行い、検査の同意が得られた場合にのみ検査を実施する。検査結果は再度、遺伝子診療部が窓口となり報告を受け、カンファレンスにて検討の上で、報告内容と今後の方針を確認し、検査前と同じ担当医が検査後のカウンセリングを行う。必要に応じて、検査後のカウンセリングは複数回行われる。

症例：生後4ヶ月、男児
現病歴：平成14年4月4日通常分娩にて出生。妊娠中の経過に特記事項なし。同年4月7日、産科にてAABR施行、35,40,70dBのいずれにおいても反応を認めない。同年8月21日当科受診。ABR施行し、両耳ともに105dBにて無反応。母親に結果を伝えた。内容としては、母親が先天性難聴であり、特徴的な容貌が母子で酷似していることより遺伝性難聴の可能性が高いこと、および聴力改善の見込みが極めて低いことを伝える。身体障害者２級を申請し、両耳補聴器装用開始。札幌聾学校乳幼児相談室に定期通学となる。その後、数年ごとに聴力測定と診断書作成目

的で当科受診。現在、両側に補聴器を装用し札幌聾学校小学部に通学中。
身体所見：前頭部毛髪色素脱失、虹彩異色
家族歴：父は3歳時に麻疹に罹患し、以後両聾となる。母は先天性両聾、前頭部毛髪色素脱失、虹彩異色、Waardenburg症候群疑い。弟は平成22年2月5日出生、現在精査中。他の親類に難聴者はいない。

（倫理面への配慮）
被験者の遺伝学的検査および遺伝カウンセリングに関する情報は遺伝子診療部の独自診療録にて保存され、耳鼻咽喉科の診療録には一般所見のみ記載され，プライバシー保護には配慮されている。難聴遺伝子診断に関する研究は2006年5月に当大学医学研究科倫理委員会の承認を受け、その内容を遵守して行われている。また、被験者には学会ならびに医学雑誌において個人情報を除く全ての所見、経過を発表することに書面にて同意を得た。

C. 研究結果

発端者である男児は早期の診断により補聴器装用と聾学校での適切な指導を受け、結果的には現在まで良好な発達が得られている。しかしながら、初診後も定期受診を促したものの母親が初診時の検査結果および遺伝性疾患疑いとの診断を十分に受け止めることができず、診断書作成以外の目的での診察を拒否する状況が続いていた。その後、第二子を妊娠したことをきっかけに確定診断のための遺伝学的検査および遺伝情報の提示を希望し、遺伝子診療部にて遺伝カウンセリングを行った。その過程で、当科初診時の検査結果の報告に大きなショックを受けていたことを伝えられた。母親は容貌の酷似などについては十分な認識をしていたが、全てが共通の遺伝子異常による疾患であり、難聴との関連があることを理解していなかった。そのため、自責の念に悩むこととなった。今回のカウンセリングにより遺伝情報を正確に伝えることが出来、母親の受け止めは良好であった。また、第２子出生後はあらためて家族全員の遺伝学的検査を希望されたため、今後検査を予定している。

D. 考察

本検討においては医師側の認識と家族側の認識の差に大きな問題があったものと考える。初回の長男受診時に十分なインフォームドコンセントを行えなかったが、その要因として平成14年当時は遺伝子カウンセリングの概念が浸透していなかったこと、および症候群性難聴のためすでに他科（産婦人科、小児科など）で十分な説明がなされていると解釈してしまったことがあげられる。
症候群性遺伝疾患の場合、症状が複数科にまたがるためそれぞれの担当科に該当する症状のみについて説明する傾向がある。そのため、当事者である患者側が疾患の全体像を把握できない状況が起こりやすい。対策として、各科で十分な連携を取ることを基本にして理想的には各科にまたがる遺伝

子診療部のような専門部署において遺伝カウンセリングが行われるべきと考える。

E. 結論

今後、遺伝学的検査の普及により優性遺伝性難聴または症候群性難聴患者が耳鼻科を受診する機会が増加することが予想される。これからは耳鼻科医として専門とする難聴に関する情報を提供するのはもちろんのこと、疾患の概要を説明するために遺伝性難聴に関する十分な知識を得る必要がある。また、施設においても遺伝カウンセリングを行う専門部署を設け、耳鼻科医も積極的に参加する必要があると考える。

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F. 研究発表

なし

G. 知的財産権の出願・登録状況

なし

優性遺伝の可能性が疑われた両側感音難聴例

研究協力者：内藤　泰（神戸市立医療センター中央市民病院）

症例報告

症例は36歳の女性で、小学生のころに学校の聴力検査で難聴を指摘され、耳鼻科で時々聴力検査を受けた。その後も近医で経過観察されていたが、少しずつ聴力が悪化したとのことである。17歳時に当科受診の記録がある。出産後に、さらに両側の聴力が悪化したとの事で、当科を受診した。現在補聴器を両耳に装用している。両側に多様な耳鳴があり、時に頭位変換でめまい感を自覚するとの事である。発音に歪はない。父親に同様の難聴があり、父親の親族にも難聴者がいる。兄弟姉妹は4名であるが、本人以外には難聴者はいない。

本患者の17歳時の聴力（3分法平均聴力）は、右側が33.7ｄＢ、左側が35.0ｄＢであった。一方、36歳時の平均聴力は右側が91.7ｄＢ、左側が83.3ｄＢであり、語音弁別能は右50%(100ｄＢ)、左75%(90ｄＢ)であった。前回受診時と今回の聴力検査結果から計算すると、1年当たりの聴力低下は、右側が3.1ｄＢ、左側が2.5ｄＢであった。

以上、優性遺伝が疑われ、長期の聴力経過が評価できた感音難聴例を報告した。

Ⅲ.　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Usami S, Miyagawa M, Suzuki N, Moteki H Nishio S Takumi Y Iwasaki S.	Genetic background of candidates for EAS (electric acoustic stimulation).	Audiological Medicine.		in press	2010
Lu SY, Nishio S, Tsukada K, Oguchi T, Kobayashi K, Abe S, Usami S.	Factors that affect hearing level in individuals with the mitochondrial 1555A>G mutation.	Clinical Genet.	75(5)	480-484	2009
宇佐美真一	難聴とウイルス感染	MB ENT	99	8-16	2009
宇佐美真一	先天性難聴	小児科	50	1182-1185	2009
武市紀人、柏村正明、中丸裕爾、津府久崇、福田諭、鈴木美華、宇佐美真一	難聴遺伝子診断が有用であった人工内耳一症例	Audiology Japan	52	214-219	2009
宇佐美真一	薬剤と遺伝子	耳鼻咽喉科・頭頸部外科	81	759-767	2009

Ⅳ. 研究成果の刊行物・別刷

Audiological Medicine, 2010; 00: 1–5

informa
healthcare

Genetic background of candidates for EAS (electric-acoustic stimulation)

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Abstract

There is a certain number of patients with so-called ski-slope hearing loss, in which there is good hearing for lower frequencies in spite of little/no hearing in high frequencies. EAS (electric-acoustic stimulation) has recently been introduced for such patients with residual hearing at lower frequencies. Ski-slope hearing loss can have either a progressive nature or can be rather stable; therefore, decisions regarding timing of surgery are sometimes hampered. One advantage of genetic testing is that the possible prognosis for hearing, i.e. progressive or not, can be predicted for individual patients. The present study was performed to estimate the frequency of ski-slope hearing loss and investigate the genetic background of candidates for EAS. Using a 2587 subject DNA database of sensorineural hearing loss patients, 1) frequency of patients with ski-slope hearing loss, 2) their clinical features including inheritance mode, onset ages, and progression, and 3) involvement of four common genes with mutations in Japanese hearing loss patients, were evaluated. One hundred and fifty-one out of 2587 subjects fulfilled the audiological criteria for EAS. The frequency of patients possibly meeting the criteria for EAS was estimated to be 9.1% by restriction to probands only (139/1520). Various inheritance modes and onset ages were noted, with earlier onset in the patients with sporadic/recessive inheritance mode. Progressiveness was recognized in 56% of the patients. Genetic analysis identified mutations in 26.6% of the patients, including the mitochondrial 1555A>G mutation, and mutations in SLC26A4, CDH23, and GJB2 genes, suggesting that at the least, these four genes may be involved in a certain group of patients, but also leaving possible genetic causes in the majority of the patients undetermined. As most of the patients showed a progressive nature in their hearing, genetic testing adds important additional information for candidates for EAS.

Key words: ski-slope hearing loss, high frequency hearing loss, partial deafness, cochlear implantation

Introduction

Cochlear implantation is currently the only available device for profound hearing loss patients and therefore has become a standard treatment choice worldwide. Although cochlear implantation has long been applied for patients with severe or profound hearing loss in all frequencies, recent advances in combined electric and acoustic stimulation (EAS) provide a chance of better speech perception for individuals with so-called ski-slope hearing loss. Selection criteria and decision making are sometimes difficult because of individual differences in progression, which is sometimes of a rather rapid progressive nature but other times rather stable. One advantage of genetic testing is that the possible prognosis for hearing, i.e. progressive or not, can be predicted for individual patients. Regarding genes responsible for hearing loss patients, to date, mutations in GJB2 and SLC26A4, and the 1555A>G mutation in the mitochondrial 12S rRNA were found to be the major causes of hearing loss in Japanese patients (1). To date, no study has treated ski-slope hearing loss from an etiological viewpoint. The present study was performed to estimate the frequency of ski-slope hearing loss, audiological characteristics, and genetic background of candidates for EAS.

Subjects and methods

A 2587 subject DNA database of bilateral sensorineural hearing loss established by Shinshu University in collaboration with 33 ENT departments (mostly university hospitals) in Japan was used in this study. The database comprises 1520 unrelated Japanese probands (who had made their initial visit to a hospital) and their family members, with various inheritance modes and ages of onset. The composition of the 1520 probands was as follows: 355 subjects from

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autosomal dominant or mitochondrial families (two or more generations affected); 282 subjects from autosomal recessive families (parents with normal hearing and two or more affected siblings); and 738 subjects with sporadic deafness (also compatible with recessive inheritance or non-genetic hearing loss). All subjects gave prior informed consent for participation in the project and the ethics committee of each hospital approved the study.

Audiological selection criteria were based on the pure tone audiogram selection criteria as follows. Pure tone hearing levels were required to be 65dB HL for 125 Hz, 250 Hz and 500 Hz; 80dB HL for 2000 Hz; 85dB HL for 4000 Hz and 8000 Hz. Subjects with one of the above mentioned frequencies being out of the criteria limits by 10dB were included as potential candidates.

Mutation screening for GJB2, SLC26A4, and the 1555A>G mutation in the mitochondrial 12S rRNA, was performed in all of the patients as follows. Direct sequencing was used for GJB2 (2), and restriction fragment length polymorphism (RFLP) was used for the 1555A>G mitochondrial mutation, as previously described (3). In patients with enlarged vestibular aqueduct, direct sequencing was used for SLC26A4 because mutations in this gene have been restricted to the patients with this particular anomaly (4,5).

For other minor responsible genes, frequencies are relatively small, and therefore one-by-one gene screening was performed in limited numbers of patients (64–319 patients depending on the gene (see reference (1)). For CDH23, 64 probands were analyzed using direct sequencing (6).

Results

One hundred and fifty-one (5.8%) out of the 2587 subjects registered in our database fulfilled the audiological criteria for EAS. The frequency of bilateral sensorineural hearing loss patients in the basic clinical population who may meet the criteria for EAS was estimated to be 9.1% by restriction to probands only (139/1520).

Regarding inheritance mode, 53% (74/139) of these patients had sporadic/recessive inheritance, 28% (39/139) dominant/mitochondrial inheritance, and in 19% (26/139) family history was unavailable (Table I).

Onset ages are shown in Table II. Onset ages were varied, and earlier onset ages were evident in the patients with sporadic/recessive inheritance mode.

Progressiveness was recognized in 56% (78/139) of the patients, regardless of inheritance mode (54% for sporadic/recessive inheritance, and 56% for dominant/mitochondrial) (Table III).

Table I. Inheritance mode of candidates for EAS (n=139).

Inheritance mode	Number (%)
Sporadic/recessive	74 (53%)
Dominant/mitochondrial	39 (28%)
Data unavailable	26 (19%)

Genetic analysis identified mutations in approximately 27% of the 145 patients, including the mitochondrial 1555A>G mutation (n=18, 12.9%), SLC26A4 (n=10, 7.2%), CDH23 (n=6, 4.3%) and GJB2 mutations (n=3, 2.2%) (Table IV). Among the 2587 subjects, 178 were associated with the 1555>G mitochondrial mutation, 153 subjects harbored biallelic GJB2 mutations, 61 subjects biallelic SLC26A4 mutations, and eight biallelic CDH23 mutations. Overlapped audiograms as well as average audiograms are shown in Figure 1A–D. Candidates rates (number of candidates/total patients with mutations) were high among the patients with the 1555A>G mitochondrial mutation (10.1%, 18/178), SLC26A4 (16.4%, 10/61) and CDH23 mutations (75%, 6/8) and low among the patients with GJB2 mutations (2.0%, 3/153).

Discussion

There is a certain number of patients with residual hearing (sometimes normal or slightly elevated thresholds) at the lower frequencies, and profound deafness at the higher frequencies (the so-called ski-slope type hearing loss or partial deafness). Most of these patients do not show any abnormal pronunciation of consonants, indicating that they likely acquired progressive hearing loss at the higher frequencies. In spite of being hard of hearing due to the high-frequency involved hearing loss, they usually do not use hearing aids or use only standard hearing aids with limited efficiency. These cases also do not meet criteria for traditional cochlear implant.

Recent advances in surgical technique, and electrode design, and newly developed devices enable preservation of residual hearing (see reference 7, for review). The concept of EAS has expanded indications for cochlear implantation from profoundly deaf patients in all frequencies to patients with residual hearing at the lower frequencies. According to the present data based on a multicenter collaborative study, 9.1% of the patients who visited the academic referral center were estimated to fulfill the audiological criteria for EAS.

There has been no etiological study of ski-slope hearing loss, and although symmetrical audiograms strongly indicate the majority of cases are due to genetic causes, there have been few reports

Table II. Onset ages of the candidates for EAS (n=139).

Inheritance mode	Number (%)					
	-2 y.o	3-10	11-30	31-50	51-	Unknown
Sporadic/recessive	24 (32%)	12 (16%)	16 (22%)	7 (9%)	5 (7%)	10 (13%)
Dominant/mitochondrial	7 (18%)	12 (30%)	9 (23%)	6 (16%)	1 (2%)	4 (11%)

discussing the genetic background. According to Liu and Xu (1994) (8), non-syndromic hearing loss can be classified into several types on the basis of audiograms. In the autosomal dominant group there are three types of audiograms – sharply sloping, flat, and gently sloping; and two types in autosomal recessive – residual and sharply sloping. The present study is in agreement with their report where cases with a sharply sloping audiogram (which may correspond with ski-slope type) are either autosomal dominantly or autosomal recessively inherited. Dominant high-frequency sensorineural hearing loss can be classified into four types – steepest, less steep, gently sloping, and horizontal (9). Together with similarity of audiograms within the same family, Higashi hypothesized heterogeneity of dominant high-frequency sensorineural hearing loss, and actually the former two types may correspond with ski-slope hearing loss.

In the present study, to understand the etiology of ski-slope hearing loss, genetic as well as clinical feature analyses were performed in the patients who fulfilled the audiological criteria. With regard to inheritance mode of these patients, 53% had sporadic/recessive inheritance, and 28% dominant/mitochondrial inheritance (Table I), indicating that various genes are involved in this category of hearing loss.

A high rate of patients with progressiveness was noted (56%) compared to overall (48%), and progressive nature was observed regardless of inheritance mode, indicating that progressiveness is one of the characteristic features of ski-slope hearing loss.

Onset ages were of great variation, also suggesting there are many responsible genes for this category of hearing loss. Earlier onset ages were noted in the patients with sporadic/recessive inheritance mode.

Ski-slope hearing loss may occur at various ages, and can have either a progressive nature or be rather stable; therefore, decisions regarding timing of surgery are sometimes hampered. There may be a great inter-individual variation regarding progressiveness, indicating that many different etiological differences may interact. Screening for commonly found responsible genes, proved at least four genes, including mitochondrial 12SrRNA, *SLC26A4*, *CDH23*, and *GJB2* are involved in this type of hearing loss, although candidate rates were different among the genes.

The 1555A>G mitochondrial mutation, which is known to result in high susceptibility to aminoglycoside antibiotics, has been identified as the most prevalent mitochondrial mutation (10). Hearing loss is usually high-frequency involved and progressive (3). Therefore, the present higher candidacy rate (10.1%) among the patients with this mutation, together with overlapped audiograms as well as average audiograms (Figure 1A), is consistent with the previously reported phenotype and there is a certain number of candidates for EAS in patients with this mutation.

The *SLC26A4* gene was initially identified as the gene responsible for Pendred syndrome, and is known to be involved in transportation of the chloride ion (11). The phenotype is known to range from Pendred syndrome to non-syndromic hearing loss associated with EVA (enlarged vestibular aqueduct) (12). Hearing is congenital/progressive, and usually high-frequency involved hearing loss (13). Patients acquire language but sometimes have incomplete pronunciation of consonants, indicating they may already have hearing loss at higher frequencies at the earlier (peri-lingual) ages. Overlapping audiogram (Figure 1B) suggested that some patients with this mutation are good candidates for EAS, but generally the slope is rather gentle. However, from the recent concept of preserving residual hearing it is still worth trying EAS for such patients with some (but not much) residual hearing at the lower frequencies.

CDH23 is known as the responsible gene for USH1D and DFNB12.

Table III. Progressiveness in the candidates for EAS (n=139).

Inheritance mode	Number (%)		
	Progressive	Non-progressive	Unknown
Overall	78 (56%)	44 (32%)	17 (12%)
Sporadic/recessive (n=74)	40 (54%)	24 (32%)	10 (14%)
Dominant/mitochondrial (n=39)	22 (56%)	10 (26%)	7 (18%)

Table IV. Responsible genes in the candidates for EAS (n=139).

Genes identified	Number (%)
Mitochondrial 1555A>G	18 (12.9%)
SLC26A4	10 (7.2%)
CDH23	6 (4.3%)
GJB2	3 (2.2%)

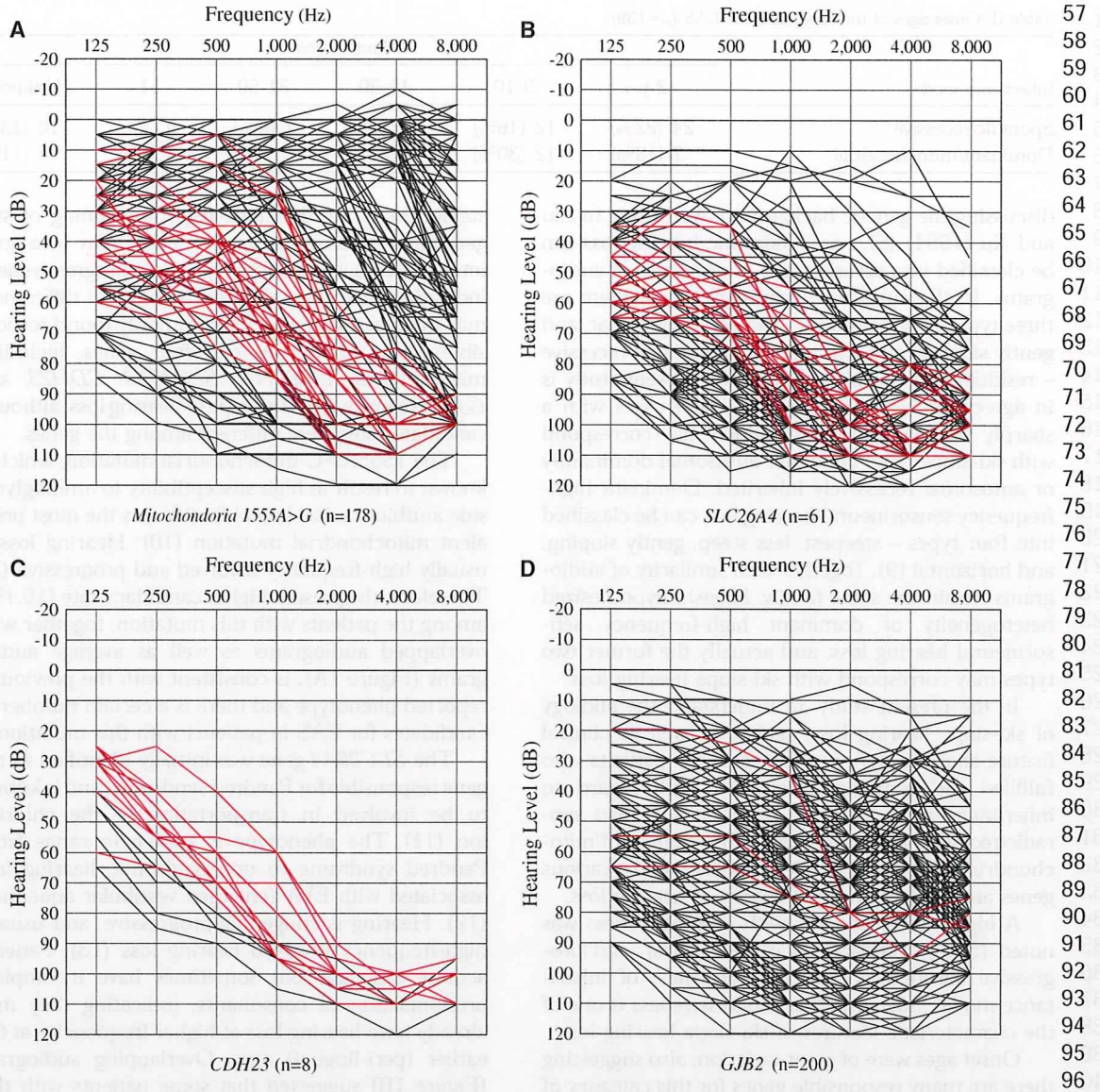

Figure 1. Overlapping audiogram of the patients with mutations. Candidates for EAS are indicated with red lines (A, mitochondrial 1555A>G; B, SLC26A4; C, CDH23; D, GJB2).

Encoded protein cadherin 23 is important for maintaining tip links (14). Patients with this mutation have high-frequency involved progressive hearing loss (6), suggesting that there is a significant number of EAS candidates. Although only a limited number of patients (n=64) with CDH23 mutations were analyzed in this study, overlapping audiograms also indicated that they are good candidates for EAS (Figure 1C).

GJB2 is known to be the most prevalent gene responsible for congenital hearing loss worldwide (see reference 15, for review). Encoded protein, Connexin 26, is known to participate in potassium ion recycling in the inner ear. Currently, more than 100 different GJB2 mutations are associated with recessive forms of non-syndromic hearing loss (see reference 15, for review). Overlapping audiograms of the 153 patients with bi-allelic GJB2 mutations showed rather flat or gently sloping audiograms (Figure 1D). As hearing loss is usually reported to be non-progressive, there may be only a small number of the patients with GJB2 mutations who are indicative for EAS. Only 2.0% of the patients with GJB2 mutations in this study fit the criteria for EAS.

The present study clearly revealed some responsible genes for ski-slope hearing loss, and genetic testing is potentially useful for estimating progressiveness and decision making for EAS in the future.

However, at the same time, in the majority of patients the cause is still unknown, and screening for various genes should be continued to understand the etiology of this type of hearing loss. In the literature, there have been many responsible genes described as having high-frequency involved hearing loss (16).

In the present study, progression is based on the anamnestic evaluation; therefore, actual speed of progression should be refined in future studies.

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Short Report

Factors that affect hearing level in individuals with the mitochondrial 1555A . G mutation

Lu SY, Nishio S, Tsukada K, Oguchi T, Kobayashi K, Abe S, Usami S. Factors that affect hearing level in individuals with the mitochondrial 1555A . G mutation.
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The mitochondrial 1555A . G mutation is one of the most common mutations responsible for hearing loss in Asians. Although the association with aminoglycoside exposure is well known, there is great variation in the severity of hearing loss. We analyzed hearing levels in 221 Japanese individuals with this mutation and attempted to identify relevant covariants including (i) age, (ii) aminoglycoside exposure, (iii) heteroplasmy ratio, and (iv) other gene mutations. At every age, average hearing levels were worse than those in normal subjects, suggesting that mitochondrial function itself may affect the severity of hearing loss. Although the hearing loss in individuals with the 1555A . G mutation progressed with age, the rate did not differ from that of the normal subjects. Those who had reported aminoglycoside exposure had moderate-to-severe hearing impairment regardless of age, confirming that such exposure is the most important environmental variable. We also confirmed the presence of heteroplasmy, which is known to modify the expression of other mitochondrial diseases, but found no evidence for a significant correlation with hearing impairment. A high prevalence of GJB2 heterozygous mutations was noted, indicating that these mutations may exhibit epistatic interaction with the 1555A . G mutation.

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The 1555A . G mutation in the mitochondrial 12S rRNA gene (1) is the commonest mitochondrial mutation associated with hearing loss. Generally associated with aminoglycoside exposure (2, 3), there are also well-documented patients without a history of exposure (4–6). Systematic screening of Japanese hearing loss patients revealed that approximately 3–5% of these subjects had the 1555A . G mitochondrial mutation, and in those patients who had reported aminoglycoside exposure, the mutation was found in 33% (1, 7). This mutation has been found not only in patients with late-onset hearing loss but also in those with congenital/early-onset sensorineural hearing loss (8). The mitochondrial 1555A . G mutation has been considered to be transmitted in the homoplasmic state, but there have been recent reports of patients with heteroplasmy (8, 9). In an effort to prevent severe deafness, we distribute a drug use warning card advising avoidance of aminoglycosides to 1555A . G mutation family members who are not yet affected (10). The hearing impairment associated with aminoglycoside exposure is usually a bilateral, progressive, high-frequency sensorineural loss. Although it is clear that the patients who report a history of aminoglycoside exposure have a more severe hearing impairment, the severity of deafness is variable (4, 6), suggesting the contribution of additional factors. Age-related expression/progression of hearing loss is one possible factor (4, 5). The existence of modifier genes has also been postulated (11–14), although no candidate genes have been identified. Finally, it was also recently reported that heteroplasmy ratios of the mitochondrial 1555A . G mutation appear to be associated with phenotype variability (9). In order to clarify the possible involvement of these factors in the severity of hearing loss, we investigated the effect of (i) age, (ii) aminoglycoside exposure, (iii) heteroplasmy ratio, and (iv) other gene mutations in 221 individuals with the 1555A . G mutation.

Materials and methods

Subjects

The subjects in this study were 221 Japanese individuals from 67 families with the 1555A . G mutation, ranging in age from 2 months to 87 years. The number of affected members in individual families ranged from 1 to 24 with an approximate average of 3.3. The control group used to determine GJB2 allele frequency was composed of 252 independent Japanese subjects with normal hearing.

Methods

Audiological analysis

Hearing level was classified using a pure-tone average over 500, 1000, 2000, and 4000 Hz in the better hearing ear. The hearing tests were performed at ages 4–87 years.

Mutation analysis

We screened for the 1555A . G mitochondrial DNA (mtDNA) mutation as described previously (4). In brief, total DNA including genome DNA and mtDNA was extracted from the blood, and the mitochondrial nucleotides 1252 through 1726 were amplified by polymerase chain reaction (PCR). To identify the Alw26I site, digestion was performed with a restriction enzyme (Alw26I). An ABI sequencer 3100XL (Perkin Elmer Co., Ltd, Waltham, MA) was used to confirm the 1555A . G mutation by direct sequencing.

To identify GJB2 mutations, a DNA fragment containing the entire coding region was amplified using the primer pair Cx48U/Cx1040L (15). PCR products were sequenced and analyzed with an ABI sequencer 3100XL (Perkin Elmer Co., Ltd). [See Abe et al. (15) for details of the sequencing analysis methods.]

Heteroplasmy ratio of the 1555A . G mitochondrial mutation

The Hitachi FMBIO II image scanning machine (Hitachi Co., Ltd, Minatoku, Tokyo, Japan), a fluorescence imaging system, was used to quantify the heteroplasmy ratio by detection of fluorescently labeled and digested PCR products as described below. A 459 bp DNA fragment was amplified with Ex Taq DNA polymerase (Takara Bio Inc., Ohtsushi, Shiga, Japan) using 200 ng of DNA from the subject as a template. Primer sequences were as follows: upper primer, 5#- GCCTATATACC-GCCATCTTC -3#; lower primer, 5#- TCTGGT-AGTAAGGTGGAGTG -3#. The upper primer was fluorescently labeled at 5# with rhodamine. PCR conditions were 95•C for 6 min, followed by 27 cycles of 95•C for 30 s, 55•C for 30 s and 72•C for 50 s and 72•C for 7 min. The PCR products were digested with restriction endonuclease Alw26I (Fermentas; 2.5 units, 37•C for 8–16 h). The subsequent PCR products were digested at 37•C for 8–16 h with 2.5 units of Alw26I (Fermentas). Two fluorescent products, wild type (300 bp) and/or mutant (459 bp), were detected because the 1555A . G mutation destroys the restriction site for Alw26I. The fluorescent intensity of the mutant bands in quantification experiments from two independent PCR amplifications was used to estimate the proportion of mutant copies in heteroplasmic subjects. We subcloned the insert including the 1555 position into the pDrive cloning vector using a QIAGEN PCR cloning kit (10) (QIAGEN, Hilden, Germany) as an appropriate standard of mutant heteroplasmy. The standard mixtures containing different amounts of wild-type and mutant synthesized oligonucleotides were used with analytical runs to quantify heteroplasmy of mtDNAs.

Statistical analyses

Student's t-test was used to compare average hearing levels of subjects with and without GJB2 mutations and with and without aminoglycoside exposure.

Results

The hearing loss of individuals with the 1555A . G mutation progressed with age; however, the rate of progression did not differ from that found in the normal population (Fig. 1a). The aminoglycoside exposure group had moderate-to-severe hearing impairment regardless of age (Fig. 1b). The existence of heteroplasmy was confirmed in 10 individuals from eight families; however, no apparent correlation was found between heteroplasmy ratio and hearing loss severity (Fig. 1c). There was a high prevalence of GJB2 heterozygous mutations in individuals bearing the 1555A . G mitochondrial mutation (Table 1), and their hearing levels tended to be worse (without GJB2 mutation, 35.4 dB; with GJB2 mutation, 42.0 dB), but the difference was not statistically significant (Fig. 1d). All the GJB2 mutations found were in heterozygous state, and no subjects were associated with biallelic mutations. There was no correlation between mutation genotype and hearing level.

Discussion

The average hearing level in people with the 1555A . G mutation was worse than that in normal populations at any age (Fig. 1a). This

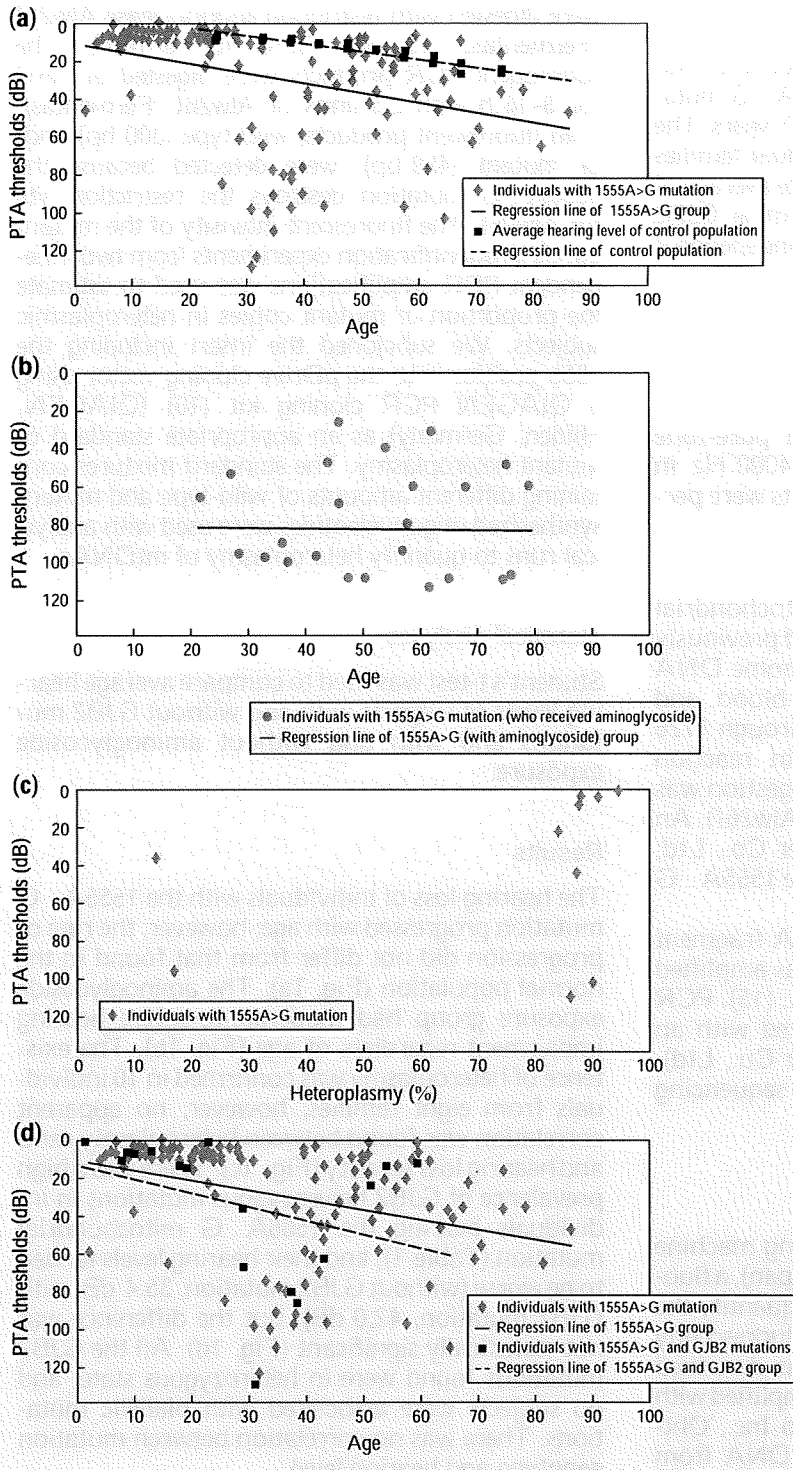

Fig. 1. Hearing levels and various parameters. (a) Correlation with age in the 1555A . G mutation group without reported aminoglycoside exposure compared with hearing levels in the normal Japanese population as described by Okamoto et al. (Pure-Tone Hearing Levels According to Age. Audiology Japan 1989: 32:82: 81–86, in Japanese). (b) Correlation with age in individuals with the 1555A . G mutation who reported aminoglycoside exposure. (c) Correlation with heteroplasmy in individuals with the 1555A . G mutation and no reported aminoglycoside exposure. (d) Comparison with age in individuals with the 1555A . G mutation with and without GJB2 mutations but with no reported aminoglycoside exposure.

suggests that the 1555A . G mitochondria mutation itself or a modifier gene may play a role in aggravating hearing loss. Hearing of the individuals with the 1555A . G mutation also worsened with age; however, the progression speed did not differ from that found in the normal population (Fig. 1a). Interestingly, most of the worst pure-tone audiometry thresholds were clustered in the age range of 30–50 years, indicating possible unreported aminoglycoside exposure as their childhoods coincided with the period in which aminoglycosides were most commonly used in

Table 1. Allele frequency of *GJB2* mutations in 1555A>G and control groups

GJB2 mutations (all hetero genotype)	Mitochondria 1555A>G (n = 26, 14 families)		Control (n = 252)	
	Family number[a]	Allele frequency (%)	Family number	Allele frequency (%)
V37I	2.85	2.13	3	0.60
G45E/Y136X	1.94	1.45	0	0.00
235 del C	1.45	1.08	2	0.40
176-191 del 16bp	0.5	0.37	0	0.00
299-300 del AT	0.19	0.14	0	0.00
Y136H	1	0.75	2	0.40
Total	7.93	5.92	7	1.40

[a]Family numbers in the 1555A>G group were calculated by the following formula: number of family members with the 1555A>G and *GJB2* mutations divided by the total number of family members.

clinical practice including for treatment of childhood infections (1960s to 1980s). Given the above, worsened hearing and mitochondrial function may be related to genetic background (the 1555A>G mitochondrial mutation itself or modifier genes), rather than environmental factors such as noise, because older persons would be expected to have had more exposure to various environmental events and therefore to have a steeper progressive curve.

One significant factor that determines the expression of mitochondrial disease is heteroplasmy. In this study, we confirmed that heteroplasmy existed in about 5% of the subjects with the 1555A>G mutation. The mitochondrial 1555A>G mutation had been thought to transmit only in a homoplasmic state, but recently, heteroplasmic cases have been found to exist and furthermore to be associated with severe hearing loss (9). Analysis of genotype–phenotype correlation indicated that subjects carrying less than 20% of mutant copies were asymptomatic or had a mild hearing loss (9). However, such correlation was not observed in our sample. It should be noted that it is difficult to determine the correlation of heteroplasmy levels with severity of hearing loss because the mutation load in blood may be different from that occurring in the inner ear.

The group that had reported aminoglycoside exposure had moderate-to-severe hearing impairment regardless of age, confirming that aminoglycoside exposure is the most important environmental factor affecting the phenotypic expression of the 1555A>G mitochondrial mutation.

A series of studies indicated that the nuclear background might be involved in modulating the phenotypic expression of the 1555A>G mitochondrial mutation (11). Genome-wide research has suggested that a region in chromosome 8p23.1 is a candidate region as a modifier gene for phenotypic expression (12). Efforts have been made by genotyping and linkage analysis to find nuclear genes that interact with the 1555A>G mutation to cause hearing loss, but no such single gene has yet been identified. Recently, mutations in TRMU were shown to modify the phenotype of the patients with the 1555A>G mutation (14). According to Guan et al., homozygous mutation in this gene leads to a marked failure in mitochondrial tRNA metabolisms, causing impaired mitochondrial protein synthesis.

We previously reported a high prevalence of *GJB2* heterozygous mutations in patients bearing the 1555A>G mitochondrial mutation and described a family in which potential interaction between *GJB2* and a mitochondrial gene appears to be the cause of hearing impairment (13). In that family, patients who are heterozygotes for the *GJB2* mutant allele showed hearing loss more severe than that seen in siblings lacking a mutant *GJB2* allele, suggesting that heterozygous *GJB2* mutations may synergistically cause hearing loss in the presence of a 1555A>G mutation. This indicates that *GJB2* mutations may sometimes be an aggravating factor in addition to aminoglycosides in the phenotypic expression in the non-syndromic hearing loss associated with the 1555A>G mitochondrial mutation (13). Our updated results in this study revealed that 5.92% of the alleles harbored the *GJB2* mutation, and this frequency is significantly (approximately) fourfold higher than that in the normal population, in line with our previous data. However, on average, in the patients without reported aminoglycoside exposure, the hearing loss severity in the 21 individuals with the *GJB2* mutation tended to be worse but not statistically significant when compared with the 165 individuals without the *GJB2* mutation.

Alternatively, it may merely be due to assortative mating having caused accelerated accumulation of various genes in one family (16).

Further study is needed to elucidate the interaction between the *GJB2* mutations and the 1555A>G mutation.

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