良好であったことを踏まえて、このガイドラインに沿って日本語を母国語とした蝸牛型耳硬化症患者に対する補聴器、アブミ骨手術、人工内耳埋め込み手術の治療方針を提案する（図4）。

その流れは以下のようである。現在の人工内耳の適応ガイドラインを満たし、補聴器装用下でCI-2004による両側の語音聴力検査（単音節、単語、日常会話文）が30%未満であれば不良聴耳側に人工内耳を勧める。語音聴力検査が30%～50%であり、CT所見で高度の脱灰像が認められれば、より蝸牛周囲の破壊や蝸牛回転の狭窄の少ない側に人工内耳を勧める。この理由は最近の細く短い残存聴力活用型人工内耳電極の保険承認、術中・術後のステロイド投与および正円窓膜からの挿入技術による聴力の保存術式の広がりにより、聴力を悪化させるリスクが減ったことを踏まえて、電極挿入の手技が困難になる前に埋め込み手術を終了しておくのがより良いと考えるからである。手術を希望しない場合は補聴器で経過観察とする。

脱灰像が高度でない場合は気導骨導差が30dB以上ある場合はアブミ骨手術を勧め、30dB未満であれば補聴器を勧める。補聴器装用下でCI-2004による語音聴力検査がそれぞれ50%以上で、気導骨導差が30dB以上あればアブミ骨手術を勧め、気導骨導差が30dB未満であれば補聴器で経過観察とする、というものである。

結　論

進行した蝸牛型耳硬化症、あるいはvan der Hoeve症候群が原因で高度の難聴となり、人工内耳埋め込み術を施行した12症例の治療成績を報告した。12症例のCI-2004の文の平均正答率（91%）は、他の原因で人工内耳手術を施行した症例（78%）にくらべ良好であった。顔面痙攣の合併症は12症例中1例のみであった。以上より、両疾患で失聴した患者への人工内耳治療は有効であると思われる。この成績を踏まえ、さらにCTによる画像診断の分類、気導骨導差も加えて、補聴器、アブミ骨手術、人工内耳手術の治療選択の流れを提案した。今後、わが国でも多施設からの日本語音声による評価成績を集めて、高度難聴を呈する両疾患に対する治療ガイドラインを作成することが望まれる。

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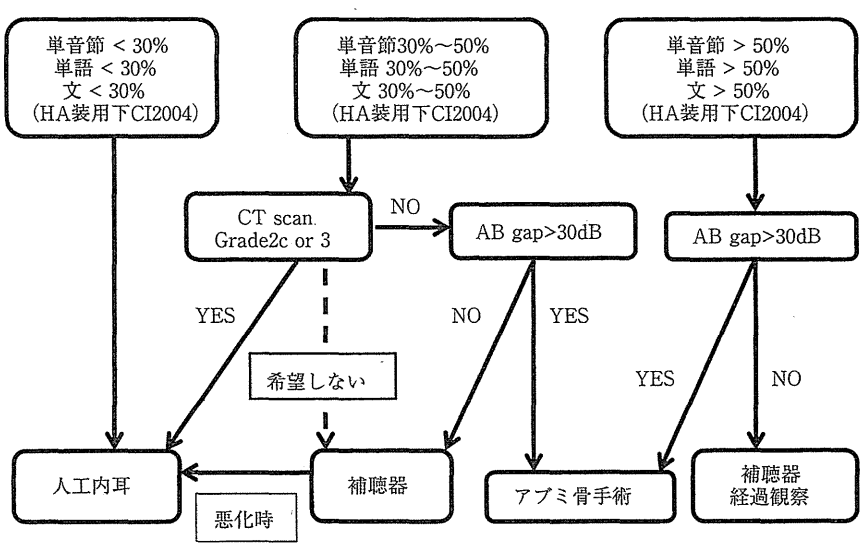

図4　当科における進行した蝸牛型耳硬化症に対する治療方針の流れ

補聴器装用下におけるCI-2004による語音聴取成績、側頭骨CTにおける蝸牛周囲の脱灰程度、気導骨導差、の3つの因子で治療方針を決定する。

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原　著

GJB2 変異例における進行性難聴の特徴と遺伝子型の検討

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【目的と方法】

先天性難聴の原因として最も頻度が高いのは *GJB2* 遺伝子変異であり，一般的に非進行性難聴を呈するとされる。今回我々は *GJB2* 変異97例について遺伝子型と難聴の進行の有無について検討した。

【結果】

遺伝子型は従来アジア人に多いとされている235 delC が最も多く，欧米人に多い35 delG は認められなかった。当初からの重度難聴例を除いた41例のうち，1年以上の間隔で聴力が2回以上測定されている症例は32例であった。明らかな難聴の進行例は1例，進行疑い例は3例であったが，遺伝子型の特定の傾向は認められなかった。

【結論】

GJB2 変異においては難聴の進行は稀であり，進行性難聴を呈する特定の遺伝子型は指摘できなかった。しかし乳幼児では特に難聴の程度が言語発達に大きく影響を与えるため，*GJB2* 遺伝子変異例であっても稀に難聴が進行するということをふまえて注意深く難聴の経過を追う必要がある。

キーワード：*GJB2* 変異，コネキシン26，進行性難聴，遺伝子型，先天性難聴

はじめに

先天性難聴は出生約1000人に1人の割合で発生すると言われており，原因のうち遺伝子要因が占める割合は，少ない報告で40%[1]，多い報告では50%以上[2,3]と言われる。遺伝性難聴

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のうち約70％が非症候性難聴であり，そのうち約80％は常染色体劣性遺伝形式である。これらの約半数を占めており，遺伝性難聴で最多の原因となっているものが *GJB2* 変異である[2]。

GJB2 遺伝子は，コネキシン26をコードする遺伝子である。蝸牛においてコネキシン26はその他のコネキシンタンパクと共にギャップジャンクションチャンネルを形成しており，細胞間のカリウムイオンの誘導的な通路となることで蝸牛内電位を維持しており，有毛細胞の興奮と機能に不可欠である[4]。そのため，*GJB2* 遺伝子に変異が生じると，生成されるタンパクの変性の度合によって軽度～高度難聴を呈する。現在世界で100種類以上の *GJB2* 変異が報告されており，遺伝子型によって難聴の程度が決まると言われる[3]。

GJB2 変異例では一般的には出生時から難聴が存在し，非進行性であると考えられているが，文献的には難聴が進行する症例の報告も散見される。特に乳幼児期における聴力の程度は，言語獲得に大きな影響を与えうるため，難聴の進行の有無は非常に重要である。今回我々の施設で経験した *GJB2* 変異例について，遺伝子型による難聴の程度，難聴の進行の有無について検討したので報告する。

方法と対象

(1)　対象

虎の門病院耳鼻咽喉科（2009年～2012年），埼玉県立小児医療センター耳鼻咽喉科（2003年～2012年）を受診した難聴患者において，*GJB2* 変異の有無について遺伝子解析を行い，*GJB2* 変異が認められた97例（女性57例，男性40例，成人例９例（21歳～77歳，平均38.3歳），小児例88例（０歳～13歳，平均5.9歳））について後方視的に遺伝子型と難聴の程度，難聴の進行の有無について検討した。本研究は，虎の門病院では同ヒトゲノム倫理委員会において2009年３月31日に承認され，先進医療として行われた。埼玉県立小児医療センターでは臨床研究としての臨床的検査として2003年７月29日付で倫理委員会で承認を受けた。

(2)　遺伝解析

GJB2 変異の解析は虎の門病院では，連結可能匿名化を行い，患者の血液からDNAを抽出した。既知の難聴遺伝子変異として高頻度であることが分かっている13遺伝子46変異を用いたAbeら[5]が開発したInvader matrixを用いて図１のフローに従って解析した。埼玉県立小児医療センターの症例については *GJB2* 遺伝子について蛋白翻訳領域全長（681塩基）をPCRで増幅後にdirect sequenceを行った[6]。Connexin Deafness Homepageに記載のある *GJB2* 変異の複合ヘテロ，またはホモ接合体を認めた場合，*GJB2* 変異による難聴と判断した。

(3)　聴力評価

乳幼児においては聴性脳幹反応検査（ABR），聴性定常反応検査（ASSR），条件詮索反応聴力検査（COR）を測定し，結果から総合的に判断して左右の聴力レベルを判定した。小児と成人においては標準純音聴力検査または遊戯聴力検査を行い，４分法（(500 Hz＋1000 Hz×2＋2000Hz)/4）で聴力レベルを決定した。難聴の程度は21–40 dBHLを軽度難聴，41–55 dBHLを中等度難聴，56–70 dBHLを中等度高度難聴，71–90 dBHLを高度難聴，91 dBHL以上を重度難聴として分類した。左右の聴力で差がある場合は良聴耳の聴力で分類した。

(4)　進行性難聴の評価

１年以上の間をあけて２回以上の聴力検査を施行されている症例については難聴の進行の有無について検討した。両側にて４分法で１dB/年以上の進行がある場合を進行例と判断した。

結　　果

(1)　遺伝子型と難聴の程度

GJB2 変異（複合ヘテロあるいはホモ接合体）を認めた97例のうち，軽度難聴例は１例，中等度難聴例は10例，中等度高度難聴例は14例，高度難聴例は12例，重度難聴例は56例，進行例は１例，進行疑い例は３例であった。それぞれの聴力における遺伝子型を表にまとめ

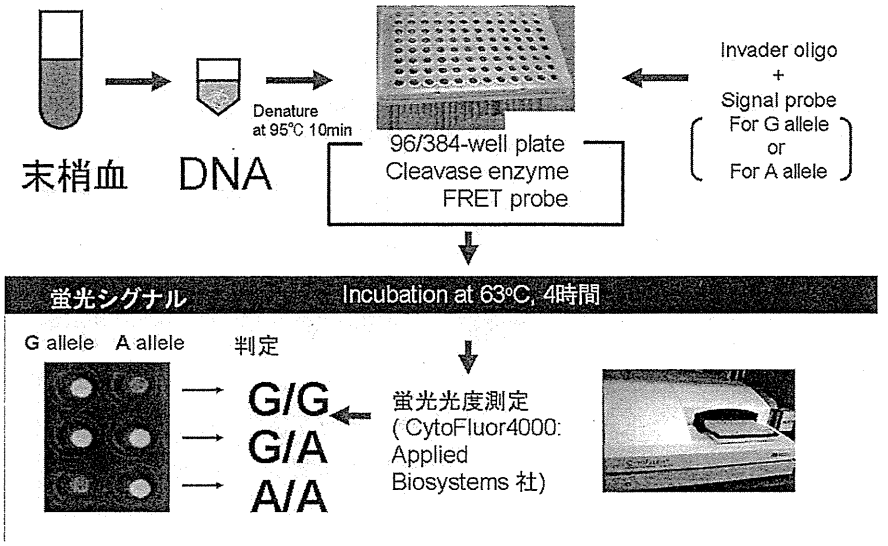

図1　Invader 法測定フロー

た。なお，進行・進行疑い例では最終聴力で分類した（表1，2）。

(2)　進行性難聴を呈した症例

当初から重度難聴であった56例を除いた41例のうち，32例で1年以上の期間をあけて2回以上の聴力検査が施行されていた。成人例が3例（31歳～70歳，平均50.0歳，追跡期間72～288カ月，平均176カ月），小児例が29例（1歳～13歳，平均6.3歳，追跡期間12カ月～116カ月，平均44カ月）であった。このうち明らかに難聴が進行したと考えられる症例は1例であり，3例においては，聴力の変化が比較的小さく「進行疑い例」とした（表3）。

以下に，進行例1例を提示する。

症例：70歳女性

既往歴：2型糖尿病

難聴の家族歴：父母，8人兄弟のうち本人以外に難聴者なし

現病歴：

幼少期の難聴歴は詳細不明であるが，37歳頃より耳鳴，めまいをはっきり自覚するようになった。徐々に難聴が進行し，45歳時より左補聴器装用を開始した。58歳時には右55 dB，左70 dB と言われていたが，その後66歳時に左耳失聴，70歳時に右耳失聴となったため，虎の門病院耳鼻咽喉科を紹介初診となった。遺伝子解析にて *GJB2* 変異（R143W，235delC の複合ヘテロ接合体）を認めた。ミトコンドリア3243変異も認められなかった。

聴力所見：右耳は6年間で62.5 dB，左耳は6年間で47.5 dB の難聴の進行を認めた（図2）。

70歳時に左人工内耳埋め込み術を施行され，経過良好である。

考　　察

1.　人種による *GJB2* 変異の遺伝子型

GJB2 変異はこれまでに100種類以上の遺伝子型が発見されており，人種によって遺伝子型の頻度が異なる。35 delG はアラブ民族・白色人種で最多[2,7,8]であり，167 delT はアシュケナージ系ユダヤ人に多い[2]。アジア民族では235 delC，V37Iが多いと言われている[2,9]。

Tsukada ら[9]は日本人における *GJB2* 変異について1511家系の3056名を対象に大規模調査を行っており，日本人においてはその他のアジア民族と同様に235 delG が最多であり，次いで V37I が多かったと報告している。また，Abe ら[10]による日本人の非症候性劣性遺伝性両側感音難聴25家系の調査によると *GJB2* 変異では235 delG のほか，Y136X，R143W が多く，白色人種に多い35 delG 変異例は認めなかった。

表1　各聴力における遺伝子型

難聴の程度	遺伝子型	例数(例)
軽度	V37I/F191L	1
中等度	235delC/235delC	3
	235delC/299–300delAT	2
	G45E/Y136X/235delC	1
	176–191del16/235delC	1
	T86R/T86R	1
	R143W/V37I	1
	G45E/G45E/Y136X/Y136X	1
中等度高度	235delC/235delC	5
	G45E/Y136X/235delC	3
	176–191del16/T86R	1
	235delC/T86R	1
	235delC/299–300delAT	1
	235delC/R143W	1
	299–300delAT/R143W	1
	G45E/Y136X/T86R	1
	G45E/Y136X/R143W	1
高度	235delC/235delC	5
	235delC/299–300delAT	3
	G45E/Y136X/235delC	2
	235delC/R143W	2
	176–191del16/235delC	1
	G45E/Y136X/299–300delAT	1
重度	G45E/Y136X/235delC	13
	235delC/235delC	11
	235delC/R143W	10
	235delC/T86R	5
	176–191del16/235delC	4
	G45E/Y136X/R143W	3
	235delC/299–300delAT	3
	R143W/605ins46	1
	V37I/R143W	1
	T86R/299–300delAT	1
	299–300delAT/R	1
	43W1235delC/V37I	1
	R143W/R143W	1
	G45E/Y136X/T86R	1
	G45E/299–300delAT/Y136X	1

表2　各遺伝子型の頻度

遺伝子型	検出数
235delC	102
G45E/Y136X	29
R143W	24
299_300delAT	14
T86R	12
176–191del(16)	7
V37I	4
605ins46	1
F191L	1

今回の検討においても235 delCが最も多く認められ，次いでG45E/Y136X，R143Wという順に多かった(表1)。Abeらの検討と同様，35 delGは皆無であった。

2.　*GJB2* 変異における難聴の程度と遺伝子型

GJB2 変異においては遺伝子型によって難聴の程度が決まるとされている。フレームシフトを起こす変異であるナンセンス変異，欠失変異，挿入変異，重複変異やスプライス部位の変異をtruncating mutation，アミノ酸置換やin-frame deletionなどの変異をnontruncating mutationと分類すると難聴の程度はtruncating mutationのホモ接合体，truncating/nontruncating mutationの複合ヘテロ，nontruncating mutationのホモ接合体の順に軽くなると報告されている[2,11]。また，遺伝子型でみるとM34T，V37I，L90Pは比較的軽度（平均25～40 dB）の難聴になると言われる[2,11,12]。欠失変異のホモ接合体は一般に高度難聴になるが，35 delG / R143W，35 delG / del（GJB6 – D13S1830）の2つの複合ヘテロに関しては35 delGホモ接合体よりさらに重度になると報告されている[2]。Tsukadaら[9]の報告においても235 delCを含む変異の中では235 delC/R143Wが最も重度の難聴を示す傾向を認めており，R143Wを含む変異は他の *GJB2* 遺伝子変異と比較して高度の難聴になる傾向があると考えら

表3　進行例・進行疑い例の遺伝子型と聴力変化

	進行例		進行疑い例					
	症例1		症例2		症例3		症例4	
年齢（歳）	70		12		49		31	
genotype	235delC deletion R143W missense 複合ヘテロ		235delC deletion R143W missense 複合ヘテロ		235delC deletion 235delC deletion ホモ		176–191del16 deletion T86R missense 複合ヘテロ	
患側	右	左	右	左	右	左	右	左
初診時聴力（dB）（4分法）	61.3	78.8	80.0	80.0	71.3	63.8	66.0	61.0
最終聴力（dB）（4分法）	123.8	126.3	110.0	83.8	75.0	75.0	83.8	68.8
初診時からの聴力変化（dB）	62.5	47.5	30.0	3.8	3.7	11.2	17.8	7.8
観察年数（年）	6	6	8.0	8.0	14	14	24.0	24.0
難聴進行開始年齢（推定）	37	37	4.0			33	4.0	
難聴進行速度（dB/年）	10.4	7.9	1.3	0.2	0.26	0.8	0.7	0.3

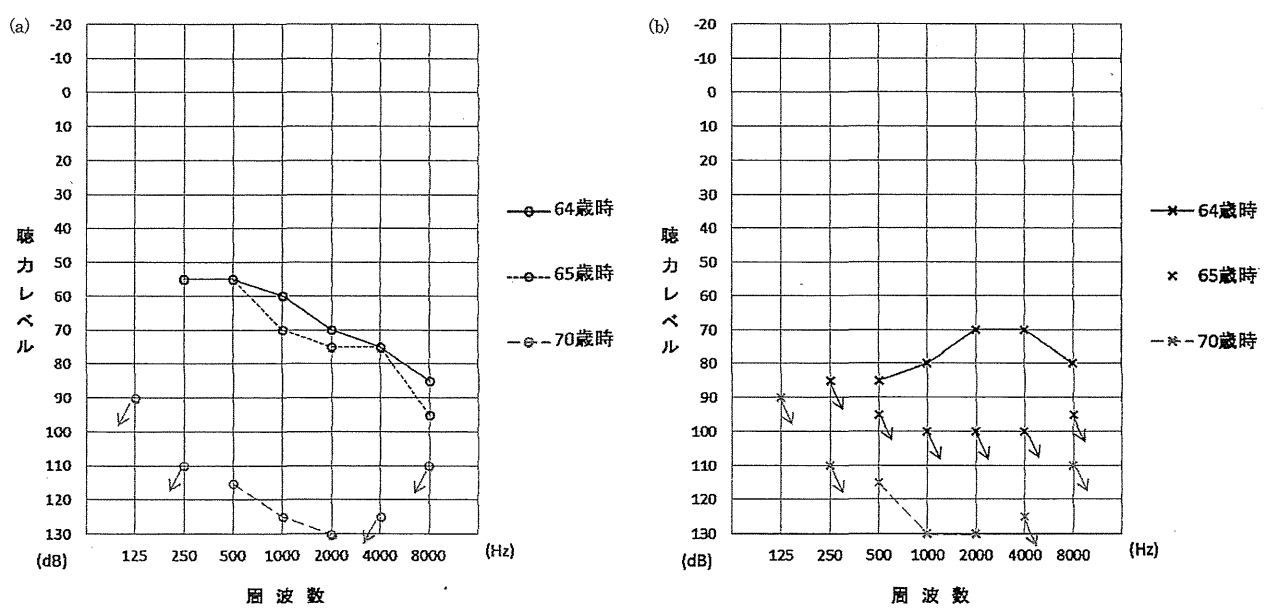

図2　症例1　聴力の推移(a)右聴力，(b)左聴力

れている。

本研究の結果でもR143Wを含まないnon-truncating mutationのみの複合ヘテロ例は2例のみであったが軽度，中等度難聴であり，難聴の程度は軽かった。重度難聴例59例のうち，57例ではtruncating mutationを含んでおり，これらを含んでいない2例はともにR143Wを含む変異であった。

3. *GJB2*変異において難聴の進行を示す割合と遺伝子型

今回認められた難聴の進行例，進行疑い例においては明らかな遺伝子型の傾向は認められず，難聴の進行開始年齢もさまざまであった。これまでの*GJB2*変異における進行性難聴についての報告（表4）からも，現在のところ進行性難聴を示す*GJB2*変異の遺伝子型は明確にはなっておらず，難聴の進行時期も乳幼児期から

表4

著　者	年	症例数	進行の基準	進行例	進行様式	進行例の追跡年数	進行開始年齢	進行例の遺伝子型
Denoyelle F, et al.[13]	1999	16	4周波数気導聴力平均で10年で10 dB以上	2	緩徐	10年以上	不明	不明
Janecke AR, et al.[12]	2002	15		3	2例：突発性難聴 1例：緩徐に進行	10年以上	1例は15歳以降に突発性難聴を反復 1例は13歳で突発性難聴を起こすも回復 1例は20年の経過で進行	突発性難聴例2例は35delG/L90P 緩徐例はL90P/314del14
Santos RL, et al.[8]	2005	5		0		7～22.7年（平均15.2年）		35delGホモ接合体
Marlin S, et al.[14]	2005	93	4周波数の気導聴力平均で10年以上離れた2回で15 dB以上，または5年以上離れた2回で8 dB以上	23	緩徐	5年以上	不明	nontruncating mutationが1つ以上含まれている症例においてはtruncating mutationのみの症例より進行しやすい
Pagarkar W, et al.[15]	2006	2		2	注1	1例：1年4カ月 1例：8カ月	1例2歳8カ月，1例6カ月以降	2例とも35delGホモ接合体
Orzan E, et al.[16]	2007	79	少なくとも4年で閾値が10–15 dB上昇	7		4年以上		
Gopalarbo D, et al.[17]	2008	7	3周波数平均で1.5 dB/年以上	3	緩徐	3～12年	進行例の初回の聴力検査は2歳6カ月，1歳10カ月，1歳4カ月	全例35delGホモ接合体
Kokotas H, et al.[18]	2008		1例：右29.3 dB 1例：右14.2 dB，左13.4 dB	2	1例：突発性難聴 1例：徐々に進行	1例：10年 1例：6年	1例：8歳で難聴発症，23歳で突発性難聴 1例：2歳で難聴発症	35delGホモ接合体
Kenna MA, et al.[11]	2010	84	2周波数以上で10 dB以上または1周波数以上で15 dB以上	47[注2]	46例は緩徐，1例は急速	平均13カ月		一定の傾向なし
Tsukada K, et al.[9]	2010	26		4[注3]		2年以上		

注1　1例：2歳8カ月時には左右ともaudiometric sweep testをパス．（20 dB）4歳2カ月で右86.3 dB，左100 dB
1例：6カ月時のABRパス（30 dB）14カ月時に90 dB no response
注2　47例中3例は難聴の進行に寄与しうる他疾患あり
注3　2例は片側の進行

成人後までさまざまであり，進行速度についても突発性難聴を呈するものから緩徐に年単位で進行するものまであると考えられている。これらの報告ではKennnaらの報告[11]を除いてはそれぞれの進行性難聴の症例数は少数であり，基本的には*GJB2*変異による難聴は非進行性であり，稀に進行する場合があると考えるのが妥当であろう。

しかし，*GJB2*遺伝子変異による難聴と診断しても，一部には進行性を呈するものがあることを前提に経過観察していくことが重要と思われる。

また，現在報告されている*GJB2*遺伝子変異例における難聴の進行については他の遺伝子や

環境因子，薬剤などが関与している可能性も否定はできない。今後は *GJB2* 変異以外の遺伝子変異についてもさらなる検索が進むことが望まれる。

結　論

GJB2 変異による難聴症例について遺伝子型，難聴の程度，進行の有無について検討した。遺伝子型は従来よりアジア人に多いと言われている235 delC が最も多く，G45E/Y136X, R143W がそれに続いていた。欧米人に多い35 delC は認めなかった。*GJB2* 変異においては従来言われているように通常は非進行性難聴を呈すると考えられるが，本論文で提示したように稀に難聴の進行を認める場合があり，特に難聴の程度が言語発達に大きく影響する乳幼児においては定期的に聴力検査を施行することは重要であろう。

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Characteristics and genotype of GJB2 mutation with progressive hearing loss

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GJB2 gene mutations are the most common cause of congenital hearing loss. These mutations generally cause non-progressive hearing loss. In the present study, we examined the relationship between genotypes and progressive hearing loss in 97 patients diagnosed with *GJB2* mutations. The genotype 235 delC, common among Asians, was the most frequent, while no 35 delG, common among Westerners, was observed. Of 41 patients, excluding those presenting with severe hearing loss from the beginning, hearing was measured twice or more at 1-year or longer intervals in 32. One apparent case and three suspected cases of advanced hearing loss were identified. No specific trend in genotypes was observed. Progressive hearing loss is rarely seen in patients with *GJB2* mutations. No specific genotype causing progressive hearing loss was identified. However, severe hearing loss significantly affects language development in infants. Hearing loss should be carefully followed up, given that it progresses in some cases with *GJB2* gene mutations.

Key words: *GJB2* mutation, connexin 26, progressive hearing loss, genotype, congenital hearing loss

161 両側小児人工内耳における臨床効果と予後良好な因子の検討

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【はじめに】

小児における両側人工内耳は海外ではすでに多くの報告がありその効果も広く伝わっている医療である。当施設では5年前に両側人工内耳を希望された9歳児のケースを皮切りに両側人工内耳の小児例が少しずつ増加、その効果を近くで見た一側人工内耳装用児やその保護者たちが更に希望するという現象が生じている。昨年我々は、両側人工内耳小児19例の語音聴取能について報告し、雑音下での聴取能および対側耳（Second CI）側からの聴取能に有意差を認めbinaural summation（加重効果）、head shadow effect（頭部陰影）、binaural squelch（両耳スケルチ機能）に極めて効果的に作用し、騒音環境下の多い小児での有効性を示した[1)]。今回は症例が増加した36症例において検討し、小児における両側人工内耳の効果、および良好となる因子について検討し報告する。

【対象】

1997年～2013年5月長崎大学医学部附属病院における人工内耳症例数は380症例でそのうち小児は258例（67.9%）。この中で両側人工内耳を行った小児は71症例（小児の27%）である。その中で、Second CI術後1年以上経過した小児が46名で、言語獲得し検査が可能であった36名について検討を行った。36名の初回人工内耳（first CI）の手術時期は1歳9ヶ月～15歳5ヶ月で、second CIの手術時期は2歳6ヶ月～15歳10ヶ月である。疾患の内訳としてはGJB2遺伝子変異：4名、MYO7A遺伝子変異：1名、Waardenburg症候群：1名、先天性サイトメガロウイルス感染症：6名、前庭水管拡大症：1名、mondini奇形：2名、家族性：5名、原因不明16名である。

【方法】

上記36名についてsecond CIの時期により、second CIが7歳未満の21名とsecond CIが7歳以上の15名に分けて検討した。また、first CI以後の対側耳の補聴器非装用期間が3年以上の8名と1年未満の28名に分けて検討した。聴覚医学的評価については語音明瞭度検査（SDS；67-S式を使用）、単語了解度検査（WRS；TY-89、3音節）、雑音下語音明瞭度検査（SDSおよびWRS）を施行した。SDSにおいては提示音圧を変えながら、また提示スピーカーを左右変えながら行った。検査室は無響室（等価騒音レベル：21.6dB（A）、残響時間：0.05s、RASTI値：0.96）を用いて行った。

【結果】

両側人工内耳での両耳聴が一側での聴取よりも全ての検査で成績が良かった。特に一側の

みの聴取よりも両耳聴において有意な差があった検査結果の主なものは、second CI側のスピーカーから1mの距離でのSDS、WRSそして雑音下SDS（S/N=80/70）、雑音下WRS（S/N=80/70）であった。これらはbinaural summation（加重効果）、head shadow effect（頭部陰影）、binaural squelch（両耳スケルチ機能）などの両耳聴効果を示していた。どの年齢においてもsecond CIは有効であったが、7歳未満で施行した群（n=21）と7歳以上で施行した群（n=15）を比較した場合、7歳未満で施行した群がsecond CIの聴取能：WRS（70dBSPL）、SDS（70dBSPL）、雑音下SDS（S/N=80/70）およびWRS（S/N=80/70）において有意な差がみられた（それぞれp=0.005、0.003、0.03、0.0003：t検定）。さらにfirst CIの手術時期の影響を鑑みる為にfirst CIを3歳未満に施行した症例に限定した場合においても結果は同様で、second CIの聴取能：WRS（70dBSPL）、SDS（70dBSPL）、雑音下SDS（S/N=80/70）およびWRS（S/N=80/70）において有意な差がみられた（それぞれp=0.002、0.007、0.04、0.0002：t検定）。また、first CI以後の対側耳の補聴器非装用期間に関する検討では、補聴器非装用が1年未満においてsecond CIの聴取能：WRS（70dBSPL）、SDS（70dBSPL）、雑音下SDS（S/N=80/70）およびWRS（S/N=80/70）が良く、雑音下WRS（S/N=80/70）において有意差がみられた（p=0.04）。second CI手術まで、できるだけ補聴器装用を続けた児の方が成績が良かった事をあらわしている。発表時間に余裕があれば、更に、良好となる因子がないかどうか、それぞれのデバイスの種類や術前術後の療育方針についても検討を加えて報告予定である。

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Long term speech perception after cochlear implant in pediatric patients with *GJB2* mutations

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ABSTRACT

Objectives: To determine the long term effect of cochlear implant (CI) in children with *GJB2*-related deafness in Japan.
Methods: Genetic testing was performed on 29 children with CI. The speech perception in 9 children with *GJB2* gene-related deafness fitted with CI was compared with those in matched 10 children who were diagnosed as having no genetic loci. The average follow-up period after CI was 55.9 months and 54.6 months, respectively.
Results: A definitive inherited hearing impairment could be confirmed in 12 (41.4%) of the 29 CI children, including 10 with *GJB2*-related hearing impairment and 2 with SLC26A4-related hearing impairment. The results of IT-MAIS, word or speech perception testing under the noise, and development of speech perception and production testing using the Enjoji scale were slightly better for the *GJB2* group after CI than for the control group without statistical significant difference.
Conclusion: The long-term results of this study show that CI is also effective in the development of speech performance after CI in Japanese children with *GJB2*-related hearing impairments as HL due to other etiologies.

1. Introduction

Recent progress in the research on hereditary hearing loss is remarkable. Since 1992, more than 125 genetic loci have been reported to be involved in nonsyndromic hearing loss (HL) [1], and over 67 of those loci are involved in autosomal recessive nonsyndromic HL [2]. Among these, the *GJB2* gene encoding the connexin (Cx) 26 protein (chromosomal 13q11-12) is the most common, of which about 100 different *GJB2* mutations have been reported globally [3]. It is reported to account for between 20 and 50% of all recessive nonsyndromic cases [4].

On the other hand, the benefits of cochlear implantation (CI) for spoken language, reading skills, and cognitive development have been clearly demonstrated [5,6]. Recently, the outcomes of CIs in patients with *GJB2* mutations have also been reported. Several studies have shown that patients with *GJB2* mutations (OMIM 121011) usually exhibit excellent speech perception and language performance after CI, when compared with those without identifiable *GJB2* mutations [7–11]. However, other studies have demonstrated that when the control group is appropriately matched with regard to age at implantation and length of post CI, there is no significant difference when comparing those with *GJB2*-related deafness to those without it [12–15]. Results analyzing post-CI speech performance in patients with *GJB2* mutations are still controversial.

In this study, in order to know whether the long term effect of CI is better in children with *GJB2*-related deafness or not, we have studied the speech perception outcome of CI in children with *GJB2* gene mutations, and compared them to those in matched children without inherited hearing loss.

2. Materials and methods

2.1. Subjects

We have performed CI in 301 cases in our clinic since 1997. Genetic testing was performed in 29 children with CI, and definitive *GJB2*- and *SLC26A4*-related hearing impairment was confirmed in 10 (34.5%) and 2 (6.9%) children with CI, respectively.

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Table 1
Clinical information of cases in the 2 groups.

Group	Control	GJB2	*P* value
Number of cases	10	9	
Sex (male:female)	3:7	2:7	
Age at CI (months)	36.7	37.4	0.5996
Post CI (months)	54.6	55.9	0.6736
Pre-CI education	Auditory-verbal/oral	Auditory-verbal/oral	

CI: cochlear implantation.

Finally, 19 children whose selection criteria were as follows were enrolled for this study.

1. Their age at CI was 6 years or less
2. Their guardian accepted gene mutation analysis
3. There was no any other apparent cause of deafness such as inner ear anomaly, central disorders/learning difficulties, or cytomegalovirus (CMV) infection

We divided them into two groups: the first, a control group consisting of 10 children who were diagnosed as having no genetic loci, while the second was the actual *GJB2* study group consisting of 9 children with *GJB2* gene-related deafness. Detail of their clinical information is shown in Table 1. HL was diagnosed at a different age in each child, but showed 90 dB or more severe HL before the age of 6 on auditory brainstem response (ABR) test. Preoperative imaging studies (CT and MR) showed no abnormal findings in any of the children in each group. None of the children showed any cognitive delay. The average age at CI in the two groups was 36.7 months (ranging from 21 to 67 months old; 3 male and 7 female) and 37.4 months (ranging from 22 to 63 months old; 2 male and 7 female), respectively. Thus, there is no significant difference between the two groups (Student's *t*-test, $t = -0.5339$, $P = 0.5996$). Their average follow-up period after CI was 54.6 months (ranging from 24 to 110 months) and 55.9 months (ranging from 47 to 62 months), respectively (Student's *t*-test, $t = -0.4278$, $P = 0.6736$). All the cases in this study had an intensive auditory-verbal education without visual information since childhood. Both the CI operation and the (re)habilitation after CI took place in the same clinic.

All patients were fitted with a CI system from either nucleus multichannel cochlear implant system (Cochlear Corporation, Englewood, CO, U.S.A.) or Combi40+ cochlear implant system (MED-EL, Innsbruck, Austria). All electrode arrays were inserted in all patients. There were no perioperative complications in any of the patients.

We examined the hearing level (both with CI and with hearing aids), the Infant-Toddler Meaningful Auditory Integration Scale (IT-MAIS), speech perception skills, and development of articulation in the two groups before and after CI several times in the postoperative period ranging from 6 months to 4 years. The best results from this period were used in evaluating the hearing level and the speech perception skills in the two groups. The speech perception skills were evaluated using CI 2004, SDS-67S, and Japanese CD SDS system (TY-89) tested at 70 dB SPL (sound pressure level) using an open-set questionnaire. We also examined the development of speech perception and production by using the Enjoji Scale of Infant Analytical Development (Enjoji Scale), which was developed in Japan and is now established as one of the standard developmental examinations for evaluating the development of children from birth to about the age of 6 [16]. In this examination, the development of a child can be assessed by checking his or her performance on the chart, in which standard developmental items at each month are described in the three fields including motor, social and language skills. The results allow us to clearly assess to what extent a child is successfully developing in each of the three fields and the six subdivided categories. These tests were conducted up to 2 years after CI.

2.2. Mutation detection

15 ml peripheral venous blood using standard procedure was sent to the Institute of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan for Genomic DNA extraction. All subjects underwent mutation screening for 47 common mutations of 10 hearing loss related genes in Japan by using invader assay [17,18].

Written informed consent was obtained from the guardians of all the subjects and the study was approved by the ethical committee of our institute (approval number: 07122106). The differences between in the two groups were analyzed statistically using the paired *t*-test and the unpaired Student's *t*-test. All the acceptance criterion for a significant addition to the explained variance was set at *P* values under 0.05.

3. Results

A definitive *GJB2*-related hearing impairment was confirmed in 9 (32.2%) of the 29 children with CI. Table 2 shows the details of detected *GJB2* gene-mutations. *GJB2 c.*235delC was observed in 3 cases, while six children each had one distinct mutation as listed in the table.

Fig. 1 shows the preoperative aided hearing thresholds. The preoperative hearing level was over 90 dB in all the cases, and the average level of preoperative aided hearing thresholds was nearly 60 dB in the two groups presenting no significant difference between the two groups.

Fig. 2 shows the postoperative hearing thresholds with CI. After CI, the hearing level improved to 25–30 dB in both groups, thus there was no significant difference between the groups.

Fig. 3 shows the results of the IT-MAIS for the two groups. Preoperative scores were worse in the *GJB2* group than in the control group, however, these improved from 1 year to 3 years after CI. The averaged IT-MAIS score in the GJB2 group was 9.8 ± 12.9 (range, 0–31) preoperatively. The averaged IT-MAIS score at 2 years after CI increased up to 33.6 ± 7.8 (range, 20–39), and this improvement was statistically significant (paired *t*-test, $P = 0.017$). The averaged IT-MAIS score in the control group at 2 years after CI

Table 2
Mutations with GJB2 gene in 9 cases.

Mutation	Number of cases
GJB2 c.[235delC];[235delC]	3
GJB2 c.[511insAACG];p.[T86R]	1
GJB2 c.[235delC];[299-300delAT]	1
GJB2 p.[G45E;Y136X];[R143W]	1
GJB2 c.[176-191del16];[299-300delAT]	1
GJB2 c.[235delC];p.[G45E;Y136X]	1
GJB2 c.[235delC];p.[R143W]	1

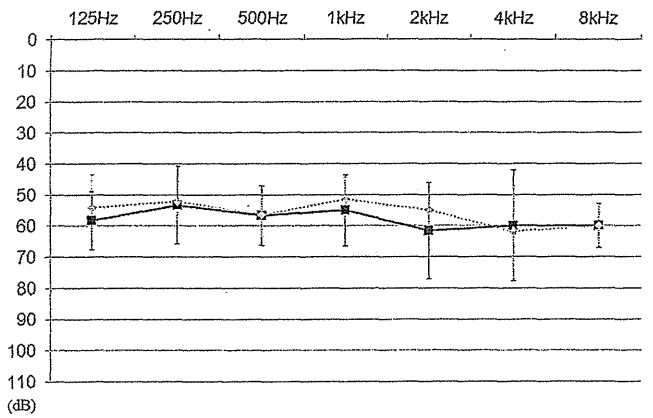

Fig. 1. Results of the average level of preoperative aided hearing thresholds at each frequency. Diamond dots and solid line: control group; square dots and solid line: *GJB2* group; bars: indicate two standard deviations.

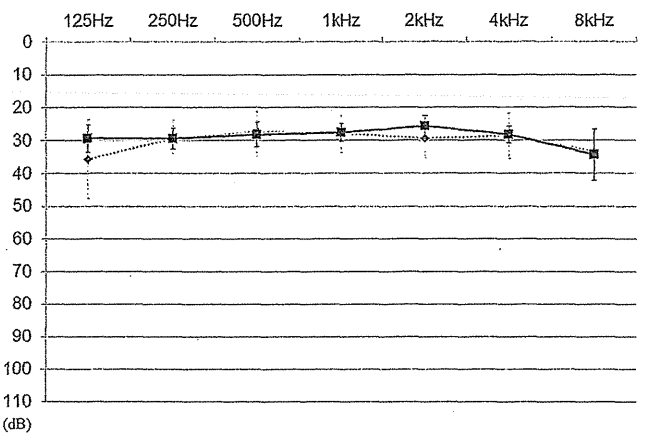

Fig. 2. Results of the average level of postoperative hearing thresholds with CI at each frequency. Diamond dots and solid line: control group; square dots and solid line: *GJB2* group; bars: indicate two standard deviations.

was 30.4 ± 7.6 (range, 19–38). There was no significant difference in the scores between the two groups at 4 years after CI.

Fig. 4 shows the results of speech perception skills in the two groups after CI. Longitudinal axis indicates the results (%) when tested at 70 dB SPL using CI 2004, SDS-67S, and Japanese CD SDS system (TY-89) in the two groups. There was no significant difference between the two groups, but the percentage of correct answers (%) examined under the noise tended to be better in the *GJB2* group.

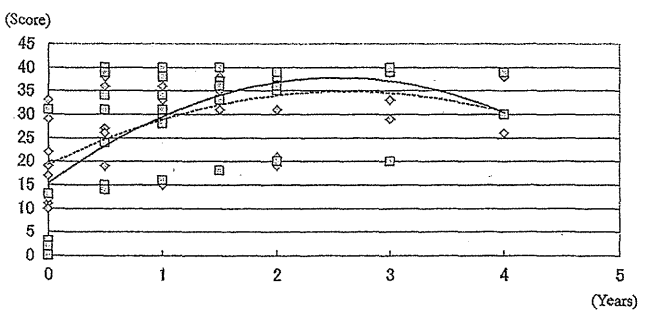

Fig. 3. Results of the difference of IT-MAIS scores from 0 years (=preoperative) to 4 years after CI. Diamond dots: scores in the control group; square dots: scores in the *GJB2* group; dotted line: trend line in the control group; solid line: trend line in the *GJB2* group.

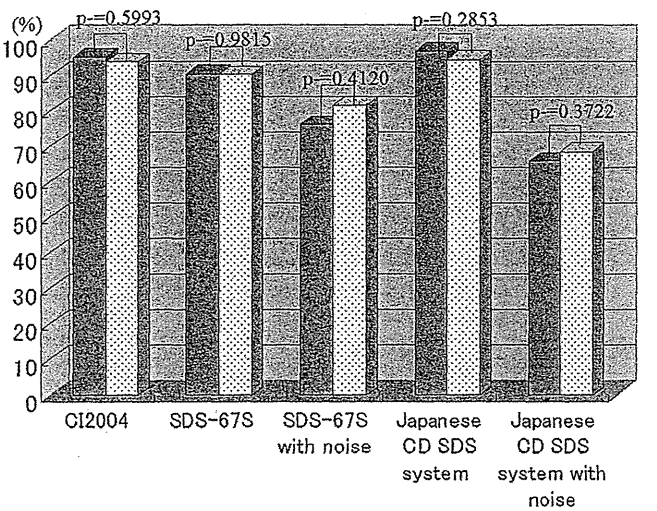

Fig. 4. Results of speech perception skills examined by using CI 2004, SDS-67S, and Japanese CD SDS system (TY-89). Longitudinal axis indicates the correct answer rate (%). Gray bars: control group; dotted bars: *GJB2* group.

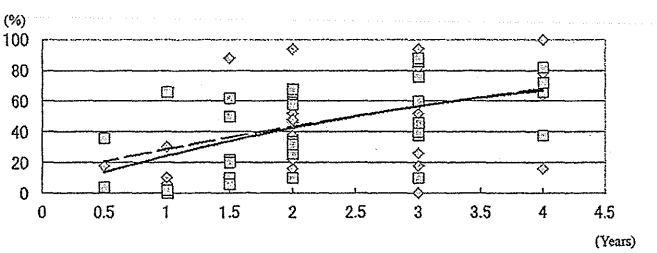

Fig. 5. Results of the development of articulation from 0.5 to 4 years after CI. Diamond dots: accuracy rates in the control group; square dots: accuracy rates in the *GJB2* group.

Fig. 5 shows the results of development of articulation in the two groups after CI. There was no significant difference in the scores between the two groups.

Fig. 6 shows the results in the development of speech perception (Fig. 6a) and production (Fig. 6b) in the two groups after CI. Values of month in the ordinate were calculated by subtracting the developmental months assessed by the Enjoji Scale from the actual age at each period, thus, smaller values indicate better development of speech perception and production. Postoperative language perception and production in the *GJB2* group tended to be slightly better, especially at one and half years after surgery, but there was no significant difference in these scores.

4. Discussion

The incidence of HL is approximately 0.1% among newborns, and hereditary HL is identified in at least 60% of patients with congenital HL, for whom the proportion of syndromic and non-syndromic is 30% and 70%, respectively [19]. The most common trait of nonsyndromic HL is autosomal recessive, which accounts for about 80% of cases [20], and *GJB2* is the gene most frequently associated with hereditary HL. The incidence of *GJB2* mutations in the Japanese population with HL is 14.2% overall and 25.2% in patients with congenital hearing loss [21], and 35 of the 119 cases (29.4%) with non-syndromic deafness [22]. In children with CI, 135 hearing-impaired patients (270 alleles) were tested, and *GJB2* mutations for the c.235delC were found in 39 alleles of 270 alleles (14%). Especially the homozygous of c.235delC was detected in 26

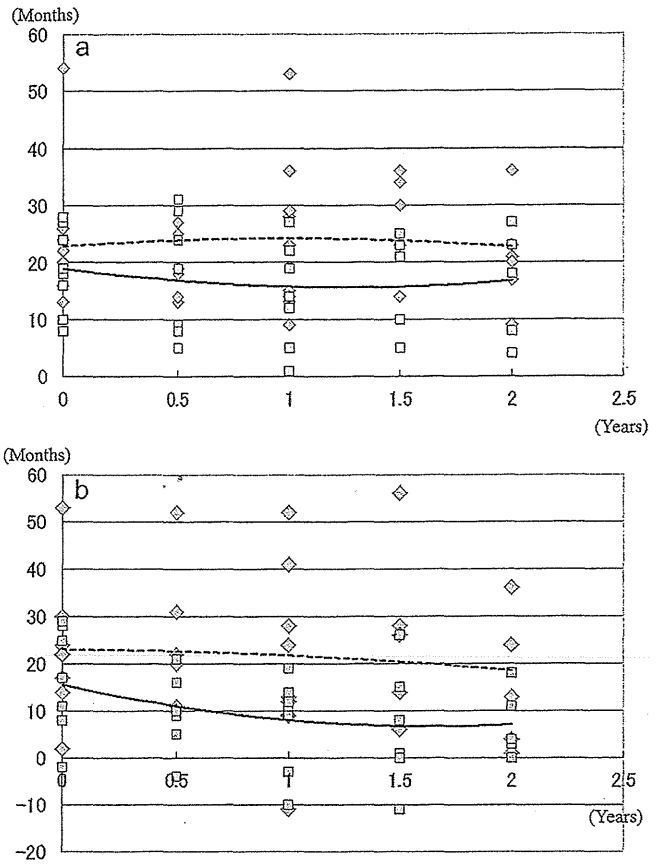

Fig. 6. Results of the developmental course of language perception (a) and production (b) in the control and *GJB2* groups examined by Enjoji Scale of Infant Analytical Development test. Diamond dots: scores in the control group; square dots: scores in the *GJB2* group; dotted line: trend line in the control group; solid line: trend line in the *GJB2* group.

alleles (9.6%), single heterozygous of c.235delC was detected in 1 allele (0.4%) and compound heterozygous of c.235delC was found in 12 alleles (4.4%) [23].

In this study, a definitive inherited hearing impairment could be confirmed in 11 (37.9%) of the 29 CI children, including 9 with *GJB2*-related hearing impairment, 2 with *SLC26A4*-related hearing impairment. These percentages are quite high and remind us of the importance of performing the mutation detection for CI patients.

The *GJB2* group underwent the IT-MAIS, word or speech perception testing under the noise, and development of speech perception and production testing using the Enjoji scale. The finally achieved performances in the two groups were not significantly different, but the averaged IT-MAIS score at 2 years after CI was significantly better in the GJB2 group than in the control group. This result may indicate that the necessary period to achieve the actual age development was shorter in the *GJB2* group than in the control group, and the difference may become smaller as they acquire language through CI in longer term. Matsushiro evaluated 4 CI children with GJB2 gene mutation and reported that the postoperative IT-MAIS score at 6 months was significantly higher in comparison with that of other prelingual CI patients [24]. In this study, children such as those having inner ear anomaly or cytomegalovirus infection, whose postoperative performance after CI is not necessarily good, were excluded from the control group. Considering that these children may also be candidates for CI in general, we can expect CI is efficient for Japanese children with *GJB2* gene mutation as well as for those reported previously [8,23,24].

GJB2 and *GJB6*, mapping to the DFNB1 locus and encoding the gap-junctions Cx 26 and 30, respectively [25]. Cx 26 and 30 are widely expressed in the cochlea at the level of the organ of Corti's supporting cells and connective tissues, and have an important role in forming homomeric or heteromeric hemichannels [26,27]. Mutations in Cx26 are presumed to result in altered potassium recirculation, leading to an accumulation of potassium in the cochlear endolymph and causing hair cell dysfunction and deafness [28]. In other words, mutations in the Cx26 protein mainly lead to the impairment of the endolymph potassium concentrations, which are required for auditory signal transduction, but may not lead to severe damage or decreasing the number of hair cells. It is generally assumed that the results of CI are poorer for inner ear malformation and in cases with neural and/or central damage than in cases with disorders within the inner ear causing the hair cells damage because the auditory pathway including the first neuron, spiral ganglion cells, may well be preserved in the latter. We speculate that the reason why the *GJB2* group had better results in this study is perhaps due to a comparatively good survival and preservation of electrical excitability of the cochlear spiral ganglion cells and the auditory nerve, which is important in the successful CI results [29].

There are some specific reports which support the present results and our speculations. In a rat model, Cx26 was shown to be expressed in nonsensory epithelial and connective tissue cells, but not in the inner or outer hair cells or cochlear nerve fibers [30]. Anatomically, Cx26 mutations result in a dysgenesis of the stria vascularis and hair cells in the organ of Corti, but with minimal neural degeneration and a normal population of spiral ganglion cells in both the apical and basal turns of the cochlea. [31] In the electrophysiological study, children with *GJB2*-related HL had greater similarities between low- and high-frequency residual hearing and between neural activity electrically evoked at apical and basal regions of the cochlea than children with non-*GJB2*-related HL [32]. These results may suggest more consistent spiral ganglion survival along the length of the cochlea in *GJB2*-related HL, which appears to involve a decreasing gradient of spiral ganglion survival from the apex to the base of the cochlea.

Most genotype-phenotype correlation studies have indicated that HL of the subjects with *GJB2* mutations shows a non-progressive pattern [33,34], however, some studies indicated a progressive pattern. [23,35,36]. Considering that early CI is well known to be one of the most important factors for the better postoperative performance for children with congenital HL, even in children with progressive hearing loss due to *GJB2* mutation, we might be able to prepare for early CI for those children if we were aware of it. The early screening of *GJB2* mutation for newborns with severe to profound HL might be advisable.

5. Conclusions

Despite the limits imposed by the small sample size, this study points to the importance of routine genetic assessments. The long-term results of this study also show that CI is also effective in the development of speech performance after CI in Japanese children with *GJB2*-related hearing impairments as HL due to other etiologies. If a child through genetic assessment is diagnosed as having a *GJB2*-related hearing impairment, CI can provide considerable benefits.

Conflict of interest

None.

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RESEARCH ARTICLE Open Access

Pathogenic substitution of IVS15 + 5G > A in *SLC26A4* in patients of Okinawa Islands with enlarged vestibular aqueduct syndrome or Pendred syndrome

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Abstract

Background: Pendred syndrome (PS) and nonsyndromic hearing loss associated with enlarged vestibular aqueduct (EVA) are caused by *SLC26A4* mutations. The Okinawa Islands are the southwestern-most islands of the Japanese archipelago. And ancestral differences have been reported between people from Okinawa Island and those from the main islands of Japan. To confirm the ethnic variation of the spectrum of *SLC26A4* mutations, we investigated the frequencies of *SLC26A4* mutations and clinical manifestations of patients with EVA or PS living in the Okinawa Islands.

Methods: We examined 22 patients,with EVA or PS from 21 unrelated families in Okinawa Islands. The patient's clinical history, findings of physical and otoscopic examinations, hearing test, and computed tomography (CT) scan of the temporal bones were recorded. To detect mutations, all 21 exons and the exon–intron junctions of *SLC26A4* were sequenced for all subjects. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for *SLC26A4* and calculations using the comparative CT ($2^{-\Delta\Delta CT}$) method were used to determine the pathogenicity associated with gene substitutions.

Results: *SLC26A4* mutations were identified in 21 of the 22 patients. We found a compound heterozygous mutation for IVS15 + 5G > A/H723R in nine patients (41%), a homozygous substitution of IVS15 + 5G > A in six patients (27%), and homozygous mutation for H723R in five patients (23%). The most prevalent types of *SLC26A4* alleles were IVS15 + 5G > A and H723R, which both accounted for 15/22 (68%) of the patients. There were no significant correlations between the types of *SLC26A4* mutation and clinical manifestations. Based on qRT-PCR results, expression of *SLC26A4* was not identified in patients with the homozygous substitution of IVS15 + 5G > A.

Conclusions: The substitution of IVS15 + 5G > A in *SLC26A4* was the most common mutation in uniquely found in patients with PS and EVA in Okinawa Islands. This suggested that the spectrum of *SLC26A4* mutation differed from main islands of Japan and other East Asian countries. The substitution of IVS15 + 5G > A leads to a loss of *SLC26A* expression and results in a phenotype of PS and EVA.

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Background

Profound hearing loss affects about 1 in 300 to 1 in 1000 newborns [1-4], and about one-half of these cases can be attributed to genetic factors [5]. About 51% of these cases are due to single nucleotide polymorphisms [5]. As to inheritance pattern among monogenic probands, about 1% is X-linked, 22% is autosomal dominant, and 77% is autosomal recessive [5]. Pendred syndrome (PS) is an autosomal recessive disorder characterized by congenital sensorineural hearing loss and goiter [6]. The causative gene for PS and EVA was identified to be *SLC26A4* [7,8]. Enlarged vestibular aqueduct (EVA) is a common inner ear malformation that can be diagnosed radiographically in patients with impaired hearing (Figure 1). EVA is frequently associated with PS [9-11]. In addition to PS, *SLC26A4* mutations also cause nonsyndromic hearing loss with EVA in the absence of a thyroid phenotype [12,13].

Previous studies revealed that the spectrum of *SLC26A4* mutations varied on the basis of ethnic background [14,15]. Tsukamoto et al. [15] demonstrated that *SLC26A4* mutations occurred in 90% of families with a history of PS and in 78% of families with a history of EVA in Japan. Among these *SLC26A4* mutations, H723R was suggested to have a founder effect in the Japanese population.

The Okinawa Islands are the southwestern-most islands of the Japanese archipelago (Figure 2). Previous studies suggested that there were substantial ancestral differences between Okinawa Islands the main islands of Japan [16]. In this study, we examined patients with EVA or PS from the Okinawa Islands to determine the frequencies and the genotypes of *SLC26A4* mutations and their clinical manifestations.

Methods

Subjects

From May 2008 to July 2012, 22 patients (8 males, 14 females; age range: 0–33 years; mean age: 5.8 years; median age: 8.5 years; Table 1) were diagnosed with PS or EVA in the Department of Otorhinolaryngology, Head and Neck Surgery of the University of the Ryukyus, Japan.

Prior to enrollment, all subjects provided a written informed consent. Our research protocol was approved by the Ethical Review Board of the University of the Ryukyus.

Clinical manifestations of PS and EVA

Clinical history of 22 patients with neuro-otologic symptoms was recorded. A physical examination, including otoscopy, hearing level test, computed tomography (CT) scan of the temporal bones, and examination for thyroid goiter was conducted.

Depending on a subject's ability, hearing level was determined using auditory brainstem response, conditioned orientated response, or pure tone audiogram. Hearing level was defined as the average of the hearing threshold at 0.5, 1.0, 2.0, and 4.0 kHz. Hearing was described as: normal, < 20 dB; mild impairment, 21–40 dB; moderate impairment, 41–70 dB; severe impairment, 71–90 dB; and profound impairment, >91 dB.

Neck palpation or echography of the neck was performed in all patients, to determine thyroid goiter. In addition, their serum levels of thyroid-stimulating hormone (TSH) and free thyroxine (FT4) were measured to evaluate thyroid function (normal values: 0.9–1.6 ng/dl and 0.5–5.0 mU/l, respectively). A perchlorate test was not performed.

High-resolution temporal bone CT was performed in all patients to determine if there were any other inner ear malformations in addition to EVA. EVA was defined as a vestibular aqueduct with a diameter of >1.5 mm at the midpoint between the common crus of the semicircular canal and the external aperture of the vestibular aqueduct on CT [17].

Mondini dysplasia was defined when the cochlea consisted of 1.5 turns in which the middle and apical turns had coalesced to form a cystic apex due to the absence of the interscalar septum [18,19].

Vestibular enlargement was defined when the ratio of the membranous vestibule diameter to the inner ear diameter of the lateral semicircular canal was >1.2 [20].

Vertigo was investigated based on spontaneous nystagmus, caloric vestibular test or patients' self-reporting of past episode. The spontaneous nystagmus was evaluated

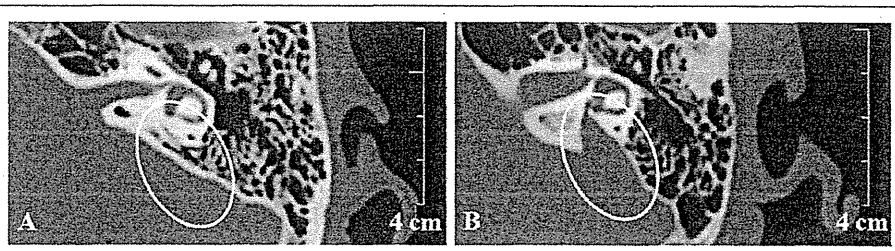

Figure 1 Computed tomography of the temporal bone showing an enlarged vestibular aqueduct. Circles show the vestibular aqueduct. The vestibular aqueduct is not identified in control subject (**A**). The enlarged vestibular aqueduct is identified in a patient with EVA (**B**).

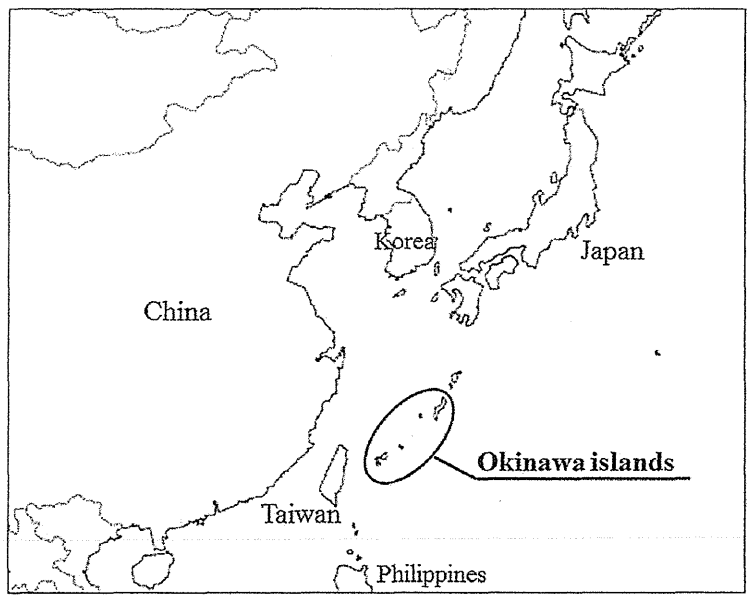

Figure 2 Location of the Okinawa islands in relation to East Asia. The Okinawa islands are located between Taiwan and the Japanese island of Kyushu. The Japanese archipelago comprises Hokkaido, Honshu, Kyusyu, and the Okinawa islands, as well as some smaller islands.

using Frenzel's glass or infrared CCD camera (IRN-1, Morita, Kyoto, Japan).

SLC26A4 genotyping

Genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). To detect mutations, all 21 exons and the exon–intron junctions of *SLC26A4* were sequenced for all subjects. A 35 step cycle of Polymerase chain reactions (PCR) was performed as follows: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 40 s, 60°C for 40 s, and 72°C for 1 min; and a final extension at 72°C for 5 min. PCR reactions were run using a programmable thermal cycler (Verti™ 96-Well Thermal Cycler, Applied Biosystems, CA, USA).

PCR products were purified using a Wizard® SV Gel and PCR Clean-Up System (Promega, WI, USA) and directly sequenced using an ABI PRISM 3130 × l Genetic Analyzer (Applied Biosystems). The sequences obtained were aligned and compared using the BLAST program with known human genome sequences available in the GenBank database.

We surveyed the substitution IVS15 + 5G > A in 100 healthy objects as control.

The genotype of the IVS15 + 5G > A was detected by digestion of the PCR product with the restriction enzyme SspI (New England Biolabs, Ipswich, MA, U.S.A).

Total RNA isolation and reverse-transcription

Total RNA was isolated from leukocytes using a QIAamp RNA Blood Mini Kit (Qiagen) according to the manufacturer's protocol. Before cDNA synthesis, residual DNA was removed by incubation with RNase-free DNase I (Ambion Inc., City, TX, USA). Then, total RNA was reverse transcribed using a TaKaRa Prime Script High Fidelity RT° Kit (TaKaRa, Tokyo, Japan) according to the manufacturer's protocol. Possible contaminating genomic DNA in RNA samples was determined by electrophoresis.

Quantitative nested real-time PCR

Nested real-time quantitative (q) PCR was performed to investigate the level of *SLC26A4* expression in the blood.

First-step PCR (conventional PCR)

A conventional PCR assay was performed in a 10 μl reaction mixture that included 2 μl of cDNA, 0.5 units of DNA Taq polymerase (TaKaRa), 2.5 mM deoxynucleotide triphosphates (dNTPs), 1 μM forward and reverse primers for first-step PCR (Table 2), 10 × buffer, and 1.875 mM $MgCl_2$, with distilled water (H_2O) for the final reaction volume of 10 μl. A 33 step cycle of PCR were performed as follows: 94°C for 5 min, 33 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 40 s, and a final extension at 72°C for 5 min.

Second-step PCR (quantitative nested PCR)

Following the first PCR, a second PCR was performed using a set of internal primers (Table 2). The reaction mixture contained 1 μl of the first PCR product (diluted 10-fold), 10 μl of SYBR Premix Ex Taq, and 0.2 μM of the internal forward and reverse primers; the final