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

難聴の遺伝子診断と次世代シークエンス解析

――保険収載された遺伝子診断からターゲットリシークエンシングとエクソーム解析

Genetic diagnosis of deafness

――From SNPs based invader analysis to the target resequencing and exome analysis

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◎先天性難聴は，新生児1,000名に1名に認められる比較的頻度の高い先天性疾患である．疫学調査の結果より先天性難聴の60～70％に遺伝子が関与することが推測されているため，正確な診断のためには遺伝子診断が重要であるが，遺伝的異質性が高く100程度の遺伝子が関与するため，効率的な遺伝子診断手法が必要である．2012年4月の診療報酬改定により日本人難聴患者に高頻度に認められる原因遺伝子変異を網羅的にスクリーニングする検査が保険収載され，日常の診断ツールとして遺伝子診断が利用可能となった．本稿では保険収載された遺伝子診断とその有用性について概説するとともに，保険収載された遺伝子診断を補完する検査として実施している次世代シークエンサーを用いたターゲットリシークエンシング解析およびエキソーム解析の現状について概説する．

Key word：遺伝学的検査，保険診療，先天性難聴，次世代シークエンサー，Usher症候群

先天性難聴は新生児1,000名に1名に認められる頻度の高い先天性疾患であり，他の先天性疾患と比較しても非常に頻度の高い疾患のひとつであるといえる．疫学調査の結果より先天性難聴あるいは小児期発症の難聴の60～70％に遺伝子が関与することが推測されているため(図1)[1]，先天性難聴の原因として最も可能性が高いのが遺伝子変異であり，正確な診断のためには遺伝子診断が必要不可欠である．

遺伝性難聴は，①難聴以外の症状を伴う“症候群性難聴”と，②難聴のみを症状とする“非症候群性難聴”に大別される．“症候群性難聴”は遺伝性難聴の約30％を占めるとされており，難聴以外に筋肉骨格系，腎尿路系，神経系，視覚障害，色素異常，代謝異常や種々の奇形を伴う400以上の疾患群が報告されている．これら“症候群性難聴”に関しては難聴以外の随伴症状より確定診断が比較的容易である．また，いくつかの症候群に関してはその原因遺伝子が明らかとなっている．一方，遺伝性難聴の約70％は難聴のみを症状とする“非症候群性難聴”である．分子遺伝学的解析手法の進歩により，“非症候群性難聴”の原因遺伝子は2013年現在で54種類報告されている[2]が(表1)，難聴以外の随伴症状を伴わないことより，臨床症状だけから原因遺伝子を特定することは困難である．

保険収載された“先天性難聴の遺伝子診断”

前述のように，先天性難聴の大部分に遺伝子が関与することが知られているが，遺伝的異質性が高く100程度の遺伝子が関与するため，効率的な遺伝子診断手法が必要となる．著者らは日本人難聴患者の遺伝子解析を精力的に行ってきたが，その結果，日本人難聴患者より見出される難聴の原因遺伝子変異部位は欧米人難聴患者に見出される部位と大きく異なっており，民族に特有の変異が存在することが明らかとなってきた[3-7]．これら日本人難聴患者に特徴的な原因遺伝子変異を網羅的かつ効率的にスクリーニングを行うことが難聴

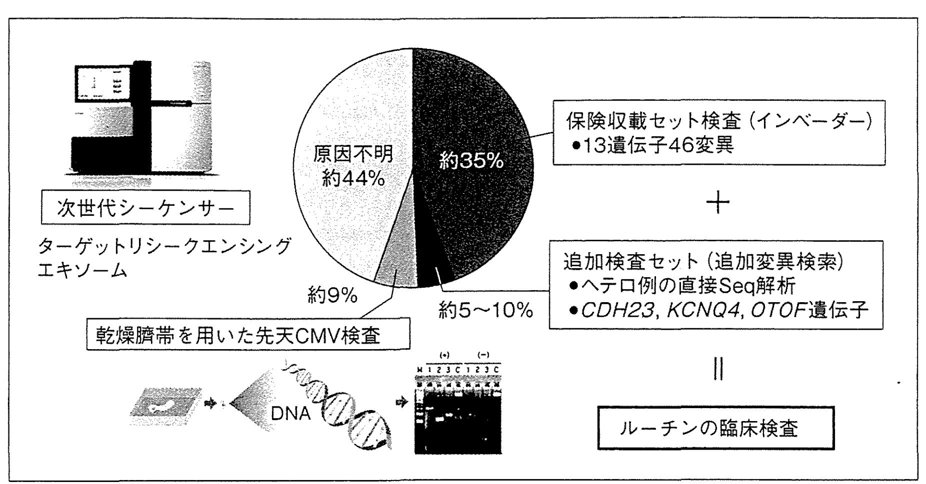

図 1　難聴遺伝子解析の現況

平成24年度より保険収載されたインベーダー法を用いた13遺伝子46変異のスクリーニング検査では，おおよそ35%の原因が特定可能である．現在，スクリーニング検査により原因遺伝子変異が同定されなかった症例を対象に，①追加変異解析の検査セット，②先天性CMV感染症の検査，③次世代シークエンサーを用いた解析を追加で行っている．※追加の検査を希望される施設は著者までご連絡いただきたい．
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の原因を特定するためには効率的であるため，日本人難聴患者に高頻度で認められる13遺伝子46変異をインベーダー法により網羅的にスクリーニングする検査を開発し，その臨床応用に関する検討を行ってきた．全国33施設と行った共同研究では，難聴患者の35%（発症年齢が6歳以下の先天性難聴に限ると44.3%）の検出率が得られ，スクリーニング検査として有用であることが明らかとなった[8]．この方法を用いた“先天性難聴の遺伝子診断”が2008年7月より先進医療として承認を受けて臨床応用が開始された．さらに，先進医療での実績数と有用性が評価され，2012年4月の診療報酬改定により“遺伝学的検査（先天性難聴）”として保険収載され，日常の診断ツールとして遺伝子診断が利用可能となった．

難聴の遺伝子診断のメリット

難聴では原因遺伝子変異の種類により臨床像がすこしずつ異なるため，遺伝子診断により原因遺伝変異を特定することで，原因遺伝子ごとにサブタイプ分類が可能となり，難聴のタイプや重症度，進行性や変動の有無，随伴症状の予防，発症・増悪の予防が可能となる．また，これら臨床像の予測に基づき，より適切な医療を提供することが可能となる．

1．難聴のタイプ・重症度の予測

遺伝子変異の種類や変異の部位によってコードする蛋白質に及ぼす影響が異なるため，最終的な表現型である難聴のタイプが異なってくることが知られている[4,9]．

新生児聴覚スクリーニングの普及により，難聴のスクリーニング検査が生後1週間以内に可能となってきたが，乳幼児の正確な聴力評価には聴性脳幹反応（ABR）や聴性定常反応（ASSR）などの聴覚検査を繰り返し行う必要がある．しかし，これら聴覚検査に加えて遺伝子診断を組み合わせて行うことにより，より多くの情報が得られ難聴のタイプや重症度に関して予測可能となるため，より早期に，より正確な診断が可能となる．

また，原因遺伝子の種類により聴力像が異なることが知られているため，遺伝子診断を行うことによりあらかじめ聴力像を予測することが可能である．とくに*CDH23*, *KCNQ4*, ミトコンドリア遺伝子1555 A>G変異による難聴の場合には高音障害型の感音難聴となるが，近年高音障害型感音難聴に対する新しい治療法として，残存聴力活用型人工内耳が臨床応用されており，遺伝子診断に基づいて人工内耳の種類を使い分けるオーダー

表 1　非症候群性難聴の原因遺伝子[2)]

常染色体優性遺伝形式

Locus (OMIM)	Gene (OMIM)	Reference
	CRYM	Abe ら (2003)
DFNA1	*DIAPH1*	Lynch ら (1997)
DFNA2A	*KCNQ4*	Kubisch ら (1999)
DFNA2B	*GJB3*	Xia ら (1998)
DFNA3A	*GJB2*	Kelsell ら (1997)
DFNA3B	*GJB6*	Grifa ら (1999)
DFNA4	*MYH14*	Donaudy ら (2004)
	CEACAM16	Zheng ら (2011)
DFNA5	*DFNA5*	Van Laer ら (1998)
DFNA6/14/38	*WFS1*	Bespalova ら (2001), Young ら (2001)
DFNA8/12	*TECTA*	Verhoeven ら (1998)
DFNA9	*COCH*	Robertson ら (1998)
DFNA10	*EYA4*	Wayne ら (2001)
DFNA11	*MYO7A*	Liu ら (1997)
DFNA13	*COL11A2*	McGuirt ら (1999)
DFNA15	*POU4F3*	Vahava ら (1998)
DFNA17	*MYH9*	Lalwani ら (2000)
DFNA20/26	*ACTG1*	Zhu ら (2003), van Wijk ら (2003)
DFNA22	*MYO6*	Melchionda ら (2001)
DFNA25	*SLC17A8*	Ruel ら (2008)
DFNA28	*GRHL2*	Peters ら (2002)
DFNA36	*TMC1*	Kurima ら (2002)
DFNA44	*CCDC50*	Modamio-Hoybjor ら (2007)
DFNA48	*MYO1A*	Donaudy ら (2003)
DFNA50	*MIRN96*	Mencia ら (2009)
DFNA51	*TJP2*	Walsh ら (2010)
DFNA64	*SMAC/ DIABLO*	Chen ら (2011)

常染色体劣性遺伝形式

Locus (OMIM)	Gene (OMIM)	Reference (OMIM)
DFNB1A	*GJB2*	Kelsell ら (1997)
DFNB1B	*GJB6*	Del Castillo ら (2002)
DFNB2	*MYO7A*	Liu ら (1997), Weil ら (1997)
DFNB3	*MYO15A*	Wang ら (1998)
DFNB4	*SLC26A4*	Li ら (1998)
DFNB6	*TMIE*	Naz ら (2002)
DFNB7/11	*TMC1*	Kurima ら (2002)
DFNB8/10	*TMPRSS3*	Scott ら (2001)
DFNB9	*OTOF*	Yasunaga ら (1999)
DFNB12	*CDH23*	Bork ら (2001)
DFNB15/72/95	*GIPC3*	Ain ら (2007), Rehman ら (2011), Charizopoulou ら (2011)
DFNB16	*STRC*	Verpy ら (2001)
DFNB18	*USH1C*	Ouyang ら (2002), Ahmed ら (2002)
DFNB21	*TECTA*	Mustapha ら (1999)
DFNB22	*OTOA*	Zwaenepoel ら (2002)
DFNB23	*PCDH15*	Ahmed ら (2003)
DFNB24	*RDX*	Khan ら (2007)
DFNB25	*GRXCR1*	Schraders ら (2010)
DFNB28	*TRIOBP*	Shahin ら (2006), Riazuddin ら (2006)
DFNB29	*CLDN14*	Wilcox ら (2001)
DFNB30	*MYO3A*	Walsh ら (2002)
DFNB31	*WHRN*	Mburu ら (2003)
DFNB35	*ESRRB*	Collin ら (2008)
DFNB36	*ESPN*	Naz ら (2004)
DFNB37	*MYO6*	Ahmed ら (2003)
DFNB39	*HGF*	Schultz ら (2009)
DFNB42	*ILDR1*	Borck ら (2011)
DFNB49	*MARVELD2*	Riazuddin ら (2006)
DFNB53	*COL11A2*	Chen ら (2005)
DFNB59	*PJVK*	Delmaghani ら (2006)
DFNB61	*SLC26A5*	Liu ら (2003)
DFNB63	*LRTOMT/ COMT2*	Ahmed ら (2008), Du ら (2008)
DFNB66/67	*LHFPL5*	Tlili ら (2005), Shabbir ら (2006), Kalay ら (2006)
DFNB74	*MSRB3*	Waryah ら (2009), Ahmed ら (2011)
DFNB77	*LOXHD1*	Grillet ら (2009)
DFNB79	*TPRN*	Rehman ら (2010), Li ら (2010)
DFNB82	*GPSM2*	Walsh ら (2010)
DFNB84	*PTPRQ*	Schraders ら (2010)
DFNB91	*GJB3*	Liu ら (2000)
	SERPINB6	Sirmaci ら (2010)

X 連鎖性

Locus (OMIM)	Gene (OMIM)	Reference
DFNX1 (DFN2)	*PRPS1*	Liu ら (2010)
DFNX2 (DFN3)	*POU3F4*	De Kok ら (1995)
DFNX4 (DFN6)	*SMPX*	Schraders ら (2011), Huebner ら (2011)

メイド医療が実現可能となってきた[10)].

2. 進行性や変動の有無の予測

通常の聴力検査ではその後の難聴の進行や変動の有無を予測することは困難であるが，遺伝子診断を行うことにより進行の有無や変動の有無を予測することが可能である．たとえば，同じ常染色体劣性遺伝形式をとる難聴原因遺伝子変異であっても，*GJB2* 遺伝子変異症例では難聴の進行を認めることは稀である[4,11)]のに対して，*SLC26A4* 遺伝子変異による難聴症例ではめまい発作を伴い，変動しながら難聴が増悪することが明らかとなっている[5)]．また，常染色体優性遺伝形式をとる

KCNQ4, *TECTA*, *WFS1* などの遺伝子変異による難聴症例およびミトコンドリア遺伝子変異による難聴症例も進行性の難聴を呈することが知られている[12-15]．進行性の難聴を特徴とする原因遺伝子変異が同定された場合には定期的に聴力を測定するとともに，補聴器・人工内耳などの機器の調整を行い，十分な聴取能を確保することが必要である．また，高度難聴への進行が予測される遺伝子変異の場合には将来的に人工内耳を視野に入れた経過観察が必要となる．

3. 随伴症状の有無の予測

Pendred症候群やUsher症候群のように症候群性の難聴であっても随伴症状の発症時期が小児期以降発症の場合には，生後〜小児期にかけては難聴以外の症状を呈さないため非症候群性難聴と区別がつかない．このような遅発性の随伴症状を伴う症候群性難聴の場合，遺伝子診断が予後の予測や適切な介入法選択のための有用な情報となる．*SLC26A4* 遺伝子変異は難聴と甲状腺腫を伴うPendred症候群の原因遺伝子でもあるため，*SLC26A4* 遺伝子変異が認められた場合には甲状腺機能を含めた経過観察が重要である[5]．

また，*MYO7A*，*CDH23*，*PCDH15* などの遺伝子変異により発症するUsher症候群では先天性の高度難聴＋後天性の網膜色素変性症を発症することが知られており，10歳前後で夜盲を自覚するまでは視覚症状に気がつかない場合が多い．遺伝子診断を行うことで，網膜色素変性症を予測可能となるため，両側人工内耳を行うなど将来の視覚障害に対応するために聴覚を積極的に活用するなどの治療計画を立てることが可能となる[16]．

4. 発症・増悪の予防

近年，分子遺伝学的・分子生物学的解析より，ミトコンドリア1555 A>G変異，1,494C>T変異をもつ場合にはアミノ配糖体抗菌薬に高感受性となることが明らかとなってきた．この変異を伴う難聴が診断された場合には，①罹患者の難聴の進行予防，および②非罹患の同胞の発症予防，が可能となる．いったん難聴を発症すると非可逆的であるが，アミノ配糖体抗菌薬を避けることで，罹患者の場合には難聴の進行を，非罹患の同胞の場合には高度難聴の発症を予防できるというメリットがあるため，薬物カードを配布して予防に努めている[15,17]．

遺伝性難聴のターゲットリシークエンシング解析

保険収載された先天性難聴の遺伝子診断は，正確な診断を行うという意味だけでなく，予後の予測，随伴症状の予測，難聴の進行予防，治療法選択に有用な情報が得られるなど多くのメリットのある検査である．遺伝子診断に基づいた難聴のサブタイプ分類と，サブタイプに応じたオーダーメイド医療の提供はこれからの難聴医療に必須となると思われるが，現在保険診療で行われているインベーダー法を用いたスクリーニング検査の診断率は30〜40%程度であり，今後の診断率の向上のためには新規原因遺伝子変異の追加が必要である．しかし，①難聴の原因遺伝子として100以上の遺伝子が関与するため，原因となる遺伝子変異の探索は容易ではない，②日本人難聴患者に高頻度で認められる主要な変異はすでにインベーダー法によるスクリーニング検査に取り込まれているため，遺伝子変異を追加することによるコストの増加に対し診断率向上の程度はわずかであるため，費用対効果に乏しい，という問題点があった．しかし，次世代シークエンサーが実用化され，多数の原因遺伝子を網羅的に解析することが可能となり，また解析費用の低下により，現実的にすべての難聴原因遺伝子を網羅的に解析することが可能となってきた．

難聴の原因を探索する際の効率を考えると，①インベーダー法に搭載されている日本人難聴患者に高頻度で認められる変異のスクリーニング検査，②既報告の難聴原因遺伝子のターゲットリシークエンシング解析，③新規の難聴原因遺伝子の探索を目的としたエクソーム解析，④エクソン領域以外に原因のある難聴遺伝子変異を見出すためのゲノム解析，の順に検索を進めるのがよいと考えられる．インベーダー法によるスクリーニング検査などで遺伝子変異が同定されないケースでは，難聴の原因遺伝子であることが報告されている54遺伝子を網羅的に解析するターゲットリシークエンシングが有用である．とくに，劣性遺伝形式を

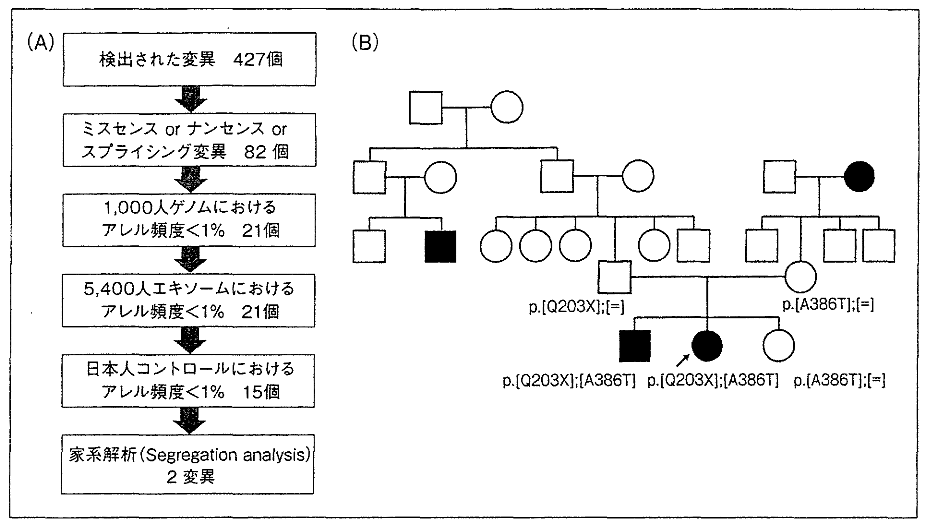

図 2 難聴のターゲットリシークエンシング解析による遺伝子変異の絞込み
実際に行われた遺伝子解析による遺伝子変異の絞込みの様子(A)と遺伝子 X の複合ヘテロ接合体変異の見出された非症候群性難聴家系(B)を示す．(Miyagawa, et al., submitted)

とる遺伝性難聴では，founder effect によると考えられる民族共通の遺伝子変異が高頻度で認められるため，インベーダー法や TaqMan Genotyping 法による診断が有効であるが，優性遺伝形式をとる遺伝性難聴の場合，家系ごとに変異の部位が異なる場合が多く，効率的なスクリーニング法が確立していないため，既知遺伝子の網羅的ターゲットリシークエンシング解析が非常に有用である．

現在，わが国も含めて既知難聴遺伝子を網羅的に解析するターゲットリシークエンシングが行われており，成果が得られつつある[18-20]．現在までに報告されている難聴の原因遺伝子をすべて合わせるとおおよそ 0.5〜0.6 Mbase となるため，エンリッチメントの方法としては SureSelect などのシークエンスキャプチャーによるものが多く用いられている．また最近では，micro droplet PCR や HaloPlex，IonAmpliSeq などの Amplicon ベースのエンリッチメント法も徐々に用いられはじめている．

Shearer ら(2010)は，既知の難聴原因遺伝子 54 種類の全エクソン領域を SureSelect により網羅的にキャプチャーする手法を利用して，難聴患者 8 例(陽性コントロール 2 例を含む)の遺伝子解析を行った．陽性コントロール 2 例とも既報告の変異が同定され，また，原因不明であった 6 家系中 5 家系より難聴の原因と考えられる遺伝子変異を同定し報告している[18]．

また著者らが，難聴患者216例(陽性コントロール 62 例を含む)を対象に，既知の非症候群性難聴の原因遺伝子 54 遺伝子および既知の症候群性難聴原因遺伝子および内耳で高発現の認められる遺伝子を含めた 121 遺伝子の全エクソン領域を SureSelect Custom＋Illumina GA Ⅱx により網羅的に解析を行ったところ，陽性コントロールにおける変異検出率は 93％以上と非常に高効率で検出可能であり，また，全体の約 70％より何らかの遺伝子変異が見出されることが明らかとなった．このことから，新規の原因遺伝子探索手法としても非常に有用であることが明らかとなってきている(Miyagawa ら．submitted)．

その後，著者らのグループでは新規の原因遺伝子変異を同定する目的で，前述 216 名に加え 296 名の追加の解析を SureSelect Custom＋Illumina HiSeq2000 で行い，合計 512 名のターゲットリシークエンシング解析を完了している．ターゲットリシークエンシング解析においては多数の SNPs が検出されるため，見出された変異が病的変異かまれな多型かを判断するのは非常に困難である．見出された遺伝子変異に関して，①1,000 人ゲノムおよび 5,400 エキソームのアレル頻度によ

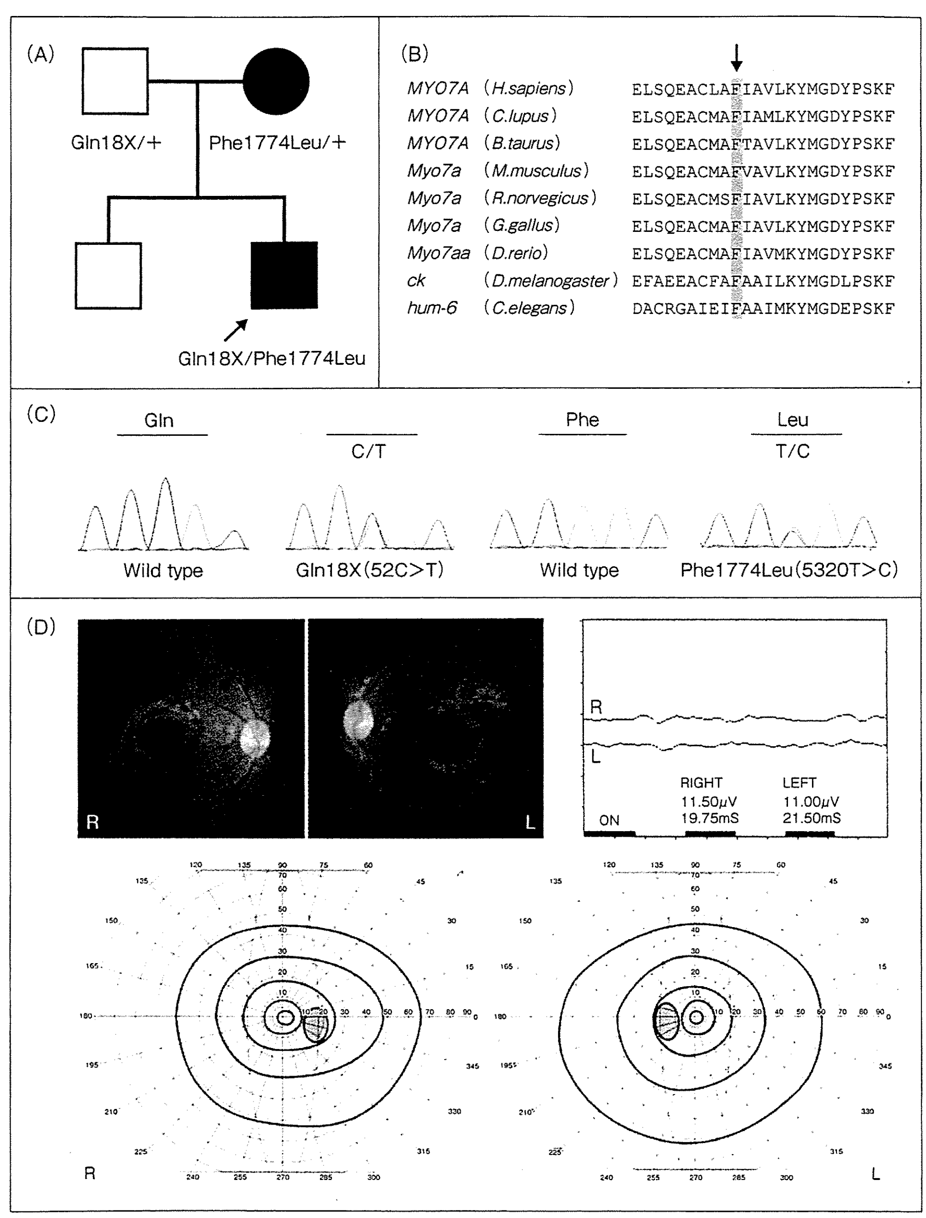

図 3　遺伝子診断により網膜色素変性症自覚前にUsher症候群を診断された症例[16)]

A：遺伝子診断により網膜色素変性症発症前に遺伝子診断された症例の家系図および見出された*MYO7A* 変異.

B：変異部位の種間の保存性.

C：サンガーシークエンスによるクロマトグラム.

D：眼底所見, 網膜電図検査(ERG検査)の結果および視野検査(Goldman visual field examination)の結果.

るフィルター, ②日本人コントロールにおけるアレル頻度によるフィルターをかけることにより候補となる遺伝子変異が10個以下のレベルまで絞り込まれる. とくに, 遺伝性難聴の場合, 極少数のケースを除き浸透率100%の単一遺伝子のMendel遺伝形式をとるため, ③家系サンプルによるセグリゲーション解析を行うことにより原因候補遺伝子変異は数個レベルまで絞り込むことが可能であり, 原因遺伝子変異を同定できる場合も多い(図2).

また著者らは, 平成22年度～24年度厚生労働科学研究「Usher症候群に関する調査研究班」と

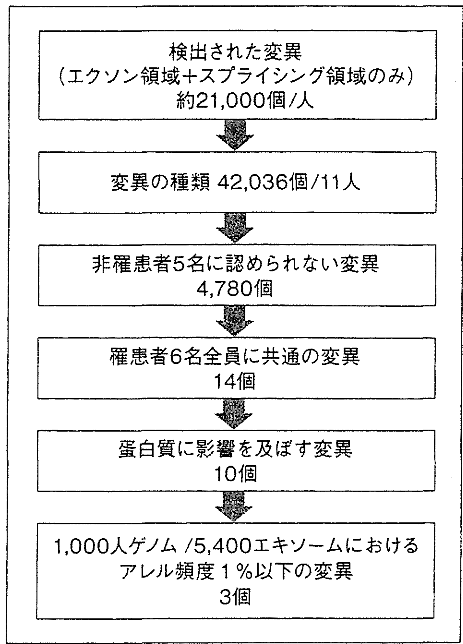

図 4　難聴のエクソーム解析による遺伝子変異の絞込み
優性遺伝形式をとる遺伝性難聴家系(罹患者6名，非罹患者5名)を対象に行ったエクソーム解析による遺伝子変異の絞り込みの様子を示す．十分に大きな家系の場合には同一家系内の複数サンプルのエクソーム解析を行うことにより候補遺伝子を数個レベルまで絞り込むことが可能である．

して，全国の共同研究施設より収集したUsher症候群症例46例中，Usher症候群type 1症例16例を対象にIonAmpliSeqを用いて，Usher症候群の原因遺伝子として知られる9遺伝子(*MYO7A*, *CDH23*, *PCDH15*, *USH1C*, *USH1G*, *USH2A*, *GPR98*, *DFNB31*, *CLRN1*)を増幅し，Ion-Torentを用いての網羅的解析を行ったところ，14例(87.5%)よりホモ接合体または複合ヘテロ接合体で原因遺伝子変異を検出し原因を同定することができた．また，検出された変異の種類は欧米人の変異とは異なるものであり民族特異性があることが明らかとなった[16)]．興味深いことに，変異の検出された遺伝子は*MYO7A*が7例(50%)，*CDH23*が4例(28%)，*PCDH15*が3例(21%)であり，海外と同程度の頻度であることが明らかとなった．このことよりUsher症候群においても難聴と同様，原因遺伝子の種類や頻度は民族間で共通しているが，変異の種類に関しては民族特異性があることが明らかとなった．Usher症候群の原因として知られる9遺伝子は数多くのエクソンからなる大きな遺伝子が多く，エクソンの合計が326にもなる．従来のサンガーシークエンス法を用いた解析では膨大な作業量が必要であったが，パーソナル型の次世代シークエンサーを用いた遺伝子解析により大幅に効率化を行うことが可能となってきた．

また，Usher症候群は先天性高度難聴に後天性の網膜色素変性症を随伴するため，生下時〜幼少期には非症候群性の難聴と診断されている症例がいるものと考えられる．そこで，非症候群性難聴症例を対象にUsher症候群の原因遺伝子を網羅的に解析したところ，網膜色素変性症を発症する前に遺伝子変異が同定され，その後眼底検査や網膜電図検査(ERG検査)により網膜色素変性症と診断された症例を経験した[16)]．このように，遺伝子診断はUsher症候群の早期診断および早期介入のために非常に有用な情報となりうることが期待される(図3)．

遺伝性難聴のエクソーム解析の実際

難聴の原因であることが報告されている遺伝子を網羅的に解析するターゲットリシークエンシング解析を行ってもなお原因遺伝子変異が同定されない場合には，全遺伝子のエクソン領域を対象としたエクソーム解析を行うことにより難聴の原因が特定可能である．

海外でも新規難聴原因遺伝子の探索の一環としてのエクソーム解析が行われはじめているが，現時点では連鎖解析やhomozygous mappingなどの他の遺伝子解析手法と組み合わせて実施することで，変異の絞り込みを行うなどの工夫を行った解析が行われている[21-23)]．

Walshら(2010)は，連鎖解析により遺伝子座DFNB85が同定されていた劣性遺伝形式をとる近親婚難聴家系1家系を対象に，SureSelect＋IlluminaGA Ⅱxを用いたエクソーム解析を行い，DFNB85座近傍の遺伝子に関してhomozygous mappingと組み合わせることにより，新規の難聴原因遺伝子GPSM2を同定し報告している．また，内耳における局在を調べるとともに発生段階での

遺伝子発現量を調べることにより，GPSM2が難聴の原因となりうる可能性を検討している．

著者らの研究室では連鎖解析が可能な規模の大家系を対象にエクソーム解析を用いた難聴遺伝子の解析を行っており，新規変異の候補が見出されている状況である．実際に解析を行った優性遺伝形式をとる遺伝性難聴1家系を例にあげて説明を行う．優性遺伝形式をとる遺伝性難聴家系のうち罹患者6名，非罹患者5名の11名を対象にIllumina TrueSeq Whole Exome kit＋Illumina HiSeq2000を用いたエクソーム解析を行った(図4)．

一般的なエクソーム解析で利用されるパイプライン(Duplication Readの除去＞QC値でのフィルタリング＞BWAによるhg19へのマッピング＞GATK toolsによる変異の検出)を用いて変異の検出を行ったところ，11名それぞれに約20,000カ所の変異が同定された．同定された変異を対象に，ANNOVARを用いてアノテーションを付けたファイルをcsvファイルとして用意し，①蛋白質に影響を及ぼす変異(ミスセンス変異，ナンセンス変異，スプライシング変異，欠失・挿入変異)，②1,000人ゲノムおよび5,400エクソームにおける頻度が0.01以下，③家系内罹患者に共通かつ非罹患者に認められない，の3条件を組み合わせて候補遺伝子の絞り込みを行ったところ3変異まで絞り込むことが可能であった．3変異とも過去に難聴との関連が報告されていない新規の遺伝子に存在するため，内耳における遺伝子発現や発現部位に関して検討を行っている．

おわりに

従来，難聴の多くは原因不明で治療法もなかったため，難聴の治療は画一的に行われていたが，遺伝子解析技術の進歩と人工内耳の登場により正確な診断に基づいた個別化医療が実現しつつある．遺伝子診断はこの根幹をなす技術であり今後の発展が期待されている．先天性難聴の遺伝子診断に関しては平成24年(2012)4月より保険診療として実施されており，今後ますます遺伝子診断の重要性が高まっていくものと思われる．また，保険診療で実施されているスクリーニング検査において原因遺伝子変異が見出されない場合には，ターゲットリシークエンシングやエクソーム解析が有用であることが明らかとなってきている．現時点ではターゲットリシークエンシングが主流であるが，解析コストとデータ量に大きな違いがあるものの解析手技には大きな違いはないため，日本人のコントロールデータの充実と解析コストの低下に従いエクソーム解析が普及してくると予測される．しかし，どのようなエンリッチメント手法を用いても全エクソン領域のカバー率が100%になることはないため，将来的には比較的均質なデータが得られるゲノム解析へとシフトすると考えられる．今後，難聴の臨床現場でこのような新しい手法を用いた正確な診断に基づいた難聴医療が定着していくことを期待している．

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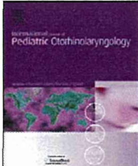

Expression of toll-like receptors in chronic otitis media and cholesteatoma

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ABSTRACT

Objective: Otitis media is one of the most common infectious diseases, especially in young children. Multiple factors affect the onset or development of otitis media. Human toll-like receptors recognize associated patterns and play a critical role in innate immune mechanisms. Toll-like receptors are considered to be important factors for clearance of infection and resolution of inflammation in otitis media. The purpose of this study was to evaluate the histological expression of toll-like receptor 2, which recognizes many kinds of pathogen-associated molecular patterns, and toll-like receptor 4, which recognizes lipopolysaccharide on Gram-negative bacteria, in tissue samples from patients with chronic otitis media and middle ear cholesteatoma.

Methods: Human middle ear tissue samples from 12 patients with chronic otitis media ($n = 7$) and acquired middle ear cholesteatoma ($n = 5$) were examined. Normal control middle ear samples without any inflammation were also included ($n = 7$). The expressions of toll-like receptors 2 and 4 in middle ear tissues were examined immunohistochemically.

Results: Only one normal control middle ear sample showed weak expression of toll-like receptor 2, and toll-like receptor 4 was not observed in all control samples. On the other hand, both toll-like receptors 2 and 4 were markedly expressed in chronic otitis media and cholesteatoma. There was a significant difference between chronic otitis media and normal controls in the expressions of both toll-like receptors. Significant up-regulation of toll-like receptors 2 and 4 was observed in cholesteatoma as compared with control samples.

Conclusions: Toll-like receptors 2 and 4 were strongly expressed in chronic otitis media and middle ear cholesteatoma. These findings suggest that toll-like receptors may play a principal role in human chronic otitis media and cholesteatoma.

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1. Introduction

Toll-like receptors are membrane proteins that play a crucial role in the induction and activation of innate immunity in the course of infection. At least ten subtypes of toll-like receptors have already been identified in humans, and they are considered to be involved in the recognition of pathogen-associated molecule patterns in the innate immune system [1]. Toll-like receptor 4 (TLR-4) is one of the toll-like receptors recognizing toxic pneumolysin ligand produced by Gram-positive bacteria, as well as binding to lipopolysaccharide. Lipopolysaccharide is the major component of Gram-negative bacteria and is frequently detected in otitis media [2,3]. Toll-like receptor 2 (TLR-2) recognizes many kinds of pathogen-associated molecular patterns.

Recent studies have shown that expression of toll-like receptors was observed in middle ear samples in acquired cholesteatoma and otitis media with effusions [1,4]. To the best of our knowledge, the comparison of toll-like receptor expressions between chronic otitis media and normal controls in human middle ear tissue has not been reported. The purpose of this study was to show the presence and localization of TLR-2 and TLR-4 in middle ear samples in patients with chronic otitis media, patients with cholesteatoma, and normal controls.

2. Materials and methods

2.1. Samples

Middle ear tissue samples were obtained from 7 patients with chronic otitis media (mean age ± standard deviation (SD), 67.1 ± 2.4 years: range, 64–70 years) and from 5 patients with middle ear cholesteatoma (mean age ± SD, 36.0 ± 27.0 years: range, 6–63 years).

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The control middle ear tissue samples were collected from 7 patients without any middle ear inflammation undergoing cochlear implant surgery (mean age ± SD, 38.0 ± 22.7 years: range, 5–61 years).

The expressions of TLR-2 and TLR-4 in middle ear tissue were examined immunohistochemically. This study was approved by the Institutional Review Board of Okayama University (IRB approval number, RINRI-1435) and was in compliance with the Declaration of Helsinki.

2.2. Immunohistochemistry

Paraffin-embedded samples were sectioned at a thickness of 4 μm. Sections were deparaffinized, rehydrated, and pretreated using Liberate Antibody Binding Solution (COSMO BIO Co., Ltd., Tokyo, Japan) for antigen retrieval. Endogenous peroxidase activity was quenched with 1% hydrogen peroxide (H_2O_2), and nonspecific protein binding was blocked with skim milk. The tissue sections were then incubated with rabbit anti-toll-like receptor 2 or 4 polyclonal antibody (TLR-2, ab24192; TLR-4, ab13556; Abcam Inc., Cambridge, UK) overnight at 4 °C. For visualization, the LSAB^{TM}2 kit, the StreptABComplex/HRP kit, and diaminobenzidine substrate (DAKO, Glostrup, Denmark) were used according to the manufacturer's instructions.

The reaction was assessed by blinded investigators under light microscopy according to the method of Szczepański et al. [4]. Briefly, the rating score was classified as: (−), no positive reaction; (+), 1–10 positive cells; (++), 11–100 positive cells; and (+++), over 100 positive cells per high power field (400×).

2.3. Statistical analysis

For statistical analysis, a Chi-square test was performed at a significance level of $p < 0.05$ using SPSS (IBM, New York, NY, USA).

3. Results

TLR-2 and TLR-4 showed similar expressions. The middle ear samples from control subjects showed no expression of TLR-4. Both TLR-2 and TLR-4 were expressed in the middle ear mucosa and granulation tissue in patients with chronic otitis media and cholesteatoma [Fig. 1]. Positive immunostaining for TLR-4 was observed in mucosal epithelial cells, infiltrating inflammatory cells, and macrophages. Positive immunostaining for TLR-2 was also observed in mucosal epithelial cells and infiltrating inflammatory cells [Fig. 2].

The expression of TLR-2 in chronic otitis media was (−) in 1 case, (+) in 2 cases, (++) in 2 cases, and (+++) in 2 cases. The cholesteatoma samples showed (−) in 0 case, (+) in 2 cases, (++) in 2 cases, and (+++) in 1 case. The control samples showed (−) in 6 cases, (+) in 1 case, (++) in 0 case, and (+++) in 0 case. The immunohistochemical staining score of TLR-2 was significantly higher in chronic otitis media and cholesteatoma than in control samples (chronic otitis media, $p = 0.048$; cholesteatoma, $p = 0.026$).

The expression of TLR-4 in chronic otitis media was (−) in 0 case, (+) in 4 cases, (++) in 2 cases, and (+++) in 1 case. The cholesteatoma samples showed (−) in 0 case, (+) in 0 case, (++) in 3 cases, and (+++) in 2 cases. The control samples showed (−) in all 7 cases. Significant expression of TLR-4 was observed both in chronic otitis media and cholesteatoma as compared with normal control samples (chronic otitis media, $p = 0.003$; cholesteatoma, $p = 0.002$).

4. Discussion

Toll-like receptors are members of the pattern-recognition receptor family that detects specific molecules associated with microbial pathogens. Toll-like receptors are key regulators of both innate and adaptive immune responses and comprise a family of germ line-encoded transmembrane receptors. The activations of toll-like receptors lead to the mobilization of other innate immune molecules, such as cytokines, chemokines, and interferons, as well as proteases, defensins, collectins, lusozyme, lactoferrin, and other antimicrobial intermediates [5]. These receptors recognize conserved microbial structures called pathogen-associated molecular patterns, which are invariant within a given class of microorganism. Many pathogen-associated molecular patterns have now been recognized and their respective toll-like receptors identified; these include peptidoglycan (which binds to TLR2), synthetic double-stranded RNA (TLR3), lipopolysaccharide (TLR4), flagellin (TLR5), and CpG DNA motifs associated with bacterial DNA (TLR9) [6]. Although the participations of toll-like receptors are necessary to defend humans against microbial invasion, the abnormal responses of toll-like receptors also cause the development of many diseases [7,8].

Lipopolysaccharide, a major component of the cell wall of Gram-negative bacteria, is a potent immune stimulator. An experimental animal study showed that injection of

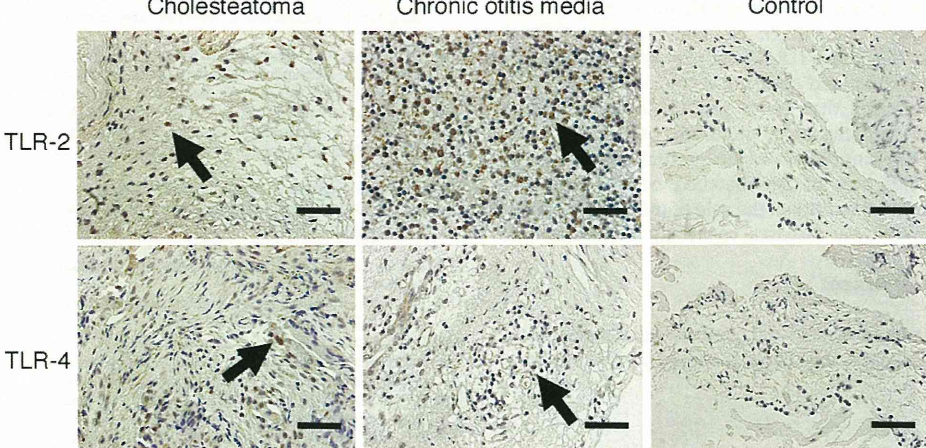

Fig. 1. The expressions of toll-like receptors 2 and 4 in patients with chronic otitis media and with cholesteatoma. Positive immunostaining of toll-like receptors (arrow) is observed both in chronic otitis media and in cholesteatoma. The normal control middle ear subjects show no positive cells (TLR-2, toll-like receptor 2; TLR-4, toll-like receptor 4; bar, 50 μm).

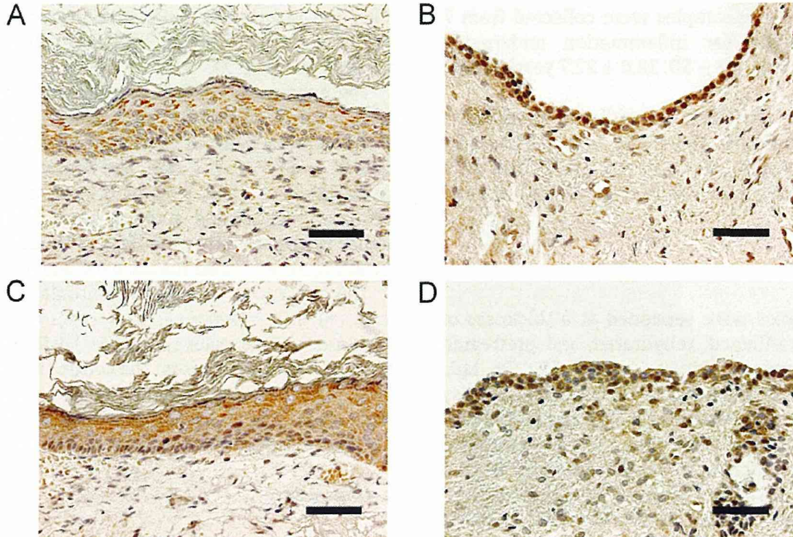

Fig. 2. Positive immunohistochemical staining for toll-like receptors is observed in middle ear mucosa of chronic otitis media and cholesteatoma epithelium. (A) Toll-like receptor 2 in cholesteatoma. (B) Toll-like receptor 2 in chronic otitis media. (C) Toll-like receptor 4 in cholesteatoma. (D) Toll-like receptor 4 in chronic otitis media (bar, 50 μm).

lipopolysaccharide into the middle ear can mimic the pathological changes of otitis media: mucosal inflammation, leukocytosis, edema, middle ear pressure abnormalities, and an infiltrate of macrophages into the subepithelial space [9–11].

Non-typeable *Haemophilus influenzae* is one of the most prominent bacterial pathogens of human otitis media and activates the TLR-4 signaling pathways [5]. Toll-like receptors are considered important factors in the pathogenesis of otitis media, but the role of toll-like receptors in chronic otitis media is controversial. The activation of toll-like receptors induces various transcription factors, including NF-κB, and subsequently results in high expression of proinflammatory cytokines such as IL-1 and TNF-α [4,5,12]. Toll-like receptor-deficient mice show reduced bacterial clearance after middle ear infection with bacterial pathogens [5]. In contrast, overexpression of toll-like receptors is observed in severe infection, and a TLR-4 antagonist (E-5564; Eisai Co., Ltd., Tokyo, Japan) is expected to be a novel remedy for sepsis [12–16].

In the present study, TLR-2 and TLR-4 were strongly expressed in the mucosal epithelium and infiltrating inflammatory cells both in chronic otitis media and middle ear cholesteatoma. These findings suggest that TLR-2 and TLR-4 may play principal roles in human chronic otitis media and middle ear cholesteatoma.

A recent study reported that TLR-2 and TLR-4 might play a different role in the pathophysiology of chronic otitis media and cholesteatoma [17]. The limitations of our preliminary study are small sample size and lack of age-matched normal controls. Further studies are needed to dissect the role of toll-like receptors in pathogenesis of middle ear diseases, especially in children.

Conflict of interest statement

All authors disclose no financial and personal relationship with other people or organization that could inappropriately influence the work.

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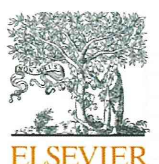

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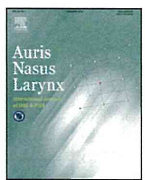

Cochlin-tomoprotein (CTP) detection test identified perilymph leakage preoperatively in revision stapes surgery

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ABSTRACT

Perilymphatic fistula (PLF) is defined as an abnormal leakage between perilymph from the labyrinth to the middle ear. Symptoms include hearing loss, tinnitus, and vertigo. The standard mode of PLF detection is intraoperative visualization of perilymph leakage and fistula, which ostensibly confirms the existence of PLF. Other possible methods of diagnosis include confirmation of pneumolabyrinth via diagnostic imaging. Recently, a cochlin-tomoprotein (CTP) detection test has been developed that allows definitive diagnosis of PLF-related hearing loss.

We report the case of a 45-year-old man who presented with right-sided tinnitus, hearing loss, and dizziness 30 years after stapes surgery. Middle ear lavage was performed after myringotomy. A preoperative diagnosis of PLF was reached using the CTP detection test. Intraoperative observations included a necrotic long process of the incus, displaced wire piston, and fibrous tissue in the oval window. Perilymph leakage was not evident. The oval window was closed with fascia, and vertigo disappeared within 2 weeks postoperatively. When PLF is suspected after stapes surgery, the CTP detection test can be a useful, highly sensitive, and less invasive method for preoperative diagnosis.

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1. Introduction

Perilymphatic fistula (PLF) is defined as abnormal leakage between perilymph from the labyrinth to the middle ear. PLF diagnosis has been made with pneumolabyrinth in the inner ear on computed tomography (CT) and T2-weighted magnetic resonance imaging (MRI) [1]. Leakage has been confirmed during open and endoscopic surgery [2,3]. However, PLF diagnosis is clinically difficult because CT, MRI, and perioperative methods are not always able to detect the leakage.

In 2001, cochlin-tomoprotein (CTP), a novel perilymph-specific protein, was identified [4]. CTP is a protein product of *COCH*, which was originally identified from the cochlea-specific cDNA library. Later, its mutation was found to be associated with DFNA9, an autosomal dominant hereditary deafness condition. Three cochlin isoforms were identified; CTP was one of these short 16-kDa isoforms. CTP is found in the functional domain of LCCL in cochlin and is secreted to the perilymph. CTP is highly specific for perilymph. Therefore, a diagnosis of PLF can be made by detection of CTP using Western blotting in lavage of the middle ear [5].

We report a case of right-sided tinnitus, hearing loss, and dizziness manifesting 30 years after stapes surgery. PLF was diagnosed preoperatively using the CTP test in middle ear washings. PLF was not suspected based on clinical manifestations, eardrum examination, and CT. Preoperative diagnosis was possible only because of the CTP test. CTP detection test is a new, highly sensitive, less invasive, and useful method to aid in the diagnosis of PLF.

2. Case report

The patient was a 45-year-old man. In 1980, right stapes surgery had been performed on him and a Teflon wire piston was placed (details of the surgery were uncertain). The patient presented at our hospital with right-sided tinnitus of idiopathic origin. In December 2009, he experienced mild dizziness, but no rotatory vertigo or awareness of hearing loss was evident. In an audiometric test, deterioration of hearing by bone conduction was detected as compared with hearing level recorded during a consultation conducted 20 years previously. Therefore acute mixed hearing loss was suspected.

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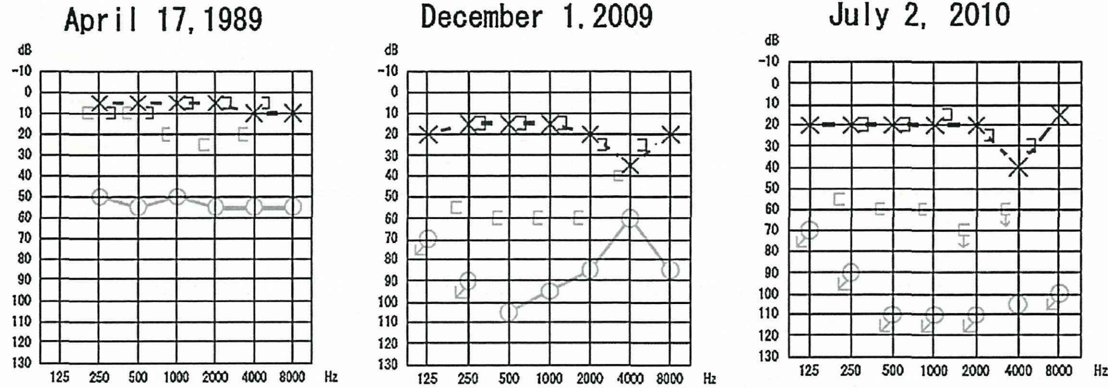

Fig. 1. Audiogram. Hearing levels in December 2009 where lower than those in 1989. In July 2010, vertigo developed, and hearing deteriorated further.

Administration of oral prednisolone (30 mg per day), ATP, and vitamin B12 was initiated. At the end of June 2010, rotatory vertigo and tinnitus appeared and hearing in the right ear deteriorated further (Fig. 1). Pure horizontal nystagmus directed to the left was observed under Frenzel glasses. On physical examination, no fluid was found in the tympanic cavity through the right tympanic membrane.

Hydrocortisone sodium succinate was administered via intravenous drip (500 mg per day) for 10 days tapering starting on July 2, 2010. Rotatory vertigo was gradually relieved, but dizziness continued. On the basis of clinical history, PLF was suspected. We obtained informed consent from him and collected middle ear washings after myringotomy under topical anesthesia and examined by the CTP detection test. The procedure of this test has been reported previously [4]. A CTP-positive signal was observed from the middle ear washings (Fig. 2), confirming the diagnosis of right PLF. After and during the test, no exacerbation of dizziness, tinnitus, or hearing loss was observed.

On November 1, 2010, surgery was performed under general anesthesia. Intraoperative observations included a necrotic long process of the incus, displaced wire piston, and fibrous tissue in the oval window. The body and short process of the incus were in the normal position. The incus and wire were transected and the wire of the piston was visible outside the oval window, but the piston was found lying deep within the vestibule. The footplate of the stapes was not found. Leakage of lymph fluid into the tympanic cavity and around the oval and round windows was not observed. Fibrous adhesions, mucosal hyperplasia, and the wire piston were removed.

positive contol CTP

perilymph

middle ear washing from this patient

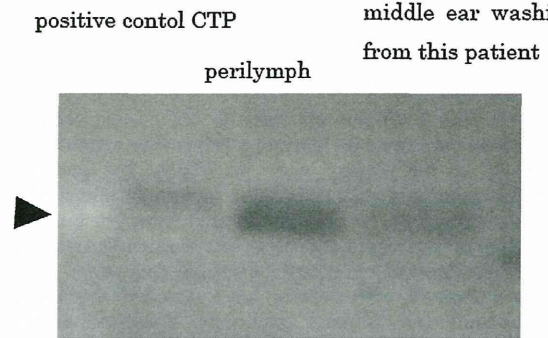

Fig. 2. Detection of cochlin-tomoprotein (CTP) in the middle ear washings. Signals represent CTP in recombinant positive control CTP (left), perilymph (middle), and middle ear washings from the patient (right) by Western blotting.

The oval and round windows were covered with the temporal fascia using fibrin glue to seal the fistula, but no prosthesis was used for the purpose of hearing improvement.

Postoperatively, mild dizziness was observed, but rotatory vertigo and nystagmus disappeared. The dizziness gradually improved and the patient was discharged 12 days after surgery.

3. Discussion

PLF causes inner ear disorders due to perilymph leakage into the tympanic cavity. PLF can be associated with a congenital anomaly, postoperative ear complications, head trauma, or barotrauma, but is most often idiopathic. PLF presents with symptoms of hearing loss, tinnitus, vestibular vertigo or dizziness, popping sounds, streaming tinnitus, and fistula signs. However, it is often indistinguishable from other inner ear diseases.

In some cases of PLF, pneumolabyrinth (air in the inner ear) and liquid leakage into the tympanic cavity can be detected by high-resolution temporal bone CT or T2-weighted MRI [1]. Although the gold standard for PLF diagnosis is intraoperative microscopic or endoscopic visualization, PLF is difficult to identify even during surgery [2,3]. Bakhos et al. [6] and Vincent et al. [7] reported that perilymphatic leakages were identified in 8% and 5.5%, respectively, of cases of revision stapes surgery. Furthermore, in their studies, PLF was suspected preoperatively in 36 cases based on clinical symptoms, but fistula was observed only in 23 cases and in 13 of them, fistula was not diagnosed due to perioperative findings [7].

Proteomic analysis of inner ear proteins identified the unique properties of CTP [4]. CTP is a protein present in perilymph, but not in other body fluids such as cerebrospinal fluid (CSF), serum, saliva, or middle ear mucosa. Therefore, CTP may be considered a specific biochemical marker for perilymph [5].

The sensitivity of the CTP test is 92.3% from middle ear lavage fluid sampled after cochlear fenestration in cochlear implant surgery [8]. While its specificity is 98.2% from middle ear lavage of non-PLF cases without middle ear infections [9]. Analysis of middle ear lavage fluid sampled from patients with middle ear infections may provide false-positive results (e.g., specificity of 93.5%) because of the high protein concentration in the thick pus [9]. In this study, CTP was detected in approximately 1 μl of perilymph present in the middle ear cavity. This method may enable diagnosis of PLF from minimal amounts of leaked perilymph, which is difficult to detect by CT and MRI or perioperatively. This method is also less invasive, as lavage can be performed by myringotomy or puncture of the tympanic membrane.

Several authors have suggested identification of an endogenous perilymph marker such as beta-2 transferrin, beta trace protein, or

intrathecal fluorescein [10–12]. Although these markers are also detectable in inner ear fluid, PLF and CSF leakage can be difficult to distinguish because they are not organ specific.

In our case, the wire piston had transferred deep into the vestibule behind the long limb of the incus necrosis. Perilymph leakage occurred, leading to rotatory vertigo and deterioration of hearing. PLF was not initially suspected because 30 years had passed since stapes surgery, and typical symptoms of PLF were not present. In addition, effusion in the tympanic cavity was not detected on examination of the tympanic membrane. Thus, diagnosis of PLF was impossible by visual inspection alone or imaging techniques such as CT and MRI. The CTP detection test was the only method for detecting perilymph leakage in this case.

Our experience suggests that the CTP detection test can be a useful, highly sensitive, specific, and less invasive method to diagnose local manifestations of PLF.

Conflict of interest

The authors report no conflicts of interest.

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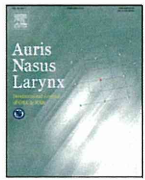

Cochlear implantation in a patient with Epstein syndrome

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ABSTRACT

Epstein syndrome is a rare disease which is accompanied by nephritis, sensorineural hearing impairment and macrothrombocytopenia. It has been suggested that this syndrome is a hereditary disease associated with mutations in MYH9, which encodes non-muscle myosin heavy chain IIA. We report a case of a patient with Epstein syndrome in whom bilateral profound hearing impairment developed and who had undergone cochlear implantation 9 years previously. Prior to this, the patient showed progressive sensorineural hearing impairment and had become completely deaf by the age of 25. A cochlear implant was successfully used with a speech discrimination score of 98% (sentence test). However, in the present case, peri- and postoperative complications occurred: tympanic perforation remained after a promontory stimulation test, followed by transitory otitis with purulent discharge. Therefore, tympanoplasty was performed simultaneously with cochlear implantation. These complications were considered to be caused by platelet dysfunction and delayed wound healing. Furthermore, cochlear destruction was observed 8 years postoperatively. In Epstein syndrome, the mechanism of osseous change remains uncertain. To the best of our knowledge, this is the first case report of Epstein syndrome in a patient with long-term use of a CI.

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1. Introduction

Epstein syndrome is a rare disease which is accompanied by nephritis, sensorineural hearing impairment and macrothrombocytopenia. It has been suggested that this syndrome is an autosomal hereditary disease associated with *MYH9* mutation, which is commonly seen in several types of macrothrombocytopenia such as May-Hegglin anomaly, Sebastian syndrome and Fechtner syndrome. Among these *MYH9*-related disorders, the presence of clinical symptoms varies according to the location of the mutation. Sensorineural hearing loss is one of the symptoms characteristic of Epstein syndrome. Hearing impairment can progress to complete deafness. We report a case of a patient with Epstein syndrome in whom bilateral profound hearing impairment developed and who had received cochlear implants (CIs) 9 years previously.

2. Case

The patient was first referred to our hospital at the age of 5 with epistaxis during investigation of idiopathic thrombocytopenic purpura. Her platelet count was 45,000/μl at that time, and 2 years later sensorineural hearing impairment was observed, although her hearing loss was mild and her recruitment phenomenon was positive. However, her hearing impairment worsened, and she was given conventional hearing aids. She had become completely deaf in her right ear by the age of 23 and in her left ear by the age of 25 (Fig. 1). During that time, she received a diagnosis of Epstein syndrome on the basis of pathological findings of a renal biopsy specimen, and of clinical symptoms such as hematuria, proteinuria, macrothrombocytopenia and sensorineural hearing loss diagnosed by pediatricians at another hospital at age 15.

Cochlear implantation was then considered owing to the limitations of hearing aids. Her computed tomography (CT) and magnetic resonance imaging findings were normal, and she showed sensitivity to a promontory stimulation test. However, a pinhole-sized perforation remained on the left tympanic membrane after the promontory stimulation test. Her platelet count decreased to 8000/μl and immunoglobulin was administered in an attempt to improve her blood dyscrasia, but it was ineffective. She had become antiplatelet antibody-positive owing to a previous platelet transfusion upon previously undergoing resection for an open cyst with endometriosis. Therefore, a human leukocyte antigen (HLA)-matched platelet transfusion was performed to prevent bleeding, and tranexamic acid was given. Her platelet count then increased to 67,000/μl. A Nucleus 24 (Cochlear Ltd., Lane Cove, Australia) CI was implanted in her left ear and myringoplasty was performed. Although her platelet count decreased to 37,000/μl after 3 days and to 14,000/μl after 11 days, perioperative bleeding was not observed. Furthermore,

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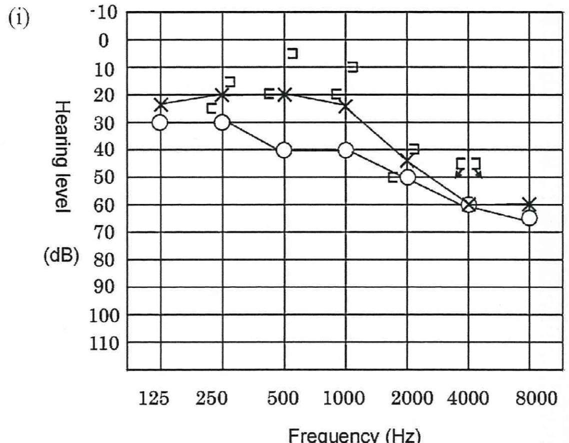


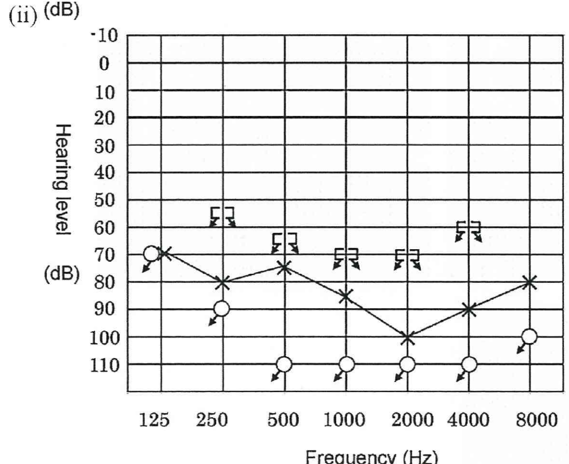


Fig. 1. Hearing levels at (i) 7 years old; (ii) 23 years old.

although the wound had healed by 4 months postoperatively, a perforation remained on the operated tympanic membrane. In addition, methicillin-resistant *Staphylococcus aureus* and a fungal infection developed and continued for 1 year (Fig. 2). However, despite the infection, the CI remained functional and her speech discrimination score showed improvement of up to 98% on a Japanese sentence recognition test. Her hearing threshold was stable between 30 and 35 dB with CIs.

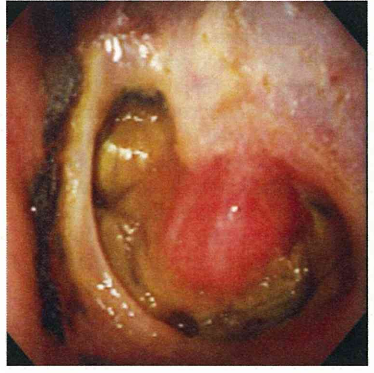
However, 7 years postoperatively, her word recognition score decreased to 76% and her MAP-threshold level (T-level) and maximum comfort level (C-level) were observed to fluctuate. Subsequently, electrode extrusion was confirmed by X-ray and CT imaging (Fig. 3), and the 2 electrodes at the apical end of the cochlea were switched off. Her speech perception was 80% (words) and 98% (sentences) at 9.3 years postoperatively.

In addition to these changes in her left ear, a perforation in the right tympanic membrane was observed 2 months postoperatively, but there was no evidence of infection in the right ear. Furthermore, cerumen accumulations were consistently observed, which were considered to be the cause of her enlarged external ear canal.

3. Discussion

Epstein syndrome was first reported in 1972 [1] and it was subsequently discovered that the disease is associated with mutations in *MYH9*, which encodes non-muscle myosin heavy chain IIA (NMMHC IIA) [2] [3]. NMMHC IIA is a type of non-muscle myosin that is distributed in many types of tissue [4]. These non-muscle myosin molecules contribute to maintaining the cytoskeleton and regulating cell adhesion, cell migration and cell division [5]. There are nearly 40 reported mutations in NMMHC IIA, some of which are considered to be associated with *MYH9*-related diseases. Epstein syndrome is one such *MYH9*-related disease, which is associated with mutations in exon 16. Hearing impairment is considered to be sensorineural because NMMHC IIA is present in the inner ear [4]. In the present case, sensorineural hearing impairment was prominent only in the high frequencies in the early stages, but progressed to bilateral severe hearing loss in all frequencies. The clinical course of the current case was consistent with Epstein syndrome [6]. However, the use of a CI was effective for the deafness due to Epstein syndrome in the present case. To the best of our knowledge, this is the first report on the long-term follow up of a CI in a patient with Epstein syndrome.

It has been reported that Epstein syndrome can be misdiagnosed as chronic autoimmune thrombocytopenia. It can be treated by splenectomy, immunosuppressive therapy and corticosteroid hormone therapy, but these treatments are presently considered to be ineffective in *MYH-9*-related diseases [6]. In the present case, Epstein syndrome was diagnosed on the basis of the clinical symptoms and findings of a renal biopsy specimen, but only after a previous period during which *MYH-9*-related diagnosis was suspected.



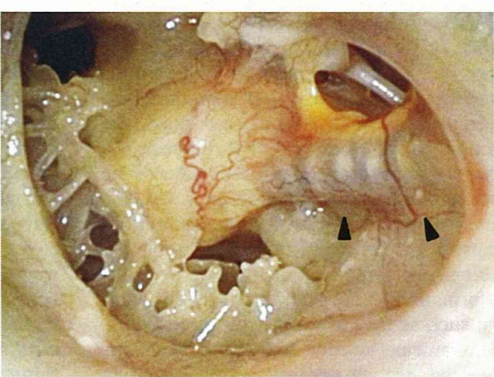


Fig. 2. Postoperative tympanic membrane. Yellowish otorrhoea can be observed in the left middle ear cavity. The electrode array, covered with fibrous tissue (arrowheads) was placed into the cochleostomy site between the round window niche and the stapedial muscle.

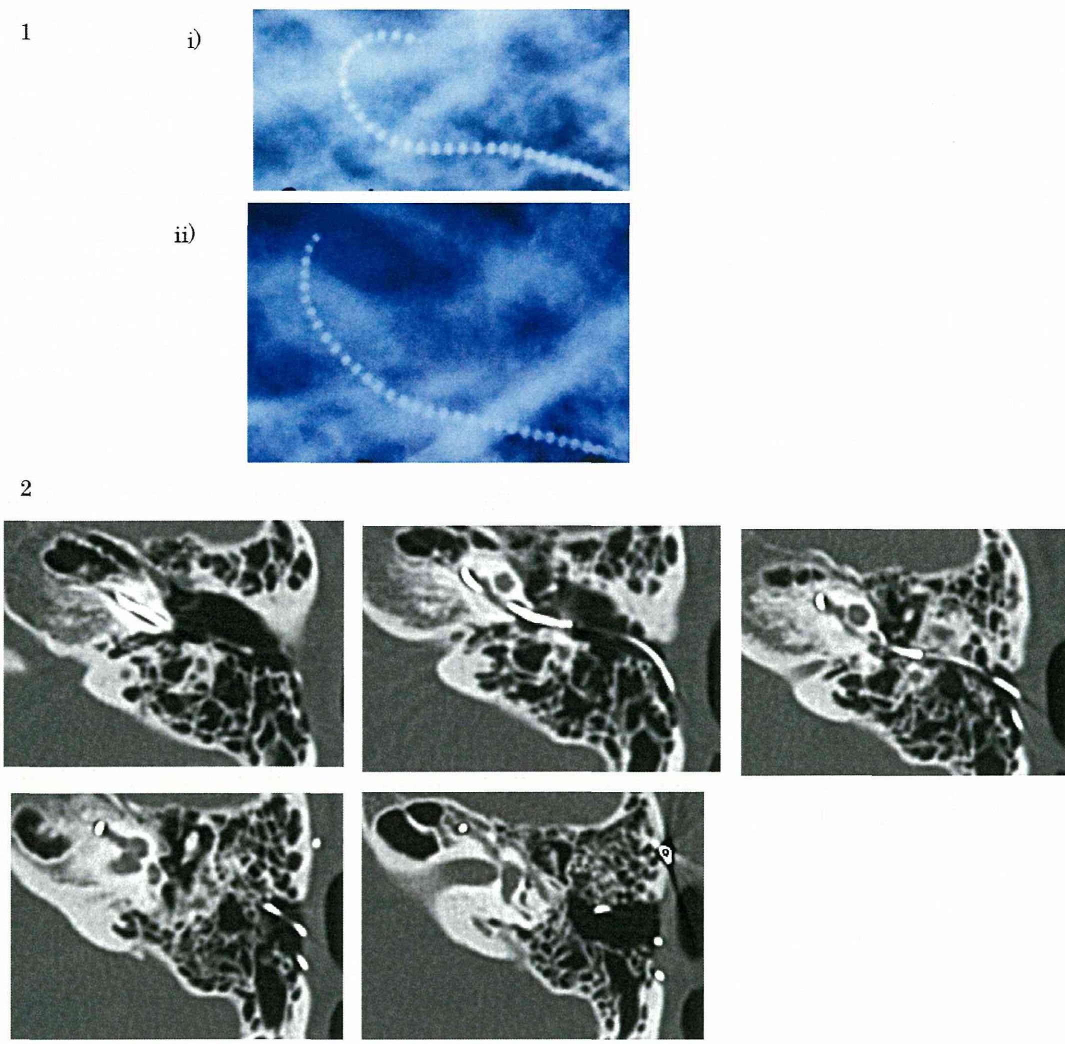


Fig. 3. (1) X-ray images of the electrode. At 4 years postoperatively, the electrode is confirmed to be in the cochlea. At 8 years postoperatively, the tip of the electrode had shifted. (2) Computed tomographic images of the electrode show osseous change in the cochlea and electrode extrusion.

In the present case, to prevent intraoperative bleeding, an HLA-matched platelet infusion was administered as this had been effective during a previous operation for endometriosis. However, as bleeding is often mild, platelet transfusion or intravenous globulin therapy may not be necessary to prevent perioperative bleeding in *MHY-9*-related diseases. In such cases, it is preferable to use desmopressin and tranexamic acid [6], but care should be taken regarding the potential risk of postoperative thrombosis [7].

In the current case, perforations remained on both sides of the tympanic membrane after promontory stimulation tests. Usually, such pinhole perforations spontaneously heal after promontory stimulation tests. The cause of the perforations in the current case appeared to be a hematological disorder. Impairment of hemostasis can result in coagula formation on the promontory, and is often followed by an infection such as that in the current case, which eventually caused the tympanic perforation to expand. It remains to be clarified if the wound healing process of the tympanic membrane is delayed by the impairment of NMMHC IIA, which has a role in cellular migration and adhesion.

Inner ear destruction after cochlear implantation is rare. In our institution, we have encountered a few cases of electrode extrusion with osteonecrosis in the cochlea following bacterial infection. It has also been speculated that chronic pressure to the outer wall of the cochlea by the electrode array of a CI can cause osseous changes. However, it remains unclear whether such osseous changes in the implanted cochlea (left) and enlarged external ear canal (right), as observed in the current case (Fig. 4), are disease-specific phenomena. A previous case report described osteoporotic changes and delayed bone age assessment in Epstein syndrome [8]. However, despite these difficulties, CIs have been shown to be an effective option for the deafness which occurs in Epstein syndrome. Considering the disruption of the inner ear and tympanic membrane, surgical repair procedures such as bilateral implantation, fistula obliteration with re-implantation of a curved electrode and myringoplasty are treatment options. However, at this stage it is crucial to prevent the occurrence of infection in the ear, and re-operation should be considered if written informed consent can be obtained.