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．

文献/URL

1) Morton, C. C. and Nance, W. E.: *N. Engl. J. Med.*, **354**: 2154-2164, 2006.
2) Van Camp, G. et al.: Hereditary Hearing loss Homepage. (http://hereditaryhearingloss.org)
3) Ohtsuka, A. et al.: *Hum. Genet.*, **112**: 329-333, 2003.
4) Oguchi, T. et al.: *J. Hum. Genet.*, **50**: 76-83, 2005.
5) Suzuki, H. et al.: *Acta Otolaryngol.*, **127**: 1292-1297, 2007.
6) Wagatsuma, M. et al.: *Clin. Genet.*, **72**: 339-344, 2007.
7) Usami, S. et al.: *Acta Otolaryngol.*, **128**: 446-454, 2008.
8) Usami, S. et al.: *PLoS ONE*, **7**: e31276, 2012.
9) Hildebrand, M. S. et al.: *Laryngoscope*, **119**: 2211-2215, 2009.
10) Usami, S. et al.: *Acta Otolaryngol.*, **132**: 377-384, 2012.
11) Tsukada, K. et al.: *Clin. Genet.*, **78**: 464-470, 2010.
12) Akita, J. et al.: *J. Hum. Genet.*, **46**: 355-361, 2001.
13) Moteki, H. et al.: *J. Hum. Genet.*, **57**: 587-592, 2012.
14) Fukuoka, H. et al.: *J. Hum. Genet.*, **52**: 510-515, 2007.
15) Lu, S. Y. et al.: *Clin. Genet.*, **75**: 480-484, 2009.
16) Yoshimura, H. et al.: *Int. J. Pediatr. Otorhinolaryngol.*, **77**: 298-302, 2013.
17) Usami, S. et al.: *J. Hum. Genet.*, **44**: 304-307, 1999.
18) Shearer, A. E. et al.: *Proc. Natl. Acad. Sci. USA*, **107**: 21104-21109, 2010.
19) Lin, X. et al.: *Hear Res.*, **288**: 67-76, 2012.
20) De Keulenaer, S. et al.: *BMC Med. Genomics*, **5**: 17, 2012.
21) Walsh, T. et al.: *Am. J. Hum. Genet.*, **87**: 90-94, 2010.
22) Gao, J. et al.: Whole exome sequencing identifies a novel DFNA9 mutation, C162Y. *Clin. Genet.*, 2012, Aug. 29. (Epub ahead of print)
23) Diaz-Horta, O. et al.: *PLoS ONE*, **7**: e50628, 2012.

International Journal of Pediatric Otorhinolaryngology 77 (2013) 674–676

Contents lists available at SciVerse ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

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

Haruka Hirai, Shin Kariya *, Mitsuhiro Okano, Kunihiro Fukushima, Yuko Kataoka, Yukihide Maeda, Kazunori Nishizaki

Department of Otolaryngology-Head and Neck Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

ARTICLE INFO

Article history:
Received 7 October 2012
Received in revised form 6 January 2013
Accepted 9 January 2013
Available online 4 February 2013

Keywords:
Otitis
Ear
Infection
Innate immunity

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.

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).

* Corresponding author at: 2-5-1, Shikata-cho, Kita-ku, Okayama 700-8558, Japan. Tel.: +81 862357307; fax: +81 862357308.
E-mail address: skariya@cc.okayama-u.ac.jp (S. Kariya).

0165-5876/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijporl.2013.01.010

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™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

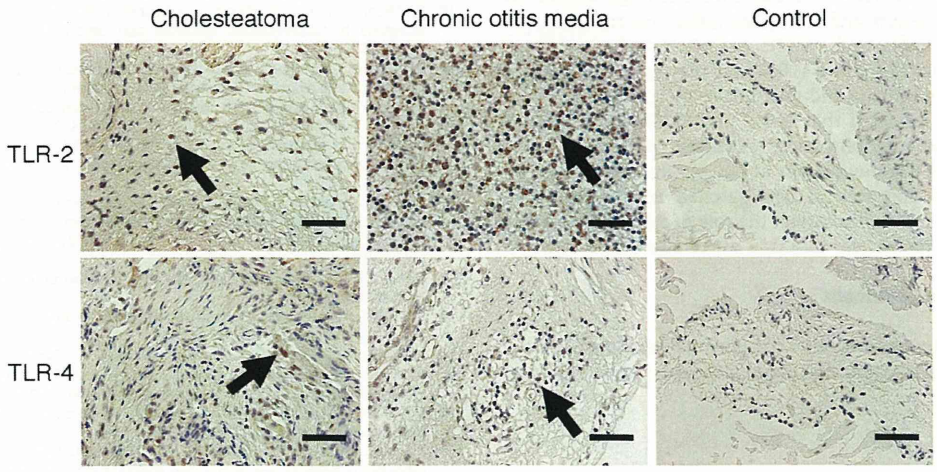

Fig. 1. The expressions of toll-like receptors 2 and 4 in patients with chronic otitis media and with cholesteatoma. Positive immunostaining of toll-like receptors (arrow) is observed both in chronic otitis media and in cholesteatoma. The normal control middle ear subjects show no positive cells (TLR-2,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

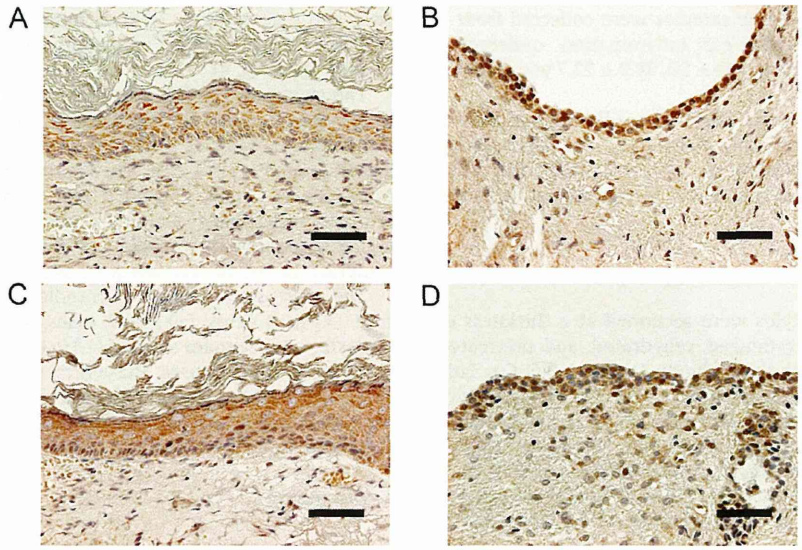

Fig. 2. Positive immunohistochemical staining for toll-like receptors is observed in middle ear mucosa of chronic otitis media and cholesteatoma epithelium. (A) Toll-like receptor 2 in cholesteatoma. (B) Toll-like receptor 2 in chronic otitis media. (C) Toll-like receptor 4 in cholesteatoma. (D) Toll-like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

Acknowledgement

This work was supported by Grants-in-Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] Y.C. Lee, C. Kim, J.S. Shim, J.Y. Byun, M.S. Park, C.I. Cha, et al., Toll-like receptors 2 and 4 and their mutations in patients with otitis media and middle ear effusion, Clin. Exp. Otorhinolaryngol. 1 (2008) 189–195.
[2] S. Kariya, M. Okano, K. Fukushima, S. Nomiya, Y. Kataoka, R. Nomiya, et al., Expression of inflammatory mediators in the otitis media induced by Helicobacter pylori antigen in mice, Clin. Exp. Immunol. 154 (2008) 134–140, Erratum in Clin. Exp. Immunol. 154 (2008) 432.
[3] S. Kariya, M. Okano, K. Aoji, M. Kosaka, E. Chikumoto, H. Hattori, et al., Role of macrophage migration inhibitory factor in otitis media with effusion in adults, Clin. Diagn. Lab. Immunol. 10 (2003) 417–422.
[4] M. Szczepański, W. Szyfter, R. Jenek, M. Wróbel, I.M. Lisewska, J. Zeromski, Toll-like receptors 2, 3 and 4 (TLR-2, TLR-3 and TLR-4) are expressed in the microenvironment of human acquired cholesteatoma, Eur. Arch. Otorhinolaryngol. 263 (2006) 603–607.
[5] A. Leichtle, Y. Lai, B. Wollenberg, S.I. Wasserman, A.F. Ryan, Innate signaling in otitis media: pathogenesis and recovery, Curr. Allergy Asthma Rep. 11 (2011) 78–84.
[6] E. Kopp, R. Medzhitov, Recognition of microbial infection by Toll-like receptors, Curr. Opin. Immunol. 15 (2003) 396–401.
[7] L.R. Prince, M.K. Whyte, I. Sabroe, L.C. Parker, The role of TLRs in neutrophil activation, Curr. Opin. Pharmacol. 11 (2011) 397–403.
[8] K. Chen, J. Huang, W. Gong, P. Iribarren, N.M. Dunlop, J.M. Wang, Toll-like receptors in inflammation, infection and cancer, Int. Immunopharmacol. 7 (2007) 1271–1285.
[9] T.F. DeMaria, M.A. Apicella, W.A. Nichols, E.R. Leake, Evaluation of the virulence of nontypeable *Haemophilus influenzae* lipooligosaccharide htrB and rfaD mutants in the chinchilla model of otitis media, Infect. Immun. 65 (1997) 4431–4435.
[10] S. Kariya, P.A. Schachern, S. Cureoglu, V. Tsuprun, M. Okano, K. Nishizaki, et al., Up-regulation of macrophage migration inhibitory factor induced by endotoxin in experimental otitis media with effusion in mice, Acta Otolaryngol. 128 (2008) 750–755.
[11] S. Kariya, M. Okano, T. Higaki, S. Makihara, T. Haruna, M. Eguchi, et al., Neutralizing antibody against granulocyte/macrophage colony-stimulating factor inhibits otitis media, Laryngoscope (November) (2012), http://dx.doi.org/10.1002/lary.23795 (Epub ahead of print).
[12] K.H. Mills, TLR-dependent T cell activation in autoimmunity, Nat. Rev. Immunol. 11 (2011) 807–822.
[13] J.H. Boyd, Toll-like receptors and opportunities for new sepsis therapeutics, Curr. Infect. Dis. Rep. 14 (2012) 455–461.
[14] M. Mullarkey, J.R. Rose, J. Bristol, T. Kawata, A. Kimura, S. Kobayashi, et al., Inhibition of endotoxin response by e5564, a novel Toll-like receptor 4-directed endotoxin antagonist, J. Pharmacol. Exp. Ther. 304 (2003) 1093–1102.
[15] D.P. Rossignol, M. Lynn, TLR4 antagonists for endotoxemia and beyond, Curr. Opin. Investig. Drugs 6 (2005) 496–502.
[16] A.C. Kalil, S.P. LaRosa, J. Gogate, M. Lynn, S.M. Opal, Eritoran Sepsis Study Group, Influence of severity of illness on the effects of eritoran tetrasodium (E5564) and on other therapies for severe sepsis, Shock 36 (2011) 327–331.
[17] Y. Si, Z.G. Zhang, C.Y. Huang, J.F. He, L.Q. Feng, Y.B. Chen, et al., Differential expression of toll-like receptors in chronic suppurative otitis media and cholesteatoma, Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 47 (2012) 388–393 (in Chinese).

Auris Nasus Larynx 40 (2013) 422–424

Contents lists available at SciVerse ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

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

Yuko Kataoka [a,*], Tetsuo Ikezono [b], Kunihiro Fukushima [a], Koji Yuen [c], Yukihide Maeda [a], Akiko Sugaya [a], Kazunori Nishizaki [a]

[a] Department of Otorhinolaryngology, Head & Neck Surgery, Okayama University Postgraduate School of Medicine, Dentistry and Pharmaceutical Science, Japan
[b] Department of Otolaryngology, Saitama Medical University, Japan
[c] Yuen Clinic, Japan

ARTICLE INFO

Article history:
Received 14 March 2012
Accepted 14 September 2012
Available online 18 October 2012

Keywords:
Perilymphatic fistula
Stapes surgery
Cochlin
Cochlin-tomoprotein

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.

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.

* Corresponding author at: Department of Otorhinolaryngology, Head & Neck Surgery, Okayama University Postgraduate School of Medicine, Dentistry and Pharmaceutical Science, 2-5-1 Shikata-cho, Kita-ku, Okayama City, Okayama 700-8558, Japan. Tel.: +81 86 235 7307; fax: +81 86 235 7308.
E-mail address: yu-kat@cc.okayama-u.ac.jp (Y. Kataoka).

0385-8146/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.anl.2012.08.001

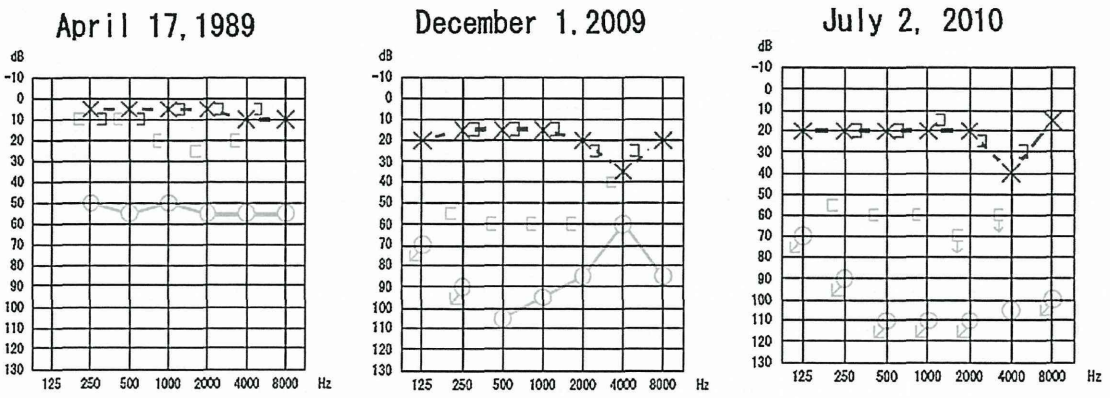

Fig. 1. Audiogram. Hearing levels in December 2009 where lower than those in 1989. In July 2010, vertigo developed, and hearing deteriorated further.

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.

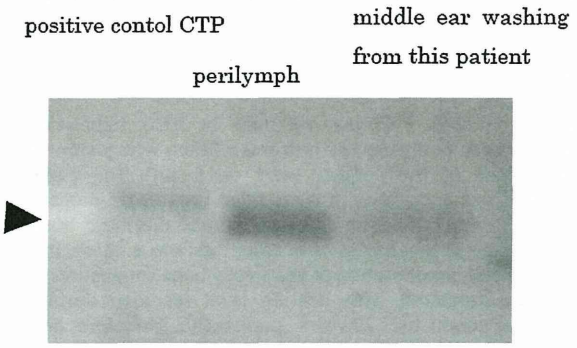

Fig. 2. Detection of cochlin-tomoprotein (CTP) in the middle ear washings. Signals represent CTP in recombinant positive control CTP (left), perilymph (middle), and middle ear washings from the patient (right) by Western blotting.

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.

Acknowledgements

This work was partly supported by a grant from the Ministry of Health, Labor, and Welfare, Japan and the Ministry of Education, Culture, Sports, Science, and Technology, Japan. The authors would like to thank Enago (http://www.enago.jp) for the English language review.

References

[1] Nishizaki K, Yamamoto T, Akagi H, Ogawa T, Masuda Y. Imaging case of the month. Pneumolabyrinth. Am J Otol 1998;19:860–1.
[2] Ogawa K, Kanzaki J, Ogawa S, Tsuchihashi N, Inoue Y, Yamamoto M. Endoscopic diagnosis of idiopathic perilymphatic fistula. Acta Otolaryngol Suppl 1994;514:63–5.
[3] Poe DS, Rebeiz EE, Pankratov MM. Evaluation of perilymphatic fistulas by middle ear endoscopy. Am J Otol 1992;13:529–33.
[4] Ikezono T, Shindo S, Li L, Omori A, Ichinose S, Watanabe A, et al. Identification of a novel Cochlin isoform in the perilymph: insights to Cochlin function and the pathogenesis of DFNA9. Biochem Biophys Res Commun 2004;314:440–6.
[5] Ikezono T, Shindo S, Sekiguchi S, Hanprasertpong C, Li L, Pawankar R, et al. Cochlin-tomoprotein: a novel perilymph-specific protein and a potential marker for the diagnosis of perilymphatic fistula. Audiol Neurootol 2009;14:338–44.
[6] Bakhos D, Lescanne E, Charretier C, Robier A. A review of 89 revision stapes surgeries for otosclerosis. Eur Ann Otorhinolaryngol Head Neck Dis 2010;127:177–82.
[7] Vincent R, Rovers M, Zingade N, Oates J, Sperling N, Devèze A, et al. Revision stapedotomy: operative findings and hearing results. A prospective study of 652 cases from the Otology–Neurotology Database. Otol Neurotol 2010;31:875–82.
[8] Ikezono T, Sugizaki K, Shindo S, Sekiguchi S, Pawankar R, Baba S, et al. CTP (cochlin-tomoprotein) detection in the profuse fluid leakage (gusher) from cochleostomy. Acta Otolaryngol 2010;130:881–7.
[9] Ikezono T, Shindo S, Sekiguchi S, Morizane T, Pawankar R, Watanabe A, et al. The performance of cochlin-tomoprotein detection test for the diagnosis of perilymphatic fistula. Audiol Neurootol 2009;15:168–74.
[10] Buchman CA, Luxford WM, Hirsch BE, Fucci MJ, Kelly RH. Beta-2 transferrin assay in the identification of perilymph. Am J Otol 1999;20:174–8.
[11] Bachmann-Harildstad G, Stenklev NC, Myrvoll E, Jablonski G, Klingenberg O. β-Trace protein as a diagnostic marker for perilymphatic fluid fistula: a prospective controlled pilot study to test a sample collection technique. Otol Neurotol 2011;32:7–10.
[12] Gehrking E, Wisst F, Remmert S, Sommer K. Intraoperative assessment of perilymphatic fistulas with intrathecal administration of fluorescein. Laryngoscope 2002;112:1614–8.

Auris Nasus Larynx 40 (2013) 409–412

Contents lists available at SciVerse ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Cochlear implantation in a patient with Epstein syndrome

Nobuhiro Nishiyama *, Atsushi Kawano, Sachie Kawaguchi, Kyouko Shirai, Mamoru Suzuki

Department of Otorhinolaryngology, Tokyo Medical University, Tokyo, Japan

ARTICLE INFO

Article history:
Received 23 February 2012
Accepted 11 July 2012
Available online 31 July 2012

Keywords:
Cochlear implantation
Epstein syndrome
Cochlear destruction

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.

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,

* Corresponding author at: Department of Otorhinolaryngology, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan. Tel.: +81 3 3342 6111x5788.
E-mail address: nobuoto@tokyo-med.ac.jp (N. Nishiyama).

0385-8146/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.anl.2012.07.005

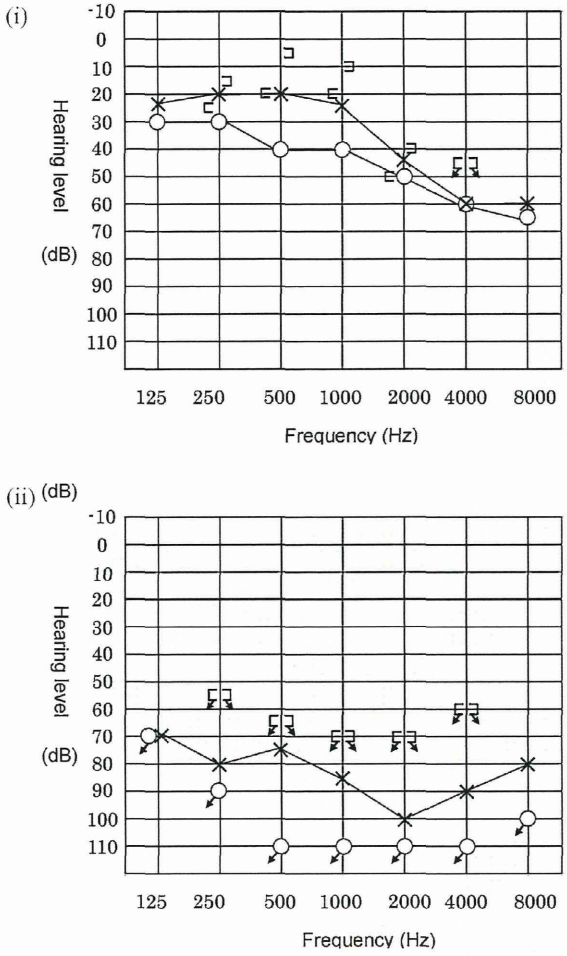

Fig. 1. Hearing levels at (i) 7 years old; (ii) 23 years old.

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.

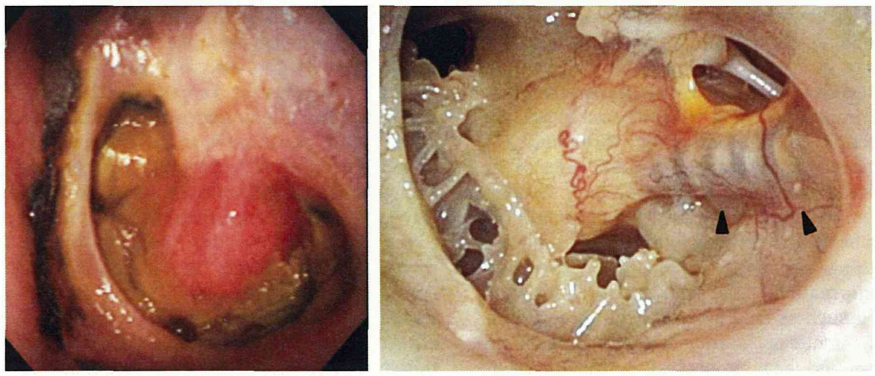

Fig. 2. Postoperative tympanic membrane. Yellowish otorrhoea can be observed in the left middle ear cavity. The electrode array, covered with fibrous tissue (arrowheads) was placed into the cochleostomy site between the round window niche and the stapedial muscle.

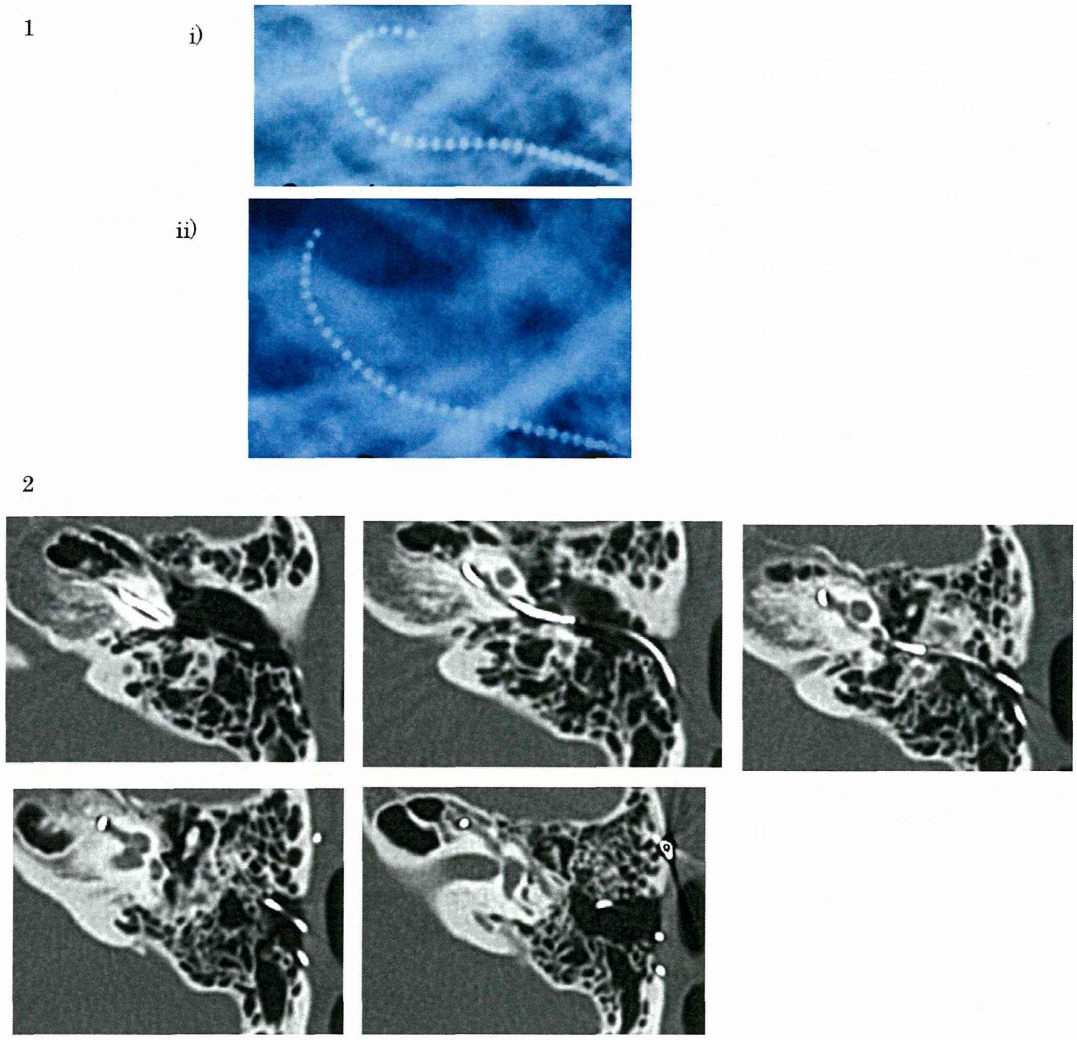

Fig. 3. (1) X-ray images of the electrode. At 4 years postoperatively, the electrode is confirmed to be in the cochlea. At 8 years postoperatively, the tip of the electrode had shifted. (2) Computed tomographic images of the electrode show osseous change in the cochlea and electrode extrusion.

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.