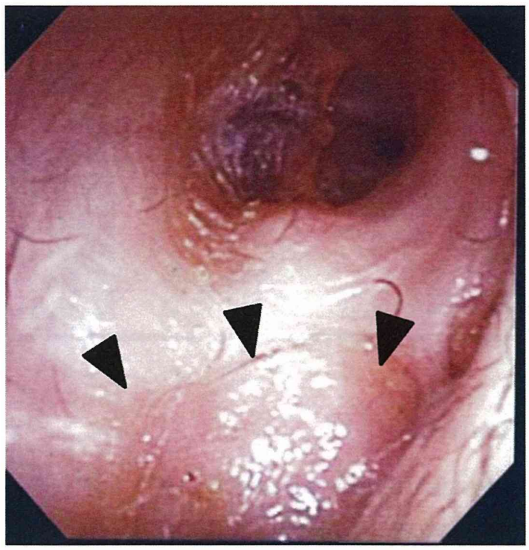

**Fig. 4.** The right external ear canal. At 8 years from implantation, the external canal widened.

## Conflict of interest statement

All authors declare that there are no conflicts of interest associated with this manuscript.

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## References

[1] Epstein CJ, Sahud MA, Piel CF, Goodman JR, Bernfield MR, Kushner JH, et al. Hereditary macrothrombocytopathia, nephritis and deafness. Am J Med 1972;52:299–310.
[2] Kunishima S, Kojima T, Matsushita T, Tanaka T, Tsurusawa M, Furukawa Y, et al. Mutations in the NMMHC-A gene cause autosomal dominant macrothrombocytopenia with leukocyte inclusions (May-Hegglin anomaly/Sebastian syndrome). Blood 2001;97:1147–9.
[3] Heath KE, Campos-Barros A, Toren A, Rozenfeld-Granot G, Carlsson LE. Nonmuscle myosin heavy chain IIA mutations define a spectrum of autosomal dominant macrothronbocytopenias: May-Hegglin anomaly and Fechtner, Sebastian, Epstein, and Alport-like syndromes. Am J Hum Genet 2001;69:1033–45.
[4] Marigo V, Nigro A, Pecci A, Montanaro D, Stazio MD, Balduini CL, et al. Correlation between the clinical phenotype of MYH9-related disease and tissue distribution of class II nonmuscle myosin heavy chains. Genomics 2004;83:1125–33.
[5] Vicente-Manzanares M, Ma X, Adelstein RS, Horowitz AR. Non-muscle myosin II takes centre stage in cell adhesion and migration. Nature 2009;10:778–90.
[6] Althaus K, Najm J, Greinacher A. MYH9-related platelet disorders – often unknown and misdiagnosed. Klin Padiatr 2011;223:120–5.
[7] Selleng K, Lubenow LE, Greinacher A, Warkentin TE. Perioperative management of *MYH9* hereditary macrothrombocytopenia (Fechtner syndrome). Eur J Haematol 2007;79:263–8.
[8] Alhindawi E, Al-Jbour S. Epstein syndrome with rapid progression to end stage renal disease. Saudi J Kidney Dis Transpl 2009;20:1076–8.

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# Long-term complications after cochlear implantation

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ABSTRACT

*Objective:* Cochlear implantation has become an effective treatment for many profoundly deaf patients. As with any surgical procedure, a proportion of patients suffer postoperative complications. The aim of this study was to analyze long-term postoperative complications in patients with cochlear implants with a view to improve clinical interventions and propose a consensus for reporting complications.

*Methods:* A total of 406 cases received cochlear implants between December 1985 and April 2007 at Tokyo Medical University (TMU) Hospital. We retrospectively reviewed case notes from 366 patients who had undergone cochlear implantation (215 adults and 151 children) after excluding 40 patients of re-implantation including 13 cases implanted initially at other hospitals. Life-threatening, major and minor complications were examined retrospectively.

*Results:* Major complications occurred following cochlear implantation in 32 patients (8.7%) who had received their initial implant at TMU Hospital. Revision surgery was required for 30 patients. The mean age at implantation was 33 years 6 months (range, 1 year 9 months to 83 years; median, 37 years). The main etiology of deafness was unknown or progressive (113, 52.6%) in adults and congenital (132, 87.4%) in children. The cause of deafness was meningitis in 41 cases (11.2%), and 26 cases (7.1%) were diagnosed with idiopathic sudden deafness.

Flap-related problems (including middle ear infection and/or flap necrosis) developed in 13 cases (3.6%), with 12 cases (7 adults, 5 children) requiring re-implantation. Electrode slip-out occurred in 8 patients (7 adults, 1 child). All adult cases in whom electrodes slipped out underwent implantation before 1994, while the child (1 pediatric case) was operated in 2003. All cases required re-implantation and most cochlear implantations were performed using the modified split-bridge technique after 1997. Six patients (4 adults, 2 children) experienced device failure. Four patients experienced electrode problems. Non-surgical major complications included 1 patient with permanent facial nerve paralysis as a result of thermal injury in 1995.

The total number of minor medical and surgical complications was 27, representing 7.4% of all operations.

*Conclusion:* Many cases of major complications, including electrode problems and facial paralysis, excluding traumatic device failure were considered avoidable by strict operative and postoperative procedures. Some cases of flap infection and traumatic device failure may not be able to be avoided completely, and every possible care should be taken by implant patients and others involved.



## 1. Introduction

Cochlear implantation is a well-established and effective treatment for many profoundly deaf patients. As with any surgical procedure, a proportion of patients experience postoperative complications. Previous studies have examined the incidence of major and minor complications following cochlear implantation. A number of reports [1–6; see Table 1] in the literature have described surgical complications following cochlear implantation in large series.

Major complications of cochlear implant surgery include electrode failure, meningitis, problems such as skin infection at the implant site, middle ear infection requiring revision surgery because of flap necrosis, and severe sequelae such as permanent facial paralysis. Minor complications are those that can be managed conservatively with medical or audiological interventions, such as wound infection or non-auditory stimulations.

Over 25 years, we have performed multichannel cochlear implantation in more than 500 cases. Postoperative courses were satisfactory in the vast majority of cases, although some postoperative problems were encountered in the 366 cochlear implant patients who were operated at Tokyo Medical University (TMU) Hospital between December 1985 and April 2007, with an implantation duration of more than 5 years.

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**Table 1**
Major and minor complications in prior studies.

| | Webb et al. [2] | Hoffman et al. [3] | | Bhatia et al. [4] | Green et al. [5] | Stratigouleas et al. [6] |
|---|---|---|---|---|---|---|
| Year of publications | 1991 | 1995 | | 2004 | 2004 | 2006 |
| | | Adults | Children | Children | Adults | |
| Number | 153 | 3064 | 1905 | 300 | 240 | 176 |
| Major | 26 (17.0%) | 208 (6.8%) | 79 (4.1%) | 7 (2.3%) | 15 (6.3%) | 7 (4.0%) |
| Flap related problem (incl. implant extrusion, skin infection, etc.) | 13 (8.5%) | 109 (3.6%) | 30 (1.6%) | 1 (0.3%) | 11 (4.6%) | |
| Electrode problem (incl. ele. failure, ele. migration, ele. slip-out, non-auditory stimulation, ele. array exposure) | 10 (6.5%) | 82 (2.7%) | 38 (1.6%) | 1 (0.3%) | 11 (4.6%) | |
| Facial paralysis (permanent) | 3 (2.0%) | 17 (0.6%) | 11 (0.6%) | | | 1 (0.6%) |
| Ear infection (incl. middle ear and external ear infection, mastoiditis, excl. flap infection) | | | | 2 (0.7%) | | 2 (1.1%) |
| Cholesteatoma | | | | 3 (1.0%) | | |
| Minor | | | | 33 (11.0%) | | |
| Facial stimulation | 1 (0.7%) | | | | | |
| Facial palsy (transient) | | | | 2 (0.7%) | 1 (0.42%) | 3 (1.7%) |
| Balance problem (permanent) | | | | | 3 (1.3%) | 4 (2.2%) |
| Flap infection | | | | 13 (4.3%) | | 2 (1.1%) |
| Minor electrode reposition | | | | | | 2 (1.1%) |
| Altered facial sensation | | | | | 4 (1.7%) | |
| Posterior cal wall defect | | | | 9 (3.0%) | | |
| Hematoma or flap swelling | | | | 14 (4.6%) | | |

## 2. Materials and methods

A total of 406 cases received cochlear implants between December 1985 and April 2007 at TMU Hospital and have been using cochlear implants for longer than 5 years. The data required for this study were collected by a retrospective review of case notes from 366 patients who had undergone cochlear implantation, excluding 40 patients of re-implantation. Seventeen patients had undergone initial implantation at other hospitals (Fig. 1).

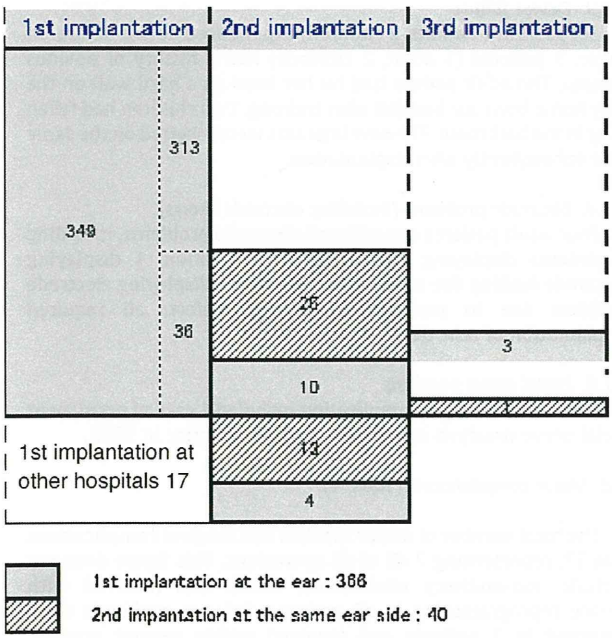


**Fig. 1.** Background of 406 cases. A total of 406 cases received cochlear implants between December 1985 and April 2007 at TMU Hospital and have been using cochlear implants for longer than 5 years. The data required for this study were collected by a retrospective review of case notes from 366 patients who had undergone cochlear implantation, excluding 40 patients of re-implantation. Seventeen patients had undergone initial implantation at other hospitals.

Life-threatening, major and minor complications were examined retrospectively.

## 3. Results

A total of 366 patients were included in this study comprising 215 adults and 151 children (including 120 infants). Patients included 190 females and 176 males. The mean age at implantation was 33 years 6 months (range, 1 year 9 months to 83 years; median, 37 years) (Fig. 2). The main etiology of deafness was unknown or progressive (113, 52.6%) in adults and congenital (132, 87.4%) in children. The cause of deafness was meningitis in 41

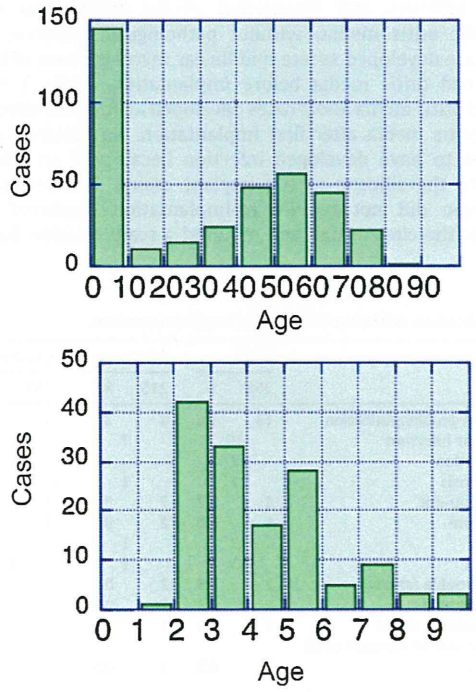


**Fig. 2.** Age at implantation (the bottom figure shows up to 10 years).

**Table 2**
Etiology of deafness.

| | Total | Total % | Adults | Children |
|---|---|---|---|---|
| Congenital | 140 | 38.3 | 8 | 132 |
| Unknown/progressive | 115 | 31.4 | 113 | 2 |
| Sudden deafness | 26 | 7.1 | 26 | |
| Meningitis | 41 | 11.2 | 27 | 14 |
| Ototoxicity | 20 | 5.5 | 20 | |
| Virus infection | 6 | 1.6 | 3 | 3 |
| Otitis media | 4 | 1.1 | 4 | |
| Trauma | 7 | 1.9 | 7 | |
| Others | 7 | 1.9 | 7 | |
| Total | 366 | | 215 | 151 |

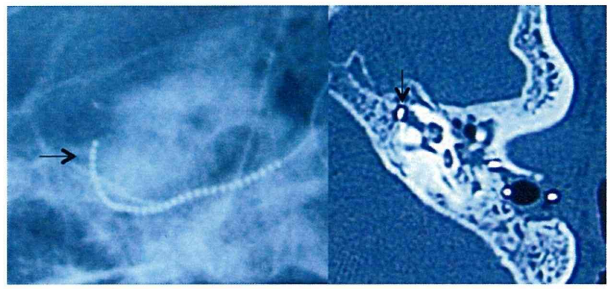

**Fig. 3.** X-ray and CT of the case of bone breaking around the cochlear with infection.

cases (11.2%), and 26 cases (7.1%) were diagnosed with idiopathic sudden deafness. The full range of etiologies for patient deafness is shown in Table 2. The majority (335, 91.5%) of patients received multichannel Nucleus devices, 20 received Advanced Bionic devices, and 11 received Med-El devices. The breakdown of devices was as follows: 1, CI22; 148, CI22M; 167, CI24M; 19, Clarion S; 1, Clarion CII; 10, C40+ and 1, C40+S.

### 3.1. Major complications (Table 3)

Major complications occurred following cochlear implantation in 32 patients (8.7%) operated initially at TMU Hospital. Revision surgery was required for 30 patients.

#### 3.1.1. Flap-related problems (including middle ear infection and/or flap necrosis)

Flap problems and/or infection developed in 13 cases (3.6%), with 12 cases (7 adults, 5 children) requiring re-implantation. All 7 adults had a previous history of otitis media before surgery, while 2 of them experienced bone breaking around the cochlea, with middle ear infection after first implantation (Fig. 3). One case had repeated infection and granulation of the middle ear (e.g., eosinophilic otitis media) without pathological evidence, and another case developed severe middle ear mycosis. None of the 5 children had otitis media before implantation, while 3 cases developed otitis media and 2 cases developed an R/S skin infection without otitis media after first implantation. No children were considered to have developed infection because of an allergic reaction to the silicone of the implant casing. The remaining patient who did not require re-implantation displayed flap necrosis at the closure line and required a scalp rotation flap to cover the implant package. No further implantation surgery has been proposed.

As for the origin bacteria, *Staphylococcus aureus* was detected in six cases, methicillin-resistant *Staphylococcus aureus* in three and *Aspergillus* in one. The period before infection occurred after operations ranged from 3 years to 20 years in adults and from 1 month to 6 years in children.

**Table 3**
Major complications following 366 cochlear implant operations.

| | Total | | Adults | | Children | |
|---|---|---|---|---|---|---|
| | 366 | % | 215 | % | 151 | % |
| Flap related problem/infection | 13 | 3.6 | 8 | 3.7 | 5 | 3.3 |
| Middle ear infection | 10 | | 7 | | 3 | |
| Flap infection | 2 | | | | 2 | |
| Flap necrosis | 1 | | 1 | | | |
| Electrode slip-out | 8 | 2.2 | 7 | 3.3 | 1 | 0.7 |
| Device failure | 6 | 1.6 | 2 | 0.9 | 4 | 2.6 |
| Trauma | 3 | | 1 | | 2 | |
| Unknown | 3 | | 1 | | 2 | |
| Electrode trouble (shorts) | 2 | 0.5 | 2 | 0.9 | | |
| Electrode kinking | 1 | 0.3 | 1 | 0.5 | | |
| Electrode trouble at the posterior wall of external canal | 1 | 0.3 | 1 | 0.5 | | |
| Facial palsy | 1 | 0.3 | 1 | 0.5 | | |
| | 32 | 8.7 | 22 | 10.3 | 10 | 6.6 |

#### 3.1.2. Electrode slip-out

Electrode slip-out occurred in 8 patients (7 adults, 1 child). All adult cases underwent implantation before 1994, while the child (1 pediatric case) was operated in 2003. All cases needed re-implantation and most cochlear implantations were performed using the modified split-bridge technique [7] after 1997. The pediatric case involved a 5-year-old boy with inner ear malformation who received implantation of Clarion S without the modified split-bridge technique and re-implantation was performed 2 years later.

#### 3.1.3. Device failure

Six patients (4 adults, 2 children) experienced device failure. Of these, 3 patients (1 adult, 2 children) had a history of obvious trauma. The adult patient had hit her head on a hard wall on the way home from our hospital after training. Two children had fallen over in the bathroom. The new implants were inserted on the same side subsequently after explantation.

#### 3.1.4. Electrode problems (including electrode shorts)

Four adult patients experienced electrode problems, including 2 patients displaying non-electrical stimulation, 1 displaying electrode kinking due to the operation, and 1 displaying electrode problem due to posterior canal wall defect; all required implantation of new devices.

#### 3.1.5. Facial nerve paralysis

Non-surgical major complication included 1 case of permanent facial nerve paralysis as a result of thermal injury in 1995.

### 3.2. Minor complications (Table 4)

The total number of minor medical and surgical complications was 27, representing 7.4% of all operations. This figure does not include non-auditory stimulation, which was resolved with device reprogramming in all patients. Balance problems were observed in 3 patients and resolved within several months. Surgical flap thinning and/or temporal skin changes were observed in 4 patients, including 2 patients with subcutaneous abscess. These minor complications were all resolved with local treatment and intravenous antibiotics. One patient developed partial facial palsy (the result of a thermal injury suffered whilst

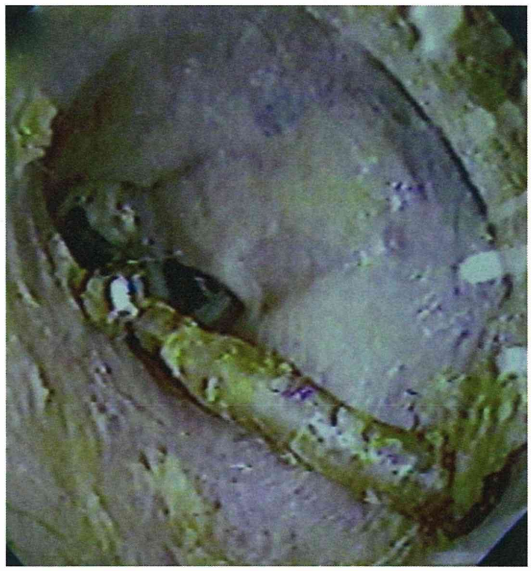

**Fig. 4.** A case of extrusion of a silver ball electrode to the external ear canal.

grading using a drill burr) that lasted for 1 month and then resolved completely. Other complications included 1 pediatric case of hematoma after surgery, 2 cases of temporary facial weakness, and 1 case of extrusion of a silver ball electrode to the external ear canal (Fig. 4). Magnet extrusion from the R/S package required repositioning in the operating theater in a 9-year-old girl (Fig. 5), but R/S migration in 1 adult patient after subcutaneous abscess did not require repositioning. The full list of minor complications is shown in Table 4.

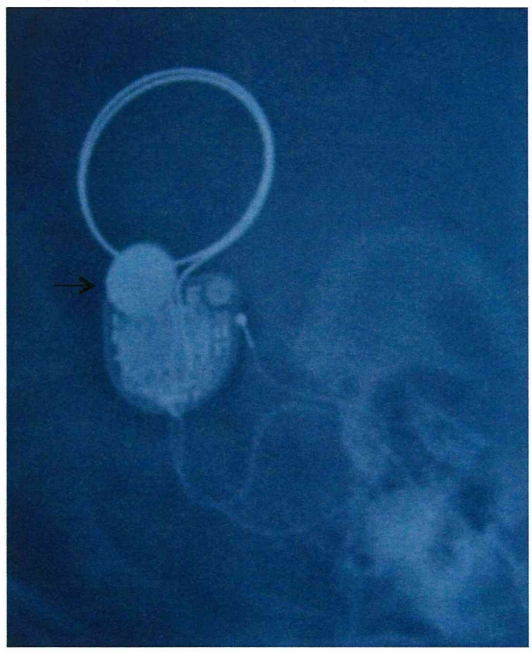

**Fig. 5.** X-ray of magnet extrusion from the R/S package after abscess magnet extrusion from the R/S package required repositioning in the operating theater in a 9-year-old girl.

**Table 4**
Minor complications following 366 cochlear implant operation.

| | Total | | Adults | | Children | |
|---|---|---|---|---|---|---|
| | 366 | % | 215 | % | 151 | % |
| Change in taste | 12 | 3.3 | 12 | 5.6 | | |
| Skin thinning and wound | 4 | 1.1 | | | 4 | 2.6 |
| Balance problems | 3 | 0.8 | 3 | 1.4 | | |
| Subcutaneous absces | 2 | 0.5 | 1 | 0.5 | 1 | 0.7 |
| Temporary facial weakness | 2 | 0.5 | 2 | 0.9 | | |
| Hematoma | 1 | 0.3 | | | 1 | 0.7 |
| R/S migration | 1 | 0.3 | 1 | 0.5 | | |
| Magnet extrusion from the R/S package | 1 | 0.3 | | | 1 | 0.7 |
| Extrusion of silver ball electrode | 1 | 0.3 | 1 | 0.5 | | |
| | 27 | 7.4 | 20 | 9.3 | 7 | 4.6 |

## 4. Discussion

Cochlear implantation has been shown to be a safe and effective treatment for the profoundly deaf. However, complications associated with implant surgery cannot be completely avoided. Previous studies [2–6] (Table 1) have shown a low incidence of major and minor complications following implantation. As with any other surgical intervention, periodic re-evaluation of potential areas of difficulty is important in developing protocols to further diminish the risk of complications. This study represents the largest single-center review of cochlear implant complications in Japan and reaffirms the impressive safety profile of implant operation and re-habilitation process before and after implantation. Recently, an increased incidence of otogenic meningitis in cochlear implant recipients has been reported [8]. However, none of these reported cases were life-threatening.

### 4.1. Major complications (Table 3)

In our study, major complications occurred in 32 patients (8.7%). Previous studies [1–6] have reported major complication rates of 5.1–17.7%. Major complications, which have gradually decreased over the years, were usually defined as electrode failure, problems requiring revision surgery, and other complications such as permanent facial paralysis. The number of cochlear implant surgeries has increased, surgical methods have improved, and operative skills have progressed. A decade ago, the main major complications included flap-related problems, electrode problems such as electrode slip-out, and facial palsy [1,3]. However, as per several reports, electrode slip-out and facial nerve paralysis have not occurred recently because of advanced surgical technique and electrode design. Therefore, in Table 5, our data have been classified according to the time of surgery, i.e., prior to 1999 and after 2000. One case of facial nerve paralysis occurred in 1995; however, that case seemed to be due to thermal damage at the time of posterior tympanotomy. Electrode slip-out had decreased using Balkany's split-bridge technique [7] after 2000 and with the recent

**Table 5**
Major complications before 1999 and after 2000.

| | Before 1999 | | After 2000 | |
|---|---|---|---|---|
| | Number | % | Number | % |
| Total | 153 | | 213 | |
| Flap infection | 6 | 3.9 | 6 | 2.8 |
| Electrode slip-out | 7 | 4.6 | 1 | 0.5 |
| Device failure with trauma | 1 | 0.7 | 6 | 2.8 |
| Device failure without trauma | 3 | 2.0 | 1 | 0.5 |
| Facial palsy | 1 | 0.7 | | |
| Total | 18 | 11.8 | 14 | 6.6 |

introduction of the curved electrode CI 24 R (CS) and other electrodes that have been designed to avoid slip out.

Flap-related problems such as flap infection and/or necrosis developed in 3.6% (13 patients), and 12 of those patients needed re-implantation. All but 1 pediatric case had a previous history of otitis media before the initial surgery; thus, every possible care needs to be taken with cases with prior otitis media. Furthermore, an explanation for the prevalence of middle ear infection in patients with a history of otitis media is needed. Many studies and our investigation may show that flap problems such as infection cannot be completely avoided. However, in some studies, flap problems have been avoided by precise operative and postoperative procedures. Stratigouleas et al. [6] reported that minimally invasive cochlear implantation (MICI) minimizes the risk of flap infection or necrosis. Several cases of flap problems had no previous confirmed middle ear infection, and these problems were considered to be caused by an allergic reaction to the silicone of the implant casing, but it was not established.

Six patients experienced device failure because of trauma, including 3 patients without obvious history. Cochlear implant candidates with advanced age and profound hearing loss frequently have balance problems and need to avoid head injury. Young children have a higher risk of head injury due to falling over and boisterous play. Two child patients (both 3 years old) had fallen over in the bathroom. The patients with device failure had new implants implanted on the same side and subsequently showed good performance.

Electrode problems included a large number of problems caused by ineffective electrodes, such as electrode kinking, electrode shorting, and electrode problems due to posterior canal wall defect except for electrode slip-out. However, most problems were due to surgical technique and it is important to know how surgical technique and experience can affect electrode problems.

### *4.2. Minor complications (Table 4)*

Minor complications were defined as those that can be managed conservatively by medical or audiological interventions, such as wound infection or non-auditory stimulation. These complications were often caused by ineffective electrodes. The total number of minor medical and surgical complications was 27, representing 7.4% of all operations. Complications such as some electrode problems, namely electrode's shorts and non-auditory stimulation, were not included as minor complications because they were resolved with reprogramming.

Flap-related problems for minor complication did not need revision surgery such as surgical flap thinning and/or subcutaneous abscess drainage. These all were resolved with local treatment and/or intravenous antibiotics. One case of partial and temporary facial paralysis, 2 cases of temporary facial weakness, 1 case of hematoma, and 1 case of extrusion of a silver ball electrode to the external ear canal could however be avoided by strict operation and postoperative procedures. One case of magnet extrusion occurred by middle ear infection and subcutaneous abscess after swimming.

### *4.3. The complication rates for two periods (before 1999 and after 2000) [Table 5]*

Most cases of electrode slip-out were experienced before 1999, and device failure cases increased after 2000. The number of flap infection cases was not largely different.

The major and minor complications resulting from operative skill and postoperative procedures can be reduced. Middle ear and flap infection may not be avoided completely, but some of them can be. It is important that medical doctors, speech therapists, educationists, cochlear implant patients, their parents, and other related people are aware of the situation.

## 5. Conclusion

The 366 implantations initially performed at TMU Hospital were investigated retrospectively in terms of major and minor complications. It shows that periodical re-evaluations are important to develop protocols for risk avoidance. Major complications were observed in 32 patients (8.7%), with minor complications in 27 patients (7.4%). Many cases of major complications, including electrode problems and facial paralysis (excluding traumatic device failure), were considered avoidable by strict operative and postoperative procedures. Some cases of flap infection and device traumatic device failure may not be avoided completely and implant patients and other involved people should take every possible care.

## Conflict of interest

None.

## References

[1] Cohen NL, Hoffman RA. Complications of cochlear implant surgery in adults and children. Ann Otol Rhinol Laryngol 1991;100:708–11.
[2] Webb RL, Lehnhardt E, Clark GM, Laszig R, Pyman BC, Franz BK. Surgical complications with the cochlear multiple-channel intracochlear implant: experience at Hannover and Melbourne. Ann Otol Rhinol Laryngol 1991;100:131–6.
[3] Hoffman RA, Cohen NL. Complications of cochlear implant surgery. Ann Otol Rhinol Laryngol Suppl 1995;166:420–2.
[4] Bhatia K, Gibbin KP, Nikolopoulos TP, O'Donoghue GM. Surgical complications and their management in a series of 300 consecutive pediatric cochlear implantations. Otol Neurotol 2004;25:730–9.
[5] Green KM, Bhatt YM, Saeed SR, Ramsden RT. Complications following adult cochlear implantation: experience in Manchester. J Laryngol Otol 2004;118:417–20.
[6] Stratigouleas ED, Perry BP, King SM, Syms 3rd CA. Complication rate of minimally invasive cochlear implantation. Otolaryngol Head Neck Surg 2006;135:383–6.
[7] Balkany TJ, Whitley M, Shapira Y, Angeli SI, Brown K, Eter E, et al. The temporalis pocket technique for cochlear implantation: an anatomic and clinical study. Otol Neurotol 2009;30:903–7 [Erratum in: Otol Neurotol 2010;31:367].
[8] Arnold W, Bredberg G, Gstöttner W, Helms J, Hildmann H, Kiratzidis T, et al. Meningitis following cochlear implantation: pathomechanisms, clinical symptoms, conservative and surgical treatments. ORL J Otorhinolaryngol Relat Spec 2002;64:382–9.

ORPHANET JOURNAL OF RARE DISEASES

RESEARCH Open Access

# Diverse spectrum of rare deafness genes underlies early-childhood hearing loss in Japanese patients: a cross-sectional, multi-center next-generation sequencing study

Hideki Mutai[1†], Naohiro Suzuki[1†], Atsushi Shimizu[2], Chiharu Torii[3], Kazunori Namba[1], Noriko Morimoto[4], Jun Kudoh[5], Kimitaka Kaga[6], Kenjiro Kosaki[3] and Tatsuo Matsunaga[1*]

## Abstract

**Background:** Genetic tests for hereditary hearing loss inform clinical management of patients and can provide the first step in the development of therapeutics. However, comprehensive genetic tests for deafness genes by Sanger sequencing is extremely expensive and time-consuming. Next-generation sequencing (NGS) technology is advantageous for genetic diagnosis of heterogeneous diseases that involve numerous causative genes.

**Methods:** Genomic DNA samples from 58 subjects with hearing loss from 15 unrelated Japanese families were subjected to NGS to identify the genetic causes of hearing loss. Subjects did not have pathogenic *GJB2* mutations (the gene most often associated with inherited hearing loss), mitochondrial m.1555A>G or 3243A>G mutations, enlarged vestibular aqueduct, or auditory neuropathy. Clinical features of subjects were obtained from medical records. Genomic DNA was subjected to a custom-designed SureSelect Target Enrichment System to capture coding exons and proximal flanking intronic sequences of 84 genes responsible for nonsyndromic or syndromic hearing loss, and DNA was sequenced by Illumina GAIIx (paired-end read). The sequences were mapped and quality-checked using the programs BWA, Novoalign, Picard, and GATK, and analyzed by Avadis NGS.

**Results:** Candidate genes were identified in 7 of the 15 families. These genes were *ACTG1*, *DFNA5*, *POU4F3*, *SLC26A5*, *SIX1*, *MYO7A*, *CDH23*, *PCDH15*, and *USH2A*, suggesting that a variety of genes underlie early-childhood hearing loss in Japanese patients. Mutations in Usher syndrome-related genes were detected in three families, including one double heterozygous mutation of *CDH23* and *PCDH15*.

**Conclusion:** Targeted NGS analysis revealed a diverse spectrum of rare deafness genes in Japanese subjects and underscores implications for efficient genetic testing.

**Keywords:** Hereditary hearing loss, Target gene capture, Deafness gene, Heterogeneity

## Background

Hearing loss is a common sensory defect, affecting approximately one in 500 to 1000 newborns [1]. Approximately 50% of congenital hearing loss cases and 70% of childhood hearing loss cases are attributed to genetic mutations [1]. The remaining 50% of congenital cases are attributable to other factors such as prenatal exposure to measles, cytomegalovirus, premature birth, and newborn meningitis. Genetic tests for hereditary hearing loss assist in the clinical management of patients and can provide the first step in the development of therapeutics [2]. For example, early diagnosis of Usher syndrome, which comprises congenital hearing loss and late-onset retinitis pigmentosa, provides important information to choose communication modalities. However, causes of hereditary hearing loss are highly heterogeneous; more than 60 genes have been identified as responsible for nonsyndromic

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hearing loss [3], and several hundreds of syndromic diseases, such as Pendred syndrome and Usher syndrome, are accompanied by hearing loss. *GJB2* mutations are the most common cause of childhood hearing loss worldwide [1], followed by *SLC26A4* mutations [4]. *OTOF* mutations are common in patients with auditory neuropathy, which is characterized by normal outer hair cell function and abnormal neural conduction [5]. The prevalence of childhood hearing loss patients with mutations in other deafness-related genes is likely to be less than 1% [1]. Such high heterogeneity of hearing loss makes it impractical to perform genetic tests by Sanger sequencing. This is also the case for some types of syndromic hearing loss. For example, nine genes have been reported to cause Usher syndrome, and all are large and difficult to analyze using Sanger sequencing.

Next-generation sequencing (NGS) technology has been applied to genetic diagnosis of nonsyndromic hearing loss [6-8] and exploring the causes of hearing loss [9-11]. These studies have revealed that it is technically feasible to identify causative genes for nonsyndromic and syndromic hearing loss using targeted NGS [6,8]. In this study, we used targeted NGS to identify the genetic basis of hearing loss in Japanese families.

## Methods

### Subjects

This was a multi-center study of 58 subjects (36 subjects with hearing loss and 22 subjects with normal hearing) from 15 unrelated Japanese families in which at least two family members had bilateral hearing loss. All subjects were patients at the National Hospital Organization Tokyo Medical Center or a collaborating hospital. Medical histories were obtained and physical, audiological, and radiological examinations were carried out for the subjects and family members. Subjects with hearing loss related to environmental factors were excluded. Subjects with *GJB2* mutations or mitochondrial m.1555A>G or 3243A>G mutations were excluded. Subjects with enlarged vestibular aqueduct, which is often associated with *SLC26A4* mutations, and subjects with clinical features that suggested syndromic hearing loss were excluded. Subjects with auditory neuropathy were tested for *OTOF* mutations, which are associated with auditory neuropathy [12], and subjects with *OTOF* mutations were excluded. The Ethics Review Committees of the National Hospital Organization Tokyo Medical Center and all collaborating hospitals approved the study procedures. All procedures were conducted after written informed consent had been obtained from each subject or their parents.

### Targeted capture and DNA sequencing

We selected coding exons and proximal flanking intronic sequences of 84 genes, including 17 genes responsible for autosomal dominant nonsyndromic hearing loss (DFNA), 32 genes responsible for autosomal recessive nonsyndromic hearing loss (DFNB), 8 genes responsible for both DFNA and DFNB, one gene responsible for auditory neuropathy, 3 genes responsible for X-linked hearing loss, and 23 genes responsible for syndromic hearing loss. A list of the targeted genes responsible for nonsyndromic or syndromic hearing loss is provided in the supporting material [Additional file 1]. More than 90% of the target genomic sequences were successfully designed to be captured by the SureSelect Target Enrichment System (Agilent Technologies, CA, USA) (data not shown). Genomic DNA was extracted from whole blood using the Genetra Puregene DNA isolation kit (QIAGEN, Hilden, Germany) and checked for quality using Qubit (Life technologies, CA, USA). Genomic DNA (3 μg) was fragmented into approximately 150 base pairs and used to capture the targeted genomic sequences. The captured DNA was subjected to the paired-end read sequencing system (GAIIx system; Illumina, CA, USA).

### Sequence analysis

Sequence analysis initially focused on the 61 genes responsible for nonsyndromic hearing loss. If no candidate mutations were detected among these genes, the 23 genes responsible for syndromic hearing loss were subjected to sequence analysis.

The sequences were mapped and quality-checked with the programs BWA, Novoalign, Picard, and GATK using the human reference sequence hg19/GRCh37. Single and multiple nucleotide variants, including small insertion or deletions that would affect amino acid sequences or could affect splice sites, were annotated by Avadis NGS v.1.4.5 (Strand Life Sciences, Bangalore, India). Variants already known as pathogenic mutations or detected with <1% frequency in public databases (dbSNP135 [13], 1000GENOME [14], NHLBI Exome Variant Server [15]) were extracted and further subjected to segregation analysis within each family. If no candidate variants were found, the 23 genes responsible for syndromic hearing loss were subjected to the same procedures.

Selected variants were classified as known mutations, possible pathogenic mutations, or variants with unknown pathogenicity; the latter classification was made if there were reports of a controversial finding of pathogenicity or >1% allele frequency in the in-house database of 95 (up to 189) Japanese subjects with normal hearing. Conservation of the corresponding mutated amino acid was compared across nine primate, 20 mammal, and 13 vertebrate species by UCSC Conservation [16]. Functional pathogenic effects of the variants were predicted by PolyPhen-2 [17] and PROVEAN [18]. Effect on splice-site mutations was predicted by NNSPLICE [19].

All the variants and their segregation in each family were confirmed by Sanger sequencing. The specific primer sets were selected from the resequencing amplicon probe sets (NCBI) or designed originally by Primer-BLAST (NCBI). The genotype of each individual and segregation in the family was characterized using DNA-SIS Pro (Hitachisoft, Tokyo, Japan).

### Structural modeling

To find sequences homologous to ACTG1 and MYO7A that could be used as the structural templates for the modeling exercise, we searched the Protein Data Bank (PDB) using Gapped BLAST [20] and PDBsum [21]. The crystal structure of *Limulus polyphemus* filamentous actin (PDB: 3B63) and the 4.1 protein-ezrin-radixin-moesin (FERM) domain of *Mus musculus* myosin VIIa in complex with Sans protein (PDB: 3PVL) were utilized as the templates to model ACTG1 with the p.G268S mutation and MYO7A with the p.W2160G mutation, respectively. The models were built using SWISS-MODEL [22-24] in the automatic modeling mode and with default parameters. The quality of the models was evaluated using the Verify_3D Structure Evaluation Server [25,26]. The α-carbon frames and ribbon models were superimposed using Chimera [27].

## Results

Pedigrees of the seven families are shown in Figure 1; clinical features are described in Table 1 and supplemental materials [Additional file 2 and Additional file 3]. In this targeted NGS study, the mean read depth of the target regions was more than 100× for all subjects (data not shown). Table 2 summarizes the number of variants detected from the 61 or 84 targeted genes for each subject. The number of variants was consistent across subjects (339–435 variants per subject for 61 genes, 539–607 variants per subject for 84 genes), which supported the reproducibility and reliability of our technical procedures and analytical pipeline. After excluding frequent variants (>1%) in public databases, 12 variants of

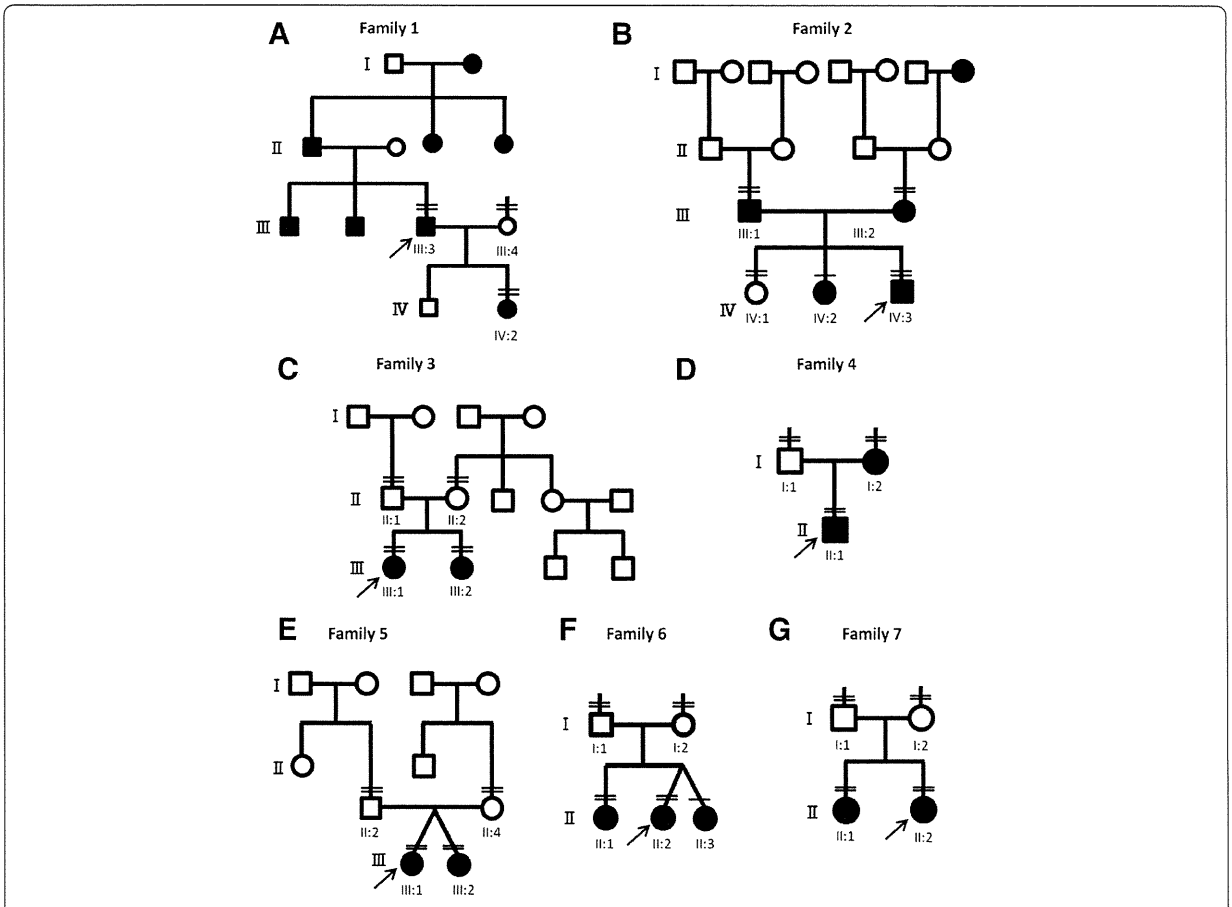


**Figure 1 Pedigrees of the seven families with hearing loss.** Double horizontal bars above a symbol indicate individuals who underwent genetic analysis by targeted next-generation sequencing. Single horizontal bars above a symbol indicate individuals who underwent analysis by Sanger sequencing. **A-G** denote pedigrees of family 1-7, respectively.

**Table 1 Summary of subjects with hearing loss**

| Family | Subject | Age at onset (years) | Age at the time of the study (years) | Hearing loss severity (left/right)* | Progression |
|---|---|---|---|---|---|
| 1 | III:3 | 45 | 53 | Moderate/Moderate | Yes |
| | IV:2 | 10 | 16 | Mild/Normal | No |
| 2 | III:1 | unknown | no data | Profound/Profound | Unknown |
| | III:2 | unknown | no data | Moderate/Severe | Unknown |
| 3 | IV:3 | 0 | 1 | Severe** | Yes |
| | III:1 | 0 | 9 | Severe** | Unknown |
| | III:2 | 0 | 6 | Moderate/Moderate | Unknown |
| 4 | I:2 | 0 | 30s | Profound/Profound | No |
| | II:1 | 0 | 2 | Profound/Profound | No |
| 5 | III:1 | 0 | 2 | Severe** | No |
| | III:2 | 0 | 2 | Profound** | No |
| 6 | II:1 | 5 | 14 | Profound/Severe | Yes |
| | II:2 | 0 | 12 | Profound/Profound | Yes |
| 7 | II:1 | 0 | 3 | Moderate (ASSR***) | Unknown |
| | II:2 | 0 | 0 | Severe (ASSR) | Unknown |

*Hearing loss severity was evaluated based on average hearing level at 500, 1,000, 2,000, and 4,000 Hz (mild, 20–40 dB; moderate, 41–70 dB; severe, 71-95dB; profound, >95 dB) according to recommendations [3]. **Binaural hearing level. ***ASSR, auditory steady state responses.

9 genes co-segregated with symptoms and were selected as possible pathogenic mutations (Table 3) or variants with uncertain pathogenicity in 7 families (Table 4).

### Candidate mutations in each family

In family 1 (Figure 1A), subjects III:3 and IV:2 with hearing loss had a unique heterozygous missense mutation of *ACTG1* (c.802G >A; p.G268S), whereas subject III:4 with normal hearing did not. *ACTG1* encodes actin gamma 1 and is responsible for DFNA20/26 (OMIM 604717) [28]. The glycine residue at 268 of actin gamma 1 is located on a hydrophobic loop that has been suggested to be critical for polymerization of the actin monomers into a filament (Figures 2A and 2B) [29]. Molecular modeling predicted that the p.G268S mutation would disrupt the hydrophobic interactions that are important for polymerization of actin gamma 1 (Figures 2C and Figure 2D). The p.G268S mutant would weaken polymerization of actin gamma 1, which could result in destabilized cytoskeletal structure of stereocilia and dysfunction of the sensory hair cells.

Family 2 (Figure 1B) had two candidate genes with possible pathogenic mutations: A unique heterozygous *POU4F3* frameshift mutation, c.1007delC (p.A336Vfs), was detected in subjects III:1 and IV:3 with hearing loss, and a unique heterozygous *DFNA5* nonsense mutation, c.781C >T (p.R261X), was detected in subjects III:2 and IV:3 with hearing loss, whereas subject IV:1 with normal hearing had neither of these mutations. Sanger sequencing revealed that subject IV:2 with hearing loss had both the heterozygous mutations. *POU4F3* is responsible for DFNA15 (OMIM 602459) [30,31], and *DFNA5* is responsible for DFNA5 (OMIM 600994) [32]. A frameshift mutation in *DFNA5*, which would lead to decreased expression, has been reported not to cause hearing loss [33]; therefore, the cause of hearing loss in subjects IV:2 and IV:3 is more likely to *POU4F3* with the p.A336Vfs mutation derived from subject III:1, rather than *DFNA5* with p.R261X mutation derived from subject III:2.

In family 3 (Figure 1C), subjects III:1 and III:2 with hearing loss had compound heterozygous *SLC26A5* with c.209G >A (p.W70X) and c.390A >C (p.R130S) mutations, whereas subjects II:1 and II:2 with normal hearing had a heterozygous p.W70X mutation and a heterozygous p.R130S mutation, respectively. *SLC26A5* encodes prestin, a member of the SLC26A/SulP transporter family, and is responsible for DFNB61 (OMIM 613865) [34].

In family 4 (Figure 1D), subjects I:2 and II:1 with hearing loss did not have candidate mutations in the first 61 genes. Analysis of the additional 23 genes indicated a heterozygous *SIX1* mutation, c.328C >T (p.R110W), in the subjects with hearing loss but not in subject I:1 with normal hearing. *SIX1* is responsible for DFNA23 (OMIM 605192) and Branchio-otic syndrome 3 (BOS3, OMIM 608389). The p.R110W mutation was previously reported in two BOS3 families [35]. To make the clinical diagnosis of branchiootorenal syndrome or branchiootic syndrome, major and minor criteria of these syndromes must be present [36]. In the affected subjects of the present study, clinical histories were thoroughly evaluated and physical examination of the ear, nose, throat, head and neck, and audiological tests were performed. In addition, CT of the temporal bone was evaluated in subject II:1. With these examinations, the affected subjects did not