Figure 2. The haplotypes around c.211delC mutation of six families constructed using SNPs are shown. Each column shows an affected allele. Each base is defined by pure segregation analysis in the family. Allele frequencies of SNPs are derived from HapMap JPT+CHB samples. Families 2, 5, 10, and 13 shared a large common region of about more than 1 Mb in their haplotypes (blue). Abbreviation: Fm, Family.
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missense variants on *KCNQ4* protein function. A missense mutation (p.F182L) was found in one control patient with normal audiogram and the results showed that it is not likely to be a pathologic mutation.

The present study identified 7 possible disease-causing mutations, including 5 novel mutations, in 19 autosomal dominant families. Based on our unbiased population-based genetic screening, the frequency is 6.62% (19/287) of the overall ADNSHL population. These data indicated that *KCNQ4* is one of the important causative genes among ADNSHL patients, particularly in patients with high frequency-involved hearing loss. This frequency is higher than our recently reported frequency (4/139:

Table 2. Clinical features of affected family members associated with KCNQ4 mutations found in this study.

Amino Acid Change	Family – Patient No.	HL onset age (years)	Age at the first visit (years)	Audiogram frequencies	Progression	Tinnitus	Vertigo
Q71fs	1–1	40	48	Ski slope	N/A	N/A	N/A
	1–2	15	15	Ski slope	+	–	–
	2–1	30	47	Ski slope	+	+	–
	3–1	N/A	31	Ski slope	N/A	–	–
	4–1	12	37	Ski slope	+	+	–
	5–1	32	42	Ski slope	–	+	–
	5–2	10	15	Ski slope	+	+	–
	6–1	14	40	Ski slope	+	+	–
	7–1	11	35	Ski slope	+	+	–
	8–1	18	25	Ski slope	+	+	–
	9–1	18	29	Ski slope	+	+	–
	10–1	17	22	Ski slope	+	+	–
	10–2	20	52	Ski slope	+	+	–
	11–1	40	43	Ski slope	+	–	–
	11–2	N/A	73	Ski slope	N/A	–	–
	12–1	22	38	Ski slope	+	+	–
	13–1	35	55	Ski slope	+	+	–
	13–2	25	33	Ski slope	+	+	+
	13–3	11	14	Ski slope	N/A	+	+
	13–4	–	6	Normal (*)	N/A	N/A	N/A
H77fs	**14**	**22**	**27**	**Ski slope**	**+**	**+**	–
V230E	**15–1**	**40**	**78**	**mid freq**	**+**	**+**	–
	15–2	**12**	**39**	**mid freq**	**+**	–	–
	15–3	**5**	**5**	**mid freq**	**+**	–	–
	15–4	**3**	**3**	**mid freq**	**N/A**	**N/A**	**N/A**
	15–5	**N/A**	**0**	**mid freq**	**N/A**	**N/A**	**N/A**
W276S	16–1	8	65	high freq	+	–	+
	16–2	12	46	high freq	+	–	–
	16–3	7	42	high freq	+	–	–
	16–4	8	8	high freq	+	–	+
	16–5	8	6	high freq	+	–	–
P291S	**17–1**	**20**	**33**	**high freq**	**+**	**N/A**	**N/A**
P291L	**18–1**	**17**	**40**	**high freq**	**N/A**	**N/A**	**N/A**
	18–2	**17**	**15**	**high freq**	**N/A**	**N/A**	**N/A**
R297S	**19–1**	**39**	**39**	**high freq**	–	**+**	–
	19–2	**5**	**5**	**high freq**	**+**	–	–

Abbreviations: HL, hearing loss; mid, middle; freq, frequency; N/A, not applicable.
(*) Six-year-old boy's hearing is normal in spite of having the mutation.
doi:10.1371/journal.pone.0063231.t002

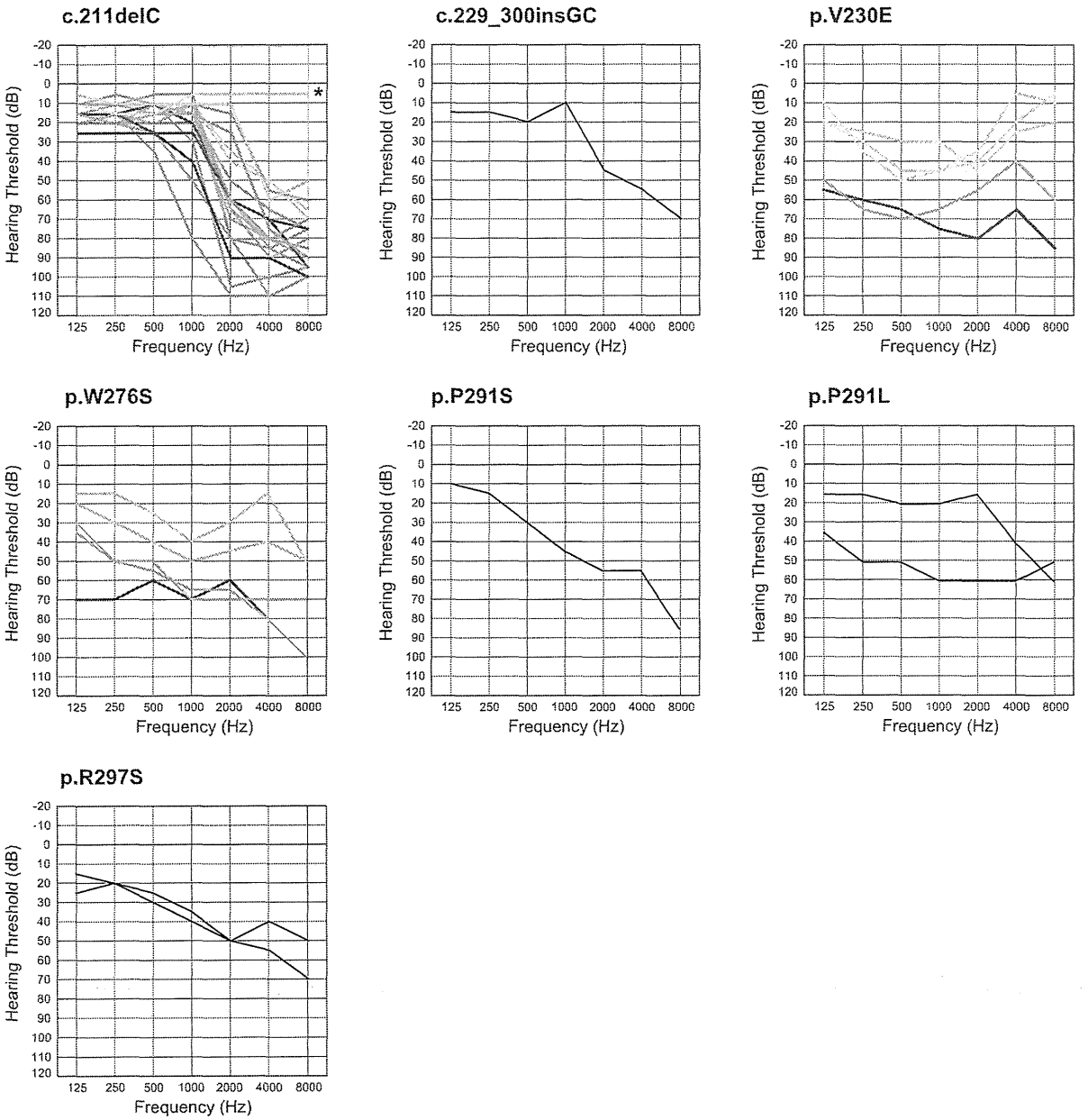

Figure 3. Overlapping audiograms from the better ear for each genotype. In cases of W276S, c.211delC, or V230E, light colored audiograms (green, blue, red) were from individuals aged 19 and under. Dark colored audiograms (green, blue, red) were from the patients aged 20–49 years old, and deep colored audiograms (green, blue, red) are from the patients in their 50 s and over. In family #13 with c.211delC, (*) a six-year-old boy's hearing is normal in spite of having the mutation.
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2.9%) of *TECTA* in Japanese ADNSHL families [4], therefore *KCNQ4* is found to be currently the most prevalent gene responsible for Japanese ADNSHL patients, and should be the first in line to be analyzed for ADNSHL patients.

Mutations lie in various domains of the *KCNQ4* protein. While the majority are private mutations, one particular recurrent mutation, c.211delC, was observed in 13 unrelated families. In this gene, we have reported that there is a hot spot mutation, p.W276S, in Belgian, Dutch, and Japanese families [5]. Based on haplotype analysis, in the case for c.211delC, it is not likely a hot spot but rather is suggested to be due to a common ancestor. Such recurrent mutations are common in recessive genes such as 235delC, 35delG, 167delT in *GJB2* [6][7], H723R in *SLC26A4* [8], and P204L in *CDH23* [9]. They are rare in dominant genes, though a mutation in *DFNA5* that causes autosomal dominant sensorineural hearing loss was reported to arise from a common ancestor [10]. Together with specific audiogram configuration, this may facilitate genetic testing for ADNSHL with a particular phenotype.

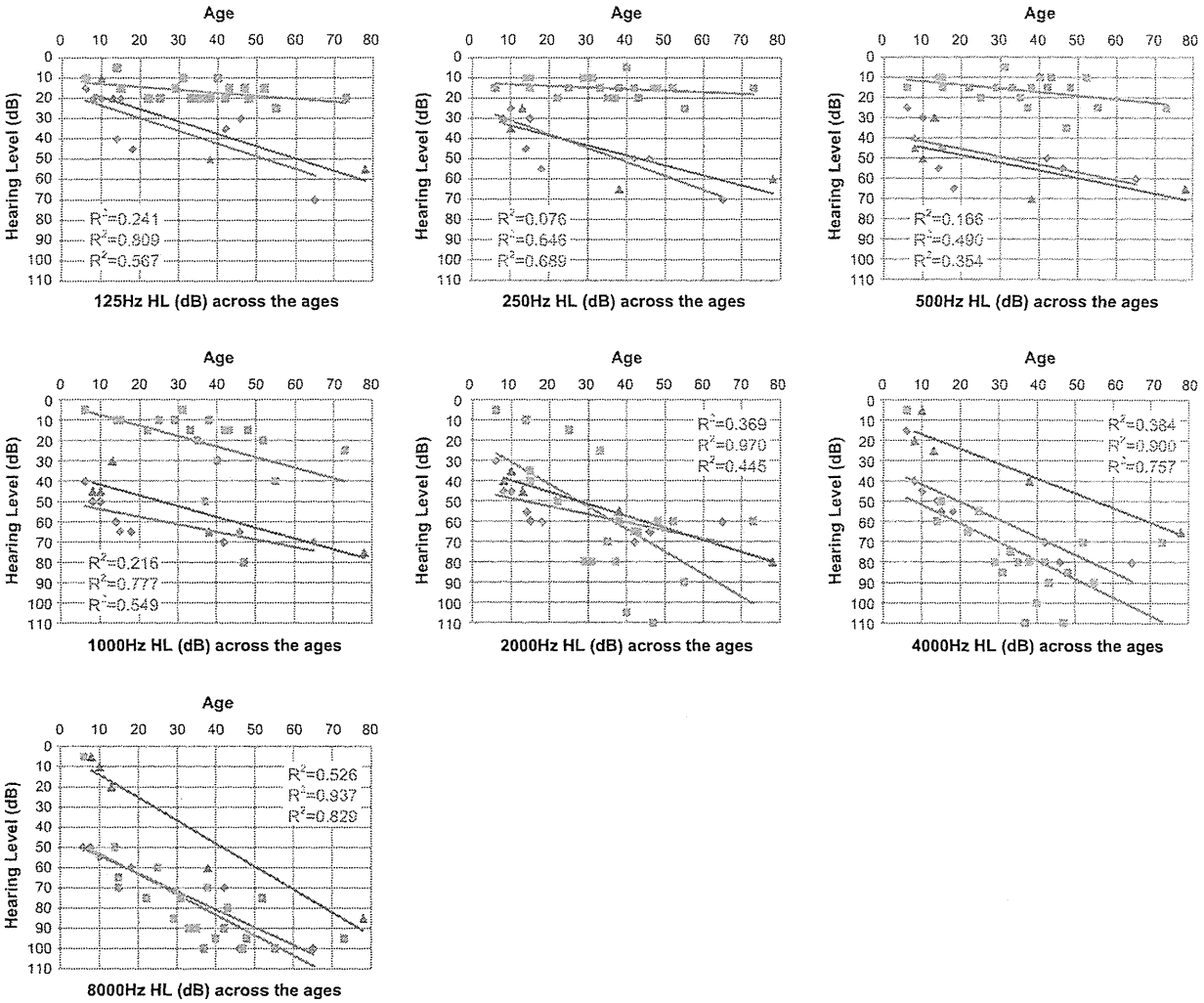

Figure 4. Detailed progression analysis in each frequency. A single audiogram (the better ear) from a single patient was plotted. Gradual progression is characterized regardless of frequency. Average progressive rates of hearing loss (db/year) for the patients with c.211delC, for 125 (0.15) and 250 Hz (0.078) were significantly stable compared to the other two mutations (ANCOVA: $p<0.05$) and they had milder hearing loss at 500 and 1 KHz (ANCOVA: $p<0.05$). In contrast, at 4 KHz and 8 KHz, patients with V230E mutations had milder hearing loss compared to the other two mutations (ANCOVA: $p<0.05$). Each color (green, blue, red) indicates W276S, c.211delC, or V230E, respectively.
doi:10.1371/journal.pone.0063231.g004

Table 2 summarizes clinical characteristics including hearing threshold, severity, onset age (age of awareness), progressiveness of hearing loss, and vestibular symptoms. Age of onset (awareness of hearing loss) ranged from 3 to 40 years old, though the majority of the patients were in their first decade of life. Many of the mutations were accumulated in the P-loop region as described before [3][11][12], but mutations were also found in the other domains (Table 1, Fig. 1). There were some correlations between genotype and phenotype (Fig. 3). Overlapped audiograms showed characteristic high frequency involved hearing loss in the majority of the patients with *KCNQ4* mutations. Unique audiograms were shown in the patients with c.211delC and p.V230E. The patients associated with c.211delC showed so-called ski slope hearing loss (high frequency involved hearing loss with nearly normal hearing at lower frequencies). Patients with p.V230E showed mid-frequency involved hearing loss.

It has been known that DFNA2 shows high-frequency involved hearing loss [3][13][14]. Based on collected audiograms from the patients with *KCNQ4*, an effective selection algorithm named "Audioprofile" has been proposed and many mutations have actually been successfully identified [13]. The present large cohort study allowed us to confirm and extend the genotype-phenotype correlations. It added a new type of audiogram configuration characterized by mid-frequency predominant hearing loss caused by a *KCNQ4* mutation (Fig. 3). Family #15 had a heterozygous T>A transition at nucleotide 689 in exon 4, which results in a Val to Glu substitution (V230E). This mutation was present in all five affected individuals, and not present in two unaffected family members. None of the 252 normal controls had this mutation. Prediction programs indicated that this mutation is likely to be pathologic. So far mid-frequency predominant hearing loss has been reported with *TECTA* mutations [4]. In this family, we sequenced for *TECTA* to find a mutation, but none were found

(data not shown). A different *KCNQ4* mutation (c.664_681del) within the same domain as this mutation was reported to cause high-frequency involved hearing loss, suggesting that the phenotype is not domain-specific [15]. The V230E mutation is a missense mutation that substitutes a nonpolar and aliphatic valine for a negatively charged glutamate. This single base substitution is located adjacent to the S4 transmembrane domain that has a key role as a voltage sensor. The V230E mutation may therefore change sensitivity of voltage sensor and have an affect on passage of potassium through the cell membrane.

The ski-slope type audiogram configuration found in the patients with c.211delC is also a striking characteristic phenotype (Fig. 3). Single families associated with c.211delC [16] and c.211_223del13 [17] have previously been reported to show ski-slope audiograms. The audiogram collection in this study further generalized this phenotype in the N-terminal site.

Analysis of the different frequencies found evident quickly progressive hearing loss in the middle frequencies, therefore those patients may be at risk for rapid deterioration of speech understanding during the time course. Patients with ski-slope type audiograms sometimes have difficultly in being fitted with hearing aids, but Electric Acoustic Stimulation (EAS) has recently been shown to be effective for those patients with high frequency involved hearing loss [18]. The present data on progression speed showed more stable hearing at low frequencies (125 and 250Hz) (Fig. 4), indicating EAS will be the potential therapeutic intervention for the patients with this particular mutation.

Progressive nature is a common feature of the patients with *KCNQ4* mutations regardless of the particular mutation (Fig. 3). Overlapped audiograms of all subjects with W276S, c.211delC, or V230E mutations showed the progressive nature of hearing loss regardless of the mutation type. However, no patients received cochlear implants in this cohort, suggesting that profound hearing loss may seldom be seen though their hearing loss has a progressive nature.

In conclusion, *KCNQ4* is frequent among ADNSHL patients, and therefore screening for this gene and molecular confirmation of *KCNQ4* mutations have become important in the diagnosis of these conditions.

Supporting Information

Figure S1 Pedigrees of the *KCNQ4* mutation families and detected mutations.
(PDF)

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Author Contributions

Conceived and designed the experiments: SU. Performed the experiments: TN SN YI TY. Analyzed the data: TN SN YI TY SU. Contributed reagents/materials/analysis tools: TN KK SA KI HK AN CO. Wrote the paper: SU TN.

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RESEARCH ARTICLE Open Access

OTOF mutation screening in Japanese severe to profound recessive hearing loss patients

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Abstract

Background: Auditory neuropathy spectrum disorder (ANSD) is a unique form of hearing loss that involves absence or severe abnormality of auditory brainstem response (ABR), but also the presence of otoacoustic emissions (OAEs). However, with age, the OAEs disappear, making it difficult to distinguish this condition from other nonsyndromic hearing loss. Therefore, the frequency of ANSD may be underestimated. The aim of this study was to determine what portion of nonsyndromic hearing loss is caused by mutations of *OTOF*, the major responsible gene for nonsyndromic ANSD.

Methods: We screened 160 unrelated Japanese with severe to profound recessive nonsyndromic hearing loss (ARNSHL) without *GJB2* or *SLC26A4* mutations, and 192 controls with normal hearing.

Results: We identified five pathogenic *OTOF* mutations (p.D398E, p.Y474X, p.N727S, p.R1856Q and p.R1939Q) and six novel, possibly pathogenic variants (p.D450E, p.W717X, p.S1368X, p.R1583H, p.V1778I, and p.E1803A).

Conclusions: The present study showed that *OTOF* mutations accounted for 3.2–7.3% of severe to profound ARNSHL patients in Japan. *OTOF* mutations are thus a frequent cause in the Japanese deafness population and mutation screening should be considered regardless of the presence/absence of OAEs.

Keywords: Auditory neuropathy spectrum disorder, DFNB9, Nonsyndromic hearing loss

Background

Auditory neuropathy (AN), a unique form of hearing loss, involves absence or severe abnormality of auditory brainstem response (ABR), but presence of otoacoustic emissions (OAE) and/or cochlear microphonic (CM). This disorder was defined by Starr [1], and also reported as "Auditory nerve disease" [2] and "Auditory dys-synchrony" [3]. AN was renamed "auditory neuropathy spectrum disorder (ANSD)" in 2008, due to the heterogeneous and multifaceted nature [4].

The prevalence of ANSD in sensorineural hearing loss is reported to be 0.5-15% [5]. The etiologies of ANSD are various; patients range from infants to adults, 42% of which are associated with hereditary neurological disorders, 10% with toxic, metabolic, immunological and infectious causes, and 48% with unknown causes [6]. Although the exact percentage of nonsyndromic ANSD is unclear, responsible genes have been gradually revealed. To date, mutations of *AUNA1, OTOF, PJVK, GJB2* and mitochondrial 12S rRNA are reported to be causal for nonsyndromic ANSD [7].

The *OTOF* gene (DFNB9) is mainly expressed in cochlear inner hair cells, and is necessary for synaptic exocytosis at the auditory ribbon synapse [8]. It encodes both long and short isoforms with the long isoform containing six C2 domains and the C-terminal transmembrane domain, and the short isoform containing only the last three C2 domains [9]. Mutations in the *OTOF* gene, encoding otoferlin, are reported to be the major causes of nonsyndromic recessive ANSD [10-12]. In Japanese, mutations in *OTOF* account for 56. 5% (13/23) of ANSD [13]. Although ANSD can be characterized by the presence of OAEs in the first two years of life, OAEs later disappear and the hearing loss then resembles other types of nonsyndromic hearing loss [14]. Because of expected good outcomes of cochlear implantation for

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patients with *OTOF* mutations [15,16], it is important to perform mutation screening for *OTOF* to select the appropriate intervention. Although some reports have described *OTOF* mutations in severe to profound autosomal recessive hearing loss patients in other populations [11,12], there has been no literature available regarding the screening of *OTOF* mutations using a large cohort in a comprehensive manner. The goal of this study was therefore to reveal the frequency of ANSD and to identify *OTOF* mutations in Japanese ARNSHL patients.

Methods

Subjects

Among the 1511 Japanese independent hearing loss patients registered in our DNA sample bank, 469 were congenital severe to profound sensorineural hearing loss (above 71 dB average over 500, 1000, 2000 and 4000 Hz in the better hearing ear) patients compatible with autosomal recessive inheritance (including sporadic cases). From those, we randomly selected 160 patients. All ANSD cases were sporadic (compatible with autosomal recessive inheritance). They were diagnosed as ANSD by evaluation of OAE response. We excluded autosomal dominant families because in previous studies *OTOF* mutations were not found in such groups [17]. Pure tone audiometry was used for adults (N= 32) and ABR, auditory steady-state responses (ASSR), and conditioned orientation response audiometry (COR) were used for pediatric patients (n=128). The control group was composed of 192 unrelated Japanese individuals who had normal hearing shown by auditory testing. All subjects gave prior informed written consent for participation in the project and the Ethical Committee of Shinshu University approved the study.

Mutation analysis

We designed 43 pairs of primers to amplify DNA fragments containing all exons in the coding regions of the *OTOF* gene (ENST00000403946). Primer3Plus (http://www.bioinformatic.nl/cgi-bin/primer3plus/primer3plus.cgi) was used to design primers to flank all the exon-intron boundaries. Each genomic DNA sample (40 ng) was amplified, using Ampli Taq Gold (Applied Biosystems, Foster City, CA), for 5 min at 95°C, followed by 30 three-step cycles of 95°C for 30s, 60°C for 30s, and 72°C for 60s, with a final extension at 72°C for 7 min, ending with a holding period at 4°C in a PCR thermal cycler (Takara, Shiga, Japan). PCR products were treated with ExoSAP-IT® (GE Healthcare Bio, Santa Clara, CA) by incubation at 37°C for 60 min, and inactivation at 80°C for 15 min. After the products were purified, we performed standard cycle-sequencing reactions with ABI Big Dye® terminators in an ABI PRISM 3100 Genetic Analyzer autosequencer (Applied Biosystems, Foster City, CA).

Computer analysis to predict the effect of missense variants on the protein function was performed with wANNOVAR [18-20] (http://wannovar.usc.edu) including functional prediction software listed below. PhyloP (http://hgdownload. cse.ucsc.edu/goldenPath/hg18/phyloP44way/), Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/), Polymorphism Phenotyping (PolyPhen2; http://genetics.bwh.harvard.edu/pph2/), LRT (http://www.genetics.wustl.edu/jflab/lrt_query.html), and MutationTaster (http://www.mutationtaster.org/).

Results

We found a total of 11 probable pathogenic variants in the patients (Table 1). Among them, five mutations were previously reported: p.D398E, p.Y474X, p.N727S, p.R1856Q and p.R1939Q. The other six probable pathogenic variants were novel: 2 nonsense mutations (p.W717X, p.S1368X) and 4 missense mutations (p.D450E, p.R1583H, p.V1778I, p. E1803A). Based on the prediction programs, it is most likely that p.D450E (c.1350C>G), p.R1583H (c.4748G>A), p.V1778I (c.5332G>A), and p.E1803A (c.5408A>C) were pathogenic. In addition, they were absent (or in very few numbers) in the controls, and located in C2 domains, which are highly conserved among species (Figure 1). In addition, polymorphic changes were also identified (Table 2). p.R1676C (c.5026C>T) was previously reported to be pathogenic [21], but we excluded p.R1676C as it is unlikely to be pathologic because of high frequencies in the control population (Table 2). Among the 16 patients with *OTOF* mutations, 4 were homozygous, 3 were compound heterozygotes, and 9 were heterozygous without second mutation (Table 3). After clinical re-evaluation, we recategorized cases with OAE as ANSD.

Discussion

So far, more than 90 pathologic mutations have been reported in *OTOF* [25]. The present study identified 11 possibly pathogenic *OTOF* variants in Japanese patients with nonsyndromic hearing loss, and 6 of them were novel mutations (p.D450E, p.W717X, p.S1368X, p.R1583H, p.V1778I, and p.E1803A). Concerning pathogenicity of the four novel missense mutations, p.R1583H is more likely to be a disease causing mutation, because 1) it was found in compound heterozygosity with p.R1939Q, 2) it was absent in controls, 3) it affects a C2 domain, and 4) the scores provided by prediction programs also agree with the pathogenicity. The pathogenic potential of the three other variants (p.D450E, p.V1778I, and p.E1803A) is less clear, because 1) all of them have been found in the heterozygous state without accompanying mutation in the other allele, and 2) p.D450E was found in controls. But it is also true that 1) they affect C2 domains, and 2) the scores of the prediction programs would support their classification as pathogenic variants.

Table 1 Probable pathogenic and uncertain pathogenic variants of OTOF identified in this study

Exon	DNA level	Protein level	Occurrence in this work (chromosome)	Control (chromosome)	Functional prediction						References
					PhyloP	SIFT (p-value)	P2 D.S.	LRT	Mutation taster	GERP ++	
Probable pathogenic variants											
Exon 14	c.1422T>A	p.Y474X	2/320	0/374	N (0.072941)	NA (0.829813)	NA (0.58309)	D (1)	A (1)	−3.78	[13]
Exon 18	c.2151G>A	p.W717X	1/320	0/344	C (0.994764)	NA (0.90345)	NA (0.734698)	D (0.999998)	A (1)	3.83	This study
Exon 34	c.4103C>G	p.S1368X	1/320	0/364	N (0.944413)	NA (0.915)	NA (0.554899)	NA (0.026679)	A (1)	0.571	This study
Exon 38	c.4748G>A	p.R1583H	1/320	0/366	C (0.997935)	D (1)	D (0.999)	D (1)	D (0.999661)	4.69	This study
Exon 44	c.5567G>A	p.R1856Q	1/320	0/380	C (0.99611)	T (0.91)	P (0.813)	D (1)	D (0.999517)	4.1	[11]
Exon 46	c.5816G>A	p.R1939Q	11/320	0/382	N (0.996658)	T (0.92)	NA (0.746672)	NA (1)	D (0.999886)	1.38	[22]
Uncertain pathogenic variants											
Exon 12	c.1194T>A	p.D398E*	1/320	1/380	N (0.232793)	T (0.77)	D (0.853)	D (1)	D (0.995165)	0.981	[23]
Exon 13	c.1350C>G	p.D450E*	1/320	1/380	C (0.986229)	T (0.74)	D (0.853)	D (1)	D (0.991594)	3.54	This study
Exon 18	c.2180A>G	p.N727S*	2/320	1/344	C (0.992986)	T (0.27)	P (0.386)	D (1)	D (0.95528)	3.98	[21]
Exon 43	c.5332G>A	p.V1778I	1/320	0/378	C (0.997116)	T (0.54)	P (0.289)	D (1)	D (0.994783)	4.38	This study
Exon 43	c.5408A>C	p.E1803A	1/320	0/378	C (0.994555)	D (1)	D (0.995)	D (1)	D (0.999914)	4.26	This study

*the variants found in controls.
Exon number was named based on ENST00000403946.
A, disease causing automatic; C, conserved; D, damaging or disease causing; N, not conserved; NA, not applicable; P, possibly damaging; T, tolerated; P2 D.S., Polyphen-2 damaging score. Polyphen-2, PhyloP, LRT, Mutation Taster, and GERP++ are functional prediction scores that indicate a probable mutation with increasing value.

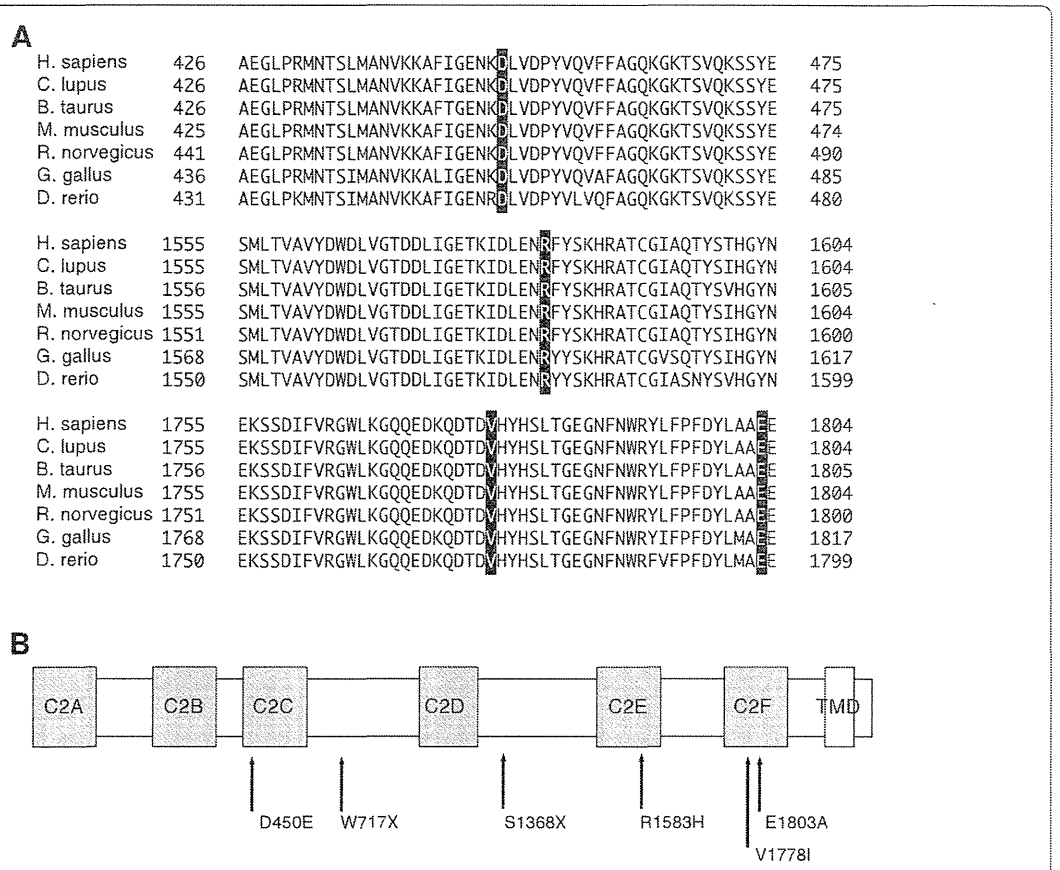

Figure 1 The location of mutations in otoferlin protein and the evolutionary conservation of the amino acids. (A) Evolutionary conservation. The locations of mutations are boxed. **(B)** Novel pathogenic *OTOF* mutations found in this work and relation to the functional domains of otoferlin. C2A-F: C2 domains. TMD: transmembrane domain.

As with other genes, the spectrum of *OTOF* mutations found in the Japanese population was quite different from those reported in Caucasians [13,26-28].

With regard to recurrent mutations, p.Q829X especially has a high frequency in Spanish people, being present in about 3% of all cases of recessive prelingual deafness [24]. C.2905-2923delinsCTCCGAGCGGCA is also common in Argentineans [12] and p.E1700Q is reported to be frequent in Taiwanese [29]. p.R1939Q, previously identified in the United States [22] and most recently reported as a frequent mutation in Japanese [13], was also frequently found in this study. Among 160 patients, 8 (5.0%) had this mutation, confirming it is indeed a recurrent mutation in Japanese.

Those recurrent mutations have been proved to be due to founder effects [13,24,29].

Out of 16 patients with *OTOF* mutations, 7 showed ANSD phenotype, confirming that *OTOF* mutations are major causes of ANSD. In this study, 9 were heterozygous without second mutation. A hallmark of recessive mutations is the detection of two mutations in the paternal and maternal alleles and the parents having normal hearing.

Table 2 Non-pathogenic variants of OTOF identified in this study

Exon	DNA level	Protein level	Occurrence in this work (chromosome)	Control (chromosome)	References
Exon 3	c.145C>T	p.R49W	5/320	10/238	[13]
Exon 3	c.157G>A	p.A53T	2/320	3/238	[23,24]
Exon 3	c.158C>T	p.A53V	42/320	110/238	[23]
Exon 4	c.244C>T	p.R82C	14/320	27/376	[23]
Exon 21	c.2452C>T	p.R818W	1/320	3/356	[12]
Exon 40	c.5026C>T	p.R1676C	1/320	3/356	[21]

Table 3 Patients who have at least one pathogenic mutation identified in this study

Patient	DNA level	Protein level	Clinical diagnosis	OAE	Age at diagnosis	Hearing loss level
1	c.1422T>A / c.5567G>A	p.Y474X / p.R1856Q	ANSD	+	1y6m	Profound
2	c.1422T>A / c.5816G>A	p.Y474X / p.R1939Q	ANSD	+	NA	Profound
3	c.5816G>A / c.5816G>A	p.R1939Q / p.R1939Q	ANSD	+	4m	Profound
4	c.5816G>A / c.5816G>A	p.R1939Q / p.R1939Q	ANSD	+	10m	Profound
5	c.5816G>A / c.5816G>A	p.R1939Q / p.R1939Q	ANSD	+	NA	Profound
6	c.4748G>A / c.5816G>A	p.R1583H / p.R1939Q	NSHL	NA	6m	Profound
7	c.2151G>A / c.5816G>A	p.W717X / p.R1939Q	NSHL	-	1y4m	Profound
8	c.5816G>A / -	p.R1939Q /-	ANSD	+	1y5m	Profound
9	c.5816G>A / -	p.R1939Q /-	ANSD	+	7m	Profound
10	c.1194T>A / -	p.D398E / -	NSHL	NA	NA	Profound
11	c.1350C>G / -	p.D450E / -	NSHL	NA	2y	Severe
12	c.2180A>G / -	p.N727S / -	NSHL	NA	6m	Profound
13	c.2180A>G / -	p.N727S / -	NSHL	NA	1y	Severe
14	c.4103C>G / -	p.S1368X / -	NSHL	NA	7m	Profound
15	c.5332G>A / -	p.V1778I / -	NSHL	NA	NA	Profound
16	c.5408A>C / -	p.E1803A / -	NSHL	NA	4m	Profound

ANSD Auditory neuropathy spectrum disorder, *NSHL* Nonsyndromic sensorineural hearing loss.

As seen in previous mutation screening reports, including those for *OTOF* [12,23,30], there were a significant number of heterozygous cases without a second mutation even after direct sequencing of the coding region of the gene. Possible explanations are: 1) the existence of a second mutation in the intron or regulatory region of *OTOF*, which has not been explored, 2) the existence of a large deletion [31], 3) contribution to hearing loss by an additional modulatory gene, and 4) the existence of a mutation in another gene and just coincidental carrying of the *OTOF* mutation.

As seen in Table 3, two heterozygous patients (#8, 9) having the ANSD phenotype, are most likely to have *OTOF* related deafness.

It is assumed that *OTOF* mutations accounted for deafness in at least 7, and possibly 16, of the 160 patients (4.4-10.0%). As described in the subject section, we excluded the subjects carrying *GJB2* and *SLC26A4* mutations. We also excluded another responsible gene (*PJVK*), because no mutations in this gene were found. Since the frequencies of *GJB2* and *SLC26A4* gene mutations among the patients with nonsyndromic severe to profound congenital SNHL are 27.0% based on our database, mutation frequency of *OTOF* among the total of severe to profound recessive nonsyndromic SNHL is considered to be about 3.2-7.3% (which is calculated by ((7-16)/160×(100/73))×100%). Although simple comparison regarding frequency is difficult because of sampling bias, it is estimated that the frequency of OTOF mutations in Japanese may be almost equal to other populations, as mutation frequency of OTOF was reported at 2.3% (13/557) in Pakistanis [11], 5.0% in Turkish [32], 1.4% (1/73) in Chinese [23], and 18.2% (4/22) in Taiwanese [29], and 3.2% (23/708) in Spanish [12]. Although simple comparison regarding frequency is difficult because of sampling bias, it is estimated that the frequency of *OTOF* mutations in Japanese may be almost equal to other populations. In Japanese, *GJB2, SLC26A4, CDH23* and the 1555A>G mutation in the mitochondrial 12S rRNA are the major causes of hearing loss [33]. Considering the frequency, the *OTOF* gene may be one of the candidate genes to be screened for recessive severe to profound recessive SNHL.

The benefits of cochlear implantation for patients with ANSD has varied [34,35], but implantation has been shown to be effective for the patients with *OTOF* mutations [15,16,36], because their auditory nerves and spiral ganglions are preserved. Consequently, if an *OTOF* mutation is identified in a deaf patient, we can anticipate a good outcome of cochlear implantation, therefore, it is important and meaningful to identify genetic mutations in patients.

Most patients with *OTOF* mutations have a phenotype of stable prelingual and severe to profound nonsyndromic hearing loss. On the other hand, other phenotypes have also been reported. For example, a Taiwanese patient with an p.E1700Q mutation displayed moderate to profound progressive hearing loss [29]. Temperature sensitive ANSD, a particular form of ANSD, has also been reported in some populations [10,23,37].

In the very young child, electrophysiological testing may indicate that *OTOF*-related deafness is ANSD, but

by age two OAEs have generally disappeared and the test results are more in accord with the findings of cochlear lesions [14]. Therefore, if OAE is not tested at a very early age, patients with *OTOF* mutations are not deemed to have ANSD (i.e., hidden ANSD). In fact, 9 out of our 16 patients were diagnosed genetically as nonsyndromic sensorineural hearing loss (NSHL). According to the present data, screening for *OTOF* is necessary not only for the patients diagnosed with ANSD, but also should be extended to ARNSHL cases. The current data indicated that OAE testing must always be conducted in addition to ABR in infants. And we should bear in mind that there may be patients with *OTOF* mutations among the patients diagnosed as having ARNSHL.

Conclusions

The present study showed that *OTOF* mutations accounted for 3.2-7.3% of recessive severe to profound SNHL patients in Japan. *OTOF* mutations are a frequent cause in the Japanese deafness population and mutation screening should be considered regardless of the presence/absence of OAEs.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YI and SN carried out the molecular genetic studies and the sequence alignment, and participated in drafting the manuscript. SU conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Original Paper

Auditory Brainstem Implantation Improves Speech Recognition in Neurofibromatosis Type II Patients

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Key Words
Acoustic neuroma · Auditory brainstem implant · Nonauditory side effects · Open-set sentence recognition · Subjective benefits · Vestibular schwannoma

Abstract
This prospective study aimed to determine speech understanding in neurofibromatosis type II (NF2) patients following implantation of a MED-EL COMBI 40+ auditory brainstem implant (ABI). Patients (n = 32) were enrolled postsurgically. Nonauditory side effects were evaluated at fitting and audiological performance was determined using the Sound Effects Recognition Test (SERT), Monosyllable-Trochee-Polysyllable (MTP) test and open-set sentence tests. Subjective benefits were determined by questionnaire. ABI activation was documented in 27 patients, 2 patients were too ill for testing and 3 patients were without any auditory perception. SERT and MTP outcomes under auditory-only conditions improved significantly between first fitting and 12-month follow-up. Open-set sentence recognition improved from 5% at first fitting to 37% after 12 months. The number of active electrodes had no significant effect on performance. All questionnaire respondents were 'satisfied' to 'very satisfied' with their ABI. An ABI is an effective treatment option in NF2 patients with the potential to provide open-set speech recognition and subjective benefits. To our knowledge, the data presented herein is exceptional in terms of the open-set speech perception achieved in NF2 patients.

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Introduction

Neurofibromatosis type II (NF2) typically leads to a clinical picture dominated by neurological symptoms caused by the development of multiple benign spinal and brain tumors (Schwann cell tumors) [1]. The hallmark of NF2 is the development of bilateral vestibular schwannomas. However, unilateral vestibular schwannomas, a family history of NF2, or any two of meningioma, glioma, neurofibroma, schwannoma or posterior subcapsular opacities are also diagnostic criteria for NF2 [2].

Vestibular schwannomas involve the internal auditory canal or cerebellopontine angle and frequently result in severe disability and reduced life expectancy. Complete loss of hearing is common in the majority of bilaterally affected patients due to the destruction of the auditory nerve, usually resulting either from tumor growth or from treatment (by surgical tumor removal or radiosurgery). After surgical treatment of the tumor the hearing preservation of patients, who showed useful preoperative hearing, ranges from 32 to 88% [3–6]. Patients with deafness and preserved function of the cochlear nerve are good candidates for cochlear implantation [7, 8]. However, in patients with complete hearing loss, following nerve degeneration or nerve loss by tumor destruction, an auditory brainstem implant (ABI) represents the only remaining therapeutic option to provide patients with auditory input [9–13].

Several studies indicate that ABIs are effective and safe in providing useful auditory sensations in most patients with NF2 who would otherwise be totally deaf [9, 14–21]. However, only a minority of the patients in the aforementioned studies achieved open-set speech discrimination and the speech recognition of individuals with an ABI varied considerably; most patients use their ABI to facilitate lip-reading and can only recognize environmental sounds [9, 15–19, 22]. Importantly, even those with strongly limited speech recognition reported being very satisfied with their implant, showing that NF2 patients can gain remarkable objective and subjective benefits from ABI use [18].

Another factor contributing to the performance of an ABI are nonauditory side effects. Typically, nonauditory side effects are produced via inadvertent stimulation of the cerebellar flocculus, the cerebellar peduncle, the long sensory tracts or the facial nerve. It is not unusual for NF2 patients with an ABI to experience nonauditory sensations [23]. Up to 42% of users experience them [24, 25] and almost all of these nonauditory effects are benign, but they cause considerable discomfort to the individual [23]. Nonauditory side effects are usually managed by selecting the configuration of the electrodes [26], i.e. programming out the stimulus. In multichannel ABIs different sites of electrode stimulation can generate different pitch percepts [27]. Therefore, changes in the frequency spectrum of sounds can be coded for by changes in electrode activation [27]. Consequently, deactivation of electrodes due to nonauditory sensations can potentially affect the performance seen in patients fitted with an ABI. Although reports indicate that the number of functional electrodes affects the performance of speech recognition tests [19, 28], opinions regarding the existence of a correlation between the number of active electrodes and patient performance remain divided.

This study aimed to determine speech understanding capabilities over time in NF2 patients following implantation of an ABI. In particular, this paper evaluated open-set speech understanding and subjective benefits in NF2 patients with an ABI, who experience some auditory sensation, over a 1-year postactivation period. In addition, the frequency and consequences of nonauditory side effects were assessed.

Table 1. Subject demographics

Subject ID	Age at implantation		Gender	Side implanted	Number of active electrodes				
	years	months			first fitting	1 month	3 months	6 months	12 months
1	41	8	M	R	10	10	–	–	–
2	34	11	F	L	10	10	11	10	–
3	42	3	M	R	5	4	5	5	2
4	63	7	M	R	10	9	12	12	12
5	49	10	M	R	12	–	12	12	12
6[1]	42	4	F	L	too sick to test				
7	36	5	M	L	7	8	–	9	9
8	22	7	M	L	7	–	8	8	8
9[1]	48	4	F	R	nonuser				
10	45	12	F	R	12	12	9	8	8
11	23	1	M	R	–	7	7	7	7
12	35	12	M	R	7	7	7	7	7
13	54	1	F	R	12	12	12	12	12
14	27	7	F	L	9	10	–	9	9
15	25	11	F	R	7	7	7	12	12
16	19	3	F	R	8	8	8	8	7
17[1]	40	12	F	R	nonuser				
18	39	12	M	L	12	8	10	10	10
19[1]	40	3	F	L	too sick to test				
20	51	1	F	L	8	8	6	6	6
21[1]	30	2	M	L	nonuser				
22	21	5	F	R	12	12	12	–	–
23	41	9	F	R	7	7	–	–	–
24	66	11	M	R	10	9	9	9	9
25	39	7	M	R	8	8	–	–	–
26	43	4	F	L	6	6	6	6	–
27	42	10	M	bilateral	6	5	5	5	5
28	31	8	M	L	–	–	–	–	–
29	42	2	M	R	–	–	–	–	–
30[2]	33	6	M	R	–	–	–	–	–
31[2]	26	8	M	L	–	7	7	7	7
32	25	7	F	R	8	8	8	8	8

[1] Not included in data analysis. [2] Revision cases.

Materials and Methods

Patients and Inclusion Criteria

Between April 2001 and July 2009, 32 patients who received a MED-EL COMBI 40+ ABI were enrolled postsurgically in this prospective multicenter study; 16 patients were treated at Würzburg (University of Würzburg, Würzburg, Germany), while the remainder were treated at 6 other centers. The mean age at implantation was 38.4 years (range: 19.0–66.1 years). Individual subject data are shown in table 1. For inclusion in this study, subjects were 15 years or older and diagnosed with NF2. All patients gave written informed consent.

All patients were implanted using the surgical procedure as described by Matthies et al. [16] (2000), Behr et al. [18] (2007) and Jackson et al. [22] (2002); 6 participants received the MED-EL COMBI 40+ and 26 the ABI. The ABI is a development of the COMBI 40+ offering an electronic platform, which allows a maximum stimulation rate of 50.760 compared to 18.180 pulses/second, which was possible with the COMBI 40+. Besides the electronics there is no difference between the 2 implants. Both feature a ceramic housing, offer the CIS+ speech-coding strategy [29], and comprise an electrode carrier with 13 (12 stimulation and 1

reference) platinum contacts partially embedded in a preshaped flat silicone paddle [18, 22]. In addition, the ABI electrode features a polyester mesh to increase the stability of the electrode array on the surface of the cochlear nucleus.

At the time of enrolment all subjects showed acceptable general health and mental stability. However, at follow-up 5 subjects could not be included in the study due to poor health, which prevented them from performing any tests (subjects 6 and 19), or they were excluded because they did not experience any auditory sensation (subjects 9, 17 and 21). Subjects 27 and 30 underwent ABI implantation a number of years earlier without success. Subject 27 was bilaterally implanted as a nonuser in the left ear, and was tested with the active right implant. Subject 26 had prior ABI experience; however, the ABI lost function following tumor regrowth. Likewise, subject 31 had prior ABI experience; however, trauma resulting in an implant defect led to implantation of a new device on the same side.

Device Fitting

All patients were fitted with the TEMPO+ BTE speech processor. In general, initial stimulation took place 6–8 weeks after surgery and was performed during a 3-day inpatient hospitalization. In some cases an extended rehabilitation period after tumor removal was required and led to delayed implant activation.

First fitting was performed in a monitored environment such as an Intensive Care Unit. Pulse oximetry, continuous echocardiography and noninvasive blood pressure were monitored during the fitting process. Emergency resuscitation equipment and drugs were available and an Advanced Cardiac Life Support certified individual was present. Activation commenced with stimulation of individual electrodes. Patients were instructed to report any auditory and nonauditory sensations. Electrodes with clear auditory percept and no or negligible nonauditory side effects were selected for an initial program.

Following 1–2 days of listening experience and refinement of the initial program the first assessment of performance was conducted. Pitch ranking of the selected electrodes was attempted during first fitting; however, in some cases this was only possible at subsequent fitting sessions. After electrodes were balanced in loudness, participants were asked to name the electrodes with the highest and lowest pitches. Successive repetition and reordering led to a tonotopic ranking of the electrodes.

Follow-up assessment took place at 1, 3, 6 and 12 months postactivation. During follow-up, individual electrode stimulation for loudness, pitch and nonauditory side effects, and speech and sound perception were used to optimize the program. The nature and subjective strength of nonauditory side effects determined which electrode contacts were activated in this study. Contacts were either checked repeatedly with regard to nonauditory side effects at follow-up or were in some instances, depending upon the nature of

Subjective Benefit Assessment

Six months after first fitting all participants were asked to complete a questionnaire specifically designed to assess the subjective impact of the ABI on the users. The questionnaire consisted of 7 questions assessing topics such as the time needed to become accustomed to the ABI, the influence of the ABI on daily life and listening capacity, as well as the subject's overall impression regarding the ABI.

Statistical Analyses

Descriptive statistics were used to report demographic data and baseline device fitting characteristics. Quantitative data are presented as mean, standard deviation and range (minimum and maximum); qualitative data are presented as absolute and relative frequencies. The Kolmogorov-Smirnov test was used to determine data distribution.

The effects between first fitting and 12-month testing for the SERT, the closed-set MTP test and the open-set sentence test were examined using the nonparametric Mann-Whitney U test. To show the benefit over lip-reading added by the ABI, the auditory gain for open-set sentence test results was calculated by subtracting the mean scores obtained under visual-only conditions from those under auditory-visual conditions.

Outcomes of the 7-item subjective questionnaire are presented as absolute and relative frequencies. Missing data were not replaced but treated as 'missing' values. Data of participating study sites were pooled. To prevent a treatment-by-center interaction, each study site followed an agreed protocol. A p value <0.05 was determined as statistically significant. IBM SPSS Statistics 19 (IBM, Armonk, N.Y., USA) was used for all analyses. Graphs were created in Microsoft Office Excel 2010 (http://www.microsoft.com).

Results

Number of Active Electrodes and Nonauditory Side Effects

At first fitting an average of 8.8 ± 2.2 out of 12 available contacts were activated to provide auditory stimuli to the subjects (table 1). None of the 27 subjects included had less than 5 active electrodes. Over time, the number of active electrodes remained essentially stable. Electrode contacts were deactivated due to several reasons: (1) contacts providing no sensation at all; (2) contacts causing unpleasant sound sensation (sound often described as faint, scratchy or persistent); (3) contacts with the same pitch rank (for optimized fitting); (4) contacts with mixed-auditory and nonauditory sensations (if nonauditory sensations were not tolerated by the subject), and (5) contacts with only nonauditory responses.

No information was available regarding nonauditory side effects for 7 subjects (25.9%); 8 subjects (29.6%) did not experience any nonauditory side effects. Of the remaining 12 subjects the body location and nonauditory sensation with the number of contacts (deactivated and active) causing the side effect are shown in figure 1. The nature of the side effect(s) led to deactivation in 48 out of 144 total contacts (33.3%); 11 out of 144 contacts (7.6%) were maintained in an active state despite subjects experiencing nonauditory sensations. Amongst these, 2 contacts were deactivated after the first fitting, while 9 were active throughout the duration of the study.

Sound Effect Recognition Test

The SERT was performed by 26 subjects in total. As shown in figure 2, a steady increase in SERT scores averaged across all data available was observed up to the 6-month test interval. The improvement between the first fitting scores and the 12-month test were significant (p = 0.009, n = 11 subjects who performed the test at the first fitting and 12-month test interval).

Monosyllabic-Trochee-Polysyllabic Tests

The closed-set MTP test was performed by 27 subjects in total. The test was scored by correct identification of syllables and words under the auditory-only and auditory-visual

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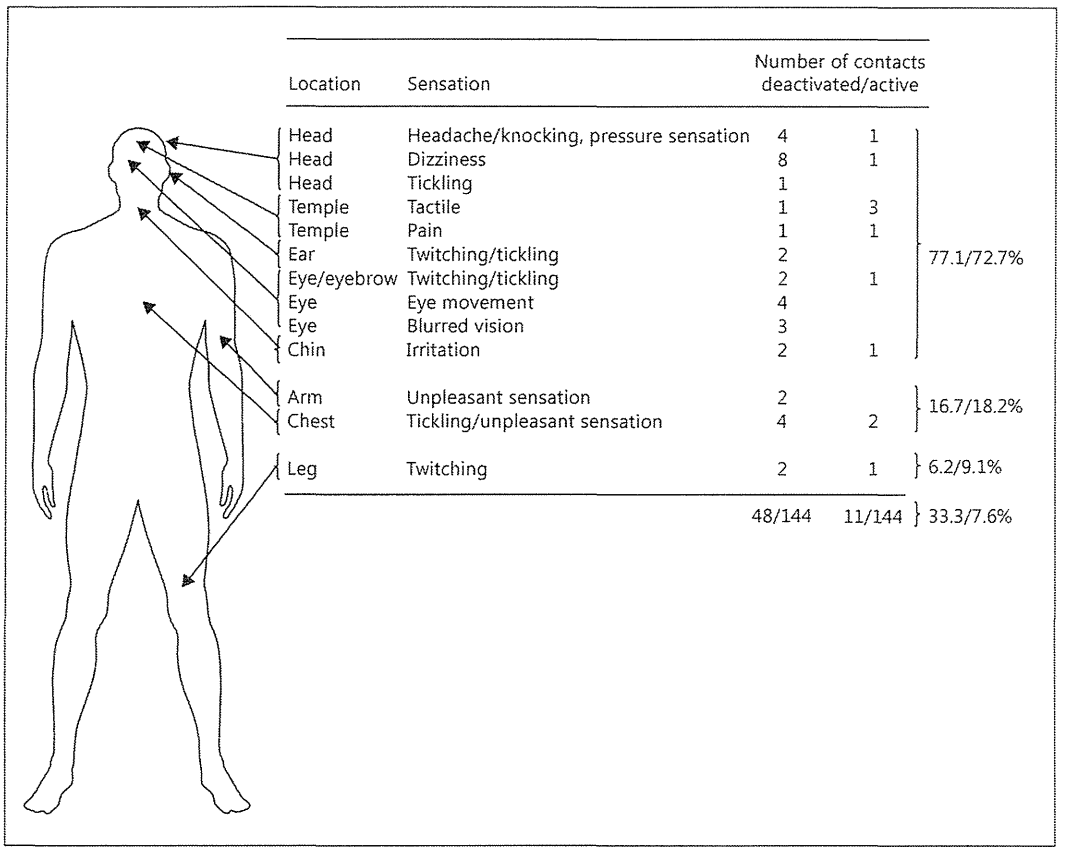

Location	Sensation	Number of contacts deactivated	Number of contacts active	
Head	Headache/knocking, pressure sensation	4	1	
Head	Dizziness	8	1	
Head	Tickling	1		
Temple	Tactile	1	3	
Temple	Pain	1	1	
Ear	Twitching/tickling	2		77.1/72.7%
Eye/eyebrow	Twitching/tickling	2	1	
Eye	Eye movement	4		
Eye	Blurred vision	3		
Chin	Irritation	2	1	
Arm	Unpleasant sensation	2		16.7/18.2%
Chest	Tickling/unpleasant sensation	4	2	
Leg	Twitching	2	1	6.2/9.1%
		48/144	11/144	33.3/7.6%

Fig. 1. Location and frequency of nonauditory sensations experienced by patients with an ABI at first fitting (n = 12).

condition. In both test conditions (auditory-only and auditory-visual) the mean syllable scores reached test ceiling immediately after device activation (data not shown). Individual word scores are shown in table 2.

Under auditory-only conditions the mean correct word score across all data available ('all data' group) was 11.7 ± 6.4 words out of 24 (48.7%) at first fitting. The mean outcome calculated from patients who were tested at all scheduled intervals ('complete' group) was 12.1 ± 6.0 words (50.4%). A steady increase in mean results was observed over the 12-month follow-up period for both the 'all data' and the 'complete' group. The improvement between first fitting scores and 12-month testing was highly significant for auditory-only word recognition (Mann-Whitney U test: $p < 0.001$, $n = 19$).

Under the auditory-visual condition the test ceiling was reached at first fitting. Likewise, no statistically significant difference was observed in the auditory-visual word score between the first fitting and 12-month testing (Mann-Whitney U test: $p = 0.106$, $n = 18$).

Sentence Tests

Sentence tests were performed by 26 subjects in the auditory-visual condition, 23 in the auditory-only condition and 22 in the visual-only condition. Results averaged across all available data under all conditions are shown in figure 3. A highly significant improvement from the first fitting to the 12-month test was observed under the auditory-only (Mann-

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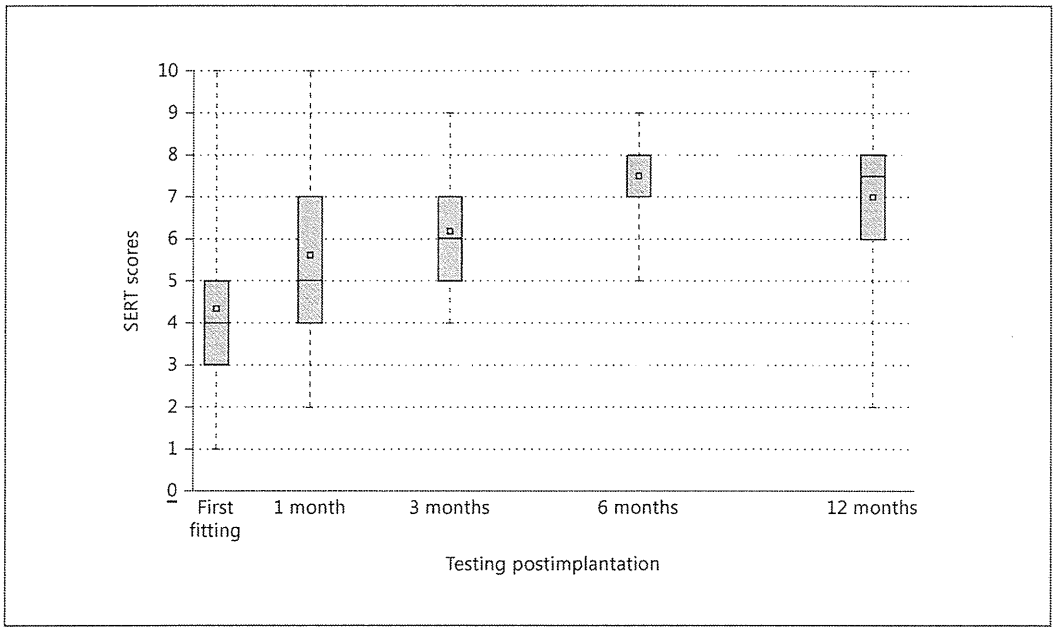

Fig. 2. Environmental sound recognition performance determined by SERT in patients with an ABI after first fitting and 1, 3, 6 and 12 months postactivation. Boxplot whiskers depict sample minimum and maximum; the white square equals the mean value.

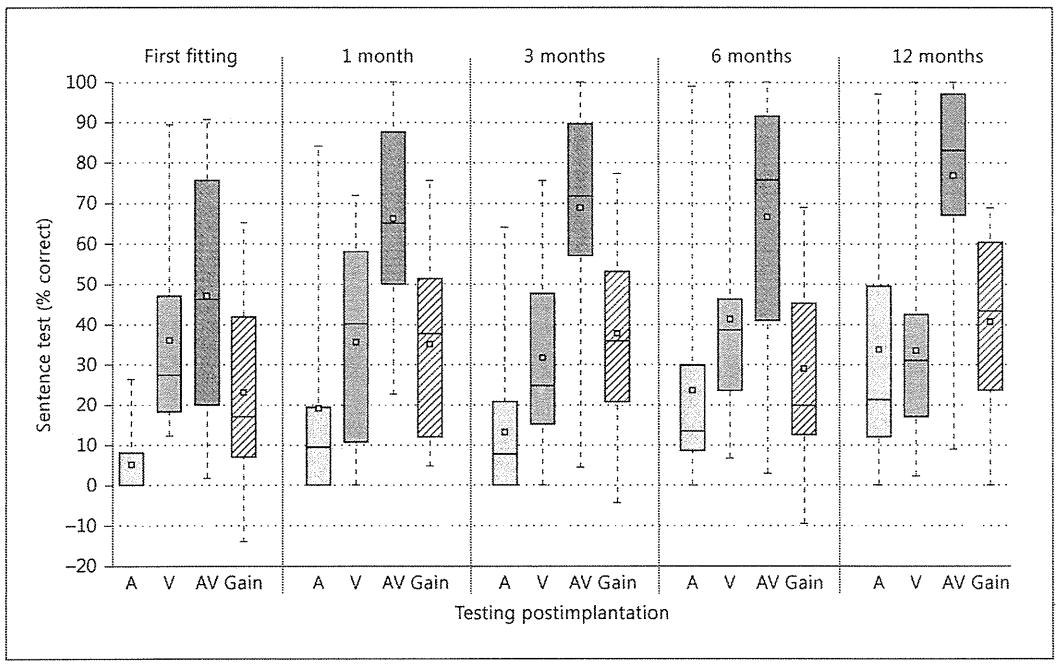

Fig. 3. Open-set speech recognition as determined by sentence testing in patients with an ABI after first fitting and 1, 3, 6 and 12 months postactivation. A: auditory only; V: visual only; AV: auditory-visual combined; Gain: AV-V. Boxplot whiskers depict sample minimum and maximum; the white square equals the mean value. Error bars represent standard deviation. Negative 'Gain' values represent patients who performed poorer in the combined auditory-only and visual-only conditions compared to the visual conditions.

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Table 2. Individual results of MTP words (words repeated correctly) of the auditory-only and auditory-visual test conditions

Subject ID	Auditory only					Auditory-visual				
	first fitting	1 month	3 months	6 months	12 months	first fitting	1 month	3 months	6 months	12 months
1	–	8	6	–	17	–	15	16	–	21
2	–	14	22	16	22	–	23	24	24	24
3	4	–	1	4	7	21	–	22	22	22
4	13	21	20	22	21	–	24	23	24	24
5	–	–	18	18	20	–	–	23	24	24
7	20	24	24	24	23	24	24	24	24	24
8	7	17	23	21	24	24	24	24	24	24
10	12	14	21	24	24	24	24	24	24	24
11	–	–	–	21	22	–	–	–	23	24
12	14	21	19	21	21	22	23	24	24	24
13	15	13	4	16	8	24	24	24	24	24
14	14	20	18	24	20	24	24	24	24	24
15	14	16	18	19	16	22	24	24	24	24
16	6	22	13	17	21	24	22	23	24	21
18	10	17	16	21	18	22	24	24	24	24
20	0	12	10	15	20	24	24	24	24	24
22	10	24	24	–	–	23	24	24	–	–
23	6	9	19	–	–	24	24	24	–	–
24	24	24	–	24	24	22	23	–	24	24
25	11	4	–	–	–	24	24	–	–	–
26	19	19	21	21	23	24	24	24	24	24
27	11	12	18	22	22	24	24	24	24	24
28	13	17	20	18	21	24	24	24	24	24
29	18	16	18	20	23	23	23	23	24	24
30[1]	19	15	16	16	12	24	24	24	24	24
31[1]	–	20	19	16	21	–	24	24	24	24
32	11	14	16	21	23	23	24	24	24	24
All data group										
n	21	24	24	23	24	21	24	24	23	24
Mean ± SD	11.7±6.4	15.4±5.3	16.1±6.2	17.9±4.8	19.6±4.2	23.2±1.0	23.2±2.1	23.2±2.1	23.8±0.6	23.6±1.0
Mean ± SD complete	12.1±6.0	15.8±3.0	16.7±3.4	18.8±2.4	19.2±3.4	23.4±0.8	23.7±0.6	23.8±0.4	24.0±0.0	23.8±0.8

Mean values ± SD are depicted for all data available (all data) and for the patients who performed the test at all test intervals (complete) under auditory-only (n = 17) and auditory-visual conditions (n = 16). SD = Standard deviation. [1] Revision cases.

Whitney U test: $p < 0.001$, n = 8) and the auditory-visual test conditions (p = 0.001, n = 12); no significant improvement was observed under visual-only test conditions (p = 0.083, n = 6).

Individual sentence test results under the auditory-only and auditory-visual conditions are shown in table 3. All subjects who performed the sentence test in the auditory-visual condition at first fitting (17 out of 26) were able to achieve at least some open-set speech understanding. Of the subjects available for the sentence test under auditory-visual conditions at first fitting and at 12-month testing, 11 out of 12 subjects performed better at the 12-month test interval. In the auditory-only condition 12 subjects performed the test at first fitting; 5 subjects achieved open-set speech understanding in this difficult test situation. After 12 months of ABI use 19 subjects could be tested, and all but 1 achieved open-set speech understanding. Overall, these data illustrate the improvement and learning ability over time

Table 3. Individual sentence test results (% correct) of the auditory-only and auditory-visual test conditions

Subject ID	Auditory only					Auditory-visual				
	first fitting	1 month	3 months	6 months	12 months	first fitting	1 month	3 months	6 months	12 months
1	–	0.0	0.0	–	35.6	–	50.0	4.4	–	8.9
2	–	0.9	22.8	22.6	16.0	–	42.5	60.4	38.7	59.4
3	–	–	–	–	–	1.8	–	–	8.8	–
4	12.3	19.3	0.0	31.6	1.8	70.2	89.5	82.5	78.9	63.2
5	–	–	25.0	35.7	21.2	–	–	26.8	48.2	21.2
7	26.4	80.2	64.2	87.7	86.8	78.3	100.0	91.5	96.2	100.0
8	–	15.1	12.3	28.3	63.4	–	34.0	69.8	91.5	98.1
10	0.0	1.9	2.8	68.3	81.1	46.2	63.2	83.0	92.5	98.1
11	–	–	–	69.8	79.2	–	–	–	100.0	100.0
12	–	–	8.3	13.3	23.3	–	75.0	81.7	75.0	93.3
13	–	–	–	–	–	44.3	40.6	43.4	59.4	–
14	6.6	28.3	12.3	12.3	–	78.3	79.2	94.3	91.5	97.2
16	–	–	–	0.0	10.4	5.1	–	–	2.8	67.0
18	–	1.7	18.3	13.3	11.7	30.0	56.7	71.7	65.0	80.0
20	–	–	–	–	–	–	65.1	–	65.1	83.0
22	0.0	94.3	–	–	86.8	91.5	100.0	–	–	–
23	0.0	0.0	7.0	–	–	75.4	87.7	96.5	–	–
24	3.8	65.1	–	99.1	97.2	90.6	94.3	–	98.1	100.0
25	0.0	–	–	–	–	15.8	–	–	–	–
26	13.2	9.4	39.6	9.4	12.3	70.8	90.6	100.0	100.0	79.2
27	–	9.6	3.8	7.5	14.2	–	65.1	87.7	91.5	87.7
28	–	–	0.0	1.9	16.0	2.8	23.8	35.8	28.3	75.5
29	0.0	0.0	0.0	0.0	5.7	19.8	29.2	56.6	37.7	73.6
30[1]	0.0	1.9	0.0	0.0	0.0	35.8	52.8	57.5	35.8	48.1
31[1]	–	0.0	22.6	11.3	38.7	–	82.1	92.5	76.4	84.9
32	0.0	0.0	0.0	11.3	36.8	53.8	69.8	70.8	84.0	93.4
All data group										
n	12	17	18	19	20	17	21	19	22	21
Mean ± SD	5.2±8.3	19.9±30.2	13.3±17.1	27.6±30.8	36.9±32.8	47.7±31.3	66.2±23.6	68.8±26.2	66.6±30.1	76.8±25.3
Mean ± SD complete	7.4±10.3	16.1±29.1	15.2±26.0	29.8±35.0	32.1±37.6	48.6±26.2	65.5±25.6	74.4±20.0	71.0±27.5	80.8±16.9

Mean values ± SD are depicted for all data and for the patients who performed the test at all test intervals (complete) under auditory-only (n = 7) and auditory-visual conditions (n = 10). SD = Standard deviation. [1] Revision cases.

both on an individual and group basis. In summary, of the 19 subjects that could be tested at 12 months, under auditory-only conditions, a mean open speech perception of 37% was achieved. Under audio-visual conditions at 12 months the percentage of speech perception achieved was 77%.

To determine the benefit of ABI use over lip-reading alone, the auditory gain was calculated for the 'all data' group (fig. 3). At all test intervals the auditory gain was greater than the auditory-only performance, i.e. the sum of the results under auditory-only and visual-only combined was less than the performance under auditory-visual conditions. Furthermore, the auditory gain increased significantly over time from the first fitting to the 12-month test ($p = 0.008$).