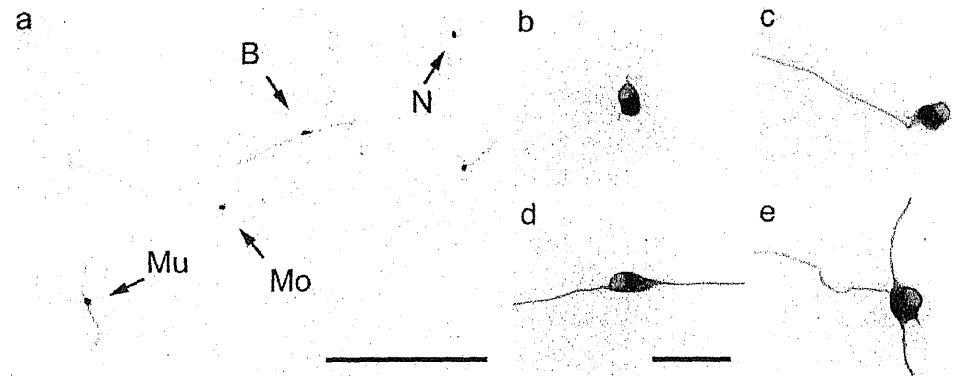

Fig. 1 **a** Example of cultured spiral ganglion neurons (SGNs) after 12 h in primary growth medium containing serum and a further 72 h in serum-free maintenance medium. SGNs were then fixed and immunostained with anti-NF200 antibody. Surviving SGNs are identified as NF200-positive cells. Cultured neurons either have no neurite (*N*) or show monopolar (*Mo*; with one neurite emanating from the cell body), bipolar (*B*; with two neurites emanating from the cell body), or multipolar (*Mu*; with three or more neurites emanating from the cell body) morphologies. High magnification views of the *N*, *Mo*, *B* and *Mu* SGNs shown in **a** are presented in **b**–**e**, respectively. *Bars* 0.5 mm (**a**), 50 μm (**b**–**e**)

Effects of NT-3, BDNF and LIF on SGN survival

Figure 3 shows representative photomicrographs of cultured SGNs in P0, P5 and P20 groups, with or without neurotrophic factor supplementation. As illustrated, increases in SGN numbers as a result of neurotrophic support are apparent for SGNs harvested at younger ages.

Figure 4 provides a quantitative analysis of SGN numbers across ages and treatment conditions. Figure 4a shows the average number of SGNs/well, uncorrected for initial seed number. In the absence of any neurotrophic factor treatment, P5 cultures obviously showed the greatest yield of surviving neurons (8.7±0.7/well), followed by P20 cultures (7.0±1.5/well) and then by P0 cultures (0.6 ±0.2/well). Moreover, the greatest sensitivity to neurotrophic treatment was observed for P5 SGNs.

Because of the difference in the initial yield of SGNs among age groups, we normalized the number of surviving SGNs/well by the initial seeding level. These data are presented in Fig. 4b, which demonstrate that the survival rate of untreated SGNs in culture increases dramatically with increasing age. In control cultures, surviving SGNs at 72 h corresponded to 0.027%, 0.98%, or 4.2% of those seeded initially in the P0, P5 and P20 groups, respectively. The normalized survival rate of SGNs in each treatment group also tended to increase for older SGNs (Fig. 4b).

In P0 cultures, neurotrophic factors had a significant effect on the survival of SGNs ($P<0.0001$; ANOVA) and this effect was additive/synergistic. When the culture was treated with N + B, the number of surviving neurons per culture (10.5±0.8) increased to 17-fold that of untreated cultures (0.6±0.2). This number almost corresponded to that of the sum of the number of surviving neurons in NT-3 (0.9±0.2) and BDNF cultures (5.3±0.5). When the culture was treated with ALL factors, the number of surviving neurons further increased to 74-fold that of untreated cultures (74.0±6.7), which is approximately 9.1-fold that of the sum of the number of surviving SGNs in NT-3-treated (0.9±0.2), BDNF-treated (5.3±0.5) and LIF-treated (1.9±0.4) cultures. The post-hoc test revealed that the treatment with N + B and ALL factors had statistically significant survival-promoting effects compared with the untreated control ($P<0.05$, $P<0.001$, respectively).

In P5 cultures, SGN survival responded to the widest range of treatments amongst the age groups; ANOVA revealed significant effect on survival ($P<0.0001$) and the post-hoc test showed that each of NT-3, BDNF, N + B and ALL factors significantly enhanced the survival of SGNs ($P<0.001$ for each; Fig. 3). In this age group, the additive/synergistic effect seen in P0 cultures was decreased under our culture conditions. When the culture was treated with N + B, the number of surviving neurons per culture (63.4±7.1), which was 6.3-fold that of untreated cultures (10.0±1.1), almost corresponded to the number of surviving neurons in BDNF cultures (5.9-fold, 59.0±4.7). When the culture was treated with ALL factors, the number of surviving neurons (nine-fold, 90.3±6.0) was slightly less than the sum of the number of SGNs treated with the individual factors: BDNF, NT-3 (3.2-fold, 31.5±2.0) and LIF(1.9-fold, 19.4±1.6).

In P20 cultures, the survival-promoting effect of each treatment was the smallest of any of the age groups ($P=0.0097$; ANOVA). The post-hoc test revealed that only treatment with NT-3 or with BDNF significantly promoted the survival of SGNs compared with the untreated control ($P<0.01$, $P<0.001$, respectively). Moreover, cultures treated with N + B or ALL factors

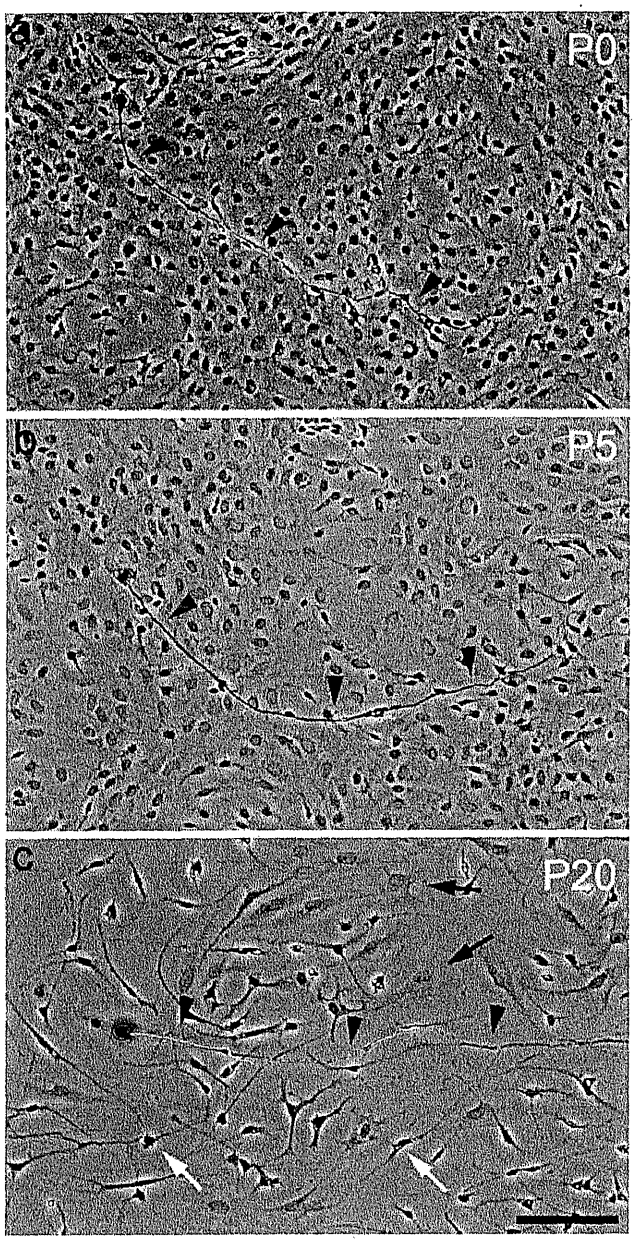

Fig. 2 Representative phase-contrast images of postnatal day 0 (P0; **a**), P5 (**b**) and P20 (**c**) control cultures maintained for 12 h in serum-containing primary growth medium and for a further 72 h in serum-free maintenance medium (*arrowheads* neurites of SGNs). Non-neuronal cells in each culture can be morphologically differentiated into two cell types, i.e., *flat-shaped* cells with large nuclei and *spindle-shaped* cells with small nuclei and prominent processes. This differentiation is most readily observed in P20 culture (*black arrows* flat-shaped cells, *white arrows* spindle-shaped cells). The density of these non-neuronal cells decreases with the increasing age of the animals. In P0 and P5 cultures, the non-neuronal cells form a confluent layer on the glass surface, whereas the glass surface is partially free of cellular covering in P20 culture. *Bar* 0.1 mm

exhibited survival no greater than that observed in controls.

Effects of NT-3, BDNF and LIF on neurite extension

The LNL of untreated SGNs was shortest for neurons harvested at P0, slightly longer for P5 neurons and longest for P20 neurons.

In P0 cultures, the overall effect of treatment on the LNL was significant (Fig. 5a; $P<0.0001$; ANOVA). However, when factors were delivered alone, only LIF elicited an enhancement of LNL compared with the control group ($P<0.001$; Dunnet post hoc test). ALL factors also showed a significant increase in LNL ($P<0.01$) but this effect was lower than that observed with LIF alone. LNL was unaffected by the presence of NT-3, BDNF, or both factors combined ($P>0.05$).

LNL of P5 SGNs responded to the widest range of treatment with neurotrophic factors among the age groups (Fig. 5b; $P<0.0001$; ANOVA). Again, LIF and ALL factors showed significantly increased LNL ($P<0.001$), although ALL factors once more produced a lower increase than LIF alone. In contrast, treatment with NT-3, BDNF, or N + B inhibited neurite extension compared with that in the control group ($P<0.05$, $P<0.001$, $P<0.001$, respectively). To test whether this inhibitory effect depended on the concentration of neurotrophic factors, we also treated P5 SGNs with BDNF and/or NT-3 at 10 ng/ml. Although a survival-promoting effect was observed at this lower concentration, as seen at 50 ng/ml (Fig. 6a), LNL in the treated groups was not significantly different from that in the untreated controls (Fig. 6b). However, the addition of LIF (ALL factors) produced a significant enhancement of both survival and LNL at this age.

In P20 cultures, the effect of neurotrophic factors on LNL was the smallest among the age groups, although it remained significant (Fig. 5c; $P=0.0008$) by ANOVA. The post-hoc test revealed that treatment with ALL factors weakly enhanced LNL ($P<0.05$), whereas N + B had a strong inhibitory effect ($P<0.001$).

Effects of NT-3, BDNF and LIF on neuronal morphology

For all age groups, monopolar neurons were the most prevalent morphological type in untreated cultures (Fig. 7). In general, neurotrophic factor treatment had modest effects on neuronal morphology. An exception was ALL factors, which enhanced the proportion of bipolar and multipolar neurons, while decreasing the monopolar and especially the "no" neurites phenotypes.

To assess these effects statistically, neurons in each age group were divided into two categories, i.e., SGN without neurites or with one neurite versus SGN with two or more neurites. In all age groups, ALL factors significantly increased the proportion of ≥2 neurite SGNs compared with the control group (Table 2; $P<0.05$ in P0,

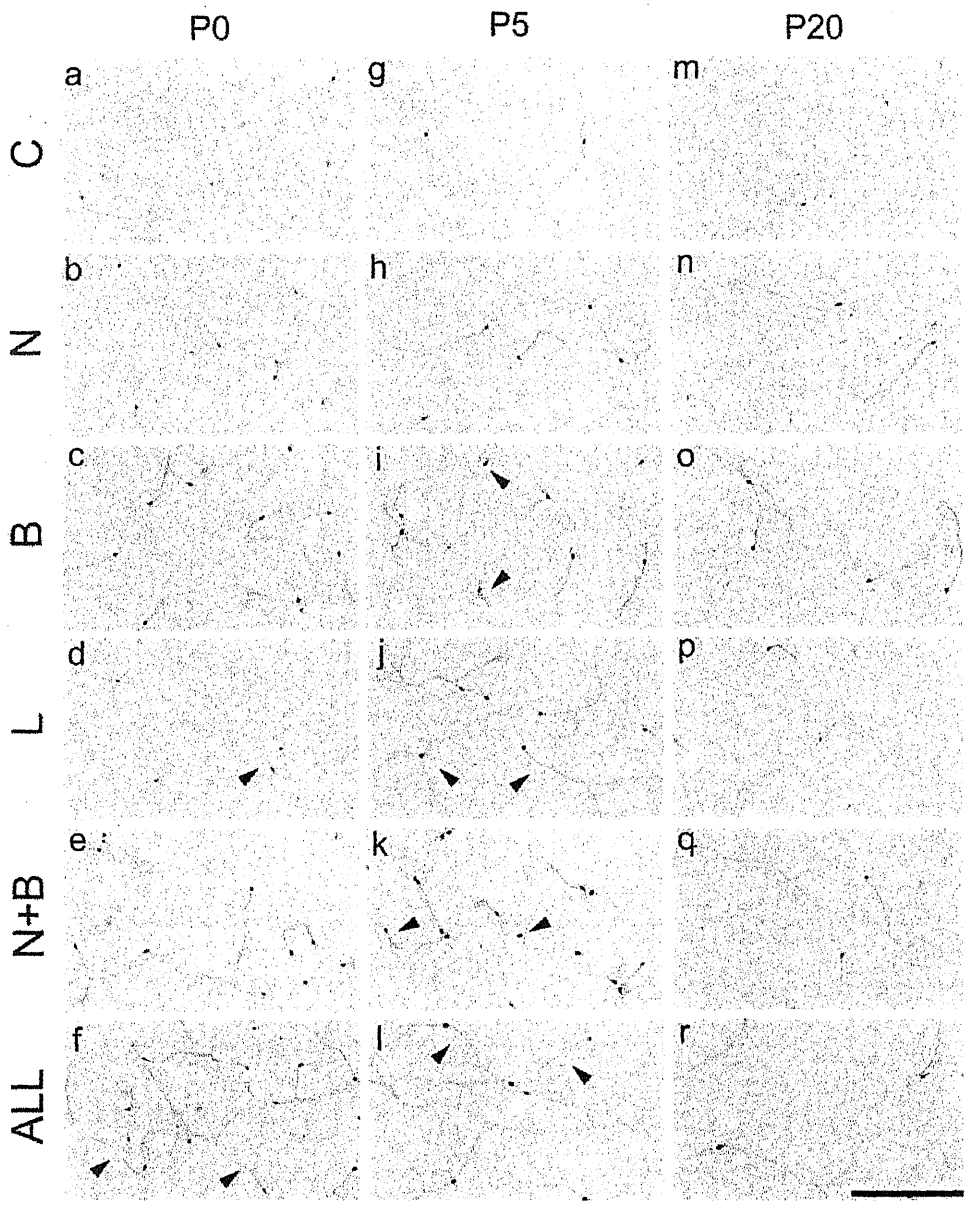

Fig. 3 Representative photomicrographs of cultured SGNs in each age group. Neurons at P0 (**a–f**), P5 (**g–l**) and P20 (**m–r**) were cultured for 12 h in serum-containing primary growth medium and for a further 72 h in serum-free maintenance medium without any neurotrophic factors (**a**, **g**, **m**) or supplemented with 50 ng/ml neurotrophin-3 (NT-3; *N*, **b**, **h**, **n**), brain-derived neurotrophic factor (BDNF; *B*, **c**, **i**, **o**), leukemia inhibitory factor (LIF; *L*, **d**, **j**, **p**), a combination of NT-3 and BDNF (50 ng/ml each; *N+B*, **e**, **k**, **q**), or a combination of NT-3, BDNF and LIF (50 ng/ml each; *ALL*, **f**, **l**, **r**) and then fixed and immunostained with anti-NF200 antibody. Survival effects of NT-3, BDNF, LIF and their combinations compared with the untreated control varied depending upon the ages of SGNs. At P0, the additive/synergistic effect of treatment in the N + B and ALL factors groups is clearly apparent, whereas this effect appears to be decreased in P5 and is not obvious in P20 cultures. The length of neurites seems to be increased by treatment with LIF or ALL factors in P0 and P5 cultures (*arrowheads* in **d**, **f**, **j**, **l**). In contrast, neurite length appears to be decreased by supplemention with BDNF or N + B in P5 cultures (*arrowheads* in **i**, **k**). *Bar* 0.5 mm

$P<0.001$ in P5 and P20; chi-square test). In the P20 group, BDNF alone and LIF alone also more modestly enhanced the proportion of ≥2 neurite SGNs compared with the control group (Table 2; $P<0.05$; chi-square test).

Discussion

The present study was designed to extend our knowledge regarding age-dependent changes in the responsiveness of SGNs to NT-3, BDNF and LIF by using dissociated cultures. Our study appears to be the first systematic in vitro study to compare the regulation of survival, neurite extension and neuronal morphology of rat SGNs among different age groups under the same culture conditions. The results demonstrate that each of these indices of SGN maturation are differentially regulated by NT-3, BDNF and/or LIF in an age-dependent manner. Our data further suggest that each of these neurotrophic factors predominantly support different ontogenetic events at different developmental stages in the innervation of the inner ear.

Effects of neurotrophic factors on SGN survival

A striking feature of our results was the dramatic age-dependent increase in the proportion of neurons that survived in culture in the absence of neurotrophic factor treatment. This finding suggests that SGNs are highly dependent for their survival on exogenous neurotrophic factors at early developmental stages but become more neurotrophic-factor-

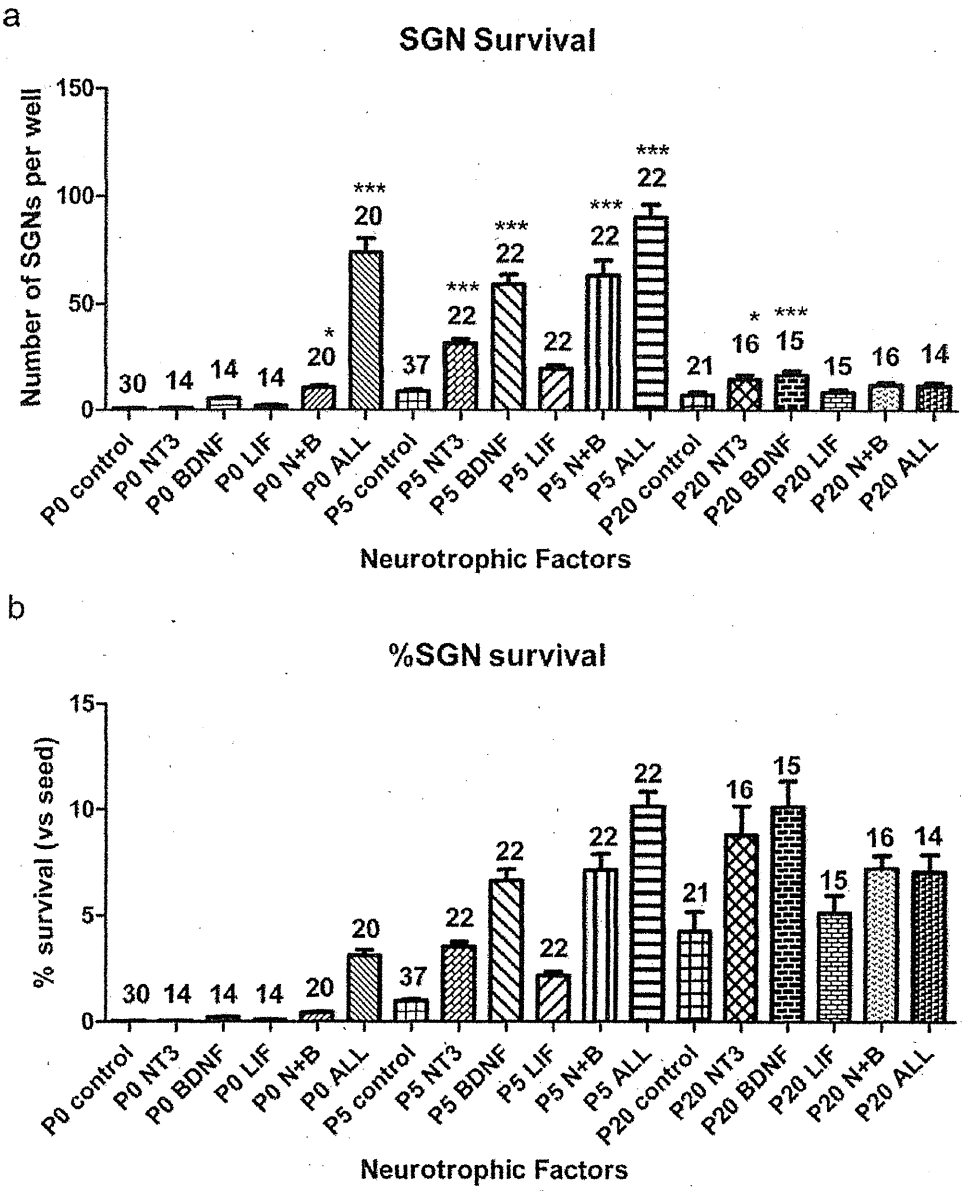

Fig. 4 **a** Effects of neurotrophic factors on SGN survival. Dissociated SG cells at P0, P5 and P20 were plated at 20,000 cells/culture well and cultured for 12 h in serum-containing primary growth medium and for a further 72 h in serum-free maintenance medium without any neurotrophic factors (*control*) or supplemented with 50 ng/ml NT-3, BDNF, LIF, or N + B at 50 ng/ml each or with ALL factors at 50 ng/ml each. The data are presented as an average number ± SE of surviving SGNs per culture well at each developmental age. Significant differences compared with control cultures are indicated: **P*<0.05, ***P*<0.01, ****P*<0.001 (ANOVA followed by Dunnett's post-hoc test). The *number above* each *bar* indicates the number of cultures analyzed. **b** Percentage of surviving SGNs divided by initial number of neurons seeded. Data are presented as an average percentage ± SE of surviving SGNs compared with the initial seed number of SGNs at each developmental stage. The *number above* each *bar* indicates the number of cultures analyzed. The survival rate of SGNs in each treatment group tended to increase with increasing age of the donor animals

independent as they approach adulthood. A similar tendency has been reported for the trigeminal ganglion (Scott and Davies 1993) and sympathetic neurons (Easton et al. 1997; Orike et al. 2001b; Putcha et al. 2000). Because the period of our P0 culture (equivalent to P0–P4 in vivo) corresponds to a period of naturally occurring SGN cell death (Echteler et al. 2005; Rueda et al. 1987), we can speculate that the higher trophic factor dependence of P0 and P5 SGNs contributes to the elimination of SGNs that fail to establish connection with a factor-producing target, as has been suggested for gerbil SGN (Mou et al. 1998).

Arguing against this interpretation is the limited survival response of P0 SGN to neurotrophin treatment, since none of the factors tested were effective in promoting the survival of SGN when applied individually. However, combinations of factors were highly effective, especially when LIF was added to BDNF plus NT-3. BDNF or NT-3 alone only became effective at older ages, whereas the synergistic effects observed at P0 declined at P5 and disappeared at P20. These data suggest that more than one factor is required to rescue SGN from apoptosis during early developmental target cell interactions, thereby serving to promote the survival of neurons that make contacts with separate sources of trophic factors, such as the peripheral and central contacts of SGN. This possibility is supported by the finding that both NT-3 and BDNF are expressed in the developing cochlea and cochlear nucleus (Sugawara et al. 2007; Tierney et al. 2001; Wiechers et al. 1999). The elements of the LIF receptor-gp130 heterodimer (Auernhammer and Melmed 2000) are expressed in the P1 mouse spiral ganglion (Oshima et al. 2007) and LIF is strongly expressed in the adult cochlear sensory epithelium after noise injury (Cho et al. 2004).

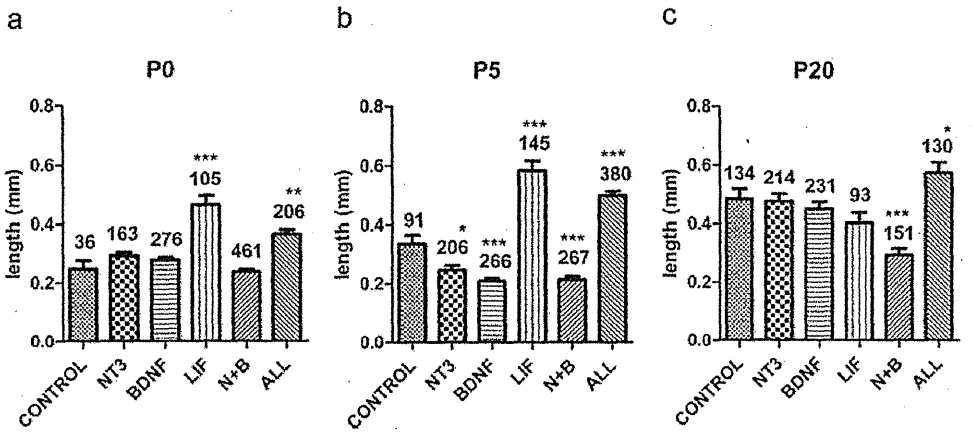

Fig. 5 Effects of neurotrophic factors on the longest neurite length (LNL) in P0 (**a**), P5 (**b**) and P20 (**c**) cultures. Data are presented as an average length ± SE of the longest neurite of each neuron at each developmental age. Significant differences compared with control cultures (*CONTROL*) are indicated: **P*<0.05, ***P*<0.01, ****P*<0.001 (ANOVA followed by Dunnett's post-hoc test). The *number above* each *bar* indicates the number of neurons analyzed. LIF supplementation of P0 and P5 cultures and ALL factors added to P0, P5 and P20 cultures significantly increased LNL compared with control SGNs. In contrast, treatment with NT-3, BDNF, or N + B in P5 cultures and with N + B in P20 cultures inhibited neurite extension

Although we could locate no published information regarding LIF expression in developing cochlear nucleus, LIF is expressed by many neurons in the brain (Lemke et al. 1996).

Notably, the initial seeding number of SGNs/well decreased dramatically with increasing age. Since the number of cells seeded/well was constant, this means that a greater proportion of the culture consisted in non-neuronal cochlear cells in older cultures. Therefore, we cannot exclude the possibility that the neurotrophic factor independence of older SGN reflects trophic support from these non-neuronal cells.

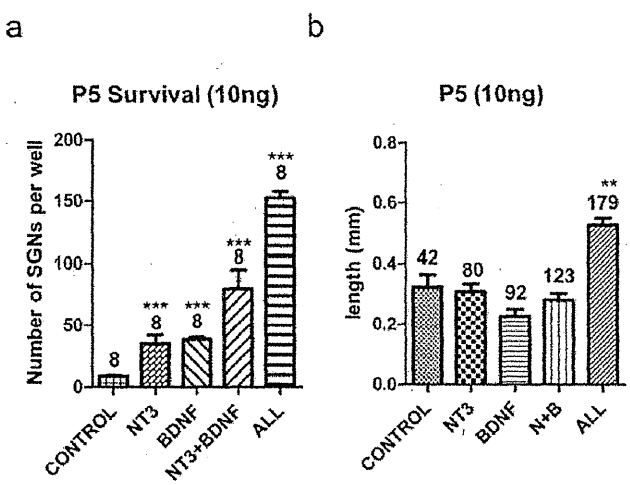

Fig. 6 Effects of NT-3 and BDNF at 10 ng/ml on survival (**a**) and LNL (**b**) in P5 cultures. Significant differences compared with control cultures are indicated: **P*<0.05, ***P*<0.01, ****P*<0.001 (ANOVA followed by Dunnett's post-hoc test). The *number above* each *bar* indicates the number of cultures (**a**) and neurons (**b**) analyzed. At 10 ng/ml, NT-3, BDNF, or N + B enhanced SGN survival, as also seen at 50 ng/ml, whereas inhibitory effects on neurite extension were not observed at this concentration

Although the density of non-neuronal cells decreased with the increasing age of the animals at the endpoint of culture period, probably reflecting the high proliferation rate of younger non-neuronal cells, the possibility that the initial support of non-neuronal cells at the early culture periods might have been associated with the higher survivial rate of SGNs at P20 cannot be excluded. The mature glial cells in P20 cultures might also have provided stronger trophic support than those in younger animals.

At P5 and P20, BDNF was more potent than NT-3 or LIF for survival. This observation is in good agreement with previous studies of dissociated early postnatal rat SGNs (Hegarty et al. 1997; Marzella et al. 1999; Zheng et al. 1995) and with the enhancement of the survival of adult SGNs by BDNF in vivo (Leake et al. 2011; Miller et al. 2007; Wise et al. 2005) and in vitro (Vieira et al. 2007; Wei et al. 2007). Although BDNF expression is almost absent in the peripheral target field of the SGNs at the later stages of development (Wheeler et al. 1994; Wiechers et al. 1999; Ylikoski et al. 1993), it is expressed by neurons in the ventral cochlear nucleus beginning on P3 (Tierney et al. 2001). BDNF is also observed in SGNs themselves until the adult stage (Ruttiger et al. 2007; Schimmang et al. 2003; Singer et al. 2008), raising the possibility of an autocrine mechanism (Schimmang et al. 2003).

With regard to the survival-promoting effects of LIF, previous in vitro studies have documented that LIF has survival-promoting effects on early postnatal rat and mouse SGNs (Gillespie et al. 2001; Whitlon et al. 2006) and adult mouse SGNs (Vieira et al. 2007). Our results suggest that the survival-promoting effects of LIF alone are not as strong as those of BDNF and NT-3. It is more potent as a synergistic enhancer of neurotrophins for the survival of P0 and, to a lesser extent, P5 rat SGNs.

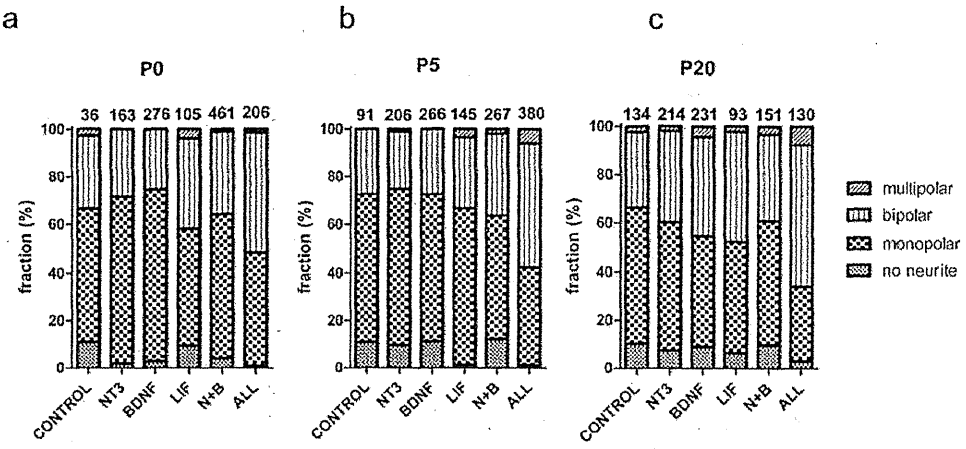

Fig. 7 Effects of NT-3, BDNF, LIF, N + B, or ALL factors on neuronal morphology. The fraction of SGNs that were without a neurite or that were monopolar, bipolar and multipolar under each culture condition are indicated. ALL factors enhanced the proportion of bipolar neurons and reduced the number of neurons without neurites at all ages. The *number above* each *bar* indicates the number of neurons analyzed

Effects of neurotrophic factors on neurite extension

The effect of neurotrophic factors on neurite extension in SGNs in vitro has been less extensively studied compared with neuronal survival or neurite number from explants and remains a relatively controversial issue. We have demonstrated that, although LNL in untreated SGNs is maximal at P20, the effect of neurotrophic factors on neurite length is greatest at P5. The period of our P5 culture (equivalent to P5–P9 in vivo) corresponds to a stage of late remodeling of afferent projections to the sensory epithelium (Echteler 1992; Wiechers et al. 1999) and cochlear nucleus (Limb and Ryugo 2000). The strong regulation of neurite extension by neurotrophic factors at P5 might be necessary to achieve the rearrangement of afferent innervation mediated by target-derived neurotrophic factors in the organ of Corti and/or brainstem.

Our study has revealed that LIF has a strong effect on neurite extension on P0 and P5 SGNs. At P0 and P5, treatment with LIF alone or with ALL factors enhanced neurite extension, whereas treatment with NT-3 and/or BDNF showed no significant effect at P0 and an inhibitory effect at P5. We can reasonably conclude that the effect of treatment with ALL factors is primarily attributable to the effect of LIF at these ages. LIF has been reported to enhance neurite extension in a variety of other neuronal types (Cafferty et al. 2001; Leibinger et al. 2009). In particular, recent studies have demonstrated that LIF mediates the enhanced intrinsic growth status after a conditioning lesion (Cafferty et al. 2001; Hyatt Sachs et al. 2010) suggesting that LIF plays a role in the regeneration of injured neurites. Therefore, our results, together with those of previous reports (Gillespie et al. 2001; Vieira et al. 2007; Whitlon et al. 2007) indicate that the application of exogenous LIF, alone or together with neurotrophins, should be clinically valuable as a treatment for central axon injury from trauma or the surgical removal of acoustic tumors and for peripheral dendrites to improve the efficacy of cochlear implants.

In contrast to LIF, NT-3 and BDNF at 50 ng/ml showed inhibitory effects for neurite extension on P5 SGNs. NT-3 and BDNF showed a survival-promoting effect on the same neuronal population at this concentration, whereas NT-3 and BDNF at 10 ng/ml did not show this inhibitory effect, suggesting that this is not a toxic effect but a specific suppression of neuritogenesis by high concentrations of NT-3 and BDNF. This finding contrasts with the extension-promoting effects of NT-3 and BDNF during in vivo and in vitro studies (Brors et al. 2008; Leake et al. 2011; Miller et al. 2007; Wise et al. 2005). The reason underlying this discrepancy is unclear but several possibilities can be proposed.

Table 2 Fraction of SGN without or with one neurite (referred to as the 0-1 neurite group) and SGN with two or more neuritis (referred to as the ≥2 neurite group). Significant differences in the fraction of ≥2 neurite group compared with control culture are indicated: **P*<0.05, ***P*<0.01, ****P*<0.001 (chi-square test)

Group	Control	NT-3	BDNF	LIF	NT-3 + BDNF	ALL
P0						
0-1 neurite group	24	115	205	61	296	100
≥2 neurite group	12	46	70	44	165	106*
P5						
0-1 neurite group	66	154	193	96	170	161
≥2 neurite group	25	52	73	48	97	219**
P20						
0-1 neurite group	89	129	127	49	92	45
≥2 neurite group	45	85	104*	44*	59	87***

One possibility is that the production of other growth factors such as LIF by SGNs themselves or by adjacent tissue is higher in explants or in vivo situations. Alternatively, the discrepancy could be attributable to the difference in the mode of exposure of SGNs to the factors: in vivo administration of neurotrophic factors by osmotic pump through the scala tympani (Leake et al. 2011; Miller et al. 2007; Wise et al. 2005) might primarily expose the SGN neurites. In contrast, in our dissociated culture system, the entire surface of the SGNs is presumably exposed to the factors and so the factors might not drive the neurites to extend. Indeed, a study of rat DRG neurons in compartmented cultures has revealed that NGF promotes neurite extension when applied to the neurite alone but suppresses neurite elongation when the cell body is exposed (Kimpinski et al. 1997). Another study of rat DRG neurons has demonstrated a dose-dependent inhibitory effect on neurite extention for NGF (≥50 ng/ml; Conti et al. 1997, 2004). The authors (Conti et al. 2004) speculate that this might be necessary to terminate axon growth when the growth cone reaches its target. Therefore, the finding that 50 ng/ml NT-3 and BDNF show greater inhibitory effects on P5 SGNs than other age groups might be associated with the observation that the period of P5 culture corresponds to the stage of afferent rearrangement. The molecular mechanisms underlying this inhibition are unknown but a higher concentration of neurotrophins might change the balance of signaling mediated through Trks and p75, the latter of which has been shown to suppress neurite extension in SGNs (Brors et al. 2008).

Effects of neurotrophic factors on neuronal morphology

SGNs are bipolar neurons possessing two neuronal processes (an axon and a dendrite), which are anatomically and functionally distinct. Previous studies have indicated that not all SGNs show this in vivo morphology in culture (Vieira et al. 2007; Whitlon et al. 2007) suggesting that the initiation of neuritogenesis is regulated differentially for SGN axons and dendrites.

In the current study, the fraction of monopolar neurons was highest for P0 SGNs, whereas the fraction showing bipolar morphology was highest at P20, especially after treatment with ALL factors. These findings suggest that the capacity of SGNs to initiate regrowth of neurites changes in an age-dependent manner and that the SGNs retain the capacity to respond to exogenous neurotrophic factors for neurite regrowth, even when the neurons are functionally mature.

A few studies have addressed the regulation of neurite morphology as modulated by growth factors. When dissociated DRG neurons are cultured in the absence of NGF, most of the neurons exhibit unipolar morphology, whereas NGF or NT-3 treatment dramatically increases the percentage of bipolar neurons (Lentz et al. 1999). NGF and NT-3 have also been demsonstrated to produce differential effects on central and peripheral neurite growth patterns of trigeminal ganglion neurons in whole-mount explant cultures (Ulupinar et al. 2000). Neonatal mouse SGNs tend to show increased bipolar morphology after LIF, ciliary neurotrophic factor, or oncostatin treatment (Whitlon et al. 2007). These observations clearly show that neurotrophic factors are involved in the initiation of neuritogenesis, although molecular mechanisms regulating this morphology remain largely unknown. The findings in our study suggest that our cocktail of neurotrophic factors should be useful in studies of the transplantation of neural stem cells into spiral ganglia, in which the development of neurons with bipolar morphology is necessary to restore the functional connection between the organ of Corti and the brainstem.

Potential limitations of the study

Several limitations need to be acknowledged with regard to the present study. First, we harvested and dissociated SGNs from along the entire length of the cochlea. Therefore, our results reflect the average response of SGNs from all cochlear turns at each developmental age. Recent studies have demonstrated that SGNs present at different cochlear positions can vary in their physiological properties in response to neurotrophins (Adamson et al. 2002; Davis and Liu 2011). SGNs located in the apex show NT-3-specific patterns in terms of their electrophysiological properties and their molecular expression in response to the higher expression of NT-3 in the apical region, whereas SGNs in the base show BDNF-specific patterns in response to the higher expression of BDNF in the basal region. This finding raises the possibility that the SGNs of different turn origin might respond to neurotrophins differently with respect to survival and neuritogenesis.

A second limitation is that our study did not differentiate the responses of type I versus type II SGNs, since the reliable differentiation of these two neuronal types is difficult based purely on their morphology in culture. Moreover, rat type I SGNs unfortunately up-regulate the type II marker peripherin in culture. Although the majority of SGNs harvested should have been type I, a recent study has demonstrated that the proportion of type II/type I in mice decreases with age (Barclay et al. 2011). In addition, BDNF more strongly supports the survival and neuritogenesis of type II SGNs in explants (Barclay et al. 2011). Therefore, similar neuronal-type-specific differences in trophic dependence might have influenced the data of our study.

A third limitation concerns the possible influence of other experimental factors that could affect the survival and neuritogenesis of SGNs in dissociated cell culture. Our data could have been influenced by mechanical and chemical stresses during dissociation, the specific coating of the glass

culture surface and the type of culture media, in addition to the supplemention of neurotrophic factors. The response of SGNs to these factors might also be age-dependent and might have influenced our results. These issues need to be addressed in further investigations.

Concluding remarks

The present study demonstrates that the responsiveness of rat postnatal SGNs to neurotrophic factors with regard to several indices of growth changes in an age-dependent manner, that various factors can exert influences that are quite distinct from one another and that strong synergistic effects are observable between factors. Improved knowledge of the processes that occur during the development of SGNs should be valuable to facilitate the maintenance of SGNs and their dendrites and also the development of regenerative therapies to improve the efficacy of cochlear implants.

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References

Adamson CL, Reid MA, Davis RL (2002) Opposite actions of brain-derived neurotrophic factor and neurotrophin-3 on firing features and ion channel composition of murine spiral ganglion neurons. J Neurosci 22:1385–1396

Agerman K, Hjerling-Leffler J, Blanchard MP, Scarfone E, Canlon B, Nosrat C, Ernfors P (2003) BDNF gene replacement reveals multiple mechanisms for establishing neurotrophin specificity during sensory nervous system development. Development 130:1479–1491

Aletsee C, Beros A, Mullen L, Palacios S, Pak K, Dazert S, Ryan AF (2001) Ras/MEK but not p38 signaling mediates NT-3-induced neurite extension from spiral ganglion neurons. J Assoc Res Otolaryngol 2:377–387

Auernhammer CJ, Melmed S (2000) Leukemia-inhibitory factor-neuroimmune modulator of endocrine function. Endocr Rev 21:313–345

Barclay M, Ryan AF, Housley GD (2011) Type I vs type II spiral ganglion neurons exhibit differential survival and neuritogenesis during cochlear development. Neural Dev 6:33

Brors D, Hansen S, Mlynski R, Volkenstein S, Aletsee C, Sendtner M, Ryan AF, Dazert S (2008) Spiral ganglion outgrowth and hearing development in p75-deficient mice. Audiol Neurootol 13:388–395

Buchman VL, Davies AM (1993) Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. Development 118:989–1001

Cafferty WB, Gardiner NJ, Gavazzi I, Powell J, McMahon SB, Heath JK, Munson J, Cohen J, Thompson SW (2001) Leukemia inhibitory factor determines the growth status of injured adult sensory neurons. J Neurosci 21:7161–7170

Chihara Y, Iwasaki S, Kondo K, Yamasoba T (2011) Responsiveness of rat vestibular ganglion neurons to exogenous neurotrophic factors during postnatal development in dissociated cultures. Brain Res 1408:1–7

Cho Y, Gong TW, Kanicki A, Altschuler RA, Lomax MI (2004) Noise overstimulation induces immediate early genes in the rat cochlea. Brain Res Mol Brain Res 130:134–148

Conti AM, Fischer SJ, Windebank AJ (1997) Inhibition of axonal growth from sensory neurons by excess nerve growth factor. Ann Neurol 42:838–846

Conti AM, Brimijoin S, Miller LJ, Windebank AJ (2004) Suppression of neurite outgrowth by high-dose nerve growth factor is independent of functional p75NTR receptors. Neurobiol Dis 15:106–114

Davis RL, Liu Q (2011) Complex primary afferents: what the distribution of electrophysiologically-relevant phenotypes within the spiral ganglion tells us about peripheral neural coding. Hear Res 276:34–43

Easton RM, Deckwerth TL, Parsadanian AS, Johnson EM Jr (1997) Analysis of the mechanism of loss of trophic factor dependence associated with neuronal maturation: a phenotype indistinguishable from Bax deletion. J Neurosci 17:9656–9666

Echteler SM (1992) Developmental segregation in the afferent projections to mammalian auditory hair cells. Proc Natl Acad Sci USA 89:6324–6327

Echteler SM, Magardino T, Rontal M (2005) Spatiotemporal patterns of neuronal programmed cell death during postnatal development of the gerbil cochlea. Brain Res Dev Brain Res 157:192–200

Ernfors P, Van De Water T, Loring J, Jaenisch R (1995) Complementary roles of BDNF and NT-3 in vestibular and auditory development. Neuron 14:1153–1164 [erratum appears in Neuron 15:739]

Farinas I, Jones KR, Tessarollo L, Vigers AJ, Huang E, Kirstein M, Caprona DC de, Coppola V, Backus C, Reichardt LF, Fritzsch B (2001) Spatial shaping of cochlear innervation by temporally regulated neurotrophin expression. J Neurosci 21:6170–6180

Fritzsch B, Farinas I, Reichardt LF (1997a) Lack of neurotrophin 3 causes losses of both classes of spiral ganglion neurons in the cochlea in a region-specific fashion. J Neurosci 17:6213–6225

Fritzsch B, Silos-Santiago I, Bianchi LM, Farinas I (1997b) The role of neurotrophic factors in regulating the development of inner ear innervation. Trends Neurosci 20:159–164

Fritzsch B, Tessarollo L, Coppola E, Reichardt LF (2004) Neurotrophins in the ear: their roles in sensory neuron survival and fiber guidance. Prog Brain Res 146:265–278

Gillespie LN, Clark GM, Bartlett PF, Marzella PL (2001) LIF is more potent than BDNF in promoting neurite outgrowth of mammalian auditory neurons in vitro. Neuroreport 12:275–279

Hartnick CJ, Staecker H, Malgrange B, Lefebvre PP, Liu W, Moonen G, Van de Water TR (1996) Neurotrophic effects of BDNF and CNTF, alone and in combination, on postnatal day 5 rat acoustic ganglion neurons. J Neurobiol 30:246–254

Hashino E, Dolnick RY, Cohan CS (1999) Developing vestibular ganglion neurons switch trophic sensitivity from BDNF to GDNF after target innervation. J Neurobiol 38:414–427

Hegarty JL, Kay AR, Green SH (1997) Trophic support of cultured spiral ganglion neurons by depolarization exceeds and is additive with that by neurotrophins or cAMP and requires elevation of $[Ca^{2+}]_i$ within a set range. J Neurosci 17:1959–1970

Hyatt Sachs H, Rohrer H, Zigmond RE (2010) The conditioning lesion effect on sympathetic neurite outgrowth is dependent on gp130 cytokines. Exp Neurol 223:516–522

Kimpinski K, Campenot RB, Mearow K (1997) Effects of the neurotrophins nerve growth factor, neurotrophin-3, and brain-derived neurotrophic factor (BDNF) on neurite growth from adult sensory neurons in compartmented cultures. J Neurobiol 33:395–410

Leake PA, Hradek GT, Hetherington AM, Stakhovskaya O (2011) Brain-derived neurotrophic factor promotes cochlear spiral ganglion cell survival and function in deafened, developing cats. J Comp Neurol 519:1526–1545

Leibinger M, Muller A, Andreadaki A, Hauk TG, Kirsch M, Fischer D (2009) Neuroprotective and axon growth-promoting effects following inflammatory stimulation on mature retinal ganglion cells in mice depend on ciliary neurotrophic factor and leukemia inhibitory factor. J Neurosci 29:14334–14341

Lemke R, Gadient RA, Schliebs R, Bigl V, Patterson PH (1996) Neuronal expression of leukemia inhibitory factor (LIF) in the rat brain. Neurosci Lett 215:205–208

Lentz SI, Knudson CM, Korsmeyer SJ, Snider WD (1999) Neurotrophins support the development of diverse sensory axon morphologies. J Neurosci 19:1038–1048

Limb CJ, Ryugo DK (2000) Development of primary axosomatic endings in the anteroventral cochlear nucleus of mice. J Assoc Res Otolaryngol 1:103–119

Malgrange B, Lefebvre P, Van de Water TR, Staecker H, Moonen G (1996) Effects of neurotrophins on early auditory neurones in cell culture. Neuroreport 7:913–917

Marzella PL, Clark GM, Shepherd RK, Bartlett PF, Kilpatrick TJ (1997) LIF potentiates the NT-3-mediated survival of spiral ganglia neurones in vitro. Neuroreport 8:1641–1644

Marzella PL, Gillespie LN, Clark GM, Bartlett PF, Kilpatrick TJ (1999) The neurotrophins act synergistically with LIF and members of the TGF-beta superfamily to promote the survival of spiral ganglia neurons in vitro. Hear Res 138:73–80

Miller JM, Le Prell CG, Prieskorn DM, Wys NL, Altschuler RA (2007) Delayed neurotrophin treatment following deafness rescues spiral ganglion cells from death and promotes regrowth of auditory nerve peripheral processes: effects of brain-derived neurotrophic factor and fibroblast growth factor. J Neurosci Res 85:1959–1969

Molliver DC, Wright DE, Leitner ML, Parsadanian AS, Doster K, Wen D, Yan Q, Snider WD (1997) IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. Neuron 19:849–861

Mou K, Hunsberger CL, Cleary JM, Davis RL (1997) Synergistic effects of BDNF and NT-3 on postnatal spiral ganglion neurons. J Comp Neurol 386:529–539

Mou K, Adamson CL, Davis RL (1998) Time-dependence and cell-type specificity of synergistic neurotrophin actions on spiral ganglion neurons. J Comp Neurol 402:129–139

Mullen LM, Pak KK, Chavez E, Kondo K, Brand Y, Ryan AF (2012) Ras/p38 and PI3K/Akt but not Mek/Erk signaling mediate BDNF-induced neurite formation on neonatal cochlear spiral ganglion explants. Brain Res 1430:25–34

Orike N, Thrasivoulou C, Cowen T (2001a) Serum-free culture of dissociated, purified adult and aged sympathetic neurons and quantitative assays of growth and survival. J Neurosci Methods 106:153–160

Orike N, Thrasivoulou C, Wrigley A, Cowen T (2001b) Differential regulation of survival and growth in adult sympathetic neurons: an in vitro study of neurotrophin responsiveness. J Neurobiol 47:295–305

Oshima K, Teo DT, Senn P, Starlinger V, Heller S (2007) LIF promotes neurogenesis and maintains neural precursors in cell populations derived from spiral ganglion stem cells. BMC Dev Biol 7:112

Pirvola U, Ylikoski J, Palgi J, Lehtonen E, Arumae U, Saarma M (1992) Brain-derived neurotrophic factor and neurotrophin 3 mRNAs in the peripheral target fields of developing inner ear ganglia. Proc Natl Acad Sci USA 89:9915–9919

Pirvola U, Arumae U, Moshnyakov M, Palgi J, Saarma M, Ylikoski J (1994) Coordinated expression and function of neurotrophins and their receptors in the rat inner ear during target innervation. Hear Res 75:131–144

Pujol R, Lavigne-Rebillard M, Lenoir M (1998) Development of sensory and neural structures in the mammalian cochlea. In: Rubel EW, Popper AN, Fay RR (eds) Development of the auditory system. Springer, New York, pp 146–192

Putcha GV, Deshmukh M, Johnson EM Jr (2000) Inhibition of apoptotic signaling cascades causes loss of trophic factor dependence during neuronal maturation. J Cell Biol 149:1011–1018

Rubel EW, Fritzsch B (2002) Auditory system development: primary auditory neurons and their targets. Annu Rev Neurosci 25:51–101

Rueda J, Sen C de la, Juiz JM, Merchan JA (1987) Neuronal loss in the spiral ganglion of young rats. Acta Otolaryngol 104:417–421

Ruttiger L, Panford-Walsh R, Schimmang T, Tan J, Zimmermann U, Rohbock K, Kopschall I, Limberger A, Muller M, Fraenzer JT, Cimerman J, Knipper M (2007) BDNF mRNA expression and protein localization are changed in age-related hearing loss. Neurobiol Aging 28:586–601

Schimmang T, Tan J, Muller M, Zimmermann U, Rohbock K, Kopschall I, Limberger A, Minichiello L, Knipper M (2003) Lack of Bdnf and TrkB signalling in the postnatal cochlea leads to a spatial reshaping of innervation along the tonotopic axis and hearing loss. Development 130:4741–4750

Scott SA, Davies AM (1993) Age-related effects of nerve growth factor on the morphology of embryonic sensory neurons in vitro. J Comp Neurol 337:277–285

Singer W, Panford-Walsh R, Watermann D, Hendrich O, Zimmermann U, Kopschall I, Rohbock K, Knipper M (2008) Salicylate alters the expression of calcium response transcription factor 1 in the cochlea: implications for brain-derived neurotrophic factor transcriptional regulation. Mol Pharmacol 73:1085–1091

Sugawara M, Murtie JC, Stankovic KM, Liberman MC, Corfas G (2007) Dynamic patterns of neurotrophin 3 expression in the postnatal mouse inner ear. J Comp Neurol 501:30–37

Tessarollo L, Coppola V, Fritzsch B (2004) NT-3 replacement with brain-derived neurotrophic factor redirects vestibular nerve fibers to the cochlea. J Neurosci 24:2575–2584

Tierney TS, P Doubell T, Xia G, Moore DR (2001) Development of brain-derived neurotrophic factor and neurotrophin-3 immunoreactivity in the lower auditory brainstem of the postnatal gerbil. Eur J Neurosci 14:785–793

Ulupinar E, Jacquin MF, Erzurumlu RS (2000) Differential effects of NGF and NT-3 on embryonic trigeminal axon growth patterns. J Comp Neurol 425:202–218

Vieira M, Christensen BL, Wheeler BC, Feng AS, Kollmar R (2007) Survival and stimulation of neurite outgrowth in a serum-free culture of spiral ganglion neurons from adult mice. Hear Res 230:17–23

Wei D, Jin Z, Jarlebark L, Scarfone E, Ulfendahl M (2007) Survival, synaptogenesis, and regeneration of adult mouse spiral ganglion neurons in vitro. Dev Neurobiol 67:108–122

Wheeler EF, Bothwell M, Schecterson LC, Bartheld CS von (1994) Expression of BDNF and NT-3 mRNA in hair cells of the organ of Corti: quantitative analysis in developing rats. Hear Res 73:46–56

Whitlon DS, Ketels KV, Coulson MT, Williams T, Grover M, Edpao W, Richter CP (2006) Survival and morphology of auditory neurons in dissociated cultures of newborn mouse spiral ganglion. Neuroscience 138:653–662

Whitlon DS, Grover M, Tristano J, Williams T, Coulson MT (2007) Culture conditions determine the prevalence of bipolar and monopolar neurons in cultures of dissociated spiral ganglion. Neuroscience 146:833–840

Wiechers B, Gestwa G, Mack A, Carroll P, Zenner HP, Knipper M (1999) A changing pattern of brain-derived neurotrophic factor expression correlates with the rearrangement of fibers during cochlear development of rats and mice. J Neurosci 19:3033–3042

Wise AK, Richardson R, Hardman J, Clark G, O'Leary S (2005) Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3. J Comp Neurol 487:147–165

Ylikoski J, Pirvola U, Moshnyakov M, Palgi J, Arumae U, Saarma M (1993) Expression patterns of neurotrophin and their receptor mRNAs in the rat inner ear. Hear Res 65:69–78

Zheng JL, Stewart RR, Gao WQ (1995) Neurotrophin-4/5 enhances survival of cultured spiral ganglion neurons and protects them from cisplatin neurotoxicity. J Neurosci 15:5079–5087

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Cochlear implantation in a patient with osteogenesis imperfecta

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ABSTRACT

Osteogenesis imperfecta (OI) is a connective tissue disorder characterized by a deficit in the synthesis of type I collagen. Hearing loss affects 42–58% of OI patients and progresses to deafness in 35–60% of these patients. For OI patients, cochlear implantation (CI) is the only promising treatment option. However, literature on CI in patients with OI is relatively rare. After CI, speech perception is generally good. However, among patients with severe demineralization of the cochlea, most patients are reported to have complications of facial nerve stimulation (FNS), preventing some patients from using the cochlear implant on a daily basis. Here we report a successful CI using a Nucleus CI24 Contour Advance cochlear implant in a patient with OI. Although high-resolution computed tomography (HRCT) showed extensive demineralization of the cochlea, intracochlear electrodes were inserted properly. The use of a modiolus-hugging device and the advance off-stylet technique contributed to the successful implantation, with no complications such as FNS or misplacement of electrodes. Therefore, CI can be used for treating deaf patients with OI.

1. Introduction

Osteogenesis imperfecta (OI) is a connective tissue disorder characterized by a deficit in the synthesis of type I collagen [1]. OI was first described by van der Hoeve and de Kleyn in 1917 [2] and, therefore, is also known as van der Hoeve–de Kleyn syndrome. The disease is characterized by brittle bones, blue sclerae, defective dentition and hearing loss [3]. Progressive hearing loss has been reported, including conductive, sensorineural, or mixed types [4]. Conductive hearing loss may be the result of a fracture or localized dehiscence of the stapedial arch, distal atrophy of the long process of the incus, or fixation of the stapedial footplate [1]. Sensorineural hearing loss is caused by microfractures, hemorrhage, and encroachment of reparative vascular and fibrous tissue in and around the cochlea [1]. Previous studies have reported hearing loss in 42–58% of OI patients and profound deafness in 35–60% of OI patients [5–9]. Hearing loss usually begins in the late teens in OI patients. The sensorineural component appears and progresses gradually in the third decade, resulting in profound deafness by the end of the fourth to fifth decade [7]. Cochlear implantation (CI) is the only treatment option for profound sensorineural hearing loss. However, the scientific and medical literature on CI in patients with OI is relatively rare [5–10]. After CI, speech perception is generally good. However, most patients with severe demineralization of the cochlea are reported to have complications of facial nerve stimulation (FNS). Several cases of electrode mis-insertion have also been reported. Some patients with such complications give up daily use of the cochlear implant [7,11].

Here, we report a successful CI using a Nucleus CI24 Contour Advance (CA) cochlear implant in a patient with OI. Although high-resolution computed tomography (HRCT) showed extensive demineralization of the cochlea, intracochlear electrodes were properly inserted without any of the common complications.

2. Case presentation

A female patient had several episodes of bone fractures due to minor trauma from childhood. At 18-years of age, she began to complain of bilateral hearing loss. A clinical examination revealed blue sclerae with hearing loss, and the patient was diagnosed as OI based on the clinical criteria [12] at the age of 21 years. The patient had no family history of OI or hearing loss, except for her grandfather who had presbycusis. At the age of 27 years, the patient underwent an ossiculoplasty of the left ear that unfortunately resulted in deafness. Subsequently, she began to wear a hearing aid in the right ear. At the age of 52 years, the patient consulted our department when her hearing acuity in the right ear worsened. An otoscopy examination revealed normal tympanic membranes in both ears. A pure-tone audiogram demonstrated

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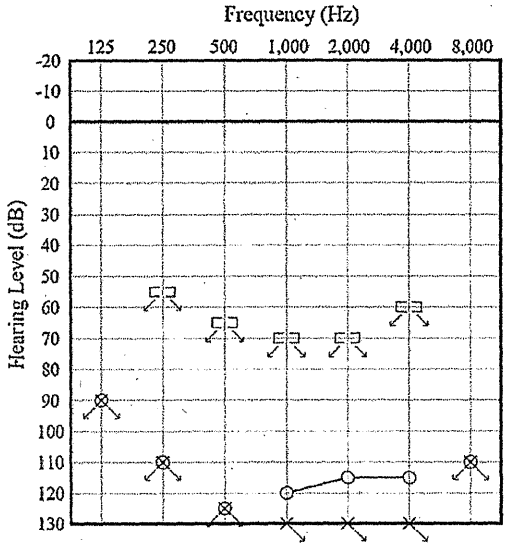

Fig. 1. Preoperative audiogram. A pure-tone audiogram demonstrated profound sensorineural hearing loss in both ears.

profound sensorineural hearing loss in both ears (Fig. 1). A speech discrimination test revealed no identification ability in both ears. Speech recognition scores with a hearing aid showed that only 10% of phonemes were recognized in the open set condition and 24% were recognized with verbal cues. The vestibular evoked myogenic potential (VEMP) was absent in both sides. A caloric test did not evoke nystagmus in either ear. A promontory stimulation test produced good auditory perception in both ears. HRCT revealed severe demineralization of the pericochlear and vestibular areas in both sides, and the outline of the cochlea was almost unrecognizable (Fig. 2). Magnetic resonance imaging (MRI) showed fluid intensity in the entire cavity of the right cochlea. However, fluid intensity in the scala tympani of the basal turn was decreased in the left cochlea (Fig. 3). The right and left cochlear nerves were well recognized on MRI.

Because there was a long period of auditory deprivation of the left ear and the MRI suggested partial occlusion in the basal turn, we decided to perform CI for the right ear. At the age of 54 years, the patient underwent surgery in the right ear to implant the Nucleus CI24R Contour Advance device. A mastoidectomy and a posterior tympanotomy were performed uneventfully. The foramen obturatum and the oval window were obliterated, and the round window was barely identified by the new bone formation of the promontory. We performed chocleostomy using the location of stapes as a landmark. The bone of the cochlear capsule was spongiotic and fragile; however, a cochleostomy was easily performed and the scala tympani was identified. All of the 22 electrodes were inserted successfully using the advanced off-stylet (AOS) technique. Postoperative neural response telemetry (NRT) showed good responses in all electrodes without FNS. Postoperative radiography and HRCT revealed the fully inserted electrodes inside the cochlea (Fig. 4). All of the electrodes showed normal impedance at first stimulation, and

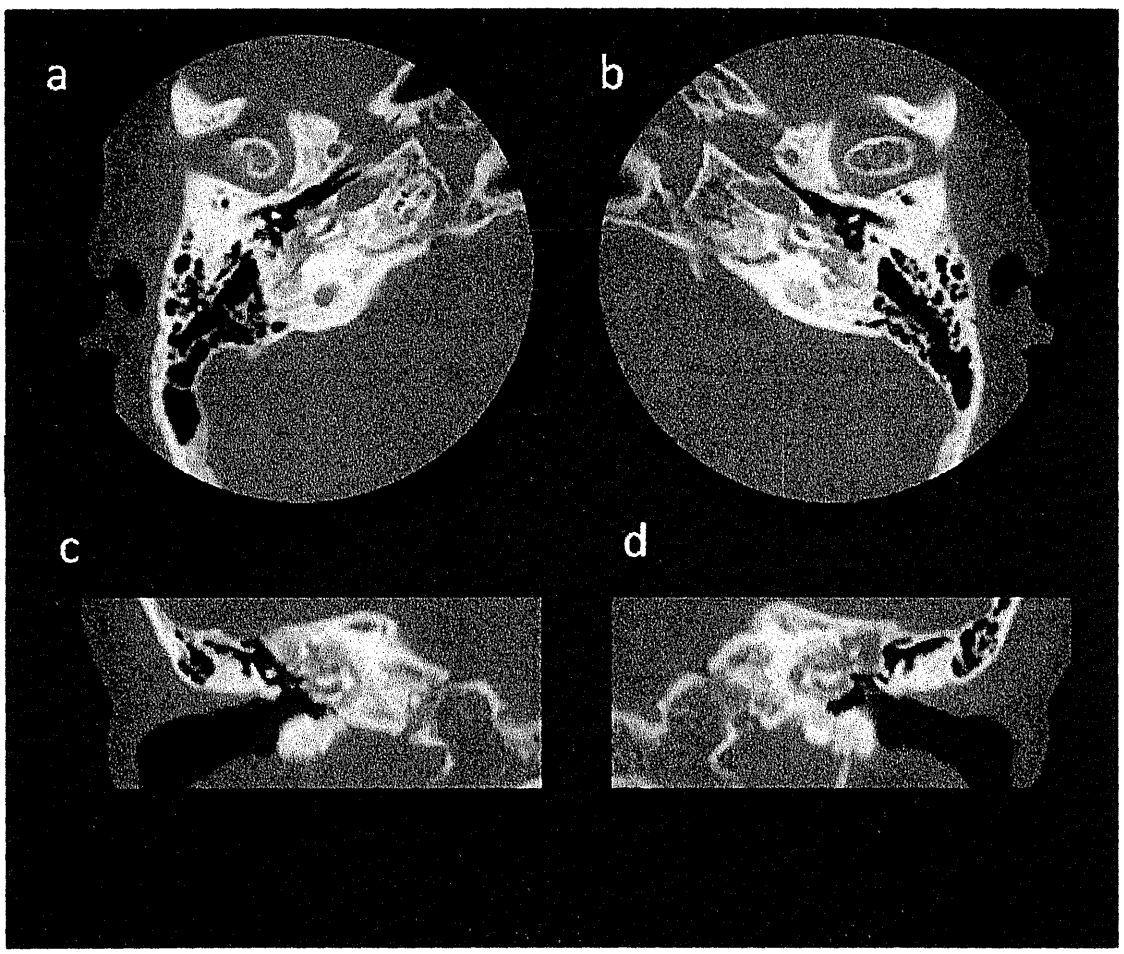

Fig. 2. Preoperative CT image of the cochlea. High resolution computed tomography (HRCT) revealed significant demineralization of the right and left pericochlear and vestibular areas, and the structures of the cochleae were almost unrecognizable.

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Fig. 3. Preoperative MRI image of the cochlea. MRI demonstrated fluid content in the entire right cochlea. However, in the left cochlea, the fluid content in the scala tympani of the basal turn showed a defect, which suggested a partial occlusion.

no FNS was found during the stimulation. At the 6-month postoperative evaluation, the perception scores of monosyllables, words, and sentences using the CI and without any other cues were 62%, 70%, and 91%, respectively. These results indicated that the patient had good speech perception.

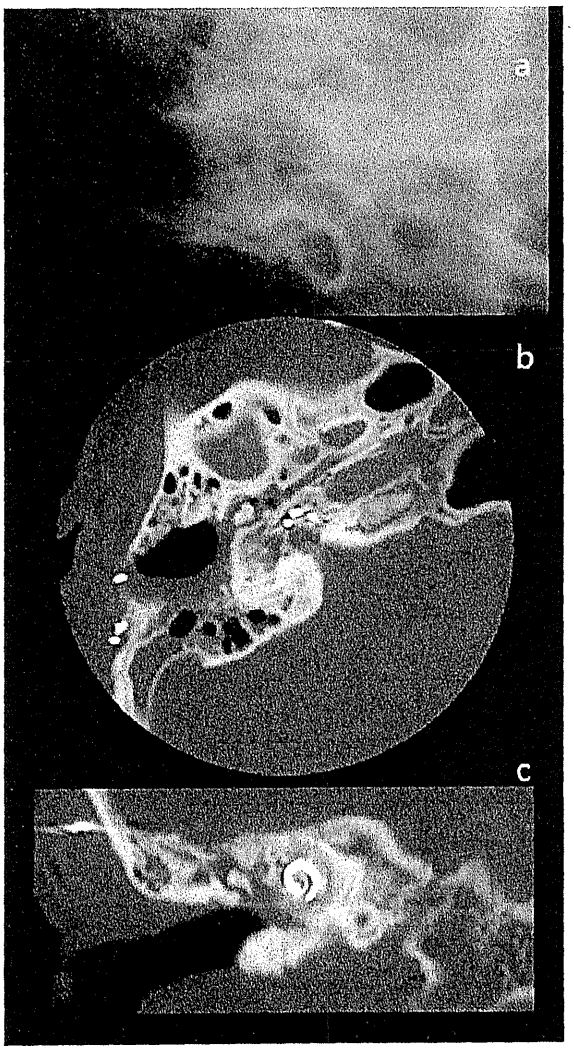

Fig. 4. Intraoperative radiograph and postoperative HRCT images of the cochlea. Intraoperative radiograph (a) and postoperative HRCT images (b and c) showed fully inserted electrodes.

3. Discussion

We have reported the case of an OI patient with a severely demineralized cochlea who underwent a successful operation for CI, resulting in good speech perception. No complications such as FNS and misplacement of electrodes were observed following the operation.

OI is a heterogeneous disease of the connective tissue caused by defective genes (*COL1A1* and *COL1A2*). *COL1A1* and *COL1A2* are responsible for the production of collagen type I, and mutations lead to defects in the bone matrix and connective tissue [7]. Recent studies have shown that reduced bone mineral density as a feature of OI and examining bone mineral density using the devices such as X-ray absorptiometry and peripheral quanitative computed tomography as well as genetic screening are becoming another powerful tool for the diagnosis of OI [13]. In this case, however, we made the diagnosis of OI based on the traditional criteria introduced by Sillence et al. [12]. Previous studies have reported various results for the prevalence of OI, ranging from 1/10,000 to 1/30,000 [6,14]. It has been reported that 2–35% of OI patients progress to deafness, with CI being the only promising treatment option [7–9]. To date, only 10 cases of CI with OI have been reported, including the present case. The HRCT findings of OI are as follows: (1) extensive dematerialized bone involving all or part of the otic capsule and extending as high as the upper margin of the superior semicircular canal; (2) fenestral manifestations caused by proliferation of bone, such as a narrow middle ear cavity, an enveloped stapes footplate, and obliterated windows with irregular and indistinct margins; and (3) involvement of the facial nerve canal in the dysplastic process [15]. These clear findings can lead surgeons to underestimate the remaining cochlear structure and space for electrodes thus improperly limiting the scope of CI. This may be the reason for the relatively small number of CIs reported for OI. In the present case, HRCT demonstrated extensive demineralization of the bony labyrinth, and the structure of cochlea was barely distinguishable. In contrast, a T2-weighted fast spin-echo (3D-FSE) MRI revealed a distinct fluid signal in the right cochlea. Based on this additional information provided by the MRI, we confidently decided to carry out CI.

In the present case, MRI showed a partial occlusion of the basal turn in the left ear. The previous ossiculoplasty might have elicited the occlusion, but we cannot deny the possibility that this occlusion was the result of ossification due to OI. Occlusion of the cochlea has also been reported in patients with otosclerosis, who have shown a similar proliferation of bones around the cochlea [16]. The genetic association with COL1A1 has been reported also in otosclerosis. Chen et al. suggested that otosclerosis has an association with single nucleotide polymorphisms in the regulatory regions of COL1A1, whereas OI is caused by a reduction in total COL1A1 mRNA secondary to mutations in COL1A1 [17]. It is possible that the association with COL1A1 in both of two diseases may cause the similar demineralization of the cochlea and that the difference in the mechanisms of COL1A1 disorder may determine the characteristics of each disease. As the present report shows, the condition of the cochlea should be carefully examined preoperatively by both HRCT and MRI. Otherwise, an evaluation by CT alone may lead to a misunderstanding of the operative indication for CI.

Because of severe demineralization of the otic capsule, CI in OI is challenging and is often accompanied by several complications. One such complication is FNS, and another is the misplacement of electrodes. Six patients with severe demineralization of the otic capsule have been reported to experience FNS as a complication after CI [5–8]. Most of the patients continued using the implant after switching off several

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electrodes that caused the FNS. However, 2 patients discontinued use of the cochlear implant due to severe discomfort. It has been postulated that FNS is induced by deviant current spread throughout dehiscent or otospongiotic bone, where impedance is low, resulting in an electrical field in the proximity of the facial nerve [18]. Perimodiolar electrodes of the Nucleus CI24R CA device are tightly shielded against the lateral spread of current and theoretically are less likely to elicit FNS [19]. A CI study of patients with otosclerosis who had histopathologically similar demineralization of the cochlea showed a higher incidence (44%) of FNS in patients implanted with non modiolus-hugging devices than in those implanted with modiolus-hugging devices (10%) [20]. In the present case, no FNS occurred despite severe demineralization of the otic capsule. This is the first report of a successful CI with a perimodiolar designed electrode in an OI patient with severe demineralization of the cochlea. Additional cases are needed to establish the efficacy of the perimodiolar electrode for preventing FNS. However, perimodiolar designed electrodes may be preferable for OI patients to prevent FNS complications.

Another significant complication of CI in OI patients is misplacement of the electrodes. Two cases of misplacement have been reported. The risk of misplacement of the electrode array in a spongiotic otic capsule has also been described in patients with otosclerosis [20]. Cochlear otosclerosis is similar to OI because otospongiotic changes of the otic capsule and abnormal bone proliferation around a promontory of the otic capsule are observed in both conditions. In an OI patient, it is difficult to identify the round window niche or oval window [5]. Therefore, cochleostomy is challenging due to a lack of anatomical landmarks. Even when cochleostomy is performed in the proper place, the electrode can easily destroy the capsule and penetrate into the surrounding structures during insertion due to the soft and brittle nature of the cochlear bone. Recently, CI devices have been developed to minimize insertion trauma. The CA electrode may be used as an alternative to the traditional straight electrode, applying the AOS insertion technique so that the electrode does not touch the outer wall of the cochlea, thus reducing the risk of trauma to the cochlea [21]. With this technique, the slower insertion speed of the electrodes is also reported to impact the insertion force and reduce the risk of trauma [22]. Therefore, using this new, less traumatic device with a slower electrode insertion speed can prevent misplacement.

Our patient could recognize of 62% of phonemes, 70% of words, and 91% of sentences. Therefore, we concluded that the CI was successful. Berger et al. [23] reviewed the histopathology of the temporal bone in OI and suggested that progressive sensorineural hearing loss results from hemorrhage into the inner ear spaces. Subsequently, the accumulated cells and plasma proteins may disturb the inner ear dynamics and alter the electrolyte gradients. In this pathological condition, the spiral ganglion cells are assumed to be well preserved; therefore, good performance can be expected after CI. Nevertheless, 3 patients had an unsuccessful result in previous report. Two of these unsuccessful results were attributed to misplacement of the electrodes and severe FNS [7]. Another case involved a 6-year-old child who had profound sensorineural hearing loss from 6 months of age [8]. The poor result observed in this case can be attributed to the late age of implantation. These results show that if the electrodes are inserted properly within the appropriate period, sufficient speech perception can be expected and that CI surgery is a promising choice of treatment for OI.

4. Conclusion

We reported the case of an OI patient showing severe demineralization of the cochlea on HRCT. Preoperative MRI showed sufficient space for the CI in the basal turn of the right cochlea. All 22 intracochlear electrodes were successfully inserted, and no complications, such as FNS and mis-insertion, occurred. We attribute this success to the use of modiolus-hugging electrodes. The results of postoperative speech perception were good and consistent with those of previous reports.

Conflict of interest

None.

References

[1] Nager GT. Ostenogenesis imperfecta of the temporal bone and its relation to otosclerosis. Ann Otol Rhinol Laryngol 1988;97:585–93.
[2] van der Hoeve J, de Kleyn A. Blaue sclerae, knochenbruechigkeit und schwerhoerighkeit. Albrecht Von Graefes Arch Klin Exp Ophthalmol 1918;95:81–93.
[3] Proscop DJ, Kuivaniemi H, Tromp G. Hereditable disorders of connective tissue. In: Isselbacher E, Braunwald JD, Wilson JB, Martin AS, Fauci DL, Kasper, editors. Harrison's principles of internal medicine. 13th ed., McGraw-Hill International; 1994. p. 2111–3.
[4] Pedersen U. Hearing loss in patients with osteogenesis imperfecta. A clinical and audiological study of 201 patients. Scand Audiol 1984;13:67–74.
[5] Streubel SO, Lustig LR. Cochlear implantation in patients with osteogenesis imperfecta. Otolaryngol Head Neck Surg 2005;132:735–40.
[6] Cohen BJ. Osteogenesis imperfecta and hearing loss. Ear Nose Throat J 1984;63:283–8.
[7] Rotteveel LJ, Beynon AJ, Mens LH, Snik AF, Mulder JJ, Mylanus EA. Cochlear implantation in 3 patients with osteogenesis imperfecta: imaging, surgery and programming issues. Audiol Neurootol 2009;13:73–85.
[8] Migirov L, Henkin Y, Hildesheimer M, Kronenberg J. Cochlear implantation in a child with osteogenesis imperfecta. Int J Pediatr Otorhinolaryngol 2003;67:677–80.
[9] Huang TS, Yen PT, Liu SY. Cochlear implantation in a patient with osteogenesis imperfecta and otospongiosis. Am J Otolaryngol 1998;19:209–12.
[10] Szilvássy J, Jóri J, Czigner J, Tóth F, Szilvássy Z, Kiss JG. Cochlear implantation in osteogenesis imperfecta. Acta Otorhinolaryngol Belg 1998;52:253–6.
[11] Mens LH, Mulder JJ. Averaged electrode voltages in users of the Clarion cochlear implant device. Ann Otol Rhinol Laryngol 2002;111:370–5.
[12] Sillence DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. J Med Genet 1979;16:101–16.
[13] Swinnen FK, De Leenheer EM, Goemaere S, Cremers CW, Coucke PJ, Dhooge IJ. Association between bone mineral density and hearing loss in osteogenesis imperfecta. Laryngoscope 2012;122:401–8.
[14] Kuurila K, Grenman R, Johansson R, Kaitila I. Hearing loss in children with osteogenesis imperfecta. Eur J Pediatr 2000;159:515–9.
[15] Tabor EK, Curtin HD, Hirsch BE, May M. Osteogenesis inperfecta tarda: appearance of the temporal bones at CT. Radiology 1990;175:181–3.
[16] Ruckenstein MJ, Rafter KO, Montes M, Bigelow DC. Management of far advanced otosclerosis in the era of cochlear implantation. Otol Neurotol 2001;22:471–4.
[17] Chen W, Meyer NC, McKenna MJ, Pfister M, McBride Jr DJ, Fukushima K, et al. Single-nucleotide polymorphisms in the COL1A1 regulatory regions are associated with otosclerosis. Clin Genet 2007;71:406–14.
[18] Bigelow DC, Kay DJ, Rafter KO, Montes M, Knox GW, Yousem DM. Facial nerve stimulation from cochlear implants. Am J Otol 1998;19:163–9.
[19] Cohen LT, Richardson LM, Saunders E, Cowan RS. Spatial spread of neural excitation on cochlear implant recipients: comparison of improved ECAP method and psychophysical forward masking. Hear Res 2003;179:72–87.
[20] Rotteveel LJ, Proops DW, Ramsden RT, Saeed SR, van Olphen AF, Mylanus EA. Cochlear implantation in 53 patients with otosclerosis: demographics, computed tomographic scanning, surgery, and complications. Otol Neurotol 2004;25:943–52.
[21] Roland Jr JT. A model for cochlear implant electrode insertion and force evaluation: results with a new electrode design and insertion technique. Laryngoscope 2005;115:1325–39.
[22] Kontorinis G, Lenarz T, Stöver T, Paasche G. Impact of the insertion speed of cochlear implant electrodes on the insertion forces. Otol Neurotol 2011;32:565–70.
[23] Berger G, Hawke M, Johnson A, Proops D. Histopathology of the temporal bone in osteogenesis imperfecta congenita: a report of 5 cases. Laryngoscope 1985;95:193–9.

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言語・耳鼻咽喉科

KeyWords
◎難聴
◎補聴器
◎人工内耳

補聴器

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Headline

1. 難聴は聴覚経路のいずれかに障害をきたし，言語音・環境音の聴取が困難となった状態をいうが，障害部位と程度，発症の時期により多様な臨床像を呈する．
2. 補聴器は適切な聴覚検査による診断のうえで，個々の生活環境に合わせた頻回の調整と聴覚リハビリテーションを併せて行うことが望ましい．
3. 人工内耳は補聴器が有効でないような重度聴覚障害児・者を対象とした埋込み型の機器である．聴取能の改善の程度は個人差が大きく，術後のリハビリテーションと機器調整は必須である．

難聴とは末梢から中枢にいたる聴覚経路のいずれかに障害をきたし，言語音・環境音の聴取が困難となった状態をいう．「難聴」という言葉を知らない者はいないが，その病態や難聴者の日常生活上の問題について社会的な理解は十分とはいえない．本稿では補聴器に代表される聴覚補償・代償機器についてその概要を述べる．

難聴とは

難聴は障害された聴覚経路の部位により，伝音難聴（外耳から中耳まで）と感音難聴（内耳とより中枢）と大きく二つに分類される．図1に標準純音聴力検査における代表的な伝音難聴と感音難聴の聴力図を示す．縦軸が音圧，横軸が周波数を表し，図の下に行くにつれて音は大きくなり，右へいくほど音色は高くなる．図2に一般的な音がこの聴力図のどこに対応しているかを示す[1]．アルファベットで示しているのは音声の分布である．母音は低音域にあり子音は高音域に分布しているのがわかる．ヒトの可聴範囲は約20 Hz～20 kHzであるが，聴力図では言語音の聴取に重要な125 Hz～8 kHzを示している．難聴には，それぞれの音の高さが一様にきこえにくくなる場合の他，音の高さによりきこえ方が異なる場合がある．

図3に伝音難聴と感音難聴のきこえの違いをイメージ化して示す．伝音難聴は耳を手で塞いで音が小さくきこえる状態に近いため，声を大きくする等の増幅により聴取の改善が見込める．しかし感音難聴の場合は音が小さいだけでなく歪んできこえるため，単純な音の増幅だけでは明瞭に聴取することは困難となる．ラジオのチューニングがずれた状態でボリュームを上げてもききとれないのと同様である．

以上のように難聴は，程度と種類，また発症の時期により多様な臨床像を呈し，日常コミュニケーションに支障をきたすこととなる．言語習得前の発症である場合は専門的な療育が必須であり，高齢の場合には加齢に伴う中枢機能の影響も考慮する必要がある．

補聴器の概要

補聴器（hearing aid；HA）は音振動を増幅さ

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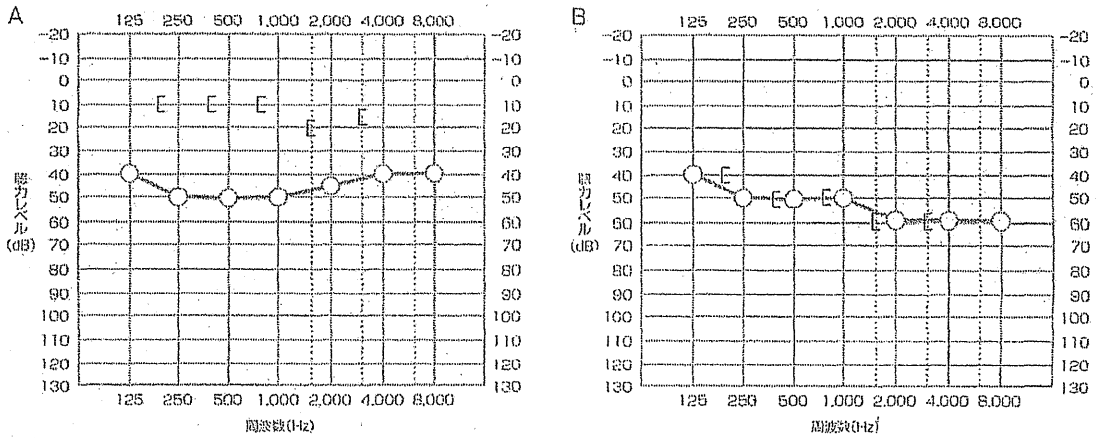


図1　代表的な伝音難聴（A）と感音難聴（B）の聴力図（いずれも右耳のみ）
[様の記号は骨導聴力閾値，○は気導聴力閾値を示す

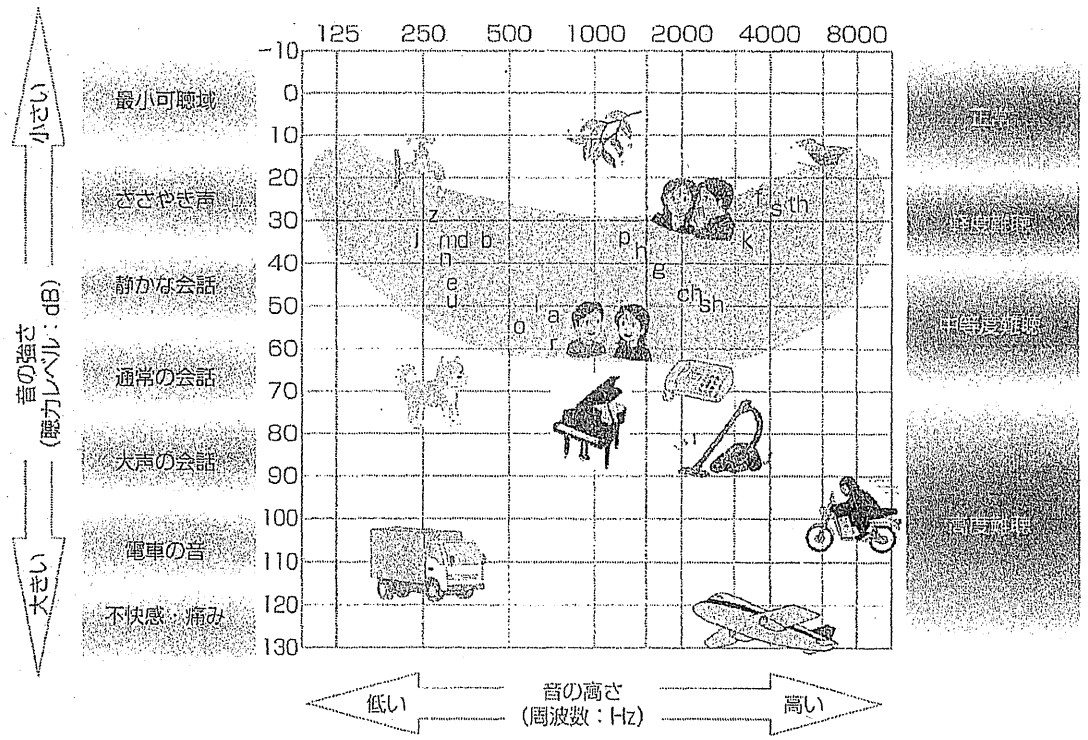


図2　聴力図　（文献1）より引用・改変）

せる機器であり，管理医療機器（クラス2）に分類される．おもにマイクロホンとアンプ，スピーカからなり，その歴史はオーディオ機器の進歩と同一である．当初は電気的増幅を使用しないラッパ様のものであったが，1900年頃の電気HAの登場以後，真空管，トランジスタ，集積回路と小型・軽量化した．近年ではデジタルHAの出現により，パーソナルコンピュータを用いて簡易に多様な調整が可能となった．

表1に代表的なHAの種類と特徴を示した．価格は数万円から数十万円以上と幅が広い

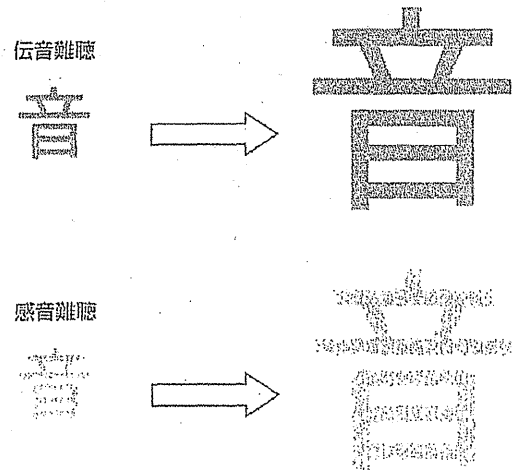
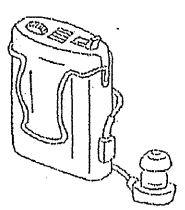
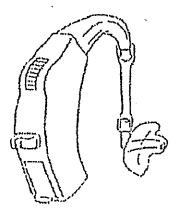
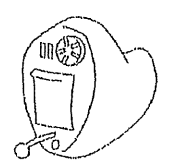


図3　きこえの違いのイメージ

表1　代表的な補聴器の種類

名称	ポケット型補聴器 Body worn	耳かけ型補聴器 Behind The Ear(BTE)	耳あな型補聴器 In The Ear(ITE) In The Canal(ITC) Completely In The Canal(CIC)
図			
伝音方法	気導・骨導	気導・骨導	気導
操作性	○	△	×
審美性	×	△	○
最大出力	○	○	×
価格の範囲 (国内主要メーカー)	約3万円~8万円	約5万円~20万円	約7万円~40万円

が，“値段＝きこえの良さ”ではない．また，現在の技術をもってしても言語音のみを100%増幅できるHAは存在しない．

補聴器の調整

先にも述べたが感音難聴の場合，音が歪んで入力されるため，HAによる音の増幅だけでは聴取能の改善に結びつかない場合が多い．HA装用の動機，HAが必要な場面等，個々の生活環境を考慮することが求められる．実際には仮調整と日常生活での試用，試用結果と評価結果を基にした再調整を頻回に行う．また家族・周囲の理解が必要であり，環境整備や，本人のコミュニケーションスキルの向上を目標とした聴覚リハビリテーションを併せて行うことが望ましい．

街中ではよくHA販売店を目にするが，わが国では実際の販売において医師の診察は義務付けられていない．現在，財団法人テクノエイド協会による「認定補聴器専門店」や，

表2　聴覚経路と対応する埋込型機器

デバイス名	適応部位	国内での供給者
埋込型骨導補聴器	おもに外耳・中耳	未
人工中耳	おもに外耳・中耳	未
人工内耳	おもに内耳	1994年
脳幹インプラント	おもに聴神経	未

「認定補聴器技能者」が整備されているが，販売する者について特に専門資格等の制限はなく，HA調整に熟練していない者もいるのが現状である．

HA装用上の最も重要な点は適切な聴覚検査による診断と，聴覚保護のための機器調整である．大学病院等では補聴器外来を設置しているところも増えており，HAの装用にあたっては，補聴器適合判定医や補聴器相談医による指導が望ましい．

補聴器の評価

HA装用が成功したかどうかの判定は，語音聴取能に代表される客観的評価と，質問紙等を用いた患者の日常生活での主観的評価を併用する．欧米ではHA装用によりいかにコミュニケーションの改善が図られたかを評価するため，ライフステージに応じた種々の質問紙が開発されている．聴覚障害による心理的影響や，音声コミュニケーション障害の状況，日常生活上の制限等を調べることで，リハビリテーションの効果やquality of life（QOL）の改善を包括的に評価するものもある[2,3]．国内でも独自の質問紙が開発されている[4]．

人工内耳

近年，聴覚補償・代償機器として，手術により機械を体内に埋め込む技術が臨床応用されている．表2に聴覚経路と対応する機器を示した．なかでも飛躍的に装用者が増大している人工内耳（cochlear implant；CI）について述べる．

CIはHAが有効でないような重度聴覚障害児・者を対象として開発された機器であり，高度管理医療機器（クラス3）に分類される．重度感音難聴では，蝸牛内の有毛細胞が損傷されて音響信号から電気信号への変換ができないが，より中枢側の聴神経の機能は保たれている場合が多い．CIはこの音響信号から電気信号への変換を代償する装置である．1960年代から欧米やオーストラリアを中心に開発研究が盛んになり，1970年代には機器の臨床応用がなされ[5～7]，現在では多数の刺激電極を持つ多チャネル方式が使用されている．

わが国で多チャネルCI手術が初めて行われたのは1985年である[8]．その後の普及に伴って1994年に保険適用となり，言語習得後に失聴した重度聴覚障害者への一般的な医療となりつつある．近年では言語習得前失聴児に対しても用いられ，有用性について多数報告されるようになった．これまでに国内では6,000人以上，世界で約18万人以上の装用者が生活しているとされる．

図4にCIの構成とX線写真を示した．機器は体内に埋め込まれるインプラントと体外に装着するスピーチプロセッサから構成される．インプラントの埋め込みは全身麻酔下に行われ，受信部を側頭部皮下に固定し，電極部を内耳に挿入する．マイクロホンから入力された音響情報がスピーチプロセッサで分析され（①）デジタルコードに変換され，送信コイルからFM無線信号のかたちで皮膚を介

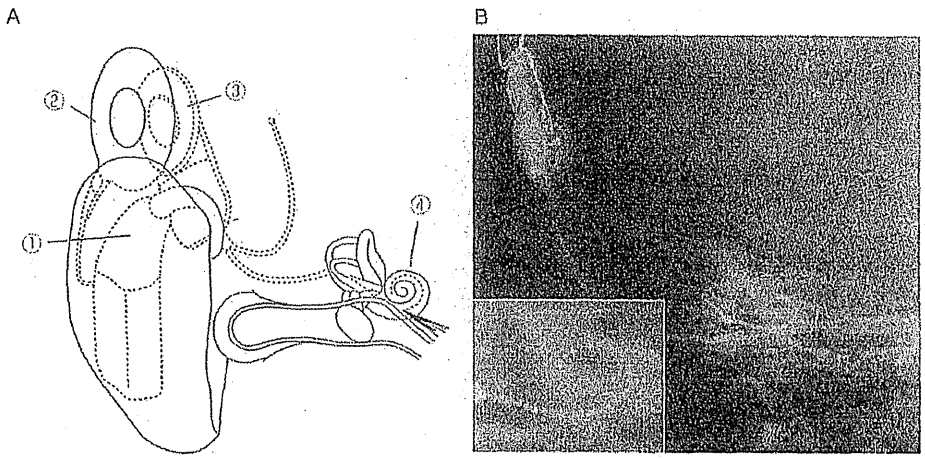


図4　人工内耳の構成（A）とX線写真（B）

して（②）インプラントの受信刺激器に送信される（③）．インプラントの受信刺激器は受信した信号に従って蝸牛内に埋め込まれた電極を選択して電流を出力する（④）．

CIの使用は手術で完結するものではなく，その後のリハビリテーションと音声処理プログラムの作成が必須となる．術後2～3週間後に，初めてプログラムを作成して機器を作動させる音入れを行う．音入れ後は数週間に一度，機器調整と聴取練習や装用指導のリハビリテーションを実施し，装用の安定後は1年に1～2度の経過観察を行う．

人工内耳聴覚について

技術進歩により，CI装用下の聴取能の改善は目覚しく，聴力レベルは軽度難聴と同等まで調整することが可能である．一方，語音聴取能については個人差があり，高度難聴程度に著しい障害を示す症例から，電話で会話聴取が可能な症例まで多様である．また，静かな場所での1対1の会話は問題なく行える症例でも，グループでの会話や大きいホール等での聴取，視覚的補助のない場面等では，急激に聴取能が低下する．このため，家族の団らんやグループ活動の際に孤独感を感じる装用者も少なくない[9]．聴取能向上に対するアプローチのほか，心理的なケアや，総合的なコミュニケーションスキルの向上がやはり重要である．

おわりに

代表的な聴覚補償機器である補聴器と人工内耳について述べた．補聴器は，適切な調整と装用指導の重要性を強調したい．人工内耳は最も成功した人工臓器の一つとされ，聴覚処理機構の解明と電気技術の進歩により，今後も適応の拡大や機器の小型化等さらなる発展が見込まれている．

文献　1) Northern JL, et al.: Hearing in children. Lippincott Williams & Wilkins, 18, 2002
2) Ewertsen HW, et al.: Social hearing handicap index. Social handicap in relation to hearing impairment. Audiology 12: 180-187, 1973
3) Tuley MR, et al.: A critical reevaluation of the Quantified Denver Scale of Communication Function. Ear & Hearing 11: 56-61, 1990
4) 鈴木恵子，他：補聴効果評価のための質問紙の作成．Audiology Japan 45: 89-101, 2002
5) House WF, et al.: Long term results of electrode implantation and electronic stimulation of the cochlea in man. Ann Otol Rhinol Laryngol 82: 504-517, 1973

6) Clark GM, et al.: A multiple-electrode hearing prosthesis for cochlea implantation in deaf patients. Med Prog Technol 5: 127-140, 1977
7) Hochmair-Desoyer IJ, et al.: Four years of experience with cochlear prostheses. Med Prog Technol 8: 107-119, 1981
8) 船坂宗太郎，他：22チャンネルCochlear Implantそのシステムと本格的言語訓練前の話声聴取能について．日本耳鼻咽喉科学会報 89: 1070-1076，1986
9) 湯川久美子：人工内耳装用患者の心理的側面についての検討．耳鼻咽喉科展望 37: 300-310，1994

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Prevalence and Clinical Features of Hearing Loss Patients with *CDH23* Mutations: A Large Cohort Study

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Abstract

Screening for gene mutations in *CDH23*, which has many exons, has lagged even though it is likely to be an important cause for hearing loss patients. To assess the importance of *CDH23* mutations in non-syndromic hearing loss, two-step screening was applied and clinical characteristics of the patients with *CDH23* mutations were examined in this study. As a first screening, we performed Sanger sequencing using 304 probands compatible with recessive inheritance to find the pathologic mutations. Twenty-six possible mutations were detected to be pathologic in the first screening. For the second screening, using the probes for these 26 mutations, a large cohort of probands (n = 1396) was screened using Taqman amplification-based mutation analysis followed by Sanger sequencing. The hearing loss in a total of 52 families (10 homozygous, 13 compound heterogygous, and 29 heterozygous) was found to be caused by the *CDH23* mutations. The majority of the patients showed congenital, high frequency involved, progressive hearing loss. Interestingly, some particular mutations cause late onset moderate hearing loss. The present study is the first to demonstrate the prevalence of *CDH23* mutations among non-syndromic hearing loss patients and indicated that mutations of the *CDH23* gene are an important cause of non-syndromic hearing loss.

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Introduction

Mutations in the *CDH23* (NM_22124) gene are known to be responsible for both Usher syndrome type ID (USH1D) and non-syndromic hearing loss (DFNB12) [1,2]. Molecular confirmation of *CDH23* mutations has become important in the diagnosis of these conditions.

This gene encodes cadherin 23, a protein of 3354 amino acids with 27 extracellular (EC) domains, a single transmembrane domain and a short cytoplasmic domain. Cadherin-specific amino acid motifs such as DRE, DXNDN, and DXD, that are highly conserved in sequence and spacing and required for cadherin dimerization and calcium binding were found in each extracelluar domain [3].

The cadherin 23 protein is known to be an important composition of the tip link that maintains the arrangement of streocilia [4].

More than 50 mutations have been reported for the Usher phenotype (USH1D) and 24 mutations reported for the non-syndromic hearing loss phenotype (DFNB12) [1,2,5–7]. As suggested by genotype–phenotype correlation study, Usher 1D, which has congenital profound hearing impairment, vestibular dysfunction, and retinitis pigmentosa, is usually associated with nonsense mutations, whereas DFNB12, which has a milder phenotype, is associated with missense mutations [1,2,5–8].

We previously reported that four pathologic mutations were identified in 5 out of 64 Japanese families compatible with autosomal recessive inheritance, suggesting that *CDH23*-caused deafness may be commonly found among non-syndromic hearing loss patients [6]. *GJB2* has been shown to be a common gene involved in congenital hearing impairment. *SLC26A4* is also frequently involved among those patients. *GJB2* and *SLC26A4* are comparatively small making Sanger sequencing relatively easy. The latter is also associated with the typical inner ear anomaly, enlarged vestibular aqueduct. Therefore, screening is relatively easy and many studies have focused on just these two genes. Clinical molecular diagnosis has been dramatically improved for these genes. However, screening strategy of other hearing loss genes is difficult and Sanger sequencing of the candidate genes, such as *CDH23*, with many exons is time consuming. Consequently, only a few reports are available for the mutation spectrum of *CDH23*.

In the present study, we performed Sanger sequencing using 304 patients whose pedigrees are compatible with recessive inheritance to find additional pathologic mutations. Also, to find the novel pathologic mutations and to clarify the frequency and clinical characteristics of patients with *CDH23* mutations, a large cohort of probands from unrelated families (n = 1396) was screened using TaqMan amplification-based mutation analysis of the variants observed in the initial 304 patients.