# Cellular development of the human cochlea and the regenerative potential of hair follicle bulge stem cells

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# 8

# DISCUSSION

# Human cochlear embryology

## On the origin of cell types in the human cochlea

The first aim of this thesis was to gain more insight into the developing human fetal cochlea. The adult cochlea contains cells derived from different embryonic regions: the otic placode, the mesoderm, and the neural crest. To establish the exact origin of a cell type one has to perform lineage-tracing experiments, either by using the classic, chimeric, transplantation and dye injection approaches or by using more modern genetic fate-mapping methods. Although lineage tracing in the human fetus is difficult due to both ethical and technical reasons, experiments on the salamander [1], chicken [2], and rodent [3] have shown similar embryonic origins of various cochlear cell types in these three species. All immunostainings presented in this dissertation on the human fetal cochlea, as shown in Chapters 2-4, match the expression patterns (when available) of the developing cochlea in other vertebrate species. This strongly supports the hypothesis that the origin of all cell types in the vertebrate cochlea, including the human, is highly conserved. Animal data in combination with the data presented in Chapters 2-4 result in a model of the origin of cell types in the human cochlea, described in the following paragraphs and summarized in Table 1.

| Cell type | Origin |
|---|---|
| **Cochlear duct epithelium** | Otic placode (cranial placode) |
| **Spiral ganglion neurons** | Otic placode (cranial placode) |
| **Peripheral glial cells** | Neural crest |
| **Strial melanocytes (intermediate cells)** | Neural crest |
| **Basal cells of the stria vascularis** | Unknown (likely mesoderm) |
| **Interdental cells** | Unknown (otic placode or neural crest?) |
| **Fibrocytes** | Periotic mesenchyme (mesoderm) |
| **Otic capsule** | Periotic mesenchyme (mesoderm) |
| **Blood vessels** | Periotic mesenchyme (mesoderm) |

Table 1. The embryonic origin of various cochlear cell types

### Cochlear duct epithelium

Most of the cochlea is derived from the otic placode and develops via the otic vesicle stage into both the vestibular labyrinth and the cochlear duct. It is therefore likely that all epithelial cells lining the cochlear duct are derived from the otic placode. Counterclockwise (Figure 1), starting at the organ of Corti, these cells are: hair cells and various types of supporting cells, Hensen's cells, Claudius' cells, outer sulcus root cells, the epithelial cells of the spiral prominence, the marginal cells of the stria vascularis, the epithelial cells of Reissner's membrane, interdental cells, and the inner sulcus cells (or Kölliker's organ in the fetus). All these different cell types develop from a more or less undifferentiated epithelium which at the 10th week of gestation (W10) expresses SOX9 and SOX10, with additional SOX2 expression in the prosensory domain (Chapter 2).



### Spiral ganglion neurons

Spiral ganglion neurons (SGNs) are, like the epithelial lining of the cochlear duct, also derived from the otic placode. Proneural cells delaminating from the

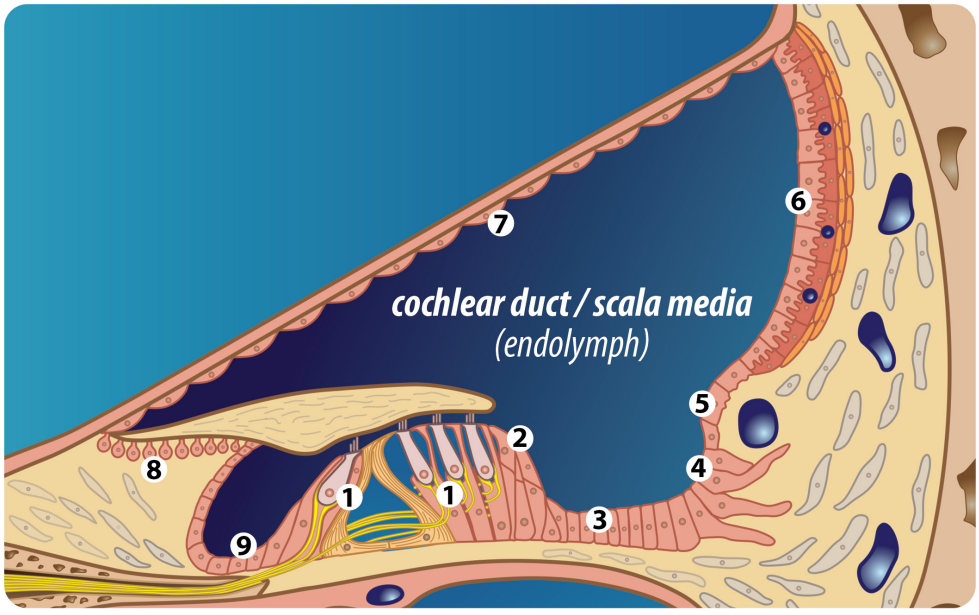


Figure 1. Schematic illustration of the cochlear duct / scala media.
1: hair cells, 2: Hensen's cells, 3: Claudius' cells, 4: outer sulcus root cells, 5: epithelial cells of the spiral prominence, 6: marginal cells of the stria vascularis, 7: epithelial cells of Reissner's membrane, 8: interdental cells in the spiral limbus, 9: inner sulcus cells. *Picture courtesy of S.B. Blankevoort.*

otic vesicle start to form the statoacoustic ganglion, which in humans presumably occurs around Carnegie stage 13 (~32 days of fetal age, during the 6th week of gestation) [4]. As maturation advances, the cochlear spiral ganglion separates from the vestibular neurons (Scarpa's ganglion) and type-I and type-II SGNs gradually appear. As we could not acquire cochlear specimens of fetal stages before W9, we were unable to visualize the key event of delamination. We did, however, shed new light on the onset of type-I versus type-II SGN identity in the human cochlea, as from W18 onwards, the intermediate filament peripherin became restrictively expressed in groups of cells representing type-II SGNs, suggesting that they share a common progenitor (Chapter 2).

**Peripheral glial cells and melanocytes**

Presently, at least two neural crest-derived cell types are known to be present in the cochlea: (1) peripheral glial cells and (2) specialized melanocytes.

A neural crest origin of the peripheral glial cells in the cochlea has been investigated in multiple studies, both recently by genetic tracing studies in the mouse [5, 6] and longer ago by tissue transplantation studies in the salamander and chicken [1, 2]. As shown in Chapter 3, the distribution and developmental pattern of peripheral glial cells in the human fetal cochlea matches with the postulate of a neural crest origin. The importance of these glial cells cannot be underestimated, as they (or their precursors) not only guide the central processes of SGNs towards their correct position within the brain [7], but are also essential for synchronous activity of SGNs in the adult cochlea, a prerequisite for normal hearing [8]. An intriguing question remaining to be answered is the exact route that is taken by the neural crest-derived peripheral glial cells to reach and enter the cochlea. The cellular distribution pattern we observed at W9 indicates that the peripheral glial cells migrate via the vestibulocochlear nerve (the 8th cranial nerve) into the human fetal cochlea. However, as SGNs originate from the otic placode, there has to be a moment during embryonic development when these two cell populations encounter one another for the first time. Interestingly, the vestibulocochlear ganglion and the geniculate ganglion (the ganglion of the facial nerve, n. VII) are in close proximity to one another early during development [9]; also see Figure 2). As the facial nerve and the geniculate ganglion receive their neural crest-derived cell population from a migratory stream of neural crest cells originating from rhombomere 4[footnote1] (Figure 3A), it is likely that the peripheral glial cells populating the cochlea also originate

from this migratory stream of neural crest cells [2, 6, 10, 11].

As proposed in Chapter 4, cochlear melanocytes might be derived from a different migratory stream of neural crest cells, this one originating from rhombomere 6. This is supported by images from several studies in mouse embryos in which, although not mentioned by the authors, neural crest (derived) cells are clearly visible on the apical side of the developing otic vesicle, i.e. the side facing rhombomere 6 [3, 5, 6, 12, 13]. In the human fetus at W9.1, melanocytes are present both around the nervous tissue at the apical side of the developing cochlea (at this stage likely part of the glossopharyngeal nerve (n. IX)), within the developing otic capsule, and in the mesenchyme bordering the developing stria vascularis (Chapter 4, Figure S1A). The glossopharyngeal nerve itself originates partly from an epibranchial placode and partly from delaminating neural crest cells, both of which are derived from rhombomere 6 [11, 14]. Taken together, we therefore believe that cochlear melanocytes originate from the stream of migrating neural crest cells from rhombomere 6 before travelling through the developing otic capsule and finding



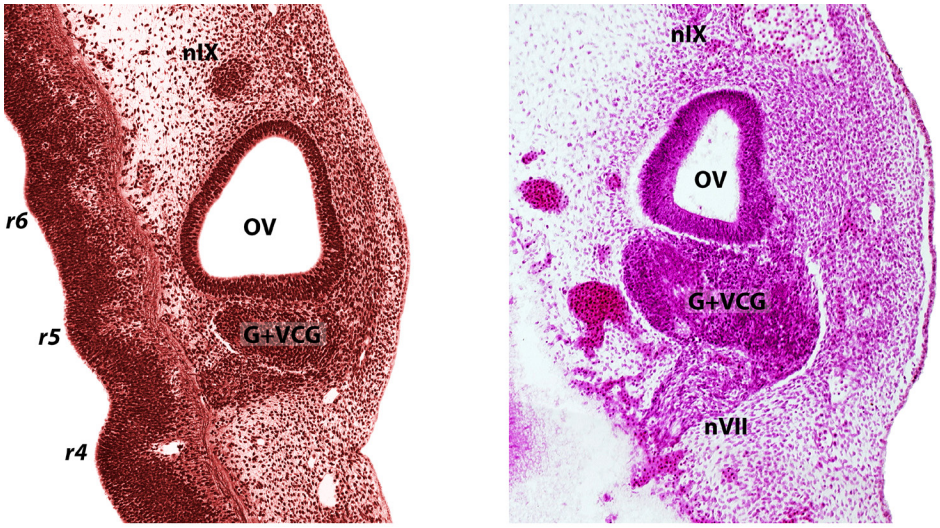


Figure 2. Sections through human fetuses at Carnegie stages 14 and 15.
r4-6: rhombomeres 4-6, G+VCG: geniculate ganglion and the vestibulocochlear ganglion, OV: otic vesicle, n. VII: facial nerve, n. IX: glossopharyngeal nerve. *Images modified and reproduced with permission from the Virtual Human Embryo website: http://www.ehd.org/virtual-human-embryo/*

their way to the lateral wall epithelium during the early embryonic development. A model of these migratory streams of neural crest cells is shown in Figure 3B. Future studies are needed to test this model.

### Cell types with unknown origin

There are two cell types residing in the cochlea whose the exact embryonic origin remains unknown: (1) the basal cells and (2) the interdental cells.

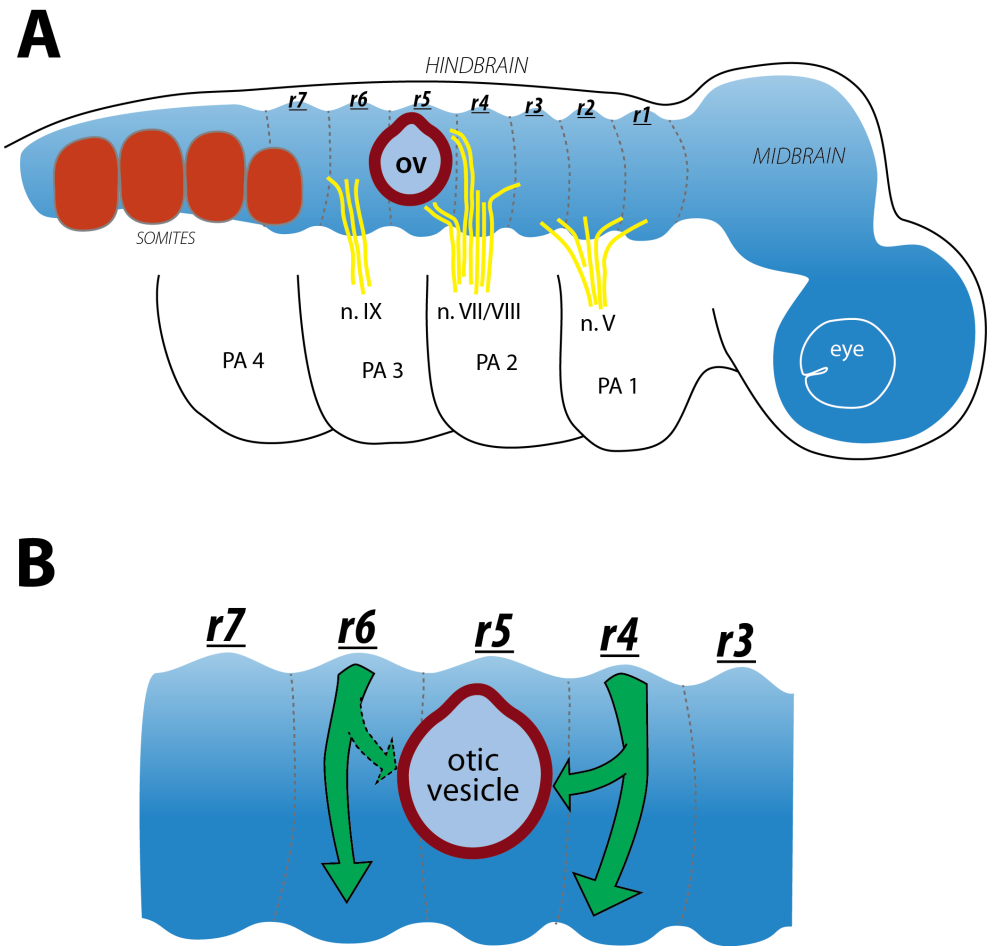


**Figure 3. Schematic illustration of the hindbrain and otic vesicle in the human fetus.**
**(A)** Overview showing the otic vesicle (OC) and its position relative to the rhombomeres (r1-7), the developing cranial nerves n. V, n. VII/VIII and n. IX and the pharyngeal arches (PA1-4). **(B)** Migratory streams of neural crest cells delaminating from the regions at rhombomeres 4 and 6, with their proposed contribution to the development of the cochlea.

### Basal cells

The origin of the basal cells in the stria vascularis remains unknown as little research has been done on these cells (the exact location of the basal cells within the cochlea is shown in Chapter 4, Figure 1). Theoretically, there are three possible sites of origin: (1) they belong to the mesenchymal portion of the cochlea (as do the bordering fibrocytes in the spiral ligament), (2) they originate from the neural crest (as the strial melanocytes), and (3) they are derived from the otic placode (differentiating from the marginal cells). The first option is the most likely one, as basal cells have never been identified in lineage-tracing studies of the otic placode or neural crest.

### Interdental cells

Interdental cells are located in the spiral limbus and most likely play an additional role in potassium homeostasis [15] as they express KCNJ10 on their apical (luminal) membranes [16]. A recent study in mice traces interdental cells to a shared lineage with spiral ganglion neurons, suggesting an otic placode origin [17]. However, the same study also groups them with the intermediate cells (melanocytes), which clearly have a different (neural crest) origin. This contradiction needs to be resolved to conclusively confirm their origin. Neural crest lineage-tracing studies in the cochlea have never observed cells that migrate to the spiral limbus, which would argue against a neural crest origin of the interdental cells. Nonetheless, they would have been easily overlooked as this specific region has not been investigated at the proper embryonic or fetal stages, and as multiple lineage markers are expressed in both the neural crest and the otic placode, hampering discrimination between the two.

## Stem cell regeneration

### Cochlear regeneration using stem cells

The second aim of this thesis was to pursue a possible stem cell strategy for the restoration of hearing. To successfully regenerate damaged or lost cochlear structures by means of stem cell-based therapies, one has first to select the appropriate stem cell source. Two general approaches may be considered: (1) taking advantage of

stem cells residing in the adult cochlea itself, and (2) introducing exogenous stem cells into the damaged cochlea.

**Cochlear stem cells**

There are several lines of evidence suggesting that the adult mammalian cochlea still contains stem cells. In the 1980s it was discovered (against expectations) that adult birds are able to regenerate hair cells in their damaged basilar papillae, i.e., the hearing organ in birds [18, 19]. Later, it became clear that in various vertebrate species, ranging from amphibians to fish, hair cells are able to regenerate or are continuously produced [20]. Hair cells in the vestibular organs likewise renew throughout life in birds as well as in most other non-mammalian vertebrates. The supporting cells in the sensory epithelium were found to be the source of the new hair cells. Unfortunately, spontaneous hair cell regeneration in mammals seems to have been lost during evolution, except for the limited capability for hair cell renewal in the vestibular organs of rodents. Although the exact reason remains unknown, it is suggested that this loss has been a trade-off with the development of the unique but intricate architecture of the organ of Corti in which regeneration has become too challenging [21].

Since the discovery that hair cells in birds can regenerate, numerous studies have focused on identifying the key regulators of hair cell specification, differentiation and regeneration in order to induce hair cell (re)generation in mammals. Major players that have been identified presently are the Notch/Jagged signaling pathway, transcription factors Atoh1 and Sox2 and cell cycle regulatory protein $p27^{Kip1}$ [22]. Since it has been shown that new hair cells are derived from Sox2-expressing supporting cells, an important question is to ask if the human organ of Corti still contains SOX2-positive cells supporting cells. Experiments from Chapter 2 showed that these supporting cells in the human fetal cochlea do express SOX2 positive up to the latest stage investigated (W19). Whether or not they retain their SOX2 expression in the adult cochlea remains to be seen. If so, successful experimental therapies in animal studies could benefit potential human trials.

Another interesting note is be made regarding regeneration of other cochlear structures. At present, there is no evidence whatsoever for regeneration of, for example, spiral ganglion neurons or cells in the stria vascularis by means of innate cochlear stem cells. Does this mean that there are no such cells? Intriguingly, we know that there are melanocytes residing in the spiral ligament (as shown in

Chapter 4) or in the modiolus (data not shown). Why exactly are those cells present at these sites, and are they or could they serve as a local source of stem cells?

**Exogenous stem cells**

The other stem cell option to consider in cochlear regeneration is the transplantation of exogenous stem cells into the damaged cochlea, which should be able to migrate to the appropriate site and differentiate into the desired cell type. Therefore, it is essential to know the embryonic origin of the cells that need to be replaced, especially since it is unlikely that a neural crest stem cell will differentiate into an otic placode-derived hair cell or that a stem cell from the central nervous system will differentiate into a myelinating Schwann cell from the peripheral nervous system. As stem cells come in multiple flavours, extensive knowledge of the molecular basis of cell fate is a prerequisite for selecting the appropriate stem cell type. For example, the transcription factors Pax2 and Pax8 are expressed in the otic placode, whereas cochlear neural crest precursors express Pax3. A Pax2-/Pax3+/Pax8- stem cell most likely will not differentiate into a hair cell, as it is destined to develop in another cell lineage (more extensively reviewed in [23]). However, this cell type could very well be the stem cell of choice for the formation of new peripheral glial cells.

As the otic placode is formed very early during embryological development, the most likely candidates for otic placode-derived cell induced regeneration (such as hair cell or spiral ganglion cell regeneration) are the pluripotent stem cells. Promising candidate stem cells are the induced-pluripotent stem cells (iPSCs) [24] as these cells resemble in many aspects an early embryonic cell type still able to differentiate into most cell lineages. An additional advantage is that iPSCs can be derived from adult tissue of the patient himself. Another option would be to use human embryonic stem cells, which have recently been shown to have a restorative effect on auditory function when introduced in an animal model with auditory neuropathy [25]. Although transplantation proved effective in that study, immune responses can be expected when using such cells in a human transplantation setting. Stem cells such as patient-specific iPSCs will most likely not have this adverse effect if used autologously. Other important reasons to consider in selecting a stem cell source for regeneration purposes is the tendency of stem cells to form tumors after transplantation, and the lineage distance between the stem cell and the desired, differentiated, cell type. A larger distance results in a higher number of

differentiation steps needed and consequently a higher chance that the stem cell will deviate from its requested path. To circumvent this problem, a stem cell type could be selected that is more differentiated but retains stem cell characteristics, such as neural crest stem cells (NCSCs). Evidently in the case of NCSCs, their suitability to regenerate hair cells or spiral ganglion neurons is limited, but they could prove to be the appropriate choice when it comes to regeneration of peripheral glial cells or cochlear melanocytes.

**Hair follicle stem cells**

Beginning in 2004, researchers began reporting that adult hair follicles contain NCSCs [26, 27]. As mentioned previously, this could have an enormous therapeutic potential, as it is thought that these cells are still able to differentiate into multiple important cell lineages and because they can be easily harvested from patients. In one of the original studies a *Wnt1*-cre/R26R compound transgenic mouse was used [26]. In hair follicle bulge cultures, TUBB3-positive cells were found showing X-gal activity, meaning that these cells (had) expressed *Wnt1*. The authors mentioned that neural crest cells express *Wnt1* transiently, and therefore concluded that the TUBB3-positive cells (which were identified as neurons) originated from NCSCs. However, as shown in Chapters 5-7, we suggest that there are no bona fide NCSCs in the adult hair follicle and that the interpretations made in their study were likely based on an extended expression of TUBB3 in multiple neural crest derivatives, including the melanocytic lineage (Chapters 5-7). Furthermore, *Wnt1* is not only expressed transiently in the neural crest but also in the melanocytic lineage, where it controls differentiation, proliferation and tumor initiation [28, 29]. Therefore, an alternative conclusion about the presence of TUBB3-positive cells in adult hair follicles could be that TUBB3-positive cells from those original studies were melanoblasts/melanocytes rather than neurons. In that perspective, the TUBB3-positive cells could originate from melanocyte stem cells residing in the bulge, or could be dedifferentiated Schwann cells (Chapter 7). Remarkably, no study so far has convincingly shown expression of other neuron-exclusive markers or recorded action potentials (Chapter 5) from *in vitro* hair follicle bulge-derived cells.

Even though it seems plausible that there is no true neural crest stem cell located in the hair follicle bulge and that these cells do not differentiate into neurons, hair-follicle bulge derived cells might retain their therapeutic potential when it comes to regeneration of other neural crest derived cell types such as melanocytes

or peripheral glial cells. This reasoning and our work from Chapters 5-7 support the hypothesis that different groups of neural crest descendants exist in the hair follicle bulge region, which may act jointly to achieve tissue restoration [30, 31].

Nevertheless, whether neural crest-derived neurons are the right candidate to replace damaged SGNs remains an open question. SGNs are derived from the otic placode and not from the neural crest like most other neurons from the peripheral nervous system. Therefore, cell regeneration with neurons derived from neural crest stem cells might not result in cells with the unique morphological and electrophysiological characteristics of SGNs.

Although more research is required, it is very likely that both Schwann cells and melanocytes can be easily cultured from hair follicle explants (from the mouse whisker pad), though it is unclear whether they originate from one multipotent stem cell or from two different (stem) cell pools (Chapter 7). In the inner ear, such cells could potentially be put to use in restoring the peripheral glial cells around the SGNs, or to renew melanocytes in the stria vascularis. As etiologic knowledge on many types of deafness is still incomplete, future insights may lead to applicability of these cell types in treating hearing loss.



## The onset of human hearing

In the mouse and rat, hearing develops predominantly after birth [32]. In contrast, the development of the cochlea in humans (as well as guinea pigs [33] and chinchillas [34]) occurs during gestation (Chapters 2-4). However, in all mammals, the cascade of developmental events that leads to hearing is largely identical. At which fetal age auditory function starts in the human remains an intriguing question, one that very few studies have investigated or have speculated on.

For simplicity's sake, hearing can be defined as the generation of an action potential in a reaction to sound, which is propageted to the brain (therefore disregarding any 'higher' features such as synchronicity and central processing, which likely continue to develop after birth). Taking this into account, there are two absolute requirements: (1) the right cells should be present at the right location, and (2) there should be a functional electrochemical environment. Unfortunately, there is a lack of knowledge on both aspects in the human fetal cochlea. Do we understand all the key cell types involved in hearing? Much insight has been gained on hair cell function, but is there hearing if interdental cells do not develop, or Claudius'

cells for that matter? Also, we do not know the exact contribution of each cell type to the cochlear ionic homeostasis. For example, do satellite glial cells play a role in potassium regulation (which they likely do)? Or, what are the exact routes of potassium recycling?

Even within these limitations, an educated guess can be made using the following assumptions: hearing is possible (1) when the spiral ganglion neurons are connected to hair cells, and (2) when there is a functional endocochlear potential (see Chapter 4 for an explanation of the term 'endocochlear potential').

As shown in Chapter 2, the first contact between hair cells and SGNs occurs in the basal turn at W12, refining in the weeks thereafter. At W18, a configuration of type-I and type-II SGNs and inner and outer hair cells can be found in the basal turn that by and large mimics the adult situation. However, the question remains whether a positive endocochlear potential is present at 18 weeks of gestation (which is, for ethical reasons, impossible to measure in an experimental setting). Based upon the morphological development of SGNs and hair cells, we have proposed, as have others, (Chapters 2-3) that hearing commences around W18-20. However, this statement has been made without taking the endocochlear potential into account (Chapter 4). There are several studies measuring auditory responses in preterm human neonates. One study measured evoked otoacoustic emissions and observed (very weak) activity in neonates born at 28 weeks of gestation [35]. Unfortunately presence of these responses was not investigated in all neonates of that particular age, and younger subjects (very preterm neonates) were not measured. In a diff erent study measuring automated auditory brainstem responses in preterm neonates, a bilateral pass was first observed at W27-28, steeply increasing to W32 where pass rates included almost all subjects [36]. Both studies suggest that the earliest week at which hearing in humans commences is around W28. However, as the used auditory tests have limitations in their sensitivity, the actual onset of hearing could occur earlier.

In mice and in gerbils, the rise of the endocochlear potential has been studied during embryonic development [37, 38]. These studies concluded concluded that a certain level of maturation of gap junctions and tight junctions is required before the endocochlear potential develops. Data from Chapter 4 studying several key potassium channels and other proteins that are thought to play a role in the generation of the endocochlear potential indicate that this system of potassium-regulating structures is still immature at W18. For example, the intermediate cells do not yet express KCNJ10 and the basal cells have not or are barely formed. A

study investigating the fetal stria vascularis by means of electron microscopy in a few human cochlear samples from stages older than W19 found that basal cells and gap junctions could be clearly observed at the end of W24 [39]. When comparing the limited amount of human immunohistochemical and electron microscopical data from all investigated developmental stages to the timelines of mouse and gerbil cochlear development, a clear pattern emerges. Extrapolating the functional onset of the endocochlear potential in these animals to the human developmental timeline indicates that hearing is unlikely to take place in a human cochlea at W18-W20. We now speculate that human hearing commences somewhere between W26 and W28 (in the 7th month of pregnancy, around the start of the third trimester).



## Future research and applications

Both basic research investigating physiology and development of auditory function and more clinically oriented research in genetics and stem cell therapies help in discriminating between normal and pathological conditions and in finding new treatment strategies for patients with hearing loss.

Investigating later gestational stages and even adult human cochlear tissue could extend the developmental insights gained by this work. However, obtaining material of sufficient quality has of course both practical and ethical issues. Other investigations could focus on mapping the expression of all genes known to be involved in SNHL. This would provide more knowledge on the involved cell types and damaged structures, which in turn will provide valuable input to the field on therapeutic interventions. Finally, regardless of whether or when gene or stem cell therapies become clinically available, there is much knowledge to be gained by investigating the causes of SNHL. For a large portion of hereditary SNHL cases, the cause of their hearing loss remains unknown. Further, patients are not yet screened for all known mutations. As knowledge on the genetics of hereditary hearing loss continues to increase and genetic screening methods become more extensive and cheaper, much progress should be expected on this topic within the next decade.

***Footnotes:***

*1: Rhombomeres are developmental segments of the neural tube that later form the rhombencephalon, the hind-brain.*

## References


1. Yntema CL: **An experimental study of the origin of the cells which constitute the VIIth and VIIIth cranial ganglia and nerves in the embryo of Amblystoma punctatum.** J Exp Zool 1937, 75:75 – 101.
2. D'Amico-Martel A, Noden DM: **Contributions of placodal and neural crest cells to avian cranial peripheral ganglia.** Am J Anat 1983, 166:445 – 68.
3. Wakaoka T, Motohashi T, Hayashi H, Kuze B, Aoki M, Mizuta K, Kunisada T, Ito Y: **Tracing Sox10-expressing cells elucidates the dynamic development of the mouse inner ear.** Hear Res 2013, 302(May):17 – 25.
4. O'Rahilly R: **The Early Development of the Otic Vesicle in Staged Human Embryos.** J Embryol Exp Morphol 1963, 11(December):741 – 55.
5. Freyer L, Aggarwal V, Morrow BE: **Dual embryonic origin of the mammalian otic vesicle forming the inner ear.** Development 2011, 138:5403 – 14.
6. Sandell LL, Butler Tjaden NE, Barlow AJ, Trainor PA: **Cochleovestibular nerve development is integrated with migratory neural crest cells.** Dev Biol 2014, 385:200 – 10.
7. Freter S, Fleenor SJ, Freter R, Liu KJ, Begbie J: **Cranial neural crest cells form corridors prefiguring sensory neuroblast migration.** Development 2013, 140:3595 – 600.
8. Zeng F-G, Kong Y-Y, Michalewski HJ, Starr A: **Perceptual consequences of disrupted auditory nerve activity.** J Neurophysiol 2005, 93:3050 – 63.
9. Bruska M, Woźniak W: **The origin of cells of the cochlear ganglion in early human embryos.** Folia Morphol (Warsz) 2000, 59:233 – 8.
10. Ayer-Le Lievre CS, Le Douarin NM: **The early development of cranial sensory ganglia and the potentialities of their component cells studied in quail-chick chimeras.** Dev Biol 1982, 94:291 – 310.
11. Lumsden A, Sprawson N, Graham A: **Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo.** Development 1991, 113:1281 – 91.
12. Steel KP, Davidson DR, Jackson IJ: **TRP-2/DT, a new early melanoblast marker, shows that steel growth factor (c-kit ligand) is a survival factor.** Development 1992, 115:1111 – 9.
13. Adameyko I, Lallemend F, Furlan A, Zinin N, Aranda S, Kitambi SS, Blanchart A, Favaro R, Nicolis S, Lübke M, Müller T, Birchmeier C, Suter U, Zaitoun I, Takahashi Y, Ernfors P: **Sox2 and Mitf cross-regulatory interactions consolidate progenitor and melanocyte lineages in the cranial neural crest.** Development 2012, 139:397 – 410.
14. Ladher RK, O'Neill P, Begbie J: **From shared lineage to distinct functions: the**

**development of the inner ear and epibranchial placodes.** Development 2010, 137:1777 – 85.

15. Spicer SS, Thomopoulos GN, Schulte BA.: **Structural evidence for ion transport and tectorial membrane maintenance in the gerbil limbus.** Hear Res 2000, 143:147 – 161.
16. Eckhard A, Gleiser C, Rask-Andersen H, Arnold H, Liu W, Mack A, Müller M, Löwenheim H, Hirt B: **Co-localisation of K(ir)4.1 and AQP4 in rat and human cochleae reveals a gap in water channel expression at the transduction sites of endocochlear K(+) recycling routes.** Cell Tissue Res 2012, 350:27 – 43.
17. Jiang H, Wang L, Beier KT, Cepko CL, Fekete DM, Brigande JV: **Lineage analysis of the late otocyst stage mouse inner ear by transuterine microinjection of a retroviral vector encoding alkaline phosphatase and an oligonucleotide library.** PLoS One 2013, 8:e69314.
18. Corwin JT, Cotanche DA: **Regeneration of sensory hair cells after acoustic trauma.** Science 1988, 240:1772 – 4.
19. Ryals BM, Rubel EW: **Hair cell regeneration after acoustic trauma in adult Coturnix quail.** Science 1988, 240:1774 – 6.
20. Rubel EW, Furrer SA, Stone JS: **A brief history of hair cell regeneration research and speculations on the future.** Hear Res 2013, 297:42 – 51.
21. Brigande JV, Heller S: **Quo vadis, hair cell regeneration?** Nat Neurosci 2009, 12:679 – 85.
22. Cotanche DA, Kaiser CL: **Hair cell fate decisions in cochlear development and regeneration.** Hear Res 2010, 266:18 – 25.
23. Okano T, Kelley MW: **Stem cell therapy for the inner ear: recent advances and future directions.** Trends Amplif 2012, 16:4 – 18.
24. Yamanaka S: **Induced pluripotent stem cells: past, present, and future.** Cell Stem Cell 2012, 10:678 – 84.
25. Chen W, Jongkamonwiwat N, Abbas L, Eshtan SJ, Johnson SL, Kuhn S, Milo M, Thurlow JK, Andrews PW, Marcotti W, Moore HD, Rivolta MN: **Restoration of auditory evoked responses by human ES-cell-derived otic progenitors.** Nature 2012, 490:278 – 282.
26. Sieber-Blum M, Grim M, Hu YF, Szeder V: **Pluripotent neural crest stem cells in the adult hair follicle.** Dev Dyn 2004, 231:258 – 69.
27. Amoh Y, Li L, Katsuoka K, Penman S, Hoffman RM: **Multipotent nestin-positive, keratin-negative hair-follicle bulge stem cells can form neurons.** Proc Natl Acad Sci U S A 2005, 102:5530 – 4.
28. O'Connell MP, Weeraratna AT: **Hear the Wnt Ror: how melanoma cells adjust to changes in Wnt.** Pigment Cell Melanoma Res 2009, 22:724 – 39.
29. Wong CE, Paratore C, Dours-Zimmermann MT, Rochat A, Pietri T, Suter U, Zimmermann DR, Dufour S, Thiery JP, Meijer D, Beermann F, Barrandon Y,

Sommer L: **Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin.** J Cell Biol 2006, 175:1005 – 15.

30. Johnston APW, Naska S, Jones K, Jinno H, Kaplan DR, Miller FD: **Sox2-Mediated Regulation of Adult Neural Crest Precursors and Skin Repair.** Stem Cell Reports 2013, 1:38 – 45.
31. Kumar A, Brockes JP: **Nerve dependence in tissue, organ, and appendage regeneration.** Trends Neurosci 2012, 35:691 – 9.
32. Jones SM, Jones TA (Eds): **Genetics, Embryology, and Development of Auditory and Vestibular Systems.** 1st edition. Plural Publishing, Inc; 2011.
33. Rubel EW, Fay RR (Eds): **Development of the Auditory System.** 1st edition. Volume 20. Springer-Verlag New York; 1998.
34. Jones HG, Koka K, Tollin DJ: **Postnatal development of cochlear microphonic and compound action potentials in a precocious species, Chinchilla lanigera.** J Acoust Soc Am 2011, 130:EL38 – L43.
35. Chabert R, Guitton MJ, Amram D, Uziel A, Pujol R, Lallemant J-G, Puel J-L: **Early maturation of evoked otoacoustic emissions and medial olivocochlear reflex in preterm neonates.** Pediatr Res 2006, 59:305 – 8.
36. Van Straaten HL, Tibosch CH, Dorrepaal C, Dekker FW, Kok JH: **Efficacy of automated auditory brainstem response hearing screening in very preterm newborns.** J Pediatr 2001, 138:674 – 8.
37. Steel KP, Barkway C: **Another role for melanocytes: their importance for normal stria vascularis development in the mammalian inner ear.** Development 1989, 107:453 – 63.
38. Souter M, Forge A: **Intercellular junctional maturation in the stria vascularis: possible association with onset and rise of endocochlear potential.** Hear Res 1998, 119:81 – 95.
39. Lavigne-Rebillard M, Bagger-Sjöbäck D: **Development of the human stria vascularis.** Hear Res 1992, 64:39 – 51.