Fibrocytes in the cochlea of the mammalian inner ear: their molecular architecture, physiological properties, and pathological relevance

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Abstract:

Fibroblasts are a cell type that dominates connective tissues in a broad array of organs and plays key roles in formation of the extracellular matrix and wound healing. The cochlea of the mammalian inner ear harbors loose connective tissues such as the spiral ligament and spiral limbus, and their cellular components are called "fibrocytes." The fibrocytes in the ligament are functionally differentiated and specialized for ion transport that is essential for proper actions of the cochlea. Molecular biological and histological assays have shown that these cells express specific types of ion channels and transporters. Results of *in vivo* electrophysiological experiments have integrated activities of individual channels and transporters into the ionic flow that circulates throughout the organ and maintains the electrochemical properties in various tissues and extracellular fluids. Moreover, analyses of deafness genes in humans as well as transgenic experiments on mice recently revealed the relevance of fibrocyte dysfunction to hearing disorders. In this review article, we not only describe molecular architecture and physiological and pathological significance of cochlear fibrocytes but also provide insights into next-generation therapies targeting these cells.

1 Introduction

Fibroblasts are mesenchymal cells that are distributed ubiquitously throughout connective tissues in most organs. These cells show branched-shape morphology and have moderately basophilic cytoplasm and an abundant rough endoplasmic reticulum.[1,2] Furthermore, they synthesize and secrete extracellular-matrix precursors including collagens, contributing to wound healing and inflammatory processes.[1-3]

There is a population of quiescent or resting fibroblasts that are less involved in collagen synthesis.[1,4] These so-called "fibrocytes" are scattered within connective tissues (e.g., tenocytes in a tendon tissue) and harbor a reduced volume of the cytoplasm and of the rough endoplasmic reticulum and more regular and smoother surface as compared to "active fibroblasts." Once activated by a tissue injury or an infection, fibrocytes can convert into fibroblasts and then migrate toward the problem focus followed by their own proliferation and collagen synthesis to assist tissue remodeling.

Currently, the term "fibrocytes" is preferably used to denote specific cell types, i.e., bone marrow-derived fibroblast progenitor cells in circulating blood, rather than to label the aforementioned quiescent or resting fibroblasts. These cells account for 0.1–0.5% of nucleated cells in peripheral blood of normal hosts and express two hematopoietic markers, CD34 and CD45, as well as an extracellular-matrix protein, collagen I.[5-7] In response to tissue injury, $CD34^+$ fibrocytes migrate out of the circulation and secrete extracellular-matrix proteins or chemokines. These cells are also involved in fibrotic diseases and tumorigenesis; therefore, they are likely to be candidates for biomarkers and therapeutic targets of diseases.[8-10]

On the other hand, in the inner ear, a "fibrocyte" represents a different cell type. In 1970, Kimura and colleagues used this terminology to describe the cells in the spiral ligament and spiral limbus, which are loose connective tissues in the cochlea.[11-13] Like fibroblasts, cochlear fibrocytes have a mesenchymal origin; nevertheless, they are mature cells that are functionally differentiated and distinct from the hematopoietic fibroblast progenitor cells that express CD34 and circulate systemically.[5]

Evidence has accumulated that cochlear fibrocytes have unique physiological profiles and are crucial for normal hearing. In this article, we briefly review the functional roles and pathological relevance of these fibrocytes.

2 An overview of the cochlea

In mammals, the cochlear bony capsule harbors a cochlear duct that contains several epithelial cell types including sensory hair cells (**Figure 1**).[14] The outside and inside of the duct are filled with two extracellular fluids, perilymph and endolymph, respectively. Whereas perilymph is similar to an ordinary extracellular fluid, endolymph contains a high K^+ concentration ($[K^+]$) of 150 mM and a highly positive potential of approximately +80 mV, called the endocochlear potential (EP). Each hair cell bathes its basolateral surface in perilymph and exposes the apical hair bundle to endolymph. When sound waves vibrate the basilar membrane, which is an elastic membranelike structure beneath the hair cells, mechanoelectrical transduction (MET) channels on top of the hair bundle are opened. This process lets K^+ from endolymph into the hair cells, inducing their depolarization (**Figure 1**). The cellular electrical excitation triggers neurotransmission that conveys the acoustic signals to auditory nerves. The highly positive EP increases the driving force behind the K^+ influx across the MET channels; therefore, it essentially contributes to the sensitivity of hair cells. The K^+ accumulated in the hair cells diffuses basolaterally to perilymph

and eventually returns to endolymph. This unidirectional K^+ transport throughout the cochlea, which is referred to as "K^+ recycling" or "K^+ circulation" (**Figure 1**), is likely to maintain the electrochemical balance within the cochlea.[15-17]

The EP and K^+ recycling depend primarily upon functions of the stria vascularis, an epithelial-like tissue that is localized to the lateral region of the cochlear duct and contains a dense capillary network.[18,19] Between the stria vascularis and bony capsule, there is a loose connective tissue that is referred to as the "spiral ligament."

3 Functional properties of cochlear fibrocytes

Previously, the spiral ligament was believed to serve only as a supportive element for anchoring the cochlear duct to the bony capsule, according to the name "ligament." Recent studies have strongly indicated that fibrocytes, the cellular components of the spiral ligament, perform key functions in cochlear K^+ recycling.[20-22] Fibrocytes in the spiral limbus seem to have similar roles.[23]

During development, the cochlear duct containing endolymph arises from the embryonic otocyst of ectodermal origin, whereas cochlear fibrocytes are derived from the periotic mesenchyme surrounding the otocyst.[24-28] Mature fibrocytes in the ligament are classified into five cell types (types I–V) in accordance with their location and profile of expression of proteins.[21,29] (**Figure 1** and **Table 1**).

A remarkable feature of the fibrocytes in the spiral ligament is an extensive gap junction network, which interconnects all five types of fibrocytes and a portion of the stria vascularis.[20,30,31] Indeed, when neurobiotin of 287 Da is injected into a fibrocyte in a cochlear slice preparation, numerous fibrocytes throughout the spiral ligament as well as the basal and intermediate cells in the stria vascularis are stained.[32] Gap junctions form channels that can freely let through ions and small molecules (up to 1,000 Da) and thereby promote communication among cells. In the cochlear K^+ recycling, the intercellular gap junctions among fibrocytes are likely to constitute the K^+ transport pathway from perilymph to the stria vascularis.[20-22] (**Figure 1**). Aberrations of connexins, the proteins constituting the gap junction, cause hearing loss, as described below.

Cochlear fibrocytes, which bathe in perilymph, are embedded in a dense and microporous collagen matrix.[11,33,34] The cochlear K^+ recycling theory has suggested that the fibrocytes in the spiral ligament continuously take up K^+ from perilymph (**Figure 1**). Subsequently, K^+ is transported to the stria vascularis through gap junctions and thereafter is secreted into endolymph by strial marginal cells. This idea arises mainly from histological findings that some K^+-transporting proteins such as Na^+/K^+ ATPase and $Na^+/K^+/2Cl^-$ cotransporter type 1 (NKCC1, also known as SLC12A2) are coexpressed in type II, IV, and V fibrocytes (**Figure 2** and **Table 1**).[35,36] Furthermore, in the strial marginal cells whose apical membrane faces endolymph and secretes K^+, Na^+/K^+ ATPase and NKCC1 occur together on the basolateral membrane and both of them strongly contribute to K^+ uptake (**Figure 2**).[37] In contrast, our *in vivo* intracellular recording experiments with cochleae of live guinea pigs have shown that the fibrocyte K^+ uptake depends primarily on Na^+/K^+ ATPase but unlikely on NKCC1.[38,39] Although fibrocytes in the ligament also express other K^+ channels and transporters such as Kir5.1, BK channels, Kv3.1b, and KCC3,[40-43] their functions remain uncertain.

One electrical property of the fibrocytes in the ligament is unusual. In general, the plasma membrane of cells in a resting state is permeated predominantly by K^+; therefore, their resting membrane potentials (RMPs) relative to the neighboring extracellular solution are negative (–30

to –90 mV).[44-46] On the other hand, these fibrocytes constantly show a positive membrane potential of +5 to +12 mV *in vivo* even without any acoustic stimuli (**Figure 2**).[37,47] This electrical component is required for the highly positive EP of approximately +80 mV (for details, see the literature[48]). The mechanisms underlying the unique RMP in the fibrocytes have remained elusive. Recently, using the aforementioned *in vivo* intracellular recording technique, we demonstrated that the fibrocyte membrane is permeable more to Na^+ than to K^+ and Cl^-, and this property is intimately involved in the maintenance of the positive RMP (**Figure 2**).[49] It is possible that the Na^+ inflow mediated by this Na^+ permeability is coupled with Na^+ outflow of fibrocyte Na^+/K^+ ATPase and contributes to the continuous K^+ uptake by this ATPase. Clearly, the fibrocyte Na^+ permeability is essential for the EP; the molecular constituents should be identified in a future study.

The fibrocytes in the spiral ligament seem to play other physiological roles. Type III fibrocytes express α-smooth muscle actin and nonmuscle myosin II and show contractility.[50] The spiral ligament is directly connected with the basilar membrane, which vibrates in response to acoustic stimuli and is surmounted by hair cells (**Figure 1**). This tissue may not only structurally maintain the basilar membrane but also actively regulate its tension.

While the stria vascularis contains a dense capillary network, the ligament is penetrated by arteriovenous anastomosing vessels. Intracellular $[Ca^{2+}]$ of type V fibrocytes correlates with the diameter of neighboring vessels.[51] Such "fibrovascular coupling" may control cochlear blood flow in response to acoustic stimuli, which alter metabolic demand in the cochlea.

4 Pathological significance of cochlear fibrocytes

In an animal model, selective disruption of the fibrocytes in the spiral ligament causes acute hearing loss.[52,53] This observation not only reinforces crucial involvement of fibrocytes in cochlear function but also implies their pathological relevance. Several studies have indicated that disorders of fibrocytes contribute to some deafness types.

4.1 Age-related and noise-induced hearing loss

Aging and noise are common causes of sensorineural hearing loss. Schuknecht has studied a number of postmortem human temporal bones and described atrophy of the spiral ligament as one of the common features of age-related changes in his articles.[54-56] Some animal models show a loss of fibrocytes in the ligament as they get older.[57-59] Furthermore, morphological changes of these fibrocytes are observed in noise-exposed animals, at much lower stimulus sound intensity than the intensity causing hair cell degeneration.[60-62] These findings suggest that degeneration or dysfunction of cochlear fibrocytes participates in presbycusis or noise-induced hearing loss even in humans.

4.2 Hereditary hearing loss

Congenital deafness occurs in one per every 1,000 births; more than a half of these disorders are attributable to genetic factors. Two-thirds of genetic deafness diseases are not accompanied by any other aberrations. Most cases (~75%) of this nonsyndromic deafness are inherited in an autosomal recessive manner.[63,64] More than 140 loci have been found to cause human deafness (Hereditary Hearing Loss Homepage: http://hereditaryhearingloss.org)[65]; genes related to cochlear fibrocytes are summarized in **Table 2**.

4.2.1 Gap junctions

The most common deafness gene is *GJB2*, which encodes a gap junction protein connexin 26.[66] Mutations of *GJB2* are responsible for 30–50% of cases of congenital hereditary deafness and 50–80% of cases of autosomal recessive nonsyndromic deafness such as DFNB1.[67-71] Genetic mutations of connexins 30, 31, and 43 are also linked with human deafness.[72-75] These observations may highlight the crucial participation of fibrocytes in K^+ recycling.

4.2.2 Fibrocytic differentiation

POU3F4 (*BRN-4*) is located in human chromosome region Xq21.1 and encodes a transcription factor of the POU domain family.[76] This gene plays key roles in mesenchymal differentiation into fibrocytes during cochlear development,[24] and its mutations cause X-linked deafness of type 2 (DFNX2).[76] The latter accounts for ~50% of all families with X-linked nonsyndromic hearing loss[77] and is clinically characterized by bilateral, progressive, and mixed conductive-sensorineural hearing loss. Moreover, this disease is accompanied by temporal-bone anomalies, stapedial fixation, and perilymphatic gusher during stapes surgery. In mice, *Pou3f4* inactivation causes profound hearing loss with degeneration of the spiral ligament and a reduction in the EP.[24,78] Similarly, mice lacking T-box transcription factor Tbx18 show deafness with severely compromised differentiation of fibrocytes in the spiral ligament and a loss of the EP[26] although the corresponding human deafness has not yet been identified. Therefore, proper differentiation and maturation of these fibrocytes is likely to be necessary for the maintenance of the EP and normal hearing.

4.2.3 Extracellular-matrix proteins

In the spiral ligament, impairments of the extracellular matrix, which is secreted by fibrocytes, are also involved in the pathogenesis of deafness. Cochlin, the most abundant noncollagen protein in the cochlea, is encoded by the *COCH* gene.[79,80] Mutations of this gene are associated with DFNA9, an autosomal dominant adult-onset progressive sensorineural hearing loss accompanied by a vestibular disorder with vertigo.[81] In mice, a knockout of the *Otos* gene—which encodes otospiralin, another extracellular-matrix protein in the ligament—causes hearing loss accompanied by degeneration of the fibrocytes.[82] It is noteworthy that in these animals, the hair cells are preserved. Physiological functions of cochlin and otospiralin remain unclear.

4.2.4 Membrane proteins

Several membrane proteins expressed in the fibrocytes of the spiral ligament may also be related to genetic deafness. These proteins include SLC12A6 (KCC3), a potassium chloride cotransporter, as well as SLC4A10 and SLC4A11, both of which belong to the bicarbonate transporter family. In animals, genetic mutation of any of the three proteins results in hearing loss[43,83-85]; the mechanisms underlying cochlear dysfunction are poorly understood.

5 The fibrocyte as a possible target of regenerative therapies

Cochlear fibrocytes are well-differentiated cells of mesenchymal origin, as already mentioned, and thereby they are likely to have limited capacity for proliferation.[62,86,87] In rats, reversal of the hearing loss induced by a selective injury of these fibrocytes is facilitated when mesenchymal stem cells, which are multipotent cells isolated from adult bone marrow, are transplanted into the semicircular canal of the inner ear.[88] The transplanted cells are detected in the injured area of the spiral ligament and express marker proteins of cochlear fibrocytes. Furthermore, hematopoietic stem cells, even when injected

systemically, can reach the ligament and start to express fibrocyte marker proteins.[89] If these stem cells were sufficiently differentiated and could fully compensate the dysfunctions of the fibrocytes, then regenerative therapies for deafness targeting these cells would be realistic.

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Competing interests

None.

Figure 1 Structure of the cochlea in the mammalian inner ear.

Shown is a schematic diagram of a cross-section of the snail-shaped cochlea, whose overview and inside architecture are depicted in the *upper left inset*. The *bold, black line* points to the cochlear duct that contains the stria vascularis and several epithelial cell types such as hair cells. The positions of the spiral ligament and other tissues and cells are also described. Areas indicated by *roman numbers* in the ligament represent locations of the five fibrocyte types. K^+ is recycled between endolymph (*E*) and perilymph (*P*) across the hair cells, ligament, and stria vascularis. Electrochemical properties of the two lymph types are denoted below the diagram.

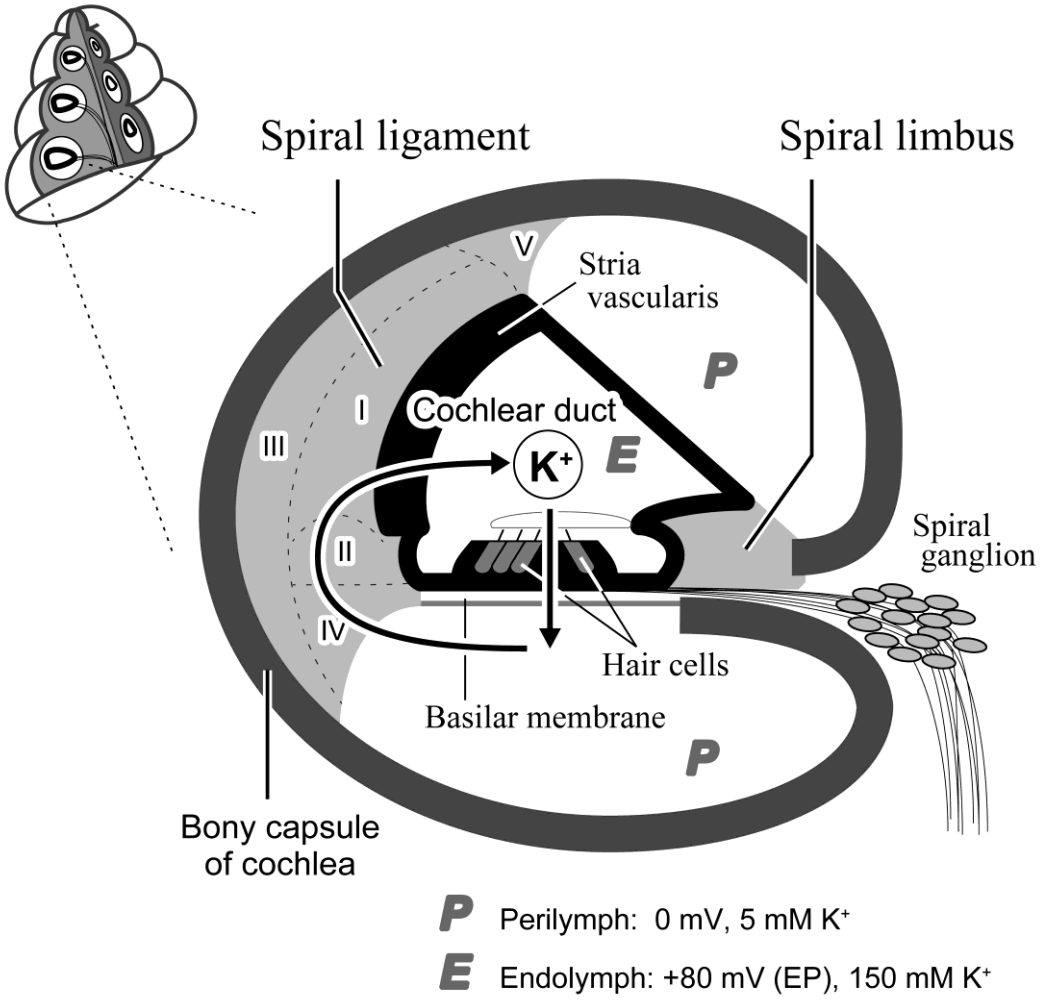

Figure 2 Cellular components of the lateral region of the cochlear duct.

The upper panel shows cells constituting the spiral ligament and stria vascularis as well as ion channels and transporters involved in K^+ transport. The collagen matrix around the fibrocytes in the ligament is omitted. These cells, which bathe in perilymph (*P*), are interconnected with each other and with strial basal and intermediate cells (BCs and ICs, respectively). This connection results in formation of a syncytium (*upper panel*). The syncytium serves as an epithelial layer due to the tight junctions among BCs. In this arrangement, the fibrocytes and ICs constitute the basolateral (*baso*) and apical (*api*) surfaces, respectively. Strial marginal cells (MCs) form the other epithelial layer. The *lower panel* indicates the potential and [K^+] in each extracellular and intracellular compartment. Fibrocytes show a positive potential of approximately +7 mV relative to perilymph (*lower panel*) because of high Na^+ permeability [Na^+ permeability value (P_{Na}) > K^+ and Cl^- permeability values (P_K, P_{Cl}); see the *upper panel*]. ClC: ClC-K-type Cl^- channels, IS: the intrastrial space, NKCC: $Na^+/K^+/2Cl^-$ cotransporter, TJ: tight junction. v_{FC}, v_{IM}, v_{MB}, and v_{MA} correspond to the membrane potentials of fibrocytes, ICs, of the basolateral surface of MCs, and of the apical surface of MCs, respectively. The illustrations are adapted from our previous work[49] with permission.

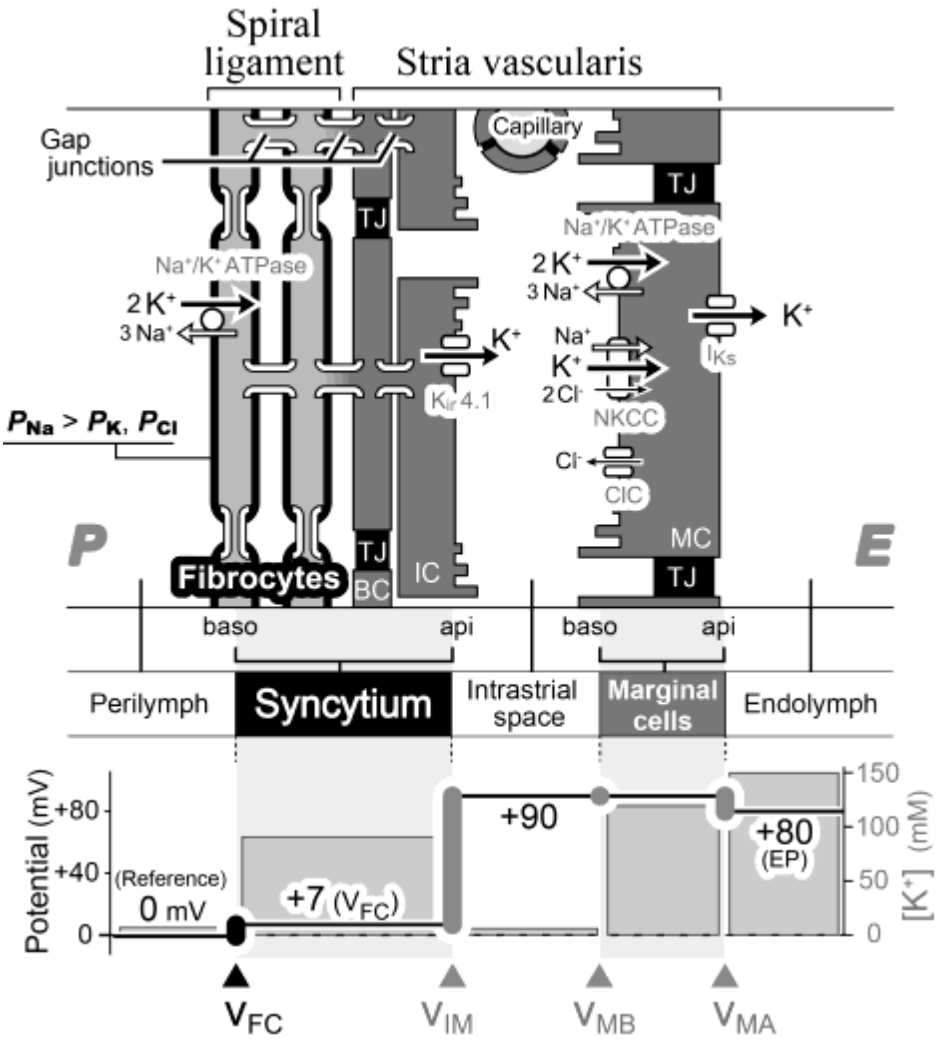

Table 1 Expression profiles of several proteins in the fibrocytes of the spiral ligament

Proteins	Fibrocyte Type				
	I	II	III	IV	V
Cytokeratin	-	-	-	-	-
Vimentin	+	-	+	+	-
Na^+/K^+ ATPase	-	+	-	+	+
Ca^{2+} ATPase (SERCA)	+	-	-	-	-
NKCC1	-	+	-	+	+
KCC3	+	-	+	-	-
Aquaporin 1	-	-	+	-	-
Carbonic anhydrase II	+	-	+	+	+

Data were obtained from Spicer and Schulte[29], Gratton, Schulte and Hazen-Martin[90] and Kelly, Forge and Jagger.[50]
KCC, K^+/Cl^- cotransporter; NKCC, $Na^+/K^+/2Cl^-$ cotransporter, SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase

Table 2 Deafness genes related to cochlear fibrocytes

Gene	**Human deafness**	Loci	Protein	Reference
Gap junction proteins				
GJA1 (CX43)	DFNA3A, DFNB1A	13q12.11	Connexin 43	74
GJB2 (CX26)	DFNA2B	1p34.3	Connexin 26	66,67
GJB3 (CX31)	DFNA3B, DFNB1B	13q12.11	Connexin 31	73
GJB6 (CX30)	AR nonsyndromic deafness	6q22.31	Connexin 30	72
Transcription factors				
POU3F4 (BRN-4)	DFNX2	Xq21.1		76
*Tbx18 **				26
Extracellular matrix proteins				
COCH	DFNA9	14q12	Cochlin (function unknown)	79
*Otos **			Otospiralin (function unknown)	82
Membrane proteins				
*Slc12a6 (Kcc3) **			KCC3 (K^+/Cl^- cotransporter)	43
*Slc4a10 **			(Bicarbonate transporter family)	85
*Slc4a11 **			(Bicarbonate transporter family)	83

*Deafness has been reported only in animal models.
DFNA, DFNB, and DFNX denote nonsyndromic deafness inherited in autosomal dominant, autosomal recessive, and X-linked patterns, respectively.

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