The Journal of Laryngology & Otology (2014), ***128***, *13–21*.

doi:10.1017/S0022215113003265

REVIEW ARTICLE

Non-syndromic hereditary sensorineural hearing loss: review of the genes involved

F STELMA[1,2], M F BHUTTA[1,3]

[1]MRC Harwell, Harwell Science and Innovation Campus, Didcot, UK, [2]Department of Otorhinolaryngology, University of Groningen, University Medical Centre Groningen, The Netherlands, and [3]Nuffield Department of Surgical Sciences (University of Oxford) and Department of Otolaryngology Head and Neck Surgery, John Radcliffe Hospital, Oxford, UK

Abstract

Background: Hereditary sensorineural hearing loss is the most frequently occurring birth defect. It has profound effects for the individual and is a substantial burden on society. Insight into disease mechanisms can help to broaden therapeutic options and considerably lower lifetime social costs. In the past few decades, the identification of genes that can cause this type of hearing loss has developed rapidly.

Objective: This paper provides a concise overview of the currently known genes involved in non-syndromic hereditary hearing loss and their function in the inner ear.

Key words: Sensorineural Hearing Loss; Ear, Inner; Anatomy; Mutations; Genetic Research

Introduction

Congenital hearing impairment affects approximately 3 in every 1000 live births and is the most frequent birth defect in developed countries.[1] Children born with hearing impairment encounter challenges in speech development, education and language acquisition. This results in decreased opportunities for the individual and a potential burden on society. It has been estimated that the lifetime social costs of untreated hearing loss can reach up to US $1.1 million per individual. Treatment and early intervention could decrease these costs by 75 per cent.[2]

More than 50 per cent of congenital sensorineural hearing impairment is hereditary and caused by genetic mutations.[3] Hearing loss can either be syndromic, as part of multiple anomalies throughout the body, or non-syndromic, being restricted to the inner ear. Non-syndromic hearing loss can be further categorised by mode of inheritance. In the largest fraction of cases (80 per cent), inheritance is autosomal recessive (often labelled 'DFNB'). This type of hearing loss is generally congenital, although some forms may present later in life. In the other 20 per cent of cases, inheritance of hearing loss is autosomal dominant ('DFNA'). In this type there is usually a delayed onset of hearing loss. The rest of non-syndromic hearing loss is either X-linked or mitochondrial (less than 1 per cent).[4]

Insight into the genes involved in congenital hearing loss and the underlying mechanisms may enable targeted genetic counselling and treatment. Screens for specific genetic mutations can be performed based on clinical features such as mode of inheritance, morphological appearance, and onset and progression of hearing loss. Genes that are a common cause of hearing loss, such as *GJB2*, *SLC26A4* and *OTOF*, are frequently included in diagnostic tests.[5] The results can be used to counsel parents about the prognosis of hearing loss in their child, the chance of recurrence in future offspring and the predicted outcomes of therapeutic options like cochlear implantation. For example, the outcome of cochlear implantation in syndromic hearing loss is less predictable than in non-syndromic hearing loss. Patients with isolated mutations in the gene *GJB2* have speech outcomes that are better than average, whereas patients with cochleovestibular dysplasia fare worse than average.[6,7] The presence of auditory nerve malformation or neuropathy also predicts inferior results.[8,9] A better understanding of underlying pathophysiology will enable us to better assess cochlear implant candidacy.

More importantly, there is the potential to exploit knowledge of underlying genetic mutations to prevent or ameliorate hereditary hearing loss. Gene therapy utilises viral vectors delivered to the inner ear to replace the defective gene with a normal copy of the gene. There are promising early results from animal studies: adenovirus-delivered *SLC17A8* (VGLUT3, vesicular

Accepted for publication 7 May 2013 First published online 14 January 2014

glutamate transporter 3) restores hearing in mice lacking this gene.[10] It may also be possible to use gene therapy to recover hair cell degeneration: delivery of the *ATOH1* gene product has been found to induce hair cell development and regeneration.[11]

This review provides an overview of the currently known genes involved in non-syndromic hereditary hearing loss (Table I).

Hearing mechanism

We begin with a brief review of the mechanism of human hearing. The cochlea is the auditory portion of the inner ear and is a spiral structure containing three compartments: the scala vestibuli and scala tympani, which are filled with perilymph, and the scala media which is filled with endolymph (Figure 1c). The organ of Corti is located within the middle compartment, the scala media, and includes the inner hair cells, which detect sound (Figure 1d). Airborne sound waves are transmitted through the external and middle ear to the oval window of the cochlea (Figure 1a). Displacement of the oval window causes a wave in the fluids of the cochlea (Figure 1b), leading to displacement of the basilar membrane. Inner hair cells attached to the basilar membrane carry on their apical surface numerous finger-like projections called stereocilia, which will deflect upon physical vibration and move relative to one another. This movement is thought to cause the opening of specific ion channels, a mechanism called mechano-transduction.[12] Opening of these channels initiates an influx of potassium and calcium ions which depolarises the hair cell. This leads to calcium-dependent exocytosis of neurotransmitter vesicles at the cell's basolateral surface in an area called the synaptic ribbon.[13] The release of neurotransmitters excites adjacent auditory neurons which signal to auditory centres in the brain, contributing to the perception of sound.

The organ of Corti also contains outer hair cells; the stereocilia of these hair cells are connected to the overlying tectorial membrane.[14] Whereas the inner hair cells function as sensory players, capturing information about the frequency, intensity and timing of sound, the outer hair cells function as cochlear amplifiers, changing the sensitivity and selectivity to sound.[15]

The structure and physiology of the inner ear is in many ways unique and unmatched at other anatomical locations. This explains why so many genes are thought to be involved in inner-ear function and why the ear is so sensitive to mutation at these loci. Mutations in genes that control the cytoskeleton of hair cells, the adhesion of hair cells, intracellular transport, neurotransmitter release or ionic homeostasis can all lead to malfunction of the cochlea (Figure 2).

Cytoskeleton

As described in the introduction, hair cells have a characteristic shape, with linear, microvilli-like projections called stereocilia arising from their apical surface. The bundles are aligned in a 'V' shape and ranked in increasing height. A number of the genes involved in the organisation of the cytoskeleton can cause non-syndromic hearing loss. These include *ACTG1* (γ-actin), *DIAPH1* (diaphanous 1), *TRIOBP* (trio-binding protein), *TPRN* (taperin), *SMPX* (small muscle protein, X-linked), *ESPN* (espin) and *RDX* (radixin).

It is known that γ-actin functions as the building block of hair cell stereocilia. These stereocilia are constantly undergoing actin polymerisation at the tip and depolymerisation at the base.[16] Mutations in *ACTG1* can interfere with this process and cause autosomal dominant hearing loss, DFNA20/26.[17,18] Other proteins are important in this constant remodelling process. For instance, diaphanous 1 regulates the polymerisation and reorganisation of actin monomers into polymers, and has been associated with autosomal dominant hearing loss (DFNA1).[19] The organisation and binding of γ-actin at the base (the so-called 'taper region') of hair cell stereocilia is regulated by two alternative splice isoforms of the *TRIOBP* gene.[20] Mutations in these isoforms, *TRIOBP4* and *TRIOBP5*, form the origin of DFNB28.[21,22] Another protein localised at this taper region is the regulating protein taperin, which is associated with DFNB79.[23] The X-linked gene *SMPX* (DFN4) encodes a protein suggested to have a function in stereocilial development and maintenance in response to the repetitive mechanical stress that these stereocilia are subjected to.[24,25]

The protein espin acts as a bundling protein, providing stability to the stereocilial cytoskeleton.[26] In 'jerker' mice that lack espin, stereocilia shorten and merge from postnatal day 11, which is simultaneous with the onset of hearing. The hair cells further degenerate over time, and after three months the whole organ of Corti is degraded, indicating the importance of a well-organised cytoskeleton.[27] In humans, mutations in *ESPN* cause DFNB36[28,29] and autosomal dominant hearing loss.[30] More stability is provided by radixin, which is present along the length of the stereocilia and links actin filaments to the plasma membrane.[31] Mutations in *RDX* cause recessive deafness, DFNB24.[32]

Adhesion proteins

Throughout their existence, stereocilia are interconnected and linked to the tectorial membrane by a set of different adhesion proteins. During maturation of the hair bundle in the mouse embryo, a set of temporary links maintain stability. These include transient lateral links (or shaft connectors) and ankle links. These links probably function to provide hair bundle integrity during maturation and/or induce signalling complexes needed for growth regulation and arrangement.[33] In the mature hair bundle, stereocilia are connected by tip links, horizontal top connectors and tectorial membrane attachment crowns (Figure 2). To date, several genes crucial for the linking apparatus have been identified. These include: DFNB31 (*WHRN* (whirlin)),[34] DFNB18 (*USH1C* (harmonin)),[35,36] DFNB66/67

https://doi.org/10.1017/S0022215113003265 Published online by Cambridge University Press

TABLE I
GENES ASSOCIATED WITH NON-SYNDROMIC HEARING LOSS

Locus	Gene	Protein	Function
Cytoskeleton			
– DFNA20/26	*ACTG1*	γ-actin	Building block of cytoskeleton
– DFNA1	*DIAPH1*	Diaphanous 1	Actin polymerisation
– DFNB36	*ESPN*	Espin	Actin cross linking & bundling
– DFNB24	*RDX*	Radixin	Actin binding to plasma membrane
– DFNB28	*TRIOBP*	Trio-binding protein	Actin binding & organisation in taper region
– DFNB79	*TPRN*	Taperin	Actin regulation in taper region
– DFN4	*SMPX*	Small muscle protein X-linked	Stereocilial development & maintenance
Adhesion			
– DFNB31	*WHRN*	Whirlin	Scaffolding protein
– DFNB18	*USH1C*	Harmonin	Scaffolding protein
– Usher syndrome	*SANS/USH1G*	SANS	Scaffolding protein
– Usher syndrome	*USH2A*	Usherin	Ankle link
– Usher syndrome	*VLGR1B*	Very large G protein-coupled receptor 1	Ankle link
– DFNB66/67	*TMHS/LHFPL5*	Tetraspan membrane protein	Transient link
– DFNB84	*PTPRQ*	Protein tyrosine phosphatase receptor Q	Transient link (shaft connector)
– DFNB16	*STRC*	Stereocilin	Horizontal top connector, TM attachment links
– DFNA4	*CAECAM16*	Carcinogenic antigen-related cell adhesion molecule 16	TM attachment crown
– DFNB22	*OTOA*	Otoancorin	TM attachment to non-sensory cells
– DFNB23	*PCDH15*	Protocadherin 15	Lateral link, tip link
– DFNB12	*CDH23*	Cadherin 23	Lateral link, tip link
Transport			
– DFNA48	*MYO1A*	Myosin Ia	Transport
– DFNB30	*MYO3A*	Myosin IIIa	Transport
– DFNA22/ DFNB37	*MYO6*	Myosin VI	Anchoring stereocilia, regulation of exocytosis
– DFNA11/DFNB2	*MYO7A*	Myosin VIIa	Transport
– DFNA17	*MYH9*	Non-muscle myosin heavy chain IX	Transport
– DFNA4	*MYH14*	Non-muscle myosin heavy chain XIV	Transport
– DFNB3	*MYO15A*	Myosin XVa	Transport
Synapse			
– DFNB9	*OTOF*	Otoferlin	Ca^{2+} dependent fusion of synaptic vesicles
– DFNA25	*SLC17A8*	VGLUT-3	Regulation of glutamate endocytosis & exocytosis
Ion Haemostasis			
– DFNB29	*CLDN14*	Claudin 14	Tight junction
– DFNB49	*MARVELD2/ TRIC*	Tricellulin	Tight junction
– DFNA51	*TJP2*	Tight junction protein 2	Binding tight junctions to membrane, cell cycle signalling
– DFNA3A/ DFNB1A	*GJB2*	Connexin 26	Gap junction
– DFNA2B/ DFNB91	*GJB3*	Connexin 31	Gap junction
– DFNA3B/ DFNB1B	*GJB6*	Connexin 30	Gap junction
– DFNA2A	*KCNQ4*	KCNQ4	Voltage-gated K^+ channel
– Modifier of DFNB12	*ATP2b2/PMCA2*	ATP2b2	ATP dependent Ca^{2+} pump
– DFNB73	*BSND*	Barttin	ClC-K channel maturation & trafficking
– DFNB4	*SLC26A4*	Pendrin	Acid–base balance of endolymph
Electromotility			
– DFNB61	*SLC26A5*	Prestin	Electromotility
Others			
Extracellular matrix			
– DFNA8/12 DFNB21	*TECTA*	A-tectorin	Structure & stability of TM
– DFNA13/ DFNB53	*COL11A2*	Type XI collagen α2	Structure & stability of TM
– DFNA9	*COCH*	Cochlin	Structure spiral limbus
Transcription factors			
– DFNA15	*POU4F3*	Class 4 POU	Regulation of transcription
– DFN3	*POU3F4*	Class 3 POU	Regulation of transcription
– DFNA10	*EYA4*	Eyes absent 4	Regulation of transcription
– DFNA50	*MIR96*	MicroRNA96	Regulation of transcription
– DFNB35	*ESRRB*	Oestrogen-related receptor β	Regulation of transcription
– DFNA28	*GRHL2/ TFCP2L3*	Transcription factor CP2-like 3	Regulation of transcription

DFNA = non-syndromic deafness, autosomal dominant; DFNB = non-syndromic deafness, autosomal recessive; TM = tectorial membrane; Ca^{2+} calcium ion; K^+ = potassium ion

https://doi.org/10.1017/S0022215113003265 Published online by Cambridge University Press

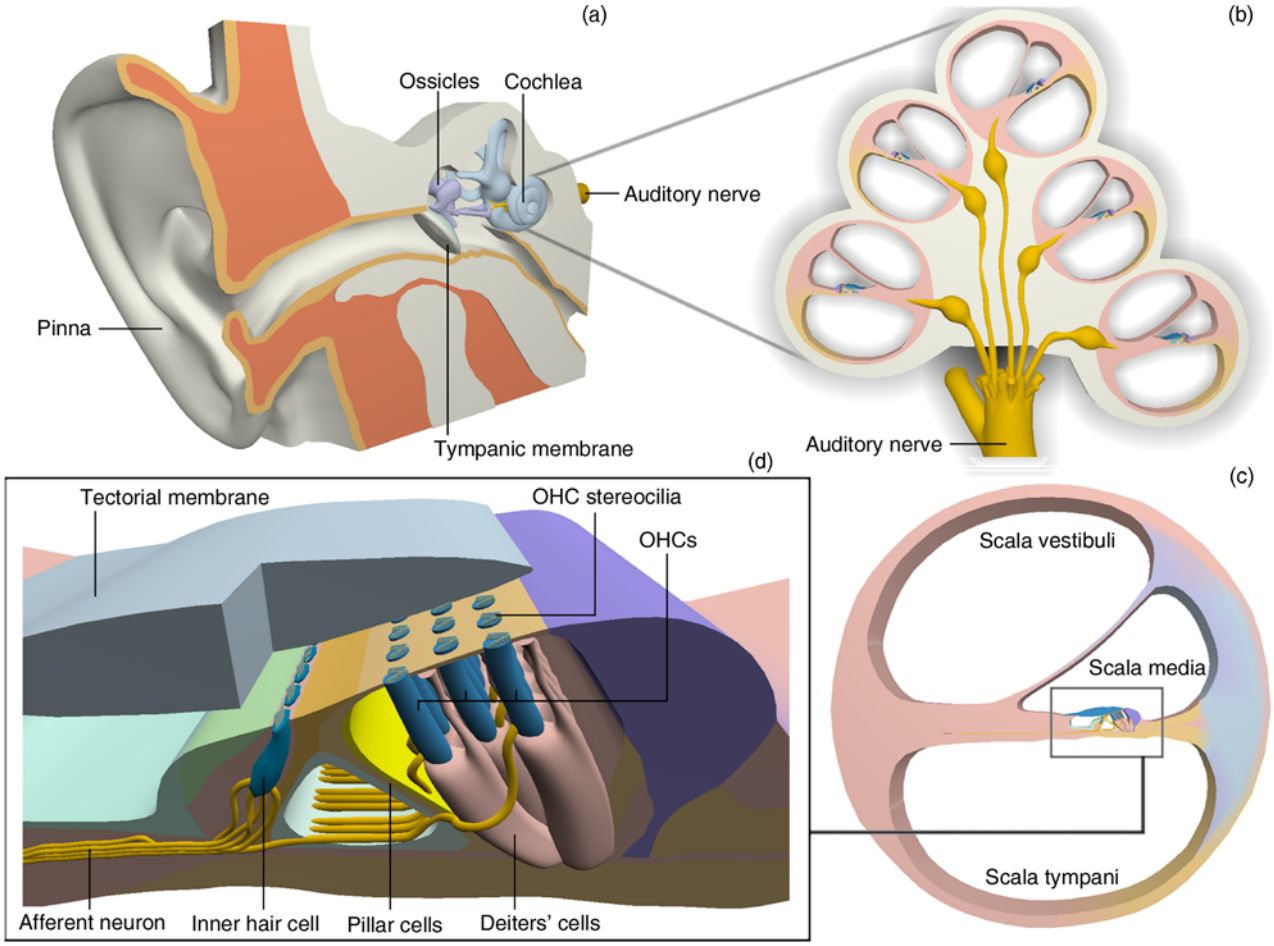

FIG. 1

Anatomical diagrams of the human ear, showing: (a) the outer ear, middle ear and inner ear; (b) a cross section of the cochlea; (c) the three compartments of the cochlea; and (d) the organ of Corti. OHC = outer hair cell

(*TMHS* (tetraspan membrane protein)),[37] DFNB84 (*PTPRQ* (tyrosine phosphate receptor Q)),[38] DFNB16 (*STRC* (stereocilin)),[39] DFNA4 (*CEACAM16* (carcinogenic antigen-related cell adhesion molecule 16)),[40] DFNB22 (*OTOA* (otoancorin)),[41] DFNB23 (*PCDH15* (protocadherin 15))[36] and DFNB12 (*CDH23* (cadherin 23)).[42]

Whirlin and harmonin are scaffolding proteins that regulate the formation of the link complexes. Through their PDZ domain binding sites (i.e. binding sites for other proteins), scaffolding proteins fulfil their role in organising multi-protein aggregates and assembling signalling complexes.[43] Mutations in whirlin and harmonin cause autosomal recessive hearing loss. A third scaffolding protein is Sans, which is associated with the complex syndromic hearing loss of Usher syndrome. *USH2a* and *VLGR1b*, two other genes associated with Usher syndrome, are part of the stereocilial ankle link.[44]

Cadherin 23 and protocadherin 15, as well as PTPRQ and TMHS, are presumably part of the transient lateral link. They prevent fusion of stereocilia by keeping them at a fixed distance from each other during development.[45–47] In the mature hair cell, cadherin 23 and protocadherin 15 become the main components of the tip link. These provide stability and gate the mechanotransduction channel; these tip links provide stability and gate the mechanotransduction channel, which plays a central role in auditory function.[48] TMHS co-localises with protocadherin 15 and is a proposed subunit of the mechanotransduction channel.[37,49]

Stereocilin is an extracellular protein that is thought to make up both horizontal top connectors and tectorial membrane attachment links. The latter, combined with the so-called attachment crown, attach the tallest stereocilia of the outer hair cell stereocilia bundle to the tectorial membrane.[50] This tectorial membrane attachment crown is probably formed by CEACAM16. In a similar way, otoancorin is thought to attach non-sensory cells to the tectorial membrane.[41]

Transport proteins

Motor proteins can be used to transport different proteins to target sites in the cell. In the inner ear, the proteins used for transport are all part of the unconventional myosin family. These proteins can bind to the actin cytoskeleton and move forward along actin filaments by using energy derived from ATP.

https://doi.org/10.1017/S0022215113003265 Published online by Cambridge University Press

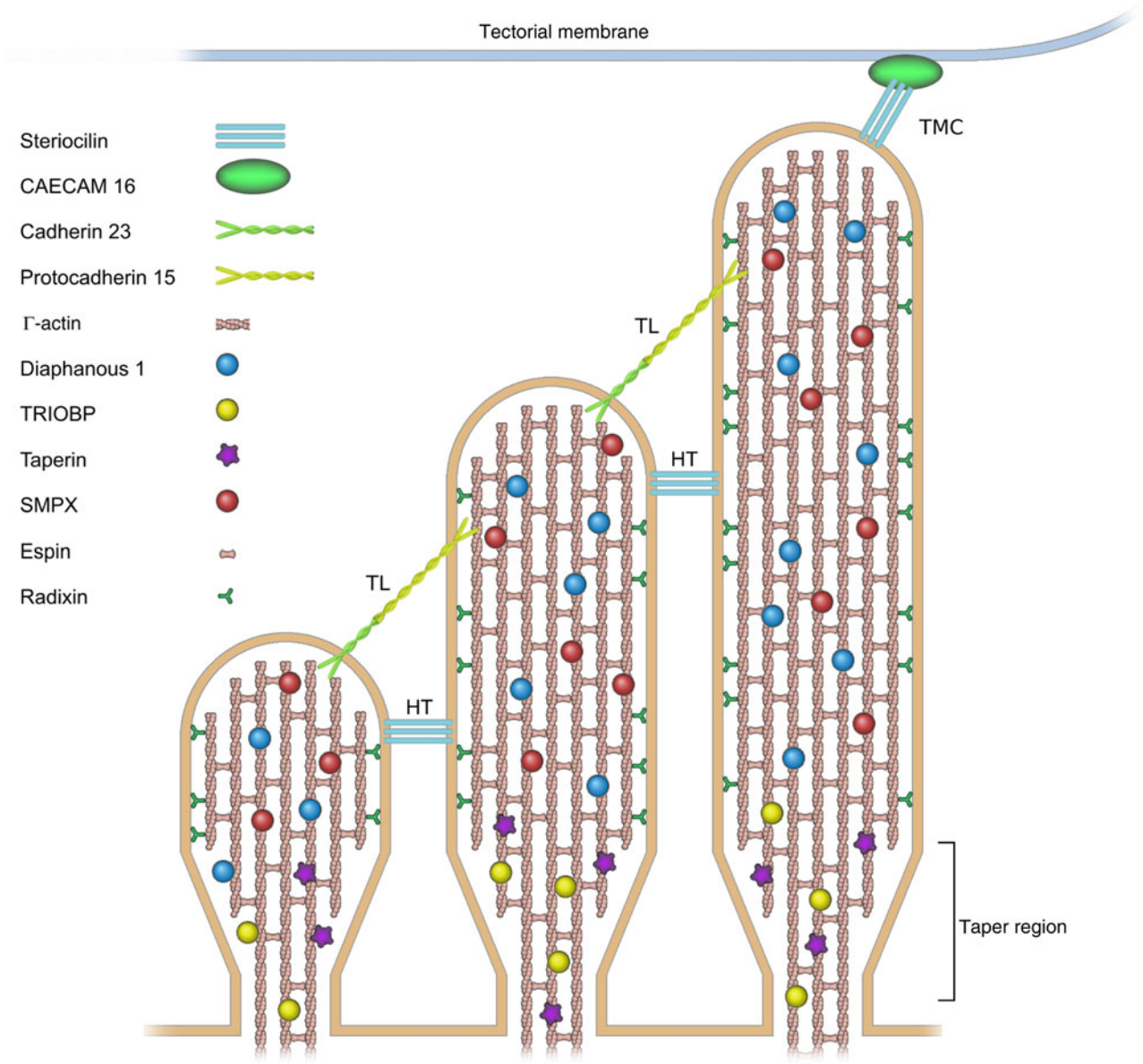

FIG. 2

Diagrammatic representation of important structural proteins in mature hair cell stereocilia. CEACAM16 = carcinogenic antigen-related cell adhesion molecule 16; TRIOBP = trio-binding protein; SMPX = small muscle protein, X-linked; TMC = tectorial membrane attachment crown; TL = tip link; HT = horizontal top connector

The carboxyl-terminal tails of the transport protein contain binding sites for the proteins they will carry.[51] Seven unconventional myosins have been associated with hereditary hearing loss: myosin Ia (DNFA48),[52] myosin IIIa (DFNB30),[53] myosin VI (DFNA22/DFNB37),[54,55] myosin VIIa (DFNA11/DFNB2),[56,57] non-muscle myosin heavy chain IX (DFNA17)[58] and XIV (DFNA4),[59] and myosin XVa (DFNB3).[60] These proteins all have their own unique transport function in the inner-ear hair cells.

Synapse

Otoferlin (*OTOF*) acts with one of the myosins, myosin VI, at the synaptic cleft of the inner hair cell. The protein is thought to be involved in the (calcium-dependent) fusion of synaptic vesicles to the plasma membrane. As a result, the neurotransmitter glutamate is released into the synaptic cleft with subsequent excitation of the afferent neuron. In *OTOF*-mutant mice, a reduction in exocytosis is detected.[61] A number of allelic variants of *OTOF* that cause DFNB9 in humans have been identified.[62]

Another player at the inner hair cell synapse is VGLUT3, a member of the vesicular glutamate receptors. VGLUT3 is encoded by *SLC17A8* and associated with autosomal recessive hearing loss DFNA25.[63] The protein is thought to regulate the endocytosis and exocytosis of glutamate. Both *OTOF* and *SLC17A8* knockout mice show a reduction in the number of post-synaptic ganglion cells, indicating that these proteins are crucial for the development and preservation of normal hearing.[64]

https://doi.org/10.1017/S0022215113003265 Published online by Cambridge University Press

Ion homeostasis

The cochlea contains two types of fluids, both different in ion composition. Perilymph is high in sodium and low in potassium, whereas endolymph is high in potassium and low in sodium. This contributes to a highly positive potential (+80 mV) called the endocochlear potential. A potassium influx from the endolymph into the hair cell causes depolarisation of the cell. Immediately after depolarisation, the hair cell repolarises, shifting cations via neighbouring structures back into the endolymph (Figure 3). This process of ion homeastasis involves claudin 14 (*CLDN14*), tricellulin (*MARVELD2/TRIC*), tight junction protein 2 (*TJP2*), a number of connexins (*GJB's*), KCNQ4 (*KCNQ4*), ATP2b2 (*ATP2b2/PMCA2*), Barttin (*BSND*) and pendrin (*SLC26A4*) all of which are related to hereditary hearing loss.

Tight junctions guard the border between endolymph and perilymph compartments. By generating a seal between two adjacent cells, a barrier is created that restricts the free diffusion of ions. In this way, the apical side of the outer hair cells and supporting cells are exposed to the endolymph, and the basolateral surface is bathed in cortilymph, a fluid which is similar to perilymph and fills up the so-called space of Nuel. This space of Nuel, which surrounds the basolateral surface of outer hair cells, might change in electric potential when the tight junction protein claudin 14 is absent or dysfunctional, as in DFNB29.[65,66] In a similar way, tricellulin, encoded by *MARVELD2/TRIC*, is presumed to function as tight junction that connects three cells together and causes DFNB49 when mutated.[67] TJP2 acts as a scaffolding protein, binding tight junctions to the actin cytoskeleton. Duplication with over-expression of the protein causes DFNA51.[68] This process occurs as a result of another role of the TJP2 protein, namely its involvement in the nucleus' signaling pathways that regulate the cell cycle. Over-expression of TJP2 disturbs the balance between pro-apoptotic and anti-apoptotic genes and will induce apoptosis.[69]

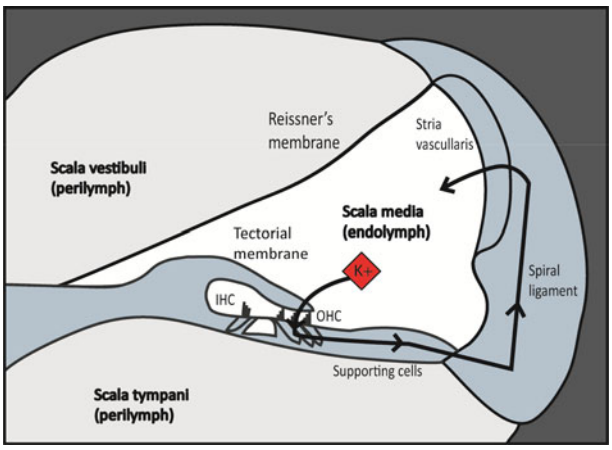

FIG. 3

Diagrammatic representation of potassium circulation within the cochlea: the opening of ion channels in the hair cell apical membrane allows a potassium influx from the endolymph into the hair cell; potassium is then moved to supporting cells and pumped back into the endolymph via spiral ligament and stria vascularis gap junction networks. K^+ = potassium ion; IHC = inner hair cells; OHC = outer hair cells

A network of gap junctions (channels that extend over two adjacent membranes) in the cochlea enables the exchange of various small molecules and ions. These gap junctions are made up of specialised proteins called connexins, which are expressed in the supporting cells of the organ of Corti and the connective tissue of the spiral ligament.[70] This gap junction network is associated with the recycling of potassium ions needed for normal hearing. The first identified gene and most common cause of non-syndromic hearing loss is *GJB2*, which encodes connexin 26 (DFNA3a/DFNB1a).[71] Mutations in the *GJB2* gene account for 30–50 per cent of all cases of childhood deafness, and 1–4 per cent of the average human population are estimated to be carriers.[70] Other connexins associated with non-syndromic hearing loss are connexin 31 (*GJB3*, DFNA2b/DFNB91)[72,73] and connexin 30 (*GJB6*, DFNA3b/DFNB1b).[74,75]

KCNQ4 encodes a protein that forms a voltage-gated potassium channel. This gene is expressed in the outer hair cells of the cochlea and is mutated in a dominant form of non-syndromic hearing loss, DFNA2a.[76] KCNQ4 is thought to aid repolarisation of the outer hair cells. Furthermore, KCNQ4 is proposed to regulate sensitivity to sound by changing the resting membrane potential of the outer hair cells.[77]

On their apical surface, in the stereocilial membrane, hair cells express *ATP2b2/PMCA2*, which is a modifier of DFNB12.[78] The protein product PMCA2 is a calcium pump that uses energy from ATP to function. Calcium, which is used (in addition to potassium) to excite the cell, is constantly pumped back into the endolymph by PMCA2, thereby ensuring a stable concentration of this ion.[79] PMCA2 fulfils a similar function in the synaptic region on the basolateral surface of the hair cell.

BSND and *SLC26A4*, which encode barttin and pendrin respectively, are genes involved in both syndromic and non-syndromic hearing loss. Barttin is a chloride channel subunit. Most mutations in *BSND* cause Bartter syndrome, which comprises hearing loss and renal abnormalities. The molecular basis of DFNB73 has been attributed to a mutation in *BSND*, which causes non-syndromic deafness.[80] The anion exchanger pendrin plays a major role in maintaining a constant acid–base balance. Both syndromic hearing loss (Pendred's syndrome, associated with goitre) and non-syndromic hearing loss (DFNB4) have been described in relation to this, the occurrence of which depends on the extent of the mutation in *SLC16A4*.[81,82]

Electromotility

The outer hair cells have a unique feature of altering sensitivity and selectivity to sound. The protein

https://doi.org/10.1017/S0022215113003265 Published online by Cambridge University Press

Prestin is thought to be responsible for this by introducing a process called electromotility. Prestin changes its configuration in reaction to changes in membrane potential, enabling the outer hair cell length to be altered. In this way, the cylindrical outer hair cell becomes shorter on depolarisation and longer on hyperpolarisation, thereby amplifying its sensitivity to sound.[15] Prestin, encoded by *SLC26A5*, was first identified by Zheng *et al.* in 2000.[83] In homozygous Prestin null mice, cochlear thresholds were found to be 40–60 dB higher than in wild-type mice.[84] In humans, mutations in *SLC26A5* are the cause of DFNB61 hearing loss.[85]

Others

Other important groups of genes involved in hereditary hearing loss that will not be discussed further are extracellular matrix proteins, including *TECTA* (α-tectorin), *COL11A2* (type XI collagen α2) and *COCH* (cochlin), and a number of transcription factors, including *POU4f3* (class 4 POU), *POU3f4* (class 3 POU), *EYA4* (eyes absent 4), *MIR96* (microRNA96), *ESRRB* (oestrogen-related receptor β) and *GRHL2* (grainy-head-like 2).

Conclusion

In the last two decades, our understanding of the basis of hereditary hearing loss has advanced significantly. This has been powered by major developments in human and mouse genetics. Improved genotyping and mapping has enabled the study of human families with multi-generational hereditary hearing loss. This has led to the identification of many genetic mutations that can cause syndromic or non-syndromic deafness. The mouse has proven to be an excellent genetic model for human hearing loss and deafness because of its structural, physiological and genetic similarities. Programmes for the systematic mutation[86] and phenotyping[87] of mice have enabled the discovery of many new genes involved in cochlear embryology or function, and many genetic loci have subsequently been found to underlie human deafness.

In this article, we have given an overview of the currently known genes involved in hereditary hearing loss (Table I). The function of these genes will become better understood with time, and no doubt many more genes that can lead to hearing loss will be discovered. With continued examination, we will build a better understanding of the function of the cochlea, and hopefully develop novel molecular therapies for human sensorineural hearing loss.

Acknowledgement

The authors would like to acknowledge Mr J Stelma for graphic design.

References

1 White KR. Early hearing detection and intervention programs: opportunities for genetic services. *Am J Med Genet A* 2004; **130A**:29–36
2 Keren R, Helfand M, Homer C, McPhillips H, Lieu TA. Projected cost-effectiveness of statewide universal newborn hearing screening. *Pediatrics* 2002;**110**:855–64
3 Smith RJ, Bale JF Jr, White KR. Sensorineural hearing loss in children. *Lancet* 2005;**365**:879–90
4 Morton NE. Genetic epidemiology of hearing impairment. *Ann N Y Acad Sci* 1991;**630**:16–31
5 Hilgert N, Smith RJ, Van Camp G. Forty-six genes causing non-syndromic hearing impairment: which ones should be analyzed in DNA diagnostics? *Mutat Res* 2009;**681**:189–96
6 Pakdaman MN, Herrmann BS, Curtin HD, Van Beek-King J, Lee DJ. Cochlear implantation in children with anomalous cochleovestibular anatomy: a systematic review. *Otolaryngol Head Neck Surg* 2012;**146**:180–90
7 Black J, Hickson L, Black B, Perry C. Prognostic indicators in paediatric cochlear implant surgery: a systematic literature review. *Cochlear Implants Int* 2011;**12**:67–93
8 Rance G, Barker EJ. Speech perception in children with auditory neuropathy/dyssynchrony managed with either hearing aids or cochlear implants. *Otol Neurotol* 2008;**29**:179–82
9 Breneman AI, Gifford RH, Dejong MD. Cochlear implantation in children with auditory neuropathy spectrum disorder: long-term outcomes. *J Am Acad Audiol* 2012;**23**:5–17
10 Akil O, Seal RP, Burke K, Wang C, Alemi A, During M *et al*. Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy. *Neuron* 2012;**75**:283–93
11 Lustig LR, Akil O. Cochlear gene therapy. *Curr Opin Neurol* 2012;**25**:57–60
12 Dror AA, Avraham KB. Hearing loss: mechanisms revealed by genetics and cell biology. *Annu Rev Genet* 2009;**43**:411–37
13 Hudspeth AJ. How hearing happens. *Neuron* 1997;**19**:947–50
14 Legan PK, Lukashkina VA, Goodyear RJ, Lukashkin AN, Verhoeven K, Van Camp G *et al*. A deafness mutation isolates a second role for the tectorial membrane in hearing. *Nat Neurosci* 2005;**8**:1035–42
15 Brownell WE, Bader CR, Bertrand D, de Ribaupierre Y. Evoked mechanical responses of isolated cochlear outer hair cells. *Science* 1985;**227**:194–6
16 Rzadzinska AK, Schneider ME, Davies C, Riordan GP, Kachar B. An actin molecular treadmill and myosins maintain stereocilia functional architecture and self-renewal. *J Cell Biol* 2004; **164**:887–97
17 van Wijk E, Krieger E, Kemperman MH, De Leenheer EM, Huygen PL, Cremers CW *et al*. A mutation in the gamma actin 1 (ACTG1) gene causes autosomal dominant hearing loss (DFNA20/26). *J Med Genet* 2003;**40**:879–84
18 Zhu M, Yang T, Wei S, DeWan AT, Morell RJ, Elfenbein JL *et al*. Mutations in the gamma-actin gene (ACTG1) are associated with dominant progressive deafness (DFNA20/26). *Am J Hum Genet* 2003;**73**:1082–91
19 Lynch ED, Lee MK, Morrow JE, Welcsh PL, Leon PE, King MC. Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the Drosophila gene diaphanous. *Science* 1997;**278**:1315–18
20 Kitajiri S, Sakamoto T, Belyantseva IA, Goodyear RJ, Stepanyan R, Fujiwara I *et al*. Actin-bundling protein TRIOBP forms resilient rootlets of hair cell stereocilia essential for hearing. *Cell* 2010;**141**:786–98
21 Riazuddin S, Khan SN, Ahmed ZM, Ghosh M, Caution K, Nazli S *et al*. Mutations in TRIOBP, which encodes a putative cytoskeletal-organizing protein, are associated with nonsyndromic recessive deafness. *Am J Hum Genet* 2006;**78**:137–43
22 Shahin H, Walsh T, Sobe T, Abu Sa'ed J, Abu Rayan A, Lynch ED *et al*. Mutations in a novel isoform of TRIOBP that encodes a filamentous-actin binding protein are responsible for DFNB28 recessive nonsyndromic hearing loss. *Am J Hum Genet* 2006;**78**: 144–52
23 Rehman AU, Morell RJ, Belyantseva IA, Khan SY, Boger ET, Shahzad M *et al*. Targeted capture and next-generation sequencing identifies C9orf75, encoding taperin, as the mutated gene in nonsyndromic deafness DFNB79. *Am J Hum Genet* 2010;**86**: 378–88
24 Huebner AK, Gandia M, Frommolt P, Maak A, Wicklein EM, Thiele H *et al*. Nonsense mutations in SMPX, encoding a protein responsive to physical force, result in X-chromosomal hearing loss. *Am J Hum Genet* 2011;**88**:621–7
25 Schraders M, Haas SA, Weegerink NJ, Oostrik J, Hu H, Hoefsloot LH *et al*. Next-generation sequencing identifies

https://doi.org/10.1017/S0022215113003265 Published online by Cambridge University Press

mutations of SMPX, which encodes the small muscle protein, X-linked, as a cause of progressive hearing impairment. *Am J Hum Genet* 2011;**88**:628–34
26 Bartles JR, Wierda A, Zheng L. Identification and characterization of espin, an actin-binding protein localized to the F-actin-rich junctional plaques of Sertoli cell ectoplasmic specializations. *J Cell Sci* 1996;**109**:1229–39
27 Zheng L, Sekerkova G, Vranich K, Tilney LG, Mugnaini E, Bartles JR. The deaf jerker mouse has a mutation in the gene encoding the espin actin-bundling proteins of hair cell stereocilia and lacks espins. *Cell* 2000;**102**:377–85
28 Boulouiz R, Li Y, Soualhine H, Abidi O, Chafik A, Nurnberg G *et al*. A novel mutation in the Espin gene causes autosomal recessive nonsyndromic hearing loss but no apparent vestibular dysfunction in a Moroccan family. *Am J Med Genet A* 2008; **146A**:3086–9
29 Naz S, Griffith AJ, Riazuddin S, Hampton LL, Battey JF Jr, Khan SN *et al*. Mutations of ESPN cause autosomal recessive deafness and vestibular dysfunction. *J Med Genet* 2004;**41**: 591–5
30 Donaudy F, Zheng L, Ficarella R, Ballana E, Carella M, Melchionda S *et al*. Espin gene (ESPN) mutations associated with autosomal dominant hearing loss cause defects in microvillar elongation or organisation. *J Med Genet* 2006;**43**:157–61
31 Pataky F, Pironkova R, Hudspeth AJ. Radixin is a constituent of stereocilia in hair cells. *Proc Natl Acad Sci U S A* 2004;**101**: 2601–6
32 Khan SY, Ahmed ZM, Shabbir MI, Kitajiri S, Kalsoom S, Tasneem S *et al*. Mutations of the RDX gene cause nonsyndromic hearing loss at the DFNB24 locus. *Hum Mutat* 2007;**28**: 417–23
33 Goodyear RJ, Marcotti W, Kros CJ, Richardson GP. Development and properties of stereociliary link types in hair cells of the mouse cochlea. *J Comp Neurol* 2005;**485**:75–85
34 Mburu P, Mustapha M, Varela A, Weil D, El-Amraoui A, Holme RH *et al*. Defects in whirlin, a PDZ domain molecule involved in stereocilia elongation, cause deafness in the whirler mouse and families with DFNB31. *Nat Genet* 2003;**34**:421–8
35 Ouyang XM, Xia XJ, Verpy E, Du LL, Pandya A, Petit C *et al*. Mutations in the alternatively spliced exons of USH1C cause non-syndromic recessive deafness. *Hum Genet* 2002; **111**:26–30
36 Ahmed ZM, Smith TN, Riazuddin S, Makishima T, Ghosh M, Bokhari S *et al*. Nonsyndromic recessive deafness DFNB18 and Usher syndrome type IC are allelic mutations of USHIC. *Hum Genet* 2002;**110**:527–31
37 Shabbir MI, Ahmed ZM, Khan SY, Riazuddin S, Waryah AM, Khan SN *et al*. Mutations of human TMHS cause recessively inherited non-syndromic hearing loss. *J Med Genet* 2006;**43**: 634–40
38 Schraders M, Oostrik J, Huygen PL, Strom TM, van Wijk E, Kunst HP *et al*. Mutations in PTPRQ are a cause of autosomal-recessive nonsyndromic hearing impairment DFNB84 and associated with vestibular dysfunction. *Am J Hum Genet* 2010;**86**:604–10
39 Verpy E, Masmoudi S, Zwaenepoel I, Leibovici M, Hutchin TP, Del Castillo I *et al*. Mutations in a new gene encoding a protein of the hair bundle cause non-syndromic deafness at the DFNB16 locus. *Nat Genet* 2001;**29**:345–9
40 Zheng J, Miller KK, Yang T, Hildebrand MS, Shearer AE, DeLuca AP *et al*. Carcinoembryonic antigen-related cell adhesion molecule 16 interacts with alpha-tectorin and is mutated in autosomal dominant hearing loss (DFNA4). *Proc Natl Acad Sci U S A* 2011;**108**:4218–23
41 Zwaenepoel I, Mustapha M, Leibovici M, Verpy E, Goodyear R, Liu XZ *et al*. Otoancorin, an inner ear protein restricted to the interface between the apical surface of sensory epithelia and their overlying acellular gels, is defective in autosomal recessive deafness DFNB22. *Proc Natl Acad Sci U S A* 2002;**99**:6240–5
42 Bork JM, Peters LM, Riazuddin S, Bernstein SL, Ahmed ZM, Ness SL *et al*. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. *Am J Hum Genet* 2001; **68**:26–37
43 Michalski N, Michel V, Caberlotto E, Lefevre GM, van Aken AF, Tinevez JY *et al*. Harmonin-b, an actin-binding scaffold protein, is involved in the adaptation of mechanoelectrical transduction by sensory hair cells. *Pflugers Arch* 2009;**459**:115–30
44 Michalski N, Michel V, Bahloul A, Lefevre G, Barral J, Yagi H *et al*. Molecular characterization of the ankle-link complex in cochlear hair cells and its role in the hair bundle functioning. *J Neurosci* 2007;**27**:6478–88
45 Goodyear RJ, Legan PK, Wright MB, Marcotti W, Oganesian A, Coats SA *et al*. A receptor-like inositol lipid phosphatase is required for the maturation of developing cochlear hair bundles. *J Neurosci* 2003;**23**:9208–19
46 Gillespie PG, Dumont RA, Kachar B. Have we found the tip link, transduction channel, and gating spring of the hair cell? *Curr Opin Neurobiol* 2005;**15**:389–96
47 Michel V, Goodyear RJ, Weil D, Marcotti W, Perfettini I, Wolfrum U *et al*. Cadherin 23 is a component of the transient lateral links in the developing hair bundles of cochlear sensory cells. *Dev Biol* 2005;**280**:281–94
48 Kazmierczak P, Sakaguchi H, Tokita J, Wilson-Kubalek EM, Milligan RA, Muller U *et al*. Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells. *Nature* 2007;**449**:87–91
49 Xiong W, Grillet N, Elledge HM, Wagner TF, Zhao B, Johnson KR *et al*. TMHS is an integral component of the mechanotransduction machinery of cochlear hair cells. *Cell* 2012;**151**: 1283–95
50 Verpy E, Leibovici M, Michalski N, Goodyear RJ, Houdon C, Weil D *et al*. Stereocilin connects outer hair cell stereocilia to one another and to the tectorial membrane. *J Comp Neurol* 2011;**519**:194–210
51 Friedman LM, Dror AA, Avraham KB. Mouse models to study inner ear development and hereditary hearing loss. *Int J Dev Biol* 2007;**51**:609–31
52 Donaudy F, Ferrara A, Esposito L, Hertzano R, Ben-David O, Bell RE *et al*. Multiple mutations of MYO1A, a cochlear-expressed gene, in sensorineural hearing loss. *Am J Hum Genet* 2003;**72**:1571–7
53 Walsh T, Walsh V, Vreugde S, Hertzano R, Shahin H, Haika S *et al*. From flies' eyes to our ears: mutations in a human class III myosin cause progressive nonsyndromic hearing loss DFNB30. *Proc Natl Acad Sci U S A* 2002;**99**:7518–23
54 Melchionda S, Ahituv N, Bisceglia L, Sobe T, Glaser F, Rabionet R *et al*. MYO6, the human homologue of the gene responsible for deafness in Snell's waltzer mice, is mutated in autosomal dominant nonsyndromic hearing loss. *Am J Hum Genet* 2001;**69**:635–40
55 Ahmed ZM, Morell RJ, Riazuddin S, Gropman A, Shaukat S, Ahmad MM *et al*. Mutations of MYO6 are associated with recessive deafness, DFNB37. *Am J Hum Genet* 2003;**72**: 1315–22
56 Liu XZ, Walsh J, Tamagawa Y, Kitamura K, Nishizawa M, Steel KP *et al*. Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. *Nat Genet* 1997;**17**: 268–9
57 Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Drira M *et al*. The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nat Genet* 1997;**16**:191–3
58 Lalwani AK, Goldstein JA, Kelley MJ, Luxford W, Castelein CM, Mhatre AN. Human nonsyndromic hereditary deafness DFNA17 is due to a mutation in nonmuscle myosin MYH9. *Am J Hum Genet* 2000;**67**:1121–8
59 Donaudy F, Snoeckx R, Pfister M, Zenner HP, Blin N, Di Stazio M *et al*. Nonmuscle myosin heavy-chain gene MYH14 is expressed in cochlea and mutated in patients affected by autosomal dominant hearing impairment (DFNA4). *Am J Hum Genet* 2004;**74**:770–6
60 Wang A, Liang Y, Fridell RA, Probst FJ, Wilcox ER, Touchman JW *et al*. Association of unconventional myosin MYO15 mutations with human nonsyndromic deafness DFNB3. *Science* 1998;**280**:1447–51
61 Heidrych P, Zimmermann U, Kuhn S, Franz C, Engel J, Duncker SV *et al*. Otoferlin interacts with myosin VI: implications for maintenance of the basolateral synaptic structure of the inner hair cell. *Hum Mol Genet* 2009;**18**:2779–90
62 Yasunaga S, Grati M, Cohen-Salmon M, El-Amraoui A, Mustapha M, Salem N *et al*. A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. *Nat Genet* 1999;**21**:363–9
63 Ruel J, Emery S, Nouvian R, Bersot T, Amilhon B, Van Rybroek JM *et al*. Impairment of SLC17A8 encoding vesicular

glutamate transporter-3, VGLUT3, underlies nonsyndromic deafness DFNA25 and inner hair cell dysfunction in null mice. *Am J Hum Genet* 2008;**83**:278–92
64 Seal RP, Akil O, Yi E, Weber CM, Grant L, Yoo J *et al*. Sensorineural deafness and seizures in mice lacking vesicular glutamate transporter 3. *Neuron* 2008;**57**:263–75
65 Ben-Yosef T, Belyantseva IA, Saunders TL, Hughes ED, Kawamoto K, Van Itallie CM *et al*. Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration. *Hum Mol Genet* 2003;**12**:2049–61
66 Wilcox ER, Burton QL, Naz S, Riazuddin S, Smith TN, Ploplis B *et al*. Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. *Cell* 2001;**104**: 165–72
67 Riazuddin S, Ahmed ZM, Fanning AS, Lagziel A, Kitajiri S, Ramzan K *et al*. Tricellulin is a tight-junction protein necessary for hearing. *Am J Hum Genet* 2006;**79**:1040–51
68 Walsh T, Pierce SB, Lenz DR, Brownstein Z, Dagan-Rosenfeld O, Shahin H *et al*. Genomic duplication and overexpression of TJP2/ZO-2 leads to altered expression of apoptosis genes in progressive nonsyndromic hearing loss DFNA51. *Am J Hum Genet* 2010;**87**:101–9
69 Lenz DR, Avraham KB. Hereditary hearing loss: from human mutation to mechanism. *Hear Res* 2011;**281**:3–10
70 Hoang Dinh E, Ahmad S, Chang Q, Tang W, Stong B, Lin X. Diverse deafness mechanisms of connexin mutations revealed by studies using in vitro approaches and mouse models. *Brain Res* 2009;**1277**:52–69
71 Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G *et al*. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature* 1997;**387**:80–3
72 Xia JH, Liu CY, Tang BS, Pan Q, Huang L, Dai HP *et al*. Mutations in the gene encoding gap junction protein beta-3 associated with autosomal dominant hearing impairment. *Nat Genet* 1998;**20**:370–3
73 Liu XZ, Xia XJ, Xu LR, Pandya A, Liang CY, Blanton SH *et al*. Mutations in connexin 31 underlie recessive as well as dominant non-syndromic hearing loss. *Hum Mol Genet* 2000;**9**:63–7
74 Grifa A, Wagner CA, D'Ambrosio L, Melchionda S, Bernardi F, Lopez-Bigas N *et al*. Mutations in GJB6 cause nonsyndromic autosomal dominant deafness at DFNA3 locus. *Nat Genet* 1999;**23**:16–18
75 del Castillo I, Villamar M, Moreno-Pelayo MA, del Castillo FJ, Alvarez A, Telleria D *et al*. A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *N Engl J Med* 2002;**346**:243–9
76 Kubisch C, Schroeder BC, Friedrich T, Lutjohann B, El-Amraoui A, Marlin S *et al*. KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. *Cell* 1999;**96**:437–46
77 Heidenreich M, Lechner SG, Vardanyan V, Wetzel C, Cremers CW, De Leenheer EM *et al*. KCNQ4 K(+) channels tune mechanoreceptors for normal touch sensation in mouse and man. *Nat Neurosci* 2011;**15**:138–45
78 Schultz JM, Yang Y, Caride AJ, Filoteo AG, Penheiter AR, Lagziel A *et al*. Modification of human hearing loss by plasma-membrane calcium pump PMCA2. *N Engl J Med* 2005;**352**:1557–64
79 Bortolozzi M, Brini M, Parkinson N, Crispino G, Scimemi P, De Siati RD *et al*. The novel PMCA2 pump mutation Tommy impairs cytosolic calcium clearance in hair cells and links to deafness in mice. *J Biol Chem* 2010;**285**:37693–703
80 Riazuddin S, Anwar S, Fischer M, Ahmed ZM, Khan SY, Janssen AG *et al*. Molecular basis of DFNB73: mutations of BSND can cause nonsyndromic deafness or Bartter syndrome. *Am J Hum Genet* 2009;**85**:273–80
81 Li XC, Everett LA, Lalwani AK, Desmukh D, Friedman TB, Green ED *et al*. A mutation in PDS causes non-syndromic recessive deafness. *Nat Genet* 1998;**18**:215–17
82 Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M *et al*. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat Genet* 1997;**17**:411–22
83 Zheng J, Shen W, He DZ, Long KB, Madison LD, Dallos P. Prestin is the motor protein of cochlear outer hair cells. *Nature* 2000;**405**:149–55
84 Liberman MC, Gao J, He DZ, Wu X, Jia S, Zuo J. Prestin is required for electromotility of the outer hair cell and for the cochlear amplifier. *Nature* 2002;**419**:300–4
85 Liu XZ, Ouyang XM, Xia XJ, Zheng J, Pandya A, Li F *et al*. Prestin, a cochlear motor protein, is defective in non-syndromic hearing loss. *Hum Mol Genet* 2003;**12**:1155–62
86 Brown SD, Hardisty-Hughes RE, Mburu P. Quiet as a mouse: dissecting the molecular and genetic basis of hearing. *Nat Rev Genet* 2008;**9**:277–90
87 Hardisty-Hughes RE, Parker A, Brown SD. A hearing and vestibular phenotyping pipeline to identify mouse mutants with hearing impairment. *Nat Protoc* 2010;**5**:177–90

Address for correspondence:
Dr F Stelma,
MRC Harwell,
Harwell Science and Innovation Campus,
Didcot OX11 0RD, UK

Fax: +44 (0) 1235 841172
E-mail: femkestelma@gmail.com

Dr F Stelma takes responsibility for the integrity of the content of the paper
Competing interests: None declared