Review of Hair Cell Synapse Defects in Sensorineural Hearing Impairment

Tobias Moser, Friederike Predoehl, and Arnold Starr

*InnerEarLab, Department of Otolaryngology, University of Göttingen Medical School; †Sensory Research Center SFB 889, ‡Bernstein Center for Computational Neuroscience, University of Göttingen, Göttingen, Germany; and §Department of Neurology, University of California, Irvine, California, U.S.A.

Objective: To review new insights into the pathophysiology of sensorineural hearing impairment. Specifically, we address defects of the ribbon synapses between inner hair cells and spiral ganglion neurons that cause auditory synaptopathy.

Data Sources and Study Selection: Here, we review original publications on the genetics, animal models, and molecular mechanisms of hair cell ribbon synapses and their dysfunction.

Conclusion: Hair cell ribbon synapses are highly specialized to enable indefatigable sound encoding with utmost temporal precision. Their dysfunctions, which we term auditory synaptopathies, impair audibility of sounds to varying degrees but commonly affect neural encoding of acoustic temporal cues essential for speech comprehension. Clinical features of auditory synaptopathies are similar to those accompanying auditory neuropathy, a group of genetic and acquired disorders of spiral ganglion neurons. Genetic auditory synaptopathies include alterations of glutamate loading of synaptic vesicles, synaptic Ca²⁺ influx or synaptic vesicle turnover. Acquired synaptopathies include noise-induced hearing loss because of excitotoxic synaptic damage and subsequent gradual neural degeneration. Alterations of ribbon synapses likely also contribute to age-related synaptic damage and subsequent gradual neural degeneration.

Key Words: Genetics—Ion channel—Sensorineural hearing impairment—Sound coding—Synapses—Synaptopathy.

Otol Neurotol 34:995–1004, 2013.
auditory pathway to be absent or abnormal (6,7). However, as cochlear amplification is functional, at least initially, OAEs and/or cochlear microphonic potentials are often present (7–10). Psychophysical findings in auditory synaptopathies vary from normal pure tone audiograms to complete deafness (6,8–14). Still, even when audibility is normal or minimally affected, speech comprehension is impaired and is often not improved by hearing aids (15). Defects of the auditory nerve (16) have similar findings as auditory synaptopathies rendering their differentiation difficult (15,16). Examination of temporal bones in subjects with neural disorders have shown both loss of auditory ganglion cells as well as demyelination of auditory nerve fibers (17). The effects of these changes are to seriously compromise the magnitude of auditory nerve activity, neural conduction speed, and to cause conduction block in affected fibers.

"Synaptopathy" is a recently introduced term for a long-known nosological concept. Myasthenic disorders such as Myasthenia gravis and Lambert-Eaton syndrome are long established synaptopathies of the neuromuscular junction (18–21). Recently, synaptic dysfunction has received much attention as a potential disease mechanism in neuropsychiatric diseases such as Huntington’s disease (22) and autism spectrum disorders (23,24). Although evidence indicates an important role of synaptic alterations in the pathophysiology of major brain diseases, their relevance as primary disease mechanism is an active topic of research (25).

Strong alterations of neuromuscular junction and synapses of the central nervous system are not compatible with life (e.g., ref. [26,27]). This is very different for ribbon synapses formed by sensory cells in the ear and retina. They are molecularly and structurally specialized and, to some extent, distinct from other synapses, such that mutations can specifically affect hearing and/or vision by impairing ribbon synapse function while sparing other synapses. The synaptic ribbon is an electron-dense structure that extends into the cytosol and tethers a halo of synaptic vesicles (Fig. 1B). Depending on the position of an inner hair cell along the tonotopic cochlear axis, it forms between 5 and 20 ribbon synapses (28) with the unbranched peripheral axons of spiral ganglion neurons (29). The exact role of this multi-protein nanomachinery is
subject of current studies (30–33). It is hypothesized to support a large pool of readily releasable vesicles and its replenishment after exocytosis. Its main molecular constituent is the protein Ribeye (34) (Fig. 1C) that is specific to ribbon synapses and thought to build the ribbon in a brick-stone like manner interacting with itself (35) and other proteins such as bassoon (36). Bassoon is a big scaffold protein (37), common to many synapses, and organizes the active zone of photoreceptors (38) and hair cells (30,33). While sharing some of the common scaffold proteins of the active zone, the hair cell ribbon synapse seems to otherwise employ different proteins than most other synapses (39–44) (Fig. 1C), some of which have been shown to be affected in hereditary synaptopathic hearing impairment.

GENETIC SYNAPTOPATHIES

Defects of Presynaptic Calcium Influx Into Inner Hair Cells

Unlike in other synapses, hair cell ribbon synapses use CaV1.3 L-type Ca$^{2+}$ channels for stimulus-secretion coupling (45–47). Their active zones cluster tens of CaV1.3 L-type Ca$^{2+}$ channels (33,48–52) (Fig. 2) that activate rapidly already at hyperpolarized potentials (53) and show only mild inactivation during ongoing stimulation (54,55). These functional properties arise from the unique molecular composition of the channel complex that involves interaction with numerous other proteins such as Ca$^{2+}$-binding proteins (56–58) (Fig. 2A). Recently, a loss of function mutation in the CACNA1D gene has been identified in a family with congenital deafness and bradycardia, signifying the importance of CaV1.3 for hearing and atrial pacemaking (12). Recently, a mutation of the CABP2 gene has been demonstrated to cause a moderate sensorineural hearing impairment, which may be related to the lower potency of the mutant CaBP2 protein to inhibit calmodulin-mediated calcium dependent inactivation of the calcium current (57). Moreover, we note for a comparison that mutations in the genes coding for the poreforming α1F (CACNA1F) subunit (59,60) of the presynaptic CaV1.4 Ca$^{2+}$ channels, the auxiliary α2δ4 subunit (61) and the interacting Ca$^{2+}$ binding protein 4 (62) (CaBP4) cause human retinal disease such as night blindness probably by disturbing synaptic transmission at the photoreceptor ribbon synapses.

The human phenotype related to the loss of function CACNA1D mutation (12) is very closely resembled in Cacna1d knock-out mice, displaying both deafness and bradycardia (45,47). The mouse model allows for analysis of Ca$^{2+}$ influx and the ensuing exocytosis in inner hair cells, which were both reduced by 90% (46) (Fig. 2C, D). This defect of hair cell transmitter release readily explains the lack of ABRs (Fig. 2E). The dramatic reduction of presensory and sensory afferent neural activity leads to

![FIG. 2. Molecular physiology and pathology of hair cell calcium influx. A: Top: a defect in Ca$^{2+}$ influx disrupts stimulus-secretion coupling, bottom: domain structures of the subunits forming the hair cell CaV1.3 Ca$^{2+}$ channel: pore-forming α1D subunit, auxiliary β2, α2δ, and γ subunits (adapted from Caterall, Pharmacol Rev 2005). B: Left: nanoanatomy of presynaptic CaV1.3 Ca$^{2+}$ channel clusters resolved by STED microscopy after immunolabeling (taken from Frank et al., Neuron 2010); right: 5 presynaptic Ca$^{2+}$ microdomains visualized as fluorescence hotspots of Fluo-5N indicator at the ribbon-occupied active zones (marked by a fluorescent Ribeye-binding peptide; taken from Frank et al., PNAS 2009). C: Representative Ca$^{2+}$ currents and (D), membrane capacitance increments (ΔCm, reflecting exocytic fusion of vesicles to the plasma membrane) of a normal IHC (black) and an IHC lacking the CaV1.3 Ca$^{2+}$ channel (gray); near-complete block of Ca$^{2+}$ influx and exocytosis (taken from Brandt et al., 2003). E: Deafness of CaV1.3 deficient mice is indicated by lack of ABRs (representative recordings in response to 100 dB clicks).](https://example.com/figure2.jpg)
substantial neurodevelopmental alterations in the auditory pathway (63–65) and to a progressive loss of hair cell afferent synapses, hair cells and spiral ganglion neurons (63,66), respectively. Interestingly, neither affected humans nor mice seem to have vestibular disorders. This is consistent with the finding of a sizable remaining Ca2+ current in vestibular hair cells of Cacna1d knock-out mice (47).

Genetic Alteration of Vesicular Glutamate Uptake in Hair Cells Disrupt Hearing

The glutamatergic ribbon synapses of hair cells use the transporter VGLUT3 to load their synaptic vesicles with glutamate (42,43,67), whereas all other glutamatergic synapses studied so far use VGLUT1 or 2 (68,69). Instead, in the CNS VGLUT3 is used by monaminergic and cholinergic neurons that co-release glutamate. Genetic ablation of Vglut3 function caused deafness in mice (42,43) and zebrafish (67) because of abolition of glutamate release (Fig. 3). Hair cell synapses remained surprisingly intact. They display robust Ca2+ influx and exocytosis of glutamate-devoid vesicles (43) (Fig. 3B), and spiral ganglion neurons exhibited robust postsynaptic receptor currents in response to application of exogenous glutamate (42). Loss of synapses, hair cells and spiral ganglion neurons proceeded at relatively slow pace (weeks rather than days as found for otoferlin mutants, see below) perhaps because of preserved release of trophic factors. Interestingly, no overt vestibular dysfunction was observed in Vglut3 knock-out mice.

![Diagram of VGLUT3-deficient hair cells](image)

**FIG. 3.** VGLUT3-deficient hair cells lack glutamate release—human vglut3 mutations are responsible for progressive deafness DFNA25. **A**, Left: representative patch-clamp recording of excitatory postsynaptic currents (EPSCs) from a SGN terminal (black), which are stimulated by superfusion with 40 mM K+ and blocked by the AMPA receptor blocker NBQX (gray); middle: lack of EPSCs in a representative recording from Vglut3-knockout mice (taken from Seal et al., Neuron 2008); right: genetic ablation Vglut3 function abolishes vesicular glutamate uptake and release. **B**, Despite ablation of Vglut3 IHCs undergo Ca2+ influx (top panel) and exocytotic fusion of vesicles (lower panel, taken from Ruel et al., Am J Hum Genet, 2008). Exocytosis of trophic factors potentially contributes to maintaining synaptic and neural integrity, such that degeneration proceeds more slowly than in Cacna1.3 or otoferlin mutants. **C**, While both acoustically evoked ABR (aABR, top panel) and electrically evoked ABR (eABR, lower panel) are regularly recorded in control mice, only eABR but not aABR are observed in Vglut3-knockout mice (adapted from Ruel et al., Am J Hum Genet, 2008). **D**, Pure tone audiogram of a DFNA25 affected boy displaying a moderate hearing impairment at 6 years of age (taken from Thirlwall et al., Head Neck Surg 2003).
First efforts toward virus-mediated transfer of Vglut3 DNA into inner hair cells of Vglut3 knock-out mice have yielded promising results: normal thresholds were restored for several weeks following viral injection into the cochlea (70). Vglut3 knock-out mice and heterozygotes littermates showed EEG abnormalities indicative of a neocortical hyperexcitability, but myoclonic activity has not been detected (42). The human hearing impairment DFNA25...
was first described in 2003 (13) and was then linked to a VGLUT3 mutation in 2008 (43). Affected subjects become progressively hearing impaired starting during adolescence (Fig. 3D) and apparently lack other symptoms. Future studies are needed to address the precise mechanism of the progressive synaptopathy DFNA25.

**Mutations in OTOF Cause Prelingual Deafness DFNB9 and Temperature-Sensitive Synaptic Hearing Impairment**

Mutations in the OTOF gene coding for otoferlin—a member of the ferlin family of transmembrane multi-C2 proteins (71,72), which is expressed in hair cells (73)—cause the prelingual deafness DFNB9 (6,8,10,74,75) and a temperature-sensitive hearing impairment (76,9,14) (Fig. 4). Since its identification, more than 50 pathogenic mutations of OTOF have been published (11) (Fig. 4A). Most mutations cause loss of otoferlin function and profound prelingual deafness (Fig. 4B), and affected individuals seem to benefit from cochlear implantation (77,78). Otoferlin is considered a synaptic vesicle protein because a direct association to synaptic vesicles was found by immuno-electron microscopy (73) and its distribution in hair cells is similar to that of the synaptic vesicle protein Vglut3 (79) (Fig. 4B). Ablation of Otof function in mice revealed a near complete abolition of hair cell exocytosis as the mechanism underlying DFNB9. Synapses were rapidly lost postnatally probably because of degeneration. However, the ultrastructure of the remaining synapses was well preserved, displaying a normal supplement of synaptic vesicles. Therefore, a role of otoferlin in a late step of exocytosis (priming and/or fusion) was postulated (73). A role of otoferlin as a Ca$^{2+}$ sensor of vesicle fusion was further suggested by the Ca$^{2+}$ and phospholipid binding of some C2 domains (73,80,81) and facilitation of fusion of SNAPRE-tagged liposomes (80). These properties are shared by the neuronal Ca$^{2+}$ sensor of fusion synaptotagmin 1, which is lacking from mature inner hair cells (82,83). However, synaptotagmin 1 if introduced as a transgene cannot replace otoferlin in hair cell exocytosis (83). Otoferlin’s role in Ca$^{2+}$ regulated fusion should be further addressed by site-directed mutagenesis of the putative Ca$^{2+}$ binding sites (84).

Mutations that do not fully inactivate function have helped further studies of the physiologic role of otoferlin and otoferlin-related synaptopathy. Three mutations were associated with a temperature-sensitive hearing impairment (9,14,76). The affected individuals become deaf when core temperature rises. ABRs at this time are absent. When afebrile, these subjects have a mild elevation of threshold. Speech perception in quiet is normal but impaired in noise. The mechanism underlying the temperature effect still awaits clarification. It might involve protein instability and subsequent degradation possibly leading to a shortage of functional otoferlin. Such a reduction of otoferlin levels (Fig. 4C) was considered as a candidate mechanism for a missense mutation in the region coding the C2F domain in the Pachanga mouse (85).

**Ca$^{2+}$-dependent vesicle fusion was surprisingly found intact, but a reduced rate of vesicle replenishment was observed (79). In physiology, synapses of inner hair cells replenish hundreds of vesicles per second to enable high rates of transmission and auditory nerve fiber spiking over prolonged periods of time. Interestingly, an additional reduction of vesicle replenishment was found with mice carrying only 1 mutant allele (Fig. 4D, E), in which otoferlin levels were reduced even further. Deafness of these mice was proposed to result from the lack of a sufficient pool of readily releasable vesicles in vivo, when spontaneous release steadily consumes vesicles in excess of the reduced capacity for vesicle replenishment (Fig. 4G). Hence, auditory nerve fibers of these mice could barely respond to sound stimulation (Fig. 4F). In conclusion, aside from being a candidate Ca$^{2+}$ sensor of fusion in hair cells, otoferlin has a function in vesicle replenishment (Fig. 4G). Additional general cell biological functions have been proposed based on protein interaction studies and the broad distribution of otoferlin in hair cells also outside the presynaptic active zones (86–88).

**Noise-Induced and Age-Related Hearing Loss**

Recent findings indicate that cochlear synaptic mechanisms may contribute to these 2 most common forms of hearing impairment. Changes in synapse number and structure have been implied in noise-induced (89–91) and age-dependent hearing loss (92). Interestingly, a human association study suggests polymorphisms in the gene coding for the metabotropic glutamate receptor mGlur7 to contribute to susceptibility for age-dependent hearing loss (93). Excitotoxic synaptic and neural damage is a key candidate mechanism for noise-induced and age-dependent hearing loss (Fig. 5A). It may result from excessive presynaptic release of glutamate, which has long been discussed for noise-induced hearing loss (see below) and has recently been implied for a human progressive hearing loss caused by mutations in the gene GIPC3 (94,95). Susceptibility to excitotoxic damage could also arise from abnormally high numbers or sensitivity of postsynaptic glutamate receptors (96), alterations of efferent innervation (97) and from interference with glutamate uptake (98,99), but the relevance of these mechanisms for human disease has not yet been demonstrated.

Excitotoxic synaptic damage due to excessive presynaptic release of glutamate has long been indicated to contribute to noise-induced hearing loss (89–91). Immunohistochemical quantification of ribbon synapse number (28,30) has now been used to establish the loss of ribbon synapses during noise exposures (100,101). Strikingly, even noise exposures that caused only temporary threshold loss were accompanied by a permanent loss of approximately 50% of the hair cell synapses and subsequent slow degeneration of spiral ganglion neurons in the high frequency region of the cochlea (Fig. 5C, D, F, G). The morphologic damage was reflected by a reduced spiral ganglion compound action potential. Measured as Jewett wave I of the auditory brainstem responses, a permanent reduction was found (Fig. 5E), despite full recovery of the physiologic threshold (Fig. 5B). One possible
hypothesis explaining this discrepancy of functional findings is that the noise-induced insult hits the low-sensitivity spiral ganglion neurons, which signal loud sounds, but spares the high-sensitivity neurons, which are responsible for sound perception near threshold. This hypothesis can well explain the finding of poor speech recognition in noisy background.

Not surprisingly synaptic insult occurs also during noise exposures that cause a permanent threshold increase (100). Current research aims to understand the presynaptic and postsynaptic changes that occur during noise damage. Moreover, studies explore the reasons why excitotoxic synapse loss is not followed by de novo synapse formation during the weeks after the insult when the disconnected inner hair cells and spiral ganglion neurons are still present. The extent, irreversibility, and functional consequences of excitotoxic synapse loss had not yet appreciated and now require studies of the relevance of this disease mechanism for human noise-induced hearing loss. If comparable to the animal findings, which is likely the case, noise exposure is much more dangerous than we have assumed. We will then have to acknowledge that noise induces synapse and progressive neuron loss and thereby impairs speech reception in noisy environments. We will need to revise noise exposure guidelines, diagnostic procedures and clinical evaluation of occupational hearing loss.

In summary, excitotoxic synaptic damage is likely a disease mechanism of noise-induced and possibly also of age-dependent hearing loss (101,102).

**DISCUSSION**

**Identification and Characterization of Auditory Synaptopathy**

Auditory synaptopathy—impaired synaptic sound encoding has only recently been appreciated as a disease mechanism of both genetic and acquired hearing impairments. The similarity of clinical expression to auditory neuropathy (15,16): preserved otoacoustic emissions and/or cochlear microphonic potentials reflecting cochlear outer hair cell function but absent or abnormal auditory brainstem responses due to the loss of cochlear synapses demonstrates the potential of synaptopathy as a disease mechanism of hearing loss. The current hypothesis explaining this discrepancy of functional findings is that the noise-induced insult hits the low-sensitivity spiral ganglion neurons, which signal loud sounds, but spares the high-sensitivity neurons, which are responsible for sound perception near threshold. This hypothesis can well explain the finding of poor speech recognition in noisy background.

**FIG. 5.** Excitotoxic irreversible loss of IHC ribbon synapses during noise-induced temporary threshold loss. A, Cartoon illustrating excitotoxic synaptic insult: loud noise induces excessive presynaptic glutamate release that causes overexcitation and massive sodium influx into the postsynaptic terminal of the SGN. The ensuing osmotic load causes swelling and finally disruption of the terminal. Work by Kujawa and Liberman (2009) in animals suggests that the SGN do not re-establish synaptic connections with IHCs after the insult and are finally lost. B, Induction and recovery of ABR threshold loss following a 100 dB octave band noise for 2 h. C, Irreversible loss of half of the synaptic ribbons in high-frequency IHCs in the same mice, despite threshold recovery after 2 weeks. D, Representative projections of confocal images of the immunolabeled IHC ribbons in control and noise-exposed mice: reduction of ribbon number. Long-term percentage reduction of (E) the amplitude of ABR wave 1 reflecting the loss of synchronously firing SGN, (F) ribbon synapse number in high frequency IHCs and (G) SGN somata: simultaneous loss of synapses and synchronously firing neurons, delayed physical loss of SGN. (B–G) were taken with permission from Kujawa and Liberman, *J Neurosci* 2009.
to impaired sound coding led to the initial denomination as auditory neuropathy or auditory neuropathy spectrum disorder. This review describes specific disease mechanisms, focusing on presynaptic alterations at the inner hair cell synapse. Human genetics has uncovered that monogenic defects and complex genetic diseases also affect sound encoding at the hair cell synapses. Starting with the identification of otoferlin (10), an increasing number of defects in genes that code for synaptic proteins and ion channels have been identified, and the list is expected to still increase. Molecular physiology in genetically manipulated mice has provided insights into gene function at the synapse and the synaptic mechanisms underlying the human disease. These studies unambiguously demonstrate the synapse as a primary site of lesion and hence support the use of auditory synaptopathy as the precise nosologic category. However, severe auditory synaptopathy sooner or later leads to degeneration of the spiral ganglion neurons and, thus, has a common final path with primary neural disorders such as hereditary motor and sensory neuropathy.

Understanding Mechanisms and Phenotypes of Auditory Synaptopathies Based on Detailed Analysis of Mouse Models

Mouse models serve as powerful tools for dissecting the precise disease mechanisms, for predicting onset and progression of degeneration and for devising therapeutic approaches. Different from the described mouse models of human auditory synaptopathy, other ‘‘synaptic’’ mouse mutants allow one to study the consequences of more subtle synaptic deficits for auditory systems function. Genetic disruption of the presynaptic protein Bassoon causes a mild synaptic hearing impairment (30,103) because of a reduction in the number of releasable synaptic vesicles and Ca2+ channels (33). ABR are present but display a reduction in the number of releasable synaptic vesicles. Starting with the identification of otoferlin (10), an increasing number of defects in genes that code for synaptic proteins and ion channels have been identified, and the list is expected to still increase. Molecular physiology in genetically manipulated mice has provided insights into gene function at the synapse and the synaptic mechanisms underlying the human disease. These studies unambiguously demonstrate the synapse as a primary site of lesion and hence support the use of auditory synaptopathy as the precise nosologic category. However, severe auditory synaptopathy sooner or later leads to degeneration of the spiral ganglion neurons and, thus, has a common final path with primary neural disorders such as hereditary motor and sensory neuropathy.

Ototerlin: Synergistic Research on Human Subjects and Animal Models Advance Our Understanding of Otoferlin Function and Dysfunction

The genetics, structure, and function of ototerlin in the context of hearing and deafness define a hot topic of auditory research. After identifying OTOF about a decade ago as the gene defect underlying autosomal recessive, nonsyndromic profound deafness DFNB9 (10), work now encompasses molecular, cellular, and systems level approaches. The presence of human subjects with temperature-sensitive OTOF mutations enables advanced electrophysiologic and psychophysical studies and promises to contribute to our understanding of otoferlin-related hearing impairment and auditory synaptopathy in general. Genetic manipulations in mice combined with comprehensive structural and functional analysis will continue to contribute. In particular, these studies will help to further test the Ca2+ sensor of vesicle fusion and vesicle replenishment hypotheses.

Presynaptic and Postsynaptic Mechanisms of Synaptopathy

Here, we have reviewed exemplary presynaptic and postsynaptic mechanisms of synaptic hearing impairment with much emphasis on the presynaptic dysfunction. Future research will reveal further genetic and acquired synaptopathies, which will likely also include other alterations of postsynaptic function. Combining specific clinical and genetic testing will likely help to distinguish primarily presynaptic and postsynaptic dysfunctions.

Acknowledgments: The authors thank Nicola Strenzke, Martin Canis and Charles M. Liberman for the comments on the manuscript. The authors also thank Regis Nouvian and Linda Hsu for contributing graphical illustrations.

REFERENCES

1. Schuknecht HF, Igasho M. Pathology of slowly progressive sensori-neural deafness. Trans Am Acad Ophthalmol Otolaryngol 1964;68:222–42.
2. Schütz M, Auth T, Gehrt A, et al. The connexin26 S17F mouse mutant represents a model for the human hereditary keratitis-ichthyosis-deafness syndrome. Hum Mol Genet 2011;20:28–39.
3. Cohen-Salmon M, Ott T, Michel V, et al. Targeted ablation of connexin26 in the inner ear epidermal gap junction network causes hearing impairment and cell death. Curr Biol 2002;12:1106–11.
4. Dallos P. Cochlear amplification, outer hair cells and preyen. Curr Opin Neurobiol 2008;18:370–6.
5. Oxenham AJ, Bacon SP. Cochlear compression: perceptual measures and implications for normal and impaired hearing. Ear Hear 2003;24:352–66.
6. Varga R, Kelley P, Keats B, et al. Non-syndromic recessive auditory neuropathy is the result of mutations in the otoferlin (OTOF) gene. J Med Genet 2003;40:45–50.
7. Santarelli R, Del Castillo I, Rodriguez-Ballesteros M, et al. Abnormal cochlear potentials from deaf patients with mutations in the otoferlin gene. J Assoc Res Otolaryngol 2009;10:545–56.
8. Rodriguez-Ballesteros M, del Castillo FJ, Martin Y, et al. Auditory neuropathy in patients carrying mutations in the otoferlin gene (OTOF). Hum Mutat 2003;22:451–6.
9. Martin S, Feldmann D, Nguyen Y, et al. Temperature-sensitive auditory neuropathy associated with an otoferlin mutation: deafening fever! Biochem Biophys Res Commun 2010;394:737–42.
10. Yasunaga S, Gritti M, Cohen-Salmon M, 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.
11. Rodriguez-Ballesteros M, Reynoso R, Olarte M, et al. A multi-center study on the prevalence and spectrum of mutations in the otoferlin gene (OTOF) in subjects with nonsyndromic hearing impairment and auditory neuropathy. Hum Mutat 2008;29:823–31.
12. Baig SM, Koschak A, Lieb A, et al. Loss of Ca(v)1.3 (CACNA1D) function in a human channelopathy with bрадycardia and congenital deafness. Nat Neurosci 2011;14:77–84.
13. Thrilwall AS, Brown DJ, McMillan PM, Barker SE, Lesperance MM. Phenotypic characterization of hereditary hearing impairment linked to DFNA25. Arch Otolaryngol Head Neck Surg 2003;129:830–5.
14. Wang D-Y, Wang Y-C, Weil D, et al. Screening mutations of OTOF gene in Chinese patients with auditory neuropathy, including a familial case of temperature-sensitive auditory neuropathy. BMC Med Genet 2010;11:79.
15. Starr A, Zeng FG, Michalewski HJ, Moser T. Perspectives on auditory neuropathy: disorders of inner hair cell, auditory nerve,
16. Starr A, Picton TW, Sinning Y, Hood LJ, Berlin CI. Auditory neuropathy. 
Brain 1996;119:741–53.

17. Starr A, Michalewski HJ, Zeng FG, et al. Pathology and physiology of auditory neuropathy with a novel mutation in the MPZ gene (Tyr145→Ser). Brain 2003;126:1604–19.

18. Finsterer J, Papić L, Auer-Grumbach M. Motor neuron, nerve, and neuromuscular junction disease. 
Curr Opin Neurol 2011;24: 469–74. doi:10.1097/WCO.0b013e3283449448.

19. Lang B, Vincent A. Autoimmune disorders of the neuromuscular junction. 
Curr Opin Pharmacol 2009;9:330–6.

20. Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. 
Lancet Neurol 2009;8:475–90.

21. Mahadeva B, Phillips LH 2nd, Juel VC. Autoimmune disorders of neuromuscular transmission. 
Semin Neurol 2008;28:212–27.

22. Li J-Y, Plomann M, Brundin P. Huntington’s disease: a synaptopathy? 
Trends Mol Med 2003;9:414–20.

23. Toro R, Konyukh M, Delorme R, et al. Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. Trends Genet 2010;26:363–72.

24. Bourgeron T. A synaptic trek to autism. 
Curr Opin Neurobiol 2009;19:231–4.

25. Brose N, O’Connor V, Skelhel P. Synaptopathy: dysfunction of synaptic function? 
Biochem Soc Trans 2010;38:40–4.

26. Verhage M, Maia AS, Plomp TJ, et al. Synaptic assembly of the brain in the absence of neurotransmitter secretion. 
Science 2000; 287:864.

27. Schoch S, Deák F, Königstorfer A, et al. SNARE function analyzed in synaptobrevin/VAMP knockout mice. 
Science 2001;294:1117–22.

28. Meyer AC, Frank T, Khimich D, et al. Tuning of synapse number, structure and function in the cochlea. 
Nat Neurosci 2009;12: 444–53.

29. Liberman M. Single-neuron labeling in the cat auditory nerve. 
Science 1982;216:1239–41.

30. Khimich D, Nouvian R, Pujol R, et al. Hair cell synaptic ribbons changes in exocytosis and the number of synaptic ribbons at active zones of an ON-type bipolar cell terminal. 
J Neurophysiol 2006;96:2025–33.

31. Frank T, Rutherford MA, S trenzke N, et al. Bassoon and the synaptic ribbon organize Ca2+ channels and vesicles to add release sites and promote refilling. 
Neuron 2010;68:724–38.

32. Schmitz F, Königstorfer A, Südhof TC. RIBEYE, a component of synaptic ribbons: a protein’s journey through evolution provides insight into synaptic ribbon function. 
Neuron 2000;28:857–72.

33. Magupalli VG, Schwarz K, Alpadi K, Natarajan S, Seigel GM, Schmitz F. Multiple RIBEYE-RIBEYE interactions create a dynamic scaffold for the formation of synaptic ribbons. 
J Neurosci 2008;28:7954–67.

34. Tom Dieck S, Altrock WD, Kessels MM, et al. Molecular dissection of the photoreceptor ribbon synapse: physical interaction of Bassoon and RIBEYE is essential for the assembly of the ribbon complex. 
J Cell Biol 2005;168:825–36.

35. Tom Dieck S, Sanmartí-Vila L, Langenau K, et al. Bassoon, a novel zinc-finger CAG/glutamine-repeat protein selectively localized at the active zone of presynaptic nerve terminals. 
J Cell Biol 1998;142:499–509.

36. Dick O, Tom Dieck S, Altrock WD, et al. The presynaptic active zone protein bassoon is essential for photoreceptor ribbon synapse formation in the retina. 
Neuron 2003;37:775–86.

37. Nouvian R, Beutner D, Parsons TD, Moser T. Structure and function of the hair cell ribbon synapse. 
J Membrane Biol 2006;209:153–65.

38. Nouvian R, Neef J, Bulankina AV, et al. Exocytosis at the hair cell ribbon synapse apparently operates without neuronal SNARE proteins. 
Nat Neurosci 2011;14:411–3.

39. Strenzke N, Chanda S, Kopp-Scheinpflug C, et al. Complexin-I is required for high-fidelity transmission at the endbulb of held auditory synapse. 
J Neurosci 2009;29:7991–8004.

40. Neal RP, Aki O, Yi E, et al. Sensorineural deafness and seizures in mice lacking vesicular glutamate transporter 3. 
Neuron 2008;57:263–75.

41. Ruel J, Emery S, Nouvian R, 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.

42. Safieddine S, Wentholt RJ. SNARE complex at the ribbon synapses of cochlear hair cells: analysis of synaptic vesicle- and synaptic membrane-associated proteins. 
Eur J Neurosci 1999;11:803–12.

43. Platzer J, Engel J, Schrott-Fischer A, et al. Congenital deafness and sinoscalar distortion in mice lacking class D L-type Ca2+ channels. 
Cell 2000;102:89–97.

44. Brandt A, Strenzke N, Moser T. CaV1.3 channels are essential for development and presynaptic activity of cochlear inner hair cells. 
J Neurosci 2003;23:10832–40.

45. Hou V, Vazquez AE, Namkung Y, et al. Null mutation of alpha1D Ca2+ channel gene results in deafness but no vestibular defect in mice. 
J Assoc Res Otolaryngol 2004;5:215–26.

46. Wu YC, Fettiplace R. A developmental model for generating frequency maps in the reptilian and avian cochlea. 
Biophys J 1996;70:2557–70.

47. Roberts WM, Jacobs RA, Hudspeth AJ. Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. 
J Neurosci 1990;10:3664–84.

48. Martinez-Dunst C, Michaels RL, Fuchs PA. Release sites and calcium channels in hair cells of the chick’s cochlea. 
J Neurosci 1997;17:9133.

49. Brandt A, Khimich D, Moser T. Few CaV1.3 channels regulate the exocytosis of a synaptic vesicle at the hair cell ribbon synapse. 
J Neurosci 2005;25:11577.

50. Frank T, Khimich D, Neef A, Moser T. Mechanisms contributing to synaptic Ca2+ signals and their heterogeneity in hair cells. 
Proc Natl Acad Sci 2009;106:4483.

51. Koschak A. alpha 1D (Cav1.3) subunits can form L-type Ca2+ channels. 
Brain 2003;126:1604–19.

52. Zeitz C, Kloeckener-Gruissem B, Forster U, et al. Mutations in nonsyndromic deafness DFNA25 and inner hair cell dysfunction encoding vesicular glutamate transporter 3. 
Science 2000;289:1056–60.

53. Koschak A. alpha 1D (Cav1.3) subunits can form L-type Ca2+ channels activating at negative voltages. 
J Biol Chem 2001;276:22100–6.

54. Schnee ME, Ricci AJ. Biophysical and pharmacological characterization of voltage-gated calcium currents in turtle auditory hair cells. 
J Physiol 2003;558:697–717.

55. Moser T, Beutner D. Kinetics of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse of the mouse. 
Proc Natl Acad Sci U S A 2000;97:883.

56. Yang PS, Alseikhan BA, Hiel H, et al. Switching of Ca2+ dependent inactivation of CaV1.3 channels by calcium binding proteins of auditory hair cells. 
J Neurosci 2006;26:10677–89.

57. Cui G, Meyer AC, Calin-Jageman I, et al. Ca2+-binding proteins tune Ca2+-feedback to Cav1.3 channels in mouse auditory hair cells. 
J Physiol 2007;585:791–803.

58. Schrauwen A, Hoffmann S, Inagaki AV, et al. Mutation in CAP22, expressed in cochlear hair cells, causes autosomal-recessive hearing impairment. 
Am J Hum Genet. 2012;91:636–45.

59. Strom TM, Nyakatura G, Apfelstedt-Sylla E, et al. An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. 
Nat Genet 1998;19:260–3.

60. Bech-Hansen NT, Naylor MJ, Maybaum TA, et al. Loss-of-function mutations in a calcium-channel alpha-subunit gene in Xpl1.23 cause incomplete X-linked congenital stationary night blindness. 
Cell 2008;134:465–78.

61. Wycisk KA, Zeitz C, Feil S, et al. Mutation in the auxiliary calcium-channel subunit CACNA2D4 causes autosomal recessive cone dystrophy. 
Am J Hum Genet 2006;79:973–7.

62. Zeitz C, Kloekenstein-Gruissem B, Forster U, et al. Mutations in CAPB4, the gene encoding the Ca2+-binding protein 4, cause
81. Ramakrishnan NA, Drescher MJ, Drescher DG. Direct interaction of otoferlin with syntaxin 1A, SNAP-25, and the L-type voltage-gated calcium channel Cav1.3. *J Biol Chem* 2006;281:1364–72.

82. Breg M, Michalski N, Safieddine S, et al. Control of exocytosis by synaptotagmins and otoferlin in auditory hair cells. *J Neurosci* 2010;30:13281–90.

83. Reisinger E, Breese C, Neef J, et al. Probing the functional equivalence of otoferlin and synaptotagmin 1 in exocytosis. *J Neurosci* 2011;31:4886.

84. Fernández-Chacón R, Königstorfer A, Gerber SH, et al. Synaptotagmin I functions as a calcium regulator of release probability. *Nature* 2001;410:41–9.

85. Schwander M, Szaneicka A, Grillot N, et al. A forward genetics screen in mice identifies recessive deafness traits and reveals that pejvakin is essential for outer hair cell function. *J Neurosci* 2007;27:2163–75.

86. Heidriech P, Zimmermann U, Kuhn S, 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.

87. Heidriech P, Zimmermann U, Bress A, et al. Rab8b GTPase, a protein transport regulator, is an interacting partner of otoferlin, defective in a human autosomal recessive deafness form. *Hum Mol Genet* 2008;17:3814–21.

88. Zak M, Pfister M, Biln N. The otoferlin interactorome in neurosensorial hair cells: significance for synaptic vesicle release and trans-Golgi network (Review). *Int J Mol Med* 2011;28:311–4.

89. Henry WR, Mulroy MJ. Affereent synaptic changes in auditory hair cells during noise-induced temporary threshold shift. *Hear Res* 1995;84:81–90.

90. Puel JL, Pujol R, Ladrech S, Eybalin M. Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid electrophysiological and neurotoxic effects in the guinea-pig cochlea. *Neuroscience* 1991;45:63–72.

91. Puel JL, Ruel J, Gervais d’Aldin C, Pujol R. Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. *Neuroreport* 1999;10:919–46.

92. Stamataki S, Francis HW, Lehar M, May BJ, Ryugo DK. Synaptic alterations at inner hair cells precede spiral ganglion cell loss in aging C57BL/6J mice. *Hear Res* 2006;221:104–18.

93. Friedman RA, Van Laer L, Huentelman MJ, et al. GMR7 variants confer susceptibility to age-related hearing impairment. *Hum Mol Genet* 2009;18:785–96.

94. Charizopoulou N, Lelli A, Schraders M, et al. Gipc3 mutations associated with audiogenic seizures and sensorineural hearing loss in mouse and human. *Nat Commun* 2011;2:201.

95. Rehman AU, Gul K, Morell RJ, et al. Mutations of GIPC3 cause nonsyndromic hearing loss DFNB72 but not DFNB81 that also maps to chromosome 19p. *Hum Mol Genet* 2011. doi:10.1007/s00439-011-1018-5.

96. Chen Z, Peppi M, Kujawa SG, Sewell WF. Regulated expression of surface AMPA receptors reduces excitotoxicity in auditory neurons. *J Neurophysiol* 2009;102:1152–9.

97. Ruel J, Nouvian R, Gervais d’Aldin C, Pujol R, Eybalin M, Puel JL. Dopamine inhibition of auditory nerve activity in the adult mammalian cochlea. *Eur J Neurosci* 2001;14:977–86.

98. Chen Z, Kujawa SG, Sewell WF. Functional roles of high-affinity glutamate transporters in cochlear afferent synaptic transmission in the mouse. *J Neurophysiol* 2010;103:2581–6.

99. Hakuba N, Koga K, Gyo K, Usami SI, Tanaka K. Exacerbation of noise-induced hearing loss in mice lacking the glutamate transporter GLAST. *J Neurosci* 2000;20:8750–5.

100. Kujawa SG, Liberman MC. Adding insult to injury: cochlear nerve degeneration after ‘temporary’ noise-induced hearing loss. *J Neurosci* 2009;29:14077–85.

101. Lin HW, Furman AC, Kujawa SG, Liberman MC. Primary neural degeneration in the guinea pig cochlea after reversible noise-induced threshold shift. *J Assoc Res Otolaryngol* 2011;12:665–16. doi:10.1007/s10162-011-0277-0.

102. Pujol R, Puel JL. Excitotoxicity, synaptic repair, and functional recovery in the mammalian cochlea: a review of recent findings. *Ann N Y Acad Sci* 2008;1144:294–54.

103. Buran BN, Strenzke N, Neef A, Gundelfinger ED, Moser T, Liberman MC. Onset coding is degraded in auditory nerve fibers from mutant mice lacking synaptic ribbons. *J Neurosci* 2010;30:7567.