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CONNEXINS IN HEARING LOSS: A COMPREHENSIVE OVERVIEW

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ABSTRACT

Connexins are a family of transmembrane proteins that form gap junctions between adjacent cells and allow intercellular communication. Connexin proteins are involved in pathological conditions in humans, mainly in hearing loss, neurodegenerative disorders and skin diseases. The association between connexin proteins and the inner ear is well established. The abundant expression of connexins in the auditory system of the inner ear demonstrates their importance in inner ear development and the hearing process. Most compelling, there are over 100 mutations in genes encoding connexins that are associated with deafness. Most prominent is the remarkable involvement of connexin 26 in hearing loss. Mutations in the gene *GJB2*, encoding connexin 26, are responsible for around 50% of genetic cases of severe to profound non-syndromic hearing loss in some parts of the world. Learning more about the connexin family in general and about connexin 26 in particular can shed light on the pathogenesis of the inner ear and bring us closer to finding clinical solutions for the hearing impaired.

KEY WORDS

genetics, mutations, hearing loss, deafness, cochlea, connexin 26, connexin 30

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INTRODUCTION

Humans are born with five senses that allow us to see, hear, taste, smell and feel the world around us. Developmental defects and/or exposure to viral infection or ototoxic drugs during pregnancy can, however, lead to profound congenital deafness, as occurs in around one in 1,000 live births /1/. Indeed, hearing loss (HL) is the most common of all human sensorineural disorders and may also occur later in life, affecting up to half the elderly population. HL is very heterogeneous with different causes, characteristics and potential methods of treatment. Despite this diversity, mutations in one gene, connexin 26 (*GJB2*), are the predominant cause of hereditary HL.

Hereditary HL may be either syndromic (SHL) or non-syndromic (NSHL). NSHL accounts for 70% of genetic deafness cases /2/. In these cases, HL is the only deficiency the patients experience. SHL involves other symptoms, such as blindness due to retinitis pigmentosis, as in the case of Usher syndrome, or skin disease, as in the case of keratitis-ichthyosis-deafness (KID) syndrome. The genetic basis of HL can be either autosomal dominant or recessive, X-linked or mitochondrial.

It is estimated that as many as 200 genes may be involved in both non-syndromic and syndromic HL. To date 65 of these genes have been cloned and 120 different loci have been mapped (Hereditary Hearing Loss Homepage; http://webhost.ua.ac.be/hhh/). Although HL is genetically heterogeneous, a single gene is responsible for around 50% of severe to profound NSHL /3/. This gene, *GJB2,* encodes the protein connexin (Cx) 26.

THE CONNEXIN FAMILY

The connexins are a family of proteins that form gap junctions between adjacent cells. There are 21 human genes in the connexin family known to date that are expressed in almost all tissues and cell types in our body. The connexins are part of a family of trans-membrane (TM) proteins. These proteins are embedded in the lipid bilayers and mediate communication between both sides of the membrane /4/. The connexin protein is comprised of four TM domains designated M1-M4, two extracellular loops (EC1, EC2) and three

intracellular domains (IC), two of which are the NH_2 and COOH termini of the protein (see Fig. 1).

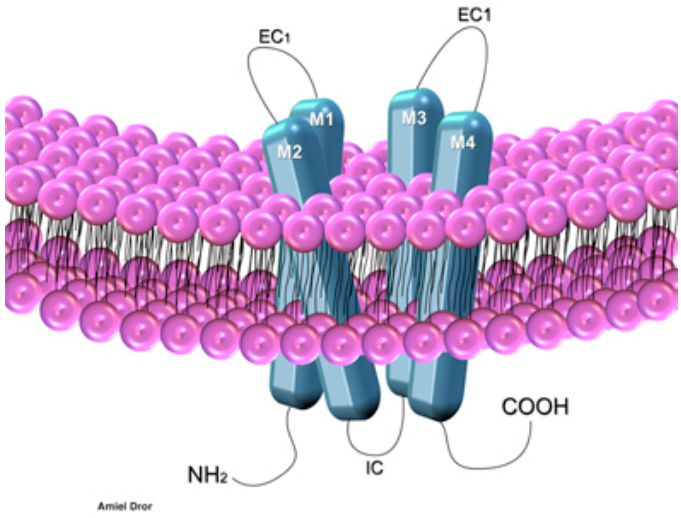

Fig. 1: Schematic presentation of connexin topology. The protein is comprised of four transmembrane domains designated M1-M4, two extracellular loops (EC1, EC2) and three intracellular domains (IC), two of which are the NH_2 and COOH termini of the protein.

The functional entity of gap junction proteins is formed by the assembly of six connexin units to form a hemichannel that is called a connexon. Connexons of two neighboring cells can travel along the plasma membrane until they dock opposite to each other, and in this way form the gap junction, a channel that allows cell communication in various tissues in our bodies. Both the connexons and the channels can be composed of more than one connexin isoform. A channel can be either homomeric (formed by six units of the same isoform) or heteromeric (formed by different isoforms). Similar connexons docking together form a homotypic junction, while different connexons form a heterotypic junction (see Fig. 2). This allows different combinations and formation of gap junctions with different permeability to ions and small molecules well-matched for particular tissues /5/. For example, it was recently reported that intercellular Ca^{2+} signaling across heteromeric gap junctions composed of Cx26 and

Cx30 was at least twice as fast as the signaling across each of the homomeric channels /6/. However, this diverse expression of different isoforms in the same tissue can lead not only to diverse functionality but also to redundancy and hence leads to the speculation that in the absence of one functional connexin due to mutations, its role can be compensated for by a different type of connexin expressed in the same tissue.

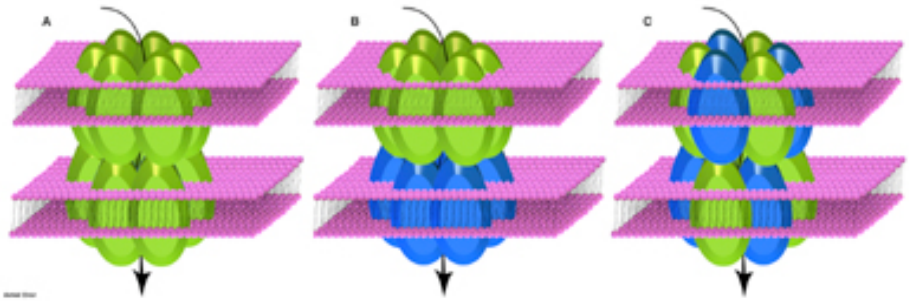

Fig. 2: Composition of gap junctions. Since a cell can express more than one isoform of connexin, a connexon can be either homomeric (formed by six units of the same isoform) or heteromeric (formed by different isoforms). Similar connexons docking together form a homotypic junction, while different connexons form a heterotypic junction. A. Homotypic junction, formed by two homomeric connexons. B. Heterotypic junction, formed by two homomeric connexons. C. Homomeric junction, formed by two heteromeric connexons.

CONNEXINS AND HUMAN DISEASE

Due to their essential roles in cellular function, connexin proteins are substantially involved in human disease. The first discovery of an inherited disease involving a connexin protein was that of Cx32, causing X-linked Charcot-Marie-Tooth disease (CMTX), a common form of inherited sensory and motor neuropathy /7/. Some cases of CMTX also involve deafness /8-10/. Several skin disorders are caused by mutations in Cx26, Cx30, Cx30.3 and Cx31. As in CMTX, some skin disorders are accompanied by impaired hearing. Two dominant forms of congenital cataract are associated with missense mutations in Cx46 and Cx50. Cx43 is involved in heart disease /11/. An interesting

aspect of disease-causing mutations in connexins is that in some cases different mutations in the same gene cause two different diseases. This is the case for *GJB3* encoding Cx31, where some mutations cause erythrokeratodermia variabilis (EKV), while others cause isolated deafness. Another example is that of *GJB6*, mutations in which result in aberrant Cx30 protein, leading to either hidrotic ectodermal dysplasia (HED) or deafness. As described, connexin proteins are expressed in many tissues and are potentially involved in numerous processes in the body, but evidently one of the major involvements of connexin proteins in human disease is in hearing loss.

CONNEXINS IN THE INNER EAR

Several connexin proteins are expressed in the ear. Along with the abundant expression of Cx26 in both the auditory and vestibular systems of the inner ear /12/, many connexin genes are expressed in the developing ear. *In situ* hybridization studies on murine sections revealed the expression of Cx26, Cx30, Cx30.2, Cx31, Cx37, Cx43, Cx46 and also low expression of Cx45 and Cx59 /13/. In a different study on the mature ear, mRNA of Cx26, Cx30, Cx31, Cx43 and Cx50 was identified by RT-PCR /14/. A recent study documented the expression of Cx43- and Cx45-encoding genes in the developing and mature murine inner ear /15/. Specific antibodies for Cx26, Cx30, Cx31 and Cx43 identified the encoded proteins in the mature inner ear /14/.

Cx26 is highly expressed in large gap junctions between adjacent supporting cells in the inner ear in both the auditory and vestibular sensory epithelia. One role of gap junctions between supporting cells may be to provide a pathway for the rapid removal of ions away from the region of the sensory cells during transduction in order to maintain sensitivity. Another possible role is to help maintain the high extra-cellular electrical potential in the cochlea by circulation of K^+ from the endolymph to perilymph and back to endolymph through the stria vascularis /12/. Cx30 is not as abundant in the inner ear as Cx26 but the two are partly co-localized in supporting cells of the sensory epithelia, fibrocytes in the spiral ligament and spiral limbus, as well as in the vestibular system /16,17/ (Fig. 3). Cx26 and Cx30 were previously reported to co-assemble in the inner ear into heteromeric channels /18/. However, several knockout experiments in either Cx26

or Cx30 revealed dramatic defects. Mice with a deletion of Cx30 are deaf. They do not generate endocochlear potentials and exhibit death of outer hair cells /19/. Mice with a targeted deletion of Cx26 in the supporting cells of the organ of Corti experience outer hair cell death as well, initiated at about the time of onsest of hearing /20/. These findings indicate that Cx26 and Cx30 do not compensate for one another in the ear.

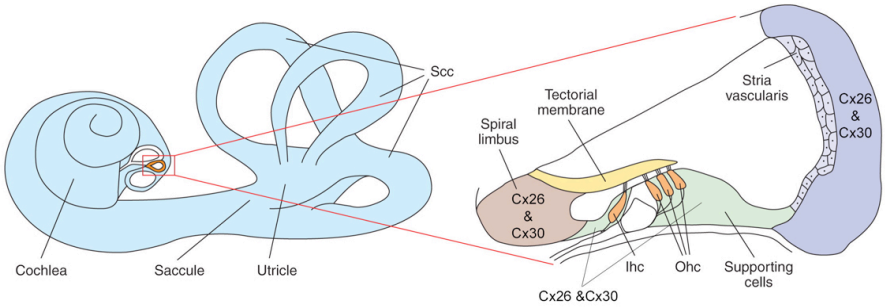

Fig. 3: Localization of connexin in the auditory system of the inner ear. The enlargement of the cochlear duct shows a cross section of the organ of Corti. Cx26 and Cx30 are the most abundant connexins in the inner ear. They are found in the supporting cells, basal cells of the stria vascularis, fibrocytes of the spiral ligament, beneath the stria vascularis and in cells of the spiral limbus (modified from /55/).

CONNEXINS AND DEAFNESS

Mutations in the genes encoding Cx30 and Cx31 are involved in either dominant or recessive NSHL. In addition, the most significant representation of deafness-causing mutations is, without a doubt, in Cx26. The reports of *GJB2* mutations associated with HL includes 92 recessive mutations and nine dominant mutations causing NSHL and nine dominant mutations causing syndromic HL reported to date (The Connexin-Deafness Homepage; http://davinci.crg.es/deafness/). However, among these mutations many were described only in a single or a few patients, while three mutations are significantly more common in many populations in the world. Two of them were first described in 1997 by Zelante *et al.* /21/. 35delG was found to be most common in the Mediterranean region /21/ and 167delT was found to be most

common in the Jewish Ashkenazi population /22,23/. In 1999, another novel mutation, 235delC, was described and found to be common among the Asian population, mostly in the Japanese /24/. Carrier frequencies for these common mutated alleles have been determined in different populations. Reports of carrier frequency of 35delG vary from none in African Americans /23,25/ to 2.8% in North European Caucasians /25/, 3.2% in Spanish and Italian Caucasians /26/ and 3.5% in Greek Caucasians /27/. Reports on 167delT indicate a carrier rate of 2.8-7.5% among Ashkenazi Jews /23,28,29/. The carrier frequency of 235delC in the Japanese population ranges from 1.0-2.1% /30,31/.

Most *GJB2* mutations lead to recessive NSHL. Three years after the first locus for recessive deafness, DFNB1, was defined and mapped to chromosome 13q11 /32/, *GJB2* was identified and found to be responsible for the high prevalence of DFNB1 mutations /33/. Since the mode of inheritance in this locus is recessive, a surprising phenomenon was the existence of only one mutant allele in a large number of autosomal recessive hearing impaired patients in several populations. The high proportion of 10-42% heterozygosity in deaf individuals could not be firmly explained /34/. This puzzle was at least partly solved with the discovery of a *GJB6* deletion in the Ashkenazi Jewish population, found on one allele in conjunction with a *GJB2* mutation /35/. The deletion was identified as 342 kb long, including the 5′-untranslated region of the *GJB6* gene and most of its coding region, but keeping the *GJB2* gene intact /34/. The deletion was found associated in *trans* with a single *GJB2* mutant allele in deaf patients. This was the missing link in around 50% of heterozygosity cases that until this point were unexplained. This deletion, named del(*GJB6*-D13S1830), was only rarely described in homozygosity /34-36/. The mechanism of a common effect between the two mutated alleles is still not clear. Some evidence supports a digenic pattern of inheritance /37/ but this hypothesis is put in doubt in light of the absence of autosomal recessive-associated *GJB6* point mutations. Another possibility is that the deletion eliminates an indispensable regulatory element for *GJB2* expression in the inner ear. Such a regulatory element has yet to be found.

IMPLICATIONS FOR GENETIC COUNSELING

In the past, a deaf individual approaching a genetic clinic would receive an empiric risk for reoccurrence, without any precise information related to the cause of HL. Today, advances in molecular biology and medical genetics have changed the picture dramatically. Much more is known about the etiology of HL and there are more possibilities available to define it. Since *GJB2* mutations are the most frequent cause of NSHL, genetic testing of *GJB2* and *GJB6* is offered to individuals either with or without a family history. It has been shown that it is more cost-effective to check for mutations in *GJB2* before proceeding through a full medical evaluation /38/. In cases where *GJB2* is not the causative mutation, additional genetic testing could be offered depending on the mode of inheritance, the nature of the HL, and the ethnic background of the family. Furthermore, the possibility of prenatal diagnosis is now a reality and can be an option for parents with hearing-impaired children, but it must be stressed that this is a very controversial topic. Moreover, a compelling question is whether deaf individuals and families are interested in genetic testing. This is one of the main questions asked by researchers in the field around the world.

In the Israeli population, a study on the interest and motivations of hearing parents of deaf children to choose genetic testing and prenatal diagnosis showed a very high interest (87%) in genetic testing among Jewish parents /39/. The study questioned parental reasons for their interest in genetic testing. The most important reasons in all groups were related to finding out the etiology of the HL. The parents expected that genetic testing would reduce the uncertainty related to the etiology of the HL in their own family. Reducing uncertainty was shown to be one of the most important roles of genetic counseling /40/ and predictive genetic testing for late onset disease /41/. Other reasons were different among the different Jewish religious sectors. For example, the possibility to utilize the test for matchmaking of hearing-impaired children and their hearing siblings was one of the most important reasons motivating the ultra-Orthodox to undertake the test, as opposed to reasons related to family planning and prenatal diagnosis, which were significantly less important in this group but high in the secular, non-religious group. The intention to utilize prenatal diagnosis was highly dependant on religious beliefs. This study demonstrates that genetic testing would be welcomed even by

communities that usually do not promote genetic counseling and testing, if it was offered in accordance with their cultural norms and beliefs.

Studies performed in the UK checked mainly adults who consider themselves part of the 'Deaf Culture'; this group views itself as a cultural group sharing the same language, identity and history. Most of the participants showed a negative attitude towards genetic testing, since they felt that it threatened their community and devalued them /42,43/. The interest in genetic testing was low and, moreover, some participants said they prefer a deaf child and would consider using prenatal genetic testing to terminate a pregnancy of a hearing fetus /43/. These studies and others show that genetic testing for deafness, though available, is a complex and sensitive issue that must be dealt with cautiously.

COCHLEAR IMPLANTATION AND CX26 MUTATIONS

Cochlear implantation is one of the most common treatments for patients with severe to profound HL. The outcome of this procedure, primarily auditory perception and speech discrimination, can vary between patients due to differences in age at implantation and on factors that influence speech perception, such as residual hearing. However, after taking these aspects into consideration, many differences in performance still remain unexplained. Psychophysiological tests can help to predict variance among users, but these tests are not always practical or reliable in very young children. It is possible that some of the differences can be explained by different etiologies of deafness. The high prevalence of *GJB2* mutations makes it possible to conduct studies that will include a large number of patients with a common etiology. Another motivation to compare the performance of Cx26-deafness patients with non-Cx26-deafness patients has to do with the nature of the ear defect. The cochlear implant sends electrical stimulus directly to the auditory nerve fibers and spiral ganglion cells and in that way bypasses the affected organ of Corti. Thus, better performance is expected when there is no neural damage. This is usually the case with sensorineural hereditary HL, which affects the cochlea and usually does not lead to neural damage or central damage to the auditory pathway. Several studies assessed speech perception of children with *GJB2*-related deafness after cochlear implantation, as

compared to a control group of *GJB2*-nonrelated deafness patients. Some studies reported better speech performance in cochlear implant patients with *GJB2*-related deafness /44-46/, and in two additional studies better results were found in the Cx26 group, but the study groups were very small /47,48/. In other studies there was no statistical difference between patients with and without *GJB2*-related HL /49-51/. It is important to point out that in the control groups the cause of deafness is not indicated and there is no uniformity within the control group and between control groups in different studies. Most likely the control groups include patients with other mutated genes, especially in participants who have hearing-impaired relatives. It is not surprising that in hereditary HL cases the central auditory pathway is substantially preserved, but the question remains whether the identity of the mutated genes has an influence on the efficacy of cochlear implants. A final consensus will have implications for counseling of pediatric patients with congenital sensorineural HL without other complications (e.g., developmental delay, inner ear malformations).

GENOTYPE-PHENOTYPE CORRELATION

There is a great deal of interest in exploring the influence of different mutations on levels and types of hearing loss, which may have implications for future treatments. Most cases of hearing impairment due to *GJB2* mutations are severe to profound, but it was observed in the clinic that some cases are mild to moderate. As a result, large-scale studies were initiated to assess the genotype-phenotype correlation of connexin mutations. The first systematic analysis indicating the influence of different *GJB2* mutations on the severity of deafness was published in 2004, showing that patients homozygous for the 35delG mutation possess the highest degree of HL compared to compound heterozygotes with only one 35delG allele, and individuals with two mutations other than 35delG possess an even lower degree of HL /52/. This study shows that, in general, inactivating mutations (frameshift and nonsense mutations) exhibit a more severe phenotype than non-inactivating (missense) mutations. Though most cases of Cx26-related deafness are severe to profound, *GJB2* mutations are involved in all severities of autosomal recessive NSHL, suggesting that a complete screening of *GJB2* mutations

should be suggested to nonsyndromic hearing-impaired patients regardless of severity. The exceptional cases that do not correlate with severe to profound degrees of Cx26-related deafness could be attributed to environmental influences and/or to the effect of modifier genes. The potential characterization of modifier genes will help to fine-tune the genotype-phenotype correlation and provide a more accurate prediction for the phenotype of *GJB2* mutations in the inner ear.

PATHOGENESIS OF MISSENSE MUTATIONS

The large number of missense mutations associated with connexin 26-deafness has remained a difficult enigma for genetic counseling, since it is not always trivial to interpret their pathogenicity. Mutations in different regions of the protein can result in different aberrations of the protein, depending on the importance of the region for protein folding, the proximity to a specific functional domain of the protein, or whether it is involved in protein-protein interaction points. In the case of connexin proteins, these influences are particularly problematic to predict since the three-dimensional (3D) structure of gap junction proteins is extremely difficult to solve by conventional methods. Recently, a computational study to explore the 3D structure of the transmembrane domains of gap junction proteins was partially completed. The first high-resolution computer model was published /53/ and provided us with a valuable tool to explore the important connection between structure and function in connexin proteins. Using this model, we can better understand why mutations in certain positions in the protein are polymorphic, while others lead to severe aberrations and various disease phenotypes. Furthermore, predicting the possible interactions between domains in the protein can shed light on the molecular basis of disease-causing mutations in connexins. The model predicts several interactions in the protein that are crucial for protein folding and assembly of functional units. Characterization of such interactions that are lost when the protein bears a disease-causing mutation provides us with a first glimpse of the consequences of the damage to the protein at the molecular level. This is the crucial first step towards attempting to repair the damage.

CONCLUSION

Exciting progress in the field of repair and regeneration was recently achieved in the Raphael laboratory, which has succeeded in regenerating hair cells in deaf cochleae of mature mammals using phenotypic transdifferentiation of non-sensory cells. Ears with new hair cells exhibited partial restoration of hearing /54/. Although many obstacles still remain, such as optimal vehicle delivery, access to the endolymph in the human inner ear and elucidation of long-term effects, this very promising step has generated optimism for the future.

The technology finally appears to be within our reach and for connexin 26, genetic repair holds great promise. Since Cx26 is the most dominant player in the etiology of hereditary HL, it is possible that the alteration of this one gene will provide a treatment for a large proportion of hearing impairment in the future.

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REFERENCES

1. Nadol JB Jr. Hearing loss. N Engl J Med 1993; 329: 1092-1102.
2. Petit C, Levilliers J, Hardelin JP. Molecular genetics of hearing loss. Annu Rev Genet 2001; 35: 589-646.
3. Denoyelle F, Marlin S, Weil D, Moatti L, Chauvin P, Garabedian EN, Petit C. Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexin-26 gene defect: implications for genetic counselling. Lancet 1999; 353: 1298-1303.
4. Sohl G, Willecke K. Gap junctions and the connexin protein family. Cardiovasc Res 2004; 62: 228-232.
5. Evans WH, Martin PE. Gap junctions: structure and function. Mol Membr Biol 2002; 19: 121-136.
6. Sun J, Ahmad S, Chen S, Tang W, Zhang Y, Chen P, Lin X. Cochlear gap junctions coassembled from Cx26 and 30 show faster intercellular Ca^{2+}

signaling than homomeric counterparts. Am J Physiol Cell Physiol 2005; 288: C613-623.
7. Bergoffen J, Scherer SS, Wang S, Scott MO, Bone LJ, Paul DL, Chen K, Lensch MW, Chance PF, Fischbeck KH. Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 1993; 262: 2039-2042.
8. Stojkovic T, Latour P, Vandenberghe A, Hurtevent JF, Vermersch P. Sensorineural deafness in X-linked Charcot-Marie-Tooth disease with connexin 32 mutation (R142Q). Neurology 1999; 52: 1010-1014.
9. Lee MJ, Nelson I, Houlden H, Sweeney MG, Hilton-Jones D, Blake J, Wood NW, Reilly MM. Six novel connexin32 (*GJB1*) mutations in X-linked Charcot-Marie-Tooth disease. J Neurol Neurosurg Psychiatry 2002; 73: 304-306.
10. Huehne K, Benes V, Thiel C, Kraus C, Kress W, Hoeltzenbein M, Ploner CJ, Kotzian J, Reis A, Rott HD, Rautenstrauss BW. Novel mutations in the Charcot-Marie-Tooth disease genes *PMP22*, *MPZ*, and *GJB1*. Hum Mutat 2003; 21: 100.
11. Kelsell DP, Dunlop J, Hodgins MB. Human diseases: clues to cracking the connexin code? Trends Cell Biol 2001; 11: 2-6.
12. Forge A, Becker D, Casalotti S, Edwards J, Evans WH, Lench N, Souter M. Gap junctions and connexin expression in the inner ear. Novartis Found Symp 1999; 219: 134-150.
13. Buniello A, Montanaro D, Volinia S, Gasparini P, Marigo V. An expression atlas of connexin genes in the mouse. Genomics 2004; 83: 812-820.
14. Forge A, Becker D, Casalotti S, Edwards J, Marziano N, Nevill G. Gap junctions in the inner ear: comparison of distribution patterns in different vertebrates and assessement of connexin composition in mammals. J Comp Neurol 2003; 467: 207-231.
15. Cohen-Salmon M, Maxeiner S, Kruger O, Theis M, Willecke K, Petit C. Expression of the connexin43- and connexin45-encoding genes in the developing and mature mouse inner ear. Cell Tissue Res 2004; 316: 15-22.
16. Kikuchi T, Kimura RS, Paul DL, Adams JC. Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. Anat Embryol (Berl) 1995; 191: 101-118.
17. Lautermann J, ten Cate WJ, Altenhoff P, Grummer R, Traub O, Frank H, Jahnke K, Winterhager E. Expression of the gap-junction connexins 26 and 30 in the rat cochlea. Cell Tissue Res 1998; 294: 415-420.
18. Ahmad S, Chen S, Sun J, Lin X. Connexins 26 and 30 are co-assembled to form gap junctions in the cochlea of mice. Biochem Biophys Res Commun 2003; 307: 362-368.
19. Teubner B, Michel V, Pesch J, Lautermann J, Cohen-Salmon M, Sohl G, Jahnke K, Winterhager E, Herberhold C, Hardelin JP, Petit C, Willecke K. Connexin30 (*GJB6*)-deficiency causes severe hearing impairment and lack of endocochlear potential. Hum Mol Genet 2003; 12: 13-21.
20. Cohen-Salmon M, Ott T, Michel V, Hardelin JP, Perfettini I, Eybalin M, Wu T, Marcus DC, Wangemann P, Willecke K, Petit C. Targeted ablation of

connexin26 in the inner ear epithelial gap junction network causes hearing impairment and cell death. Curr Biol 2002; 12: 1106-1111.

21. Zelante L, Gasparini P, Estivill X, Melchionda S, D'Agruma L, Govea N, Mila M, Monica MD, Lutfi J, Shohat M, Mansfield E, Delgrosso K, Rappaport E, Surrey S, Fortina P. Connexin26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. Hum Mol Genet 1997; 6: 1605-1609.
22. Kelley PM, Harris DJ, Comer BC, Askew JW, Fowler T, Smith SD, Kimberling WJ. Novel mutations in the connexin 26 gene (*GJB2*) that cause autosomal recessive (DFNB1) hearing loss. Am J Hum Genet 1998; 62: 792-799.
23. Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fisher R, Van Camp G, Berlin CI, Oddoux C, Ostrer H, Keats B, Friedman TB. Mutations in the connexin 26 gene (*GJB2*) among Ashkenazi Jews with nonsyndromic recessive deafness. N Engl J Med 1998; 339: 1500-1505.
24. Fuse Y, Doi K, Hasegawa T, Sugii A, Hibino H, Kubo T. Three novel connexin26 gene mutations in autosomal recessive non-syndromic deafness. NeuroReport 1999; 10: 1853-1857.
25. Gasparini P, Rabionet R, Barbujani G, Melchionda S, Petersen M, Brondum-Nielsen K, Metspalu A, Oitmaa E, Pisano M, Fortina P, Zelante L, Estivill X. High carrier frequency of the 35delG deafness mutation in European populations. Eur J Hum Genet 2000; 8: 19-23.
26. Estivill X, Fortina P, Surrey S, Rabionet R, Melchionda S, D'Agruma L, Mansfield E, Rappaport E, Govea N, Mila M, Zelante L, Gasparini P. Connexin-26 mutations in sporadic and inherited sensorineural deafness. Lancet 1998; 351: 394-398.
27. Antoniadi T, Rabionet R, Kroupis C, Aperis GA, Economides J, Petmezakis J, Economou-Petersen E, Estivill X, Petersen MB. High prevalence in the Greek population of the 35delG mutation in the connexin 26 gene causing prelingual deafness. Clin Genet 1999; 55: 381-382.
28. Sobe T, Erlich P, Berry A, Korostichevsky M, Vreugde S, Avraham KB, Bonne-Tamir B, Shohat M. High frequency of the deafness-associated 167delT mutation in the connexin 26 (*GJB2*) gene in Israeli Ashkenazim. Am J Med Genet 1999; 86: 499-500.
29. Lerer I, Sagi M, Malamud E, Levi H, Raas-Rothschild A, Abeliovich D. Contribution of connexin 26 mutations to nonsyndromic deafness in Ashkenazi patients and the variable phenotypic effect of the mutation 167delT. Am J Med Genet 2000; 95: 53-56.
30. Kudo T, Ikeda K, Kure S, Matsubara Y, Oshima T, Watanabe K, Kawase T, Narisawa K, Takasaka T. Novel mutations in the connexin 26 gene (*GJB2*) responsible for childhood deafness in the Japanese population. Am J Med Genet 2000; 90: 141-145.
31. Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ. Prevalent connexin 26 gene (*GJB2*) mutations in Japanese. J Med Genet 2000; 37: 41-43.

32. Guilford P, Ben Arab S, Blanchard S, Levilliers J, Weissenbach J, Belkahia A, Petit C. A non-syndrome form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. Nat Genet 1994; 6: 24-28.
33. Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Mueller RF, Leigh IM. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature 1997; 387: 80-83.
34. del Castillo I, Villamar M, Moreno-Pelayo MA, del Castillo FJ, Alvarez A, Telleria D, Menendez I, Moreno F. A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. N Engl J Med 2002; 346: 243-249.
35. Lerer I, Sagi M, Ben-Neriah Z, Wang T, Levi H, Abeliovich D. A deletion mutation in *GJB6* cooperating with a *GJB2* mutation in trans in non-syndromic deafness: a novel founder mutation in Ashkenazi Jews. Hum Mutat 2001; 18: 460.
36. Pallares-Ruiz N, Blanchet P, Mondain M, Claustres M, Roux AF. A large deletion including most of *GJB6* in recessive non syndromic deafness: a digenic effect? Eur J Hum Genet 2002; 10: 72-76.
37. del Castillo I, Moreno-Pelayo MA, del Castillo FJ, Brownstein Z, Marlin S, Adina Q, Cockburn DJ, Pandya A, Siemering KR, Chamberlin GP, Ballana E, Wuyts W, Maciel-Guerra AT, Alvarez A, Villamar M, Shohat M, Abeliovich D, Dahl HH, Estivill X, Gasparini P, Hutchin T, Nance WE, Sartorato EL, Smith RJ, Van Camp G, Avraham KB, Petit C, Moreno F. Prevalence and evolutionary origins of the del(GJB6-D13S1830) mutation in the DFNB1 locus in hearing-impaired subjects: a multicenter study. Am J Hum Genet 2003; 73: 1452-1458.
38. Preciado DA, Lim LH, Cohen AP, Madden C, Myer D, Ngo C, Bradshaw JK, Lawson L, Choo DI, Greinwald JH Jr. A diagnostic paradigm for childhood idiopathic sensorineural hearing loss. Otolaryngol Head Neck Surg 2004; 131: 804-809.
39. Dagan O, Hochner H, Levi H, Raas-Rothschild A, Sagi M. Genetic testing for hearing loss: different motivations for the same outcome. Am J Med Genet 2002; 113: 137-143.
40. Lippman-Hand A, Fraser FC. Genetic counseling: parents' responses to uncertainty. Birth Defects Orig Artic Ser 1979; 15: 325-339.
41. van der Steenstraten IM, Tibben A, Roos RA, van de Kamp JJ, Niermeijer MF. Predictive testing for Huntington disease: nonparticipants compared with participants in the Dutch program. Am J Hum Genet 1994; 55: 618-625.
42. Middleton A, Hewison J, Mueller RF. Attitudes of deaf adults toward genetic testing for hereditary deafness. Am J Hum Genet 1998; 63: 1175-1180.
43. Middleton A, Hewison J, Mueller R. Prenatal diagnosis for inherited deafness–what is the potential demand? J Genet Couns 2001; 10: 121-131.
44. Green GE, Scott DA, McDonald JM, Teagle HF, Tomblin BJ, Spencer LJ, Woodworth GG, Knutson JF, Gantz BJ, Sheffield VC, Smith RJ. Performance of cochlear implant recipients with *GJB2*-related deafness. Am J Med Genet 2002; 109: 167-170.

45. Bauer PW, Geers AE, Brenner C, Moog JS, Smith RJ. The effect of *GJB2* allele variants on performance after cochlear implantation. Laryngoscope 2003; 113: 2135-2140.
46. Sinnathuray AR, Toner JG, Geddis A, Clarke-Lyttle J, Patterson CC, Hughes AE. Auditory perception and speech discrimination after cochlear implantation in patients with connexin 26 (*GJB2*) gene-related deafness. Otol Neurotol 2004; 25: 930-934.
47. Fukushima K, Sugata K, Kasai N, Fukuda S, Nagayasu R, Toida N, Kimura N, Takishita T, Gunduz M, Nishizaki K. Better speech performance in cochlear implant patients with *GJB2*-related deafness. Int J Pediatr Otorhinolaryngol 2002; 62: 151-157.
48. Matsushiro N, Doi K, Fuse Y, Nagai K, Yamamoto K, Iwaki T, Kawashima T, Sawada A, Hibino H, Kubo T. Successful cochlear implantation in prelingual profound deafness resulting from the common 233delC mutation of the *GJB2* gene in the Japanese. Laryngoscope 2002; 112: 255-261.
49. Dahl HH, Wake M, Sarant J, Poulakis Z, Siemering K, Blamey P. Language and speech perception outcomes in hearing-impaired children with and without connexin 26 mutations. Audiol Neurootol 2003; 8: 263-268.
50. Cullen RD, Buchman CA, Brown CJ, Copeland BJ, Zdanski C, Pillsbury HC 3rd, Shores CG. Cochlear implantation for children with *GJB2*-related deafness. Laryngoscope 2004; 114: 1415-1419.
51. Taitelbaum-Swead RB, Brownstein Z, Muchnik C, Kishon-Rabin L, Kronnenberg J, Hildesheimer M, Avraham KB. Speech perception performance of implanted children with connexin-related deafness. J Basic Clin Physiol Pharmacol 2004; 15: 127 (Abst).
52. Cryns K, Orzan E, Murgia A, Huygen PL, Moreno F, del Castillo I, Chamberlin GP, Azaiez H, Prasad S, Cucci RA, Leonardi E, Snoeckx RL, Govaerts PJ, Van de Heyning PH, Van de Heyning CM, Smith RJ, Van Camp G. A genotype-phenotype correlation for *GJB2* (connexin 26) deafness. J Med Genet 2004; 41: 147-154.
53. Fleishman SJ, Unger VM, Yeager M, Ben-Tal N. A Calpha model for the transmembrane alpha helices of gap junction intercellular channels. Mol Cell 2004; 15: 879-888.
54. Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, Brough DE, Raphael Y. Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nature Med 2005; 11: 271-276.
55. Ahituv N, Avraham KB. Mouse models for human deafness: current tools for new fashions. Trends Mol Med 2002; 8: 447-451.

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