Functional analysis of human Cx26 mutations associated with deafness

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Abstract

Mutations in the connexin26 (Cx26) gene are not only a major cause of nonsyndromic deafness, but can also cause syndromic forms of hearing loss that are associated with palmoplantar keratoderma (PPK, i.e., Vohwinkel’s syndrome). It is not clear how two very distinct pathologies can arise from different mutations within the same connexin gene. This review summarizes the available data on wildtype and mutant Cx26 channel behavior that has been obtained in the paired Xenopus oocyte assay. These results suggest that dominant and recessive loss of function mutations in Cx26 can cause nonsyndromic deafness, but cannot easily explain the syndromic forms exhibiting PPK. Dominant Cx26 mutations that can cause both PPK and deafness must show some additional alteration of function beyond a simple inhibition of Cx26 activity.

Autosomal recessive (DFNB1) and autosomal dominant (DFNA3) forms of genetic deafness have been associated with coding region mutations in Connexin26 (Cx26) [9,21,6,10,3,12,8]. Cx26 is a member of the connixin family of gap junction proteins, which facilitate intercellular communication by encoding channels that directly link the cytoplasm of adjacent cells [1]. Intercellular channels are structurally more complex than other ion channels, because a complete cell-to-cell channel spans two plasma membranes and results from the association of two half channels, or connexons, contributed separately by each of the two participating cells. Each connexon, in turn, is a hexameric assembly of connexin subunits. Thus, connexin mutations could cause hearing loss by several mechanisms, including interference with the proper oligomerization or intracellular transport of connexons, impairment of interactions between connexons in opposing cells, or the formation of structurally complete channels with altered permeation or gating properties.

The most frequent recessive Cx26 mutation is a single base deletion 35delG that results in a frameshift at the 12th amino acid and premature termination of the protein. Other common recessive Cx26 mutations include nonsense mutations and small deletions/insertions, most of which also lead to premature termination of protein translation. Therefore, most of the frequently observed recessive mutations result in severely truncated connexin proteins that are unlikely to retain any channel activity and recessive hearing loss may largely be a result of null mutations in Cx26. In contrast, the role of Cx26 mutations in dominant forms of deafness is less obvious. Currently three different missense mutations have been associated with dominant deafness, M34T, R75W, and W44C, which are similar in that they all encode full length products containing non-conservative amino acid substitutions [9,5,13].

The functional consequences of missense Cx26 mutations associated with deafness have been explored by comparing the channel activity of wildtype and mutant proteins using an in vitro expression system composed of paired Xenopus oocytes [17]. In this assay, RNAs encoding connexins are transcribed in vitro and microinjected into single oocytes. The injected cells are then brought into physical contact to form pairs, providing a cell-to-cell interface where intercellular channels can form. Each cell in a pair is then impaled with current and voltage electrodes so that intercellular channel activity between the cells can be precisely quantified using the dual voltage clamp technique [16]. Before pairing, oocytes can be injected either with a single connexin RNA species, or with an equimolar mixture of two connexin RNAs, so that either recessive connexin mutations, or interactions between dominant mutants and wildtype connexins can be studied in this system.

In one such investigation, dominant deafness and a skin disorder, palmoplantar keratodema (PPK), segregated with a heterozygous missense mutation of Cx26 causing the
non-conservative amino acid substitution R75W [13]. This mutation is located at the junction between the first extra-cellular and second transmembrane domain of Cx26. The sequences of both domains are highly conserved between all connexins and arginine is invariably present at residue 75, suggesting a common functional requirement at this position [13,18]. The dominant inhibitory effect of the R75W variant on channel function was demonstrated using the paired oocyte expression system. In this study, wild-type Cx26 was able to form intercellular channels, as evidenced by the induction of robust electrical conductance between paired oocytes. In contrast, oocyte pairs injected with only R75W were not electrically coupled, demonstrating that R75W lacked channel activity. Moreover, the heterozygous R75W mutation identified in a pedigree with autosomal dominant deafness had a deleterious effect on the function of wildtype Cx26, inhibiting intercellular conductance of wildtype Cx26 by >99% when co-expressed at an equal ratio of wildtype to mutant connexin. These results provided compelling evidence for a functional dominant-negative effect of the R75W mutant, and implicated heterozygous Cx26 mutations in the etiology of autosomal dominant deafness.

For comparison, Richard et al. [13], also tested a second mutation that has been reported to segregate with autosomal recessive deafness, and which produced a non-conservative amino acid change in a neighboring residue, W77R [3]. Like R75W, the W77R mutant failed to induce intercellular channel activity when expressed alone. Unlike R75W, W77R did not significantly inhibit the ability of wildtype Cx26 to form functional channels when co-expressed [13]. These results demonstrated that homozygous W77R mutations of Cx26 resulted in a complete loss of connexin function consistent with its recessive pattern of inheritance. The W77R mutation in a heterozygous state however, did not significantly interfere with the channel activity of coexpressed wildtype Cx26, suggesting that the disease phenotype only results when wildtype Cx26 activity is completely absent. These functional results corresponded with the clinical presence of deafness only in individuals homozygous for W77R, or individuals who were compound heterozygotes of W77R and 35delG [3].

Another Cx26 point mutation (W44C) has recently been found in the original pedigree used to map autosomal dominant deafness near the Cx26 locus [4]. All 10 of the deaf individuals, but none of the 17 normal hearing individuals within the large kindred were heterozygous for the W44C allele [5]. While the functional consequences of the W44C mutation remain to be tested, its identification in this relatively large pedigree support the identification of Cx26 mutations as underlying both dominant and recessive forms of deafness.

In contrast to the functional evidence discussed above, the role of the first Cx26 mutation associated with dominant deafness has been more enigmatic. Kelsell et al. [9] originally reported a heterozygous point mutation (M34T) in a family with dominant deafness and PPK and proposed that the mutant allele inhibited the activity of the wildtype Cx26 allele [9]. This hypothesis was subsequently supported by functional expression studies. The M34T variant, which did not form functional channels when expressed alone, also acted as a dominant inhibitor of wildtype Cx26 channel activity when co-expressed in pairs of Xenopus oocytes [19]. However, the ability of the M34T variant to cause dominant deafness has been brought into question by the frequent finding of heterozygous M34T mutations in individuals with normal hearing [6,10,14]. In light of the in vitro data demonstrating dominant inhibition, it is difficult to explain why heterozygous carriers of the M34T exhibit normal hearing. One possible mechanism would be second-site mutations that silence the allele, and analysis of M34T allelic expression in carriers with normal hearing would help to resolve this issue. Alternatively, it is possible that M34T only causes deafness when associated with a second connexin mutation in Cx26 or Cx31, which is also expressed in the cochlea. Mutations in the human Cx31 gene have been associated with high frequency hearing loss [20]. Re-screening the original M34T deafness pedigree for additional connexin mutations could examine this possibility.

It is also worth considering that the Xenopus oocyte expression system may not exactly mimic the cellular conditions in the supporting epithelial cells of the cochlea where Cx26 is highly expressed [11], and that the dominant negative activity of M34T may be restricted to the oocyte cellular environment. Differences in the intracellular trafficking behavior of Cx32 mutations associated with X-linked Charcot–Marie–Tooth disease have been reported, depending on whether the mutant protein was exogenously expressed in Xenopus oocytes or mammalian cells [2,7]. Functional analysis of the M34T mutant in transfected mammalian cells would clarify this possibility. Regardless of its role in dominant deafness, the M34T mutant has been identified as contributing to recessive deafness in compound heterozygotes [10] consistent with the functional data demonstrating loss of channel activity.

In conclusion, analysis of the functional consequences of mutations in Cx26 strongly suggest that complete loss of channel activity leads to deafness. While the precise role of Cx26 in the etiology of deafness is not known, it is likely that junctional communication influences the ionic environment of inner ear sensory epithelia. Cells coupled by gap junctions in the mammalian cochlea fall into two groups, the non-sensory epithelial cells among which hair cells are dispersed, and the connective tissue cells at locations more distal to the hair cells. Cx26 expression is abundant in both groups of cells, and it has been proposed that serially arranged gap junctions of epithelial and connective tissue cells serve as a mechanism for recycling potassium ions that pass through sensory cells during auditory transduction [11]. A conceptually similar role for junctional communication has been proposed in the spatial
buffering of potassium by astrocytes in the central nervous system [15]. Further evaluation of this model in terms of nonsyndromic deafness awaits the development of transgenic animals expressing dominant Cx26 mutations.

References