Connexinopathies: a structural and functional glimpse

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Abstract

Mutations in human connexin (Cx) genes have been related to diseases, which we termed connexinopathies. Such hereditary disorders include nonsyndromic or syndromic deafness (Cx26, Cx30), Charcot Marie Tooth disease (Cx32), occludentodigital dysplasia and cardiopathies (Cx43), and cataracts (Cx46, Cx50). Despite the clinical phenotypes of connexinopathies have been well documented, their pathogenic molecular determinants remain elusive. The purpose of this work is to identify common/uncommon patterns in channels function among Cx mutations linked to human diseases. To this end, we compiled and discussed the effect of mutations associated to Cx26, Cx32, Cx43, and Cx50 over gap junction channels and hemichannels, highlighting the function of the structural channel domains in which mutations are located and their possible role affecting oligomerization, gating and perm-selectivity processes.

Keywords: Connexins, hemichannels, gap junction channels, structure and function, human genetic disease

Background

Connexin gap junction channels (GJCs) and hemichannels (HCs) are critical for cellular communication. GJCs allow the intercellular exchange of ions and small molecules (e.g., IP3, cAMP, cGMP, ATP) and diverse metabolites (e.g., sugars, amino acids, glutathione) (reviewed in [1]). The same molecules and ions can pass through HCs, but in this case to take part as autocrine and paracrine signals (reviewed by [2, 3]). Mutations in connexins (Cx) genes are associated to genetic disorders such as skin abnormalities, cardiopathies, neurodegenerative and developmental diseases, cataracts, and most cases of hereditary deafness (reviewed by [4–6]).

Each HC is formed by the oligomerization of six Cxs subunits and the end-to-end docking of two HCs forms GJCs. The membrane topology of Cxs includes four transmembrane domains (designated as TM1-TM4) connected by two extracellular loops (ECL) and one intracellular loop (ICL). The amino terminus (NT) and the carboxyl terminus (CT) segments are cytoplasmic (Fig. 1a). Despite Cxs share high homology, there are important differences in the amino acid sequence of the ICL and CT. These segments contain motifs for regulatory kinases and cytoskeletal binding proteins [7, 8]. Oligomerization between suited isoforms also contributes to the assortment of Cx-based channels; for instance, heteromeric GJCs (HCs constituted by more than one Cxs type) and/or heterotypic channels (two homomorphic HCs each made by a different Cxs type). These combinations may produce GJCs with particular functional and regulatory properties. Several works pointed out to TM3 in Cx32 [9–11] and Cx43 [12], and TM1 and NT segments in Cx26 [12, 13] as critical to regulate oligomerization of Cxs. In addition, a salt bridge between residues Glu-146 (TM3) and Arg-32 (TM1) in
Cx32; and between Lys-22 (TM1) and Glu-209 (TM4) in Cx26, might sustain intraprotomer stability [14]. Nevertheless, the crystal structure of Cx26 showed that the main interactions between protomers occur at the extracellular side of the TM2 and TM4. Moreover, an aromatic cluster formed by the extracellular loops and TM3 also participates in inter-protomer interaction [15]. However, the oligomerization compatibility between Cxs has been associated to specific residues in the NT region [13, 16].

Cxs oligomerize to form a pore whose narrowest part is observed at the ECLs, near the docking zone [15, 17]. As mentioned above, the differences in the amino acid sequences among Cxs may influence the channel properties. It has been proposed that the membrane-spanning regions of Cxs are not only important for intra- and inter-protomer interactions, but they also might determine functional properties such as gating, permeability and the pore’s structure. Concerning the pore composition, there is some controversy about which TMs domains are involved. Some works pointed out the TM3 in Cx32 channels [11, 18] and TM1 in Cx46 channels [19, 20] as principal pore helix components. In support of the role of TM1 as a pore lining segment, it has been proposed that the voltage dependent loop-gating mechanism in the Cx32*Cx43 EL1 chimera (in which the ECL1 of Cx43 replaced the ECL1 of Cx32), involves a rotation of TM1 together with an inward tilt of the six protomers [21]. The 3.5 Å resolution of the Cx26 crystal structure revealed that TM1 is the main constituent of the pore [15] (Fig. 1). The structure also showed that the TM2 lines the pore but in a minor extent, whereas TM3 and TM4 face the hydrophobic membrane environment. The TM1 is tilted, which narrow the pore diameter to 14 Å from the cytoplasmic to the extracellular side of the membrane [15]. More recently, performing molecular dynamic refinements of the crystal structure of Cx26, Kwon and co-workers (2011) [22], shown that the narrow part of the pore could be even smaller.

As it was proposed previously for Cx32 [23] and then confirmed by Maeda and co-workers for Cx26 [15], the Cx-NT domain is located inside the pore, facing the TM1s and forming a funnel like structure that might restricts the pore diameter during gating process [15]. The intra-pore stabilization of the NT is achieved by

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**Fig. 1** Localization of loss-of-function mutations for Cx26 GJC. a Cartoon representation of a Cx26 monomer, colored with a blue-green gradient from the N- to the CT region. Localization of loss-of-function mutations are colored in red. b Lateral (c) Top (d) Bottom view of the same subunit of (a), in the context of the HC assemble. The HC surface is transparent and white. The figure was generated with PyMol and edited with Gimp.
hydrophobic interactions between residues Trp-3 (NT) and Met-34 (TM1) from neighboring protomers [15]. This interaction was previously proposed by Oshima and co-workers (2007), which found a prominent pore electron-density in the middle of the pore generated by the deafness mutant Cx26M34A. A reduction of this pore electron-density was observed when residues 2-7 (Cx26M34A-del2–7) were deleted [24], confirming the NT as major contributor to the pore occlusion.

Experiments using a chimeric HC of Cx32∗Cx43ECL1, have provided more insight about the gating-mechanism of Cx-based channels [21]. In this chimera, the Cys substitution of the Ala residues in positions 40 and 43, located at the TM1/E1 border, form disulphide bonds with adjacent protomers when the cells are bathed in solutions expected to keep HCs closed (5 mM Ca²⁺ or 10 µM Cd²⁺). These results strongly support a role for these residues in the “loop-gating” mechanism and extracellular Ca²⁺ regulation of HCs [21, 25].

**GJCs and HCs gating regulation**

How gating and permeability are regulated in Cxs- based channels is a matter of intense debate. To date, three types of gating mechanism have been proposed: 1) **The NT as a voltage-sensor domain**: that plugs the channel vestibule and contribute to the fast or V₀-gating [15, 26], 2) **The Loop gating**: in which extracellular divalent cations (p.g., Ca²⁺) binds to the extracellular loops and blocks HCs by stabilizing the closed loop gate conformation [25, 27], and 3) **The ball-and-chain model**: which proposes that the CT as part of a ball-and-chain mechanism to regulate the gating of HCs. The last mechanism involves CT conformational rearrangements elicited by voltage or chemical (pH, redox, phosphorylation) stimuli, which promote a link between this segment and the ICL, and regulates the fast V₀-gating mechanism [28–33]. This interaction requires the formation of alpha helical structures on the ICL peptide, in which the CT binds upon intracellular acidification [30].

Considering the relevance of the aforementioned mechanisms for channel function, it is critical to understand how Cxs mutations linked to diseases impair these processes. In the next sections, we describe genetic diseases associated to four Cxs that we used as models for the purpose of this review. For space reason, we did not include information about other important Cxs with mutations associated to disease, like Cx46 mutations linked to congenital cataracts [6, 34] or increased risk to developing diseases, like in polymorphisms in Cx37 genes associated to cardiovascular diseases [35].

**Disease associated to Cx26 mutations**

Genetic sensorineural hearing loss is associated mainly to mutations in Cx26 [36] (Table 1). Two clinical phenotypes derive from Cx26 mutations: 1) non-syndromic deafness, in which patients evince moderated to severe deafness with absence of other pathological manifestation; and 2) syndromic deafness, in which profound sensorineural hearing loss is accompanied by a range of severe tissue defects such as the observed in palmoplantar keratoderma [37, 38], keratitis ichthyosis deafness syndrome (KID) [39–42], Vohwinkel syndrome [43], histrix-like ichthyosis with deafness syndrome and Bart-Pumphrey syndrome [44, 45].

Among the attempts to identify the pathogenic mechanism of KID syndrome, two transgenic animal models have been developed. They express the Cx26S17F and Cx26G45E mutations in the skin and/or cochlea [46, 47] and exhibit similar phenotypes than humans. Experimental results strongly support that the possible mechanisms in the skin might include the impairment of the epidermal calcium homeostasis and the disruption of the water barrier due to abnormal lipid composition of the stratum corneum [48]. For hearing loss, several hypotheses have been proposed. They include loss of Ca²⁺ homeostasis and ATP release [49, 50], impaired permeability to Ins(1,3,4) P3 [51], loss of the endocochlear potential by deficient K⁺ recycling between the epithelial GJ network and the stria vascularis [52], and developmental malformation or cochlear degeneration induced by massive cell death [53, 54]. For comprehensive reviews see [4, 55].

**Disease associated to Cx32 mutations**

Cx32 is expressed in several cell types, including the myelin-forming cells in both the peripheral and central nervous systems (CNS); the Schwann cells and oligodendrocytes, respectively. Mutations in this protein are associated to the most common X-linked inheritance form of the Charcot-Marie-Tooth disease (CMT), a pathology referred as CMT1X that accounts for the 10 % of all the CMT cases [56–58]. Due to its X-linkage, males display moderate to severe symptoms [59, 60], while milder phenotypes are observed in heterozygous females [61, 62].

In the peripheral nervous system, mutations in Cx32 induce progressive muscular atrophy and variable sensory loss, symptoms associated to slow axonal conduction and distal axonal loss [63]. However, prolonged central conductions times of sensory inputs also arise as consequence of Cx32 missense mutations [64–66].

Cx32 localizes in the axonal paranodes and Schmidt-Lantermann incisures [67–69] of the peripheral nerves. Hence, GJC made by this protein do not connect adjacent cells but contiguous loops of non-compact myelin. These channels likely act as a preferential diffusion pathway, significantly decreasing the distance between the nucleus and the adaxonal membrane of the myelin sheaths [67, 70].
Table 1 Effect of mutations in Cx26 (GJB2) on the functional state of HCs and GJCs evaluated in a heterologous expression system, the domain that is affected and its phenotype

| Domain | Mutation                         | GJCs Function | HCs Function | Deafness Phenotype       |
|--------|----------------------------------|---------------|--------------|-------------------------|
| NT     | M1V, T8M, G12V [13, 123, 132–136] | (−)           | n.d.         | NS, Profound, Moderate  |
|        |                                  |               |              |                         |
|        | G11E [130, 136, 137]             | n.d.          | (+)          | S, Profound; KID        |
|        | G12R<sup>+</sup>, N14K [13, 123, 136, 138] | (−) | (+) | S, Mild, Severe; KID/EKV |
|        | N14D [139]                       | n.d.          | (−)          | NS, Moderate            |
|        | N14Y<sup>+</sup> [13, 39, 136]   | (−)           | (+)          | S, Profound; KID        |
|        | S17F<sup>+</sup> [13, 40, 123, 136] | (−) | (−) | NS, SNHL; KID          |
| TM1    | V27I [140]                       | Normal        | Normal       | NS, HL and Normal       |
|        |                                  | (−)           | n.d.         | NS, Severe to Profound  |
|        | M34T [36, 115, 142–146]          | (−)           | (−)          | NS, Mild to Moderate; S, Profound; PPK |
|        | V37I, A40G [113, 143, 147–149]   | (−)           | (−)          | NS, Mild-Moderate, Severe |
|        | A40V [124, 136, 150, 151]        | Normal        | (−)          | S, Profound; KID        |
| ECL1   | DelE42, D66H [152–159]           | (−)           | n.d.         | S, Profound, Moderate to Profound; PPK |
|        | W44C, W44S, D46E, T55N [142, 143, 152, 153, 159–163] | (−) | (−) | NS, Severe to Profound, HL, Moderate, Severe |
|        |                                  | Normal        | (+)          | S, Profound; KID        |
|        |                                  | (−)           | (−)          | NS, Severe to Profound  |
|        |                                  | n.d.          | (+)          | S, Profound; KID        |
|        |                                  | (−)           | (+)          | S, Profound; KID        |
|        |                                  | n.d.          | (−)          | NS, Profound            |
|        |                                  | (−)           | (−)          | S, Severe to Profound; PPK |
| TM2    | W77R, F83L, L90V, V95M [37, 135, 142, 143, 147, 173, 175, 176] | (−) | n.d. | NS, Moderate to Profound, Moderate, Profound |
|        |                                  | n.d.          | (−)          | NS, Profound            |
|        |                                  | Normal/No IP3 transfer | (−) | (−) | NS, Profound, Moderate to Profound, Mild to Moderate |
|        |                                  | n.d.          | (−)          | S, Severe to Profound; KID |
|        |                                  | n.d.          | (−)          | NS, Severe to Profound, Profound |
|        |                                  | n.d.          | (−)          | NS, Severe to Profound |
| ICL    | E114G, R127H [115, 140, 144, 147, 173, 178, 182, 183] | (−) | (−) | NS, Severe to Profound, Profound |
|        | DeElE120 [141, 143, 147]         | (−)           | n.d.         | NS, Severe to Profound  |
| TM3    | R143Q, R153 [133, 152, 153, 183, 184] | (−) | n.d. | NS, Profound            |
|        | R143W [133, 144, 178, 185, 186]  | (−)           | n.d.         | NS, Profound            |
| ECL2   | F161S, P173R, D179N, R165W, R184P, R184Q [132, 141, 143, 147, 152, 153, 187–190] | (−) | n.d. | NS, HL, Severe to Profound, Profound |
|        | M163L [191]                      | n.d.          | (+)          | NS, Moderate to Profound |
|        |                                  | (−)           | (−)          | NS, Moderate to Profound |
|        |                                  | n.d.          | (+)          | S, High Frequency HL; PPK |
| TM4    | M195T, A197S,206S, L214P [133, 135, 190, 193, 194] | (−) | n.d. | NS, HL, Moderate, Profound |
|        |                                  | n.d.          | (−)          | NS, Mild to Moderate    |
|        |                                  | (−)           | (−)          | NS, HL, Profound        |

NS: Non-syndromic; S: Syndromic; KID: Keratitis-Ichthyosis-Deafness; EKV: Erythrokeratodermia variabilis; PPK: Palmoplantar Keratoderma-deafness; HL: Hearing loss. 

(+*) = Generate gain of HC function when they are coexpressed with wild type Cx26 or Cx43 [13]  
(−) = Loss of function. (+) = Gain of function. n.d. = not determined
The peripheral pathological mechanisms associated to Cx32 mutations possibly involve the loss of function of the GJC (Table 2), either by intracellular retention or the production of channels with aberrant properties [70–72]. This lack of functionality might reduce the transfer of signaling molecules, like cAMP, between the adaxonal portions and the nucleus of the Schwann cell [73].

Furthermore, at least some effects of Cx32 mutations have been associated to a gain of function of the GJC (Table 2). Nevertheless, this is based on indirect electrophysiological studies performed in two patients who do not express Cx32; these patients display visual and auditory evoked responses with normal central conduction times [74, 75]. However, the absence of central functional disruptions in most CMT1X patients and Cx32-KO animals supports the hypothesis of gain of function of GJC in patients where disease also affects CNS [61, 76, 77]. However, further studies about the functional properties of the Cx32 channels are required to support these hypothesis.

### Disease associated to Cx43 mutations

Oculodentodigital Dysplasia (ODDD) is the most important human disease related to Cx43 mutations (Table 3). ODDD is an autosomal inherited developmental disorder affecting face, eyes, teeth and limbs (reviewed in [1, 78]). This pathology was linked to a germ line Cx43 gene (GJA1) mutation [79]. The phenotype varies from syndactyli type III alone, to ODDD without syndactyli [80, 81], camptodactyli [79], cardiac impairments, mild cognitive retardation [82] and skeletal abnormalities, which could be associated to impaired osteoblast differentiation [83].

Currently, over 74 mutations related with ODDD have been reported. However, less than a half of these mutations have been characterized. Missense mutations of

### Table 2

| Domain | Mutation | GJC Function | HCs Function | CMTX Phenotype |
|--------|----------|--------------|--------------|----------------|
| NT     | W3A, W3S, W3Y, G12S, W13L, V13L, R15W, R22G, R22X [127, 197–203] | (−) | n.d. | Mild to Severe, Severe, Mild to Moderate, Not described |
| TM1    | S26L, M34K, A39V, A40V [70, 71, 204, 205] | (−) | n.d. | Mild, Not Described |
| TM1    | M34T, V35M, V38M [70, 205, 206] | (−) | n.d. | Mild to Moderate, Severe |
| ECL1   | G45E [207] | n.d. | (+) | Not Described |
| ECL1   | C53S, C60F, Y65C, R75P [203, 205, 208–210] | (−) | n.d. | Not Described |
| TM2    | S85C [127, 211] | n.d. | (+) | Severe, Mild |
| TM2    | T86A, T86S, T86N, T87A [70, 212] | (−) | (−) | Not Described, Mild |
| TM2    | H94Y, H94Q [127, 206] | (−) | n.d. | Mild to Moderate |
| TM2    | M93V, V95M [203, 204, 206] | (−) | n.d. | Not Described, Mild to Moderate |
| ICL    | E102G, Null111-116 [71, 198, 202, 213] | (−) | n.d. | Mild, Mild to Moderate |
| ICL    | R107W, R129H [203, 214] | (−) | n.d. | Mild to Moderate, Not described |
| TM3    | V139M, V140E, R142W [127, 197, 209, 215–219] | (−) | n.d. | Mild to Moderate, Mild to Severe, Moderate to Severe |
| ECL2   | L143P, L156R [203, 218] | (−) | n.d. | Mild to Moderate |
| ECL2   | R164Q, V181A, E186K [197, 204, 206, 213, 214, 216, 219] | (−) | n.d. | Moderate to Severe |
| ECL2   | R164W, P172R, S182T, R183H [72, 198, 203, 204, 206, 208] | (−) | n.d. | Mild to Moderate, Not described |
| TM4    | G199R, R203C, N205I [203, 205, 206, 214] | (−) | n.d. | Moderate to Severe, Not described |
| TM4    | E208K, R208K [197, 202, 203, 216, 220, 221] | (−) | (−) | Moderate to Severe |
| TM4    | Y211X [203, 222] | (−) | n.d. | Severe |
| CT     | R215W [206, 209, 221] | (−) | (−) | Mild to Moderate |
| CT     | C217X [198, 220] | n.d. | (−) | Severe |
| CT     | R220X [71, 197, 198, 206, 220] | (−) | n.d. | Moderate to Severe |
| CT     | F235C [126] | (−) | (+) | Severe |
| CT     | R265X [198] | (−) | (−) | Severe |

(−) = Loss of function. (+) = Gain of function. n.d. = not determined
CX43 associated to ODDD are spread through CX43 amino acid sequence without a clear pattern (Table 3). However, most mutations concentrate in the first half of the protein, with few localized at the CT region (Table 3). The CT domain has several residues that may be phosphorylated, and these allow the regulation of processes like communication, trafficking to the plasma membrane and assembly and degradation of the gap junction protein [84]. The CT also interacts with the ZO-1 [85], v-Scr [86] and other proteins, including cytoskeletal proteins [87].

Several mutations associated to ODDD are located in the ICL region of CX43 (Table 3), demonstrating the importance of this domain for CX43 based channels functionality. ICL is critical for both, the pH-mediated gating and the architecture of the channel pore [88]. For example, the ODDD mutant CX43G138R, which is located in this domain, results in unfunctional GJCs when expressed in N2A cells [89–92]. In contrast, the mutation increases the HC activity determined by ATP release measurements [91]. Moreover, a mouse model carrying this mutation (CX43G138R) exhibits a phenotype that resembled the observed in humans, i.e., craniofacial alterations, bilateral syndactyli, smaller teeth (microdontia), unspcialized enamel hypoplasia, osteopenia and sparse hair [93].

A principal role of CX43 GJCs in the myocardium is to allow a rapid and coordinated electrical excitation important for the cardiac-action potential propagation. CX43 is mainly located at the intercalated discs in the ventricular myocardium. The geometrical arrangement of the discs, as well as the total number of GJCs, seems to be determinant for the characteristic anisotropic conduction of the ventricular myocardium. The atrial myocardium expresses high levels of CX43 and CX40 in addition to small quantities of CX45 [94]. In addition, it has been reported that cells forming the conduction system (responsible for rapid electrical signal localization from the sinoatrial node to the ventricles), express CX43, CX45, CX40, and CX30 [95, 96]. However, patients with mutation in CX43 rarely exhibit cardiac problems (Table 3). In addition, congenital heart diseases are not commonly associated to CX43 mutations [97]. Until now, only a few cases of CX43 mutations linked to heart diseases have been reported. For example Ser364Pro, which results in viscera atrial heterotaxia [98] restrict GJCs communication in transfected cells. A subsequent work of Thibodeau et al. [99] showed a frameshift mutation in a patient with atrial fibrillation. This modification involves a single nucleotide deletion (c.932delC) with 36 aberrant amino acids with a consecutive stop codon. Interestingly, the mutation was absent in peripheral blood lymphocytes and the

| Domain | Mutant | GJCs Function | HC Function | Phenotype |
|--------|--------|---------------|-------------|-----------|
| NT     | G2V, D3N, W4A, L7V, L11P, S18P [79, 92, 223–225] | (–) | n.d. | ODDD |
|        | G12R, Y17S [79, 90, 92, 223, 226–228] | (–) | (–) | ODDD |
| TM1    | I31M [91, 229] | (–) | (+) | ODDD |
|        | R33X [81, 230] | (–) | n.d. | Small deep-set eyes, syndactyli, dental abnormalities |
| ECL1   | A40V, L90V, F52dup [79, 226, 227, 229, 231] | (–) | (–) | ODDD |
|        | E42K [232, 233] | (–) | n.d. | Sudden infant death, lethal ventricular arrhythmias |
|        | Q49K [79, 227, 231, 234] | (–) | n.d. | ODDD |
|        | S69P [235] | (–) | n.d. | Nonsyndromic Hearing Loss |
|        | R76H [230, 236] | (–) | n.d. | Hallermann-Streiff syndrome: small stature, hypotrichosis, teeth and skeletal abnormalities |
| ICL    | I13OT [79, 89, 226, 227] | (–) | (–) | ODDD |
|        | K134E, T154A [89, 226, 236–239] | (–) | n.d. | ODDD |
|        | G138R, G143S [79, 89–92] | (–) | (+) | ODDD |
|        | H194P [80, 91] | (–) | Normal | ODDD |
| ECL2   | R202H, V216L [79, 92, 226, 228, 229, 231] | (–) | n.d. | ODDD |
| TM4    | F5230, F5260 [92, 240] | (–) | n.d. | ODDD |
|        | S272P [232] | (–) | Normal | ODDD |
|        | S272P [232] | (–) | Normal | Sudden infant death |
| CT     | T326I [235] | (–) | (–) | ODDD |
|        | S364P [98, 241] | (–) | n.d. | Viscero-atrial heterotaxia/heart malformations |

(–) = Loss of function. (+) = Gain of function. n.d. = not determined

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immunohistological analysis from left atrial tissue showed areas with normal GJCs localization but at the same time, areas with predominant intracellular retention of Cx.

Disease associated to Cx50 mutations
Fibers and epithelial cells in the eye lens are connected through Cx50 GJCs [100–102]. This communication is required to maintain the ionic conditions necessary to avoid the formation of cataract [103], a pathology resulting in the opacity of the lens, restricting the amount of light reaching the retina. The Cx50 mutations (Table 4) have been identified in members of families with inherited cataracts. The phenotype may vary across patients, in which missense locations and frame shifts have been commonly identified (reviewed in [6]).

All Cx50 mutations produce loss of function GJCs, except G46V that produce gain of function GJCs [104]. These mutations could generate both, mislocalization and impaired function of GJCs and HC (e.g., gating or charge selectivity) [105–107]. At cellular level, it is possible that Cx50 mutations affect the intercellular communication mediated by heteromeric Cx46-Cx50 GJCs. This idea is based on results demonstrating that these Cxs co-localize at GJCs plaques [108–110]. The defective GJCs activity could alter the solute transport between cells and disrupt the Ca²⁺ homeostasis [111, 112]. The abnormal ion transport, especially Na⁺ ions, causes lens swelling and ameliorates the fluid circulation inside the structure. These abnormal processes might affect the nutrient transport and the clearance of noxious metabolites, triggering the cataract formation [112].

**Table 4** Effect of mutations in Cx50 (GJA8) on the functional state of HCs and GJCs evaluated in a heterologous expression system, the domain that is affected and its phenotype

| Domain | Mutation | GJCs Function | HCs Function | Cataract Phenotype |
|--------|----------|---------------|--------------|-------------------|
| NT     | R23T [242] | (-)           | n.d.         | Bilateral nuclear |
| TM1/ECL1 | V44A [243] | n.d.          | (-)          | Suture-sparing nuclear |
|        | V44E [110] | (-)           | n.d.         | Whole lens |
| ECL1   | W45S [106, 244] | (-) | (-) | Jellyfish-like appearance, Micro cornea |
|        | G46V [104, 106] | (+) | (+) | Total |
|        | D47N [110, 117] | (-) | n.d. | Nuclear Pulverulent |
|        | E48K [116, 245] | (-) | Normal | Zonular Nuclear Pulverulent |
|        | S50P [114, 118] | (-) | (-) | Altered fiber cell formation, dense cataract and posterior capsule rupture |
| TM2    | V79L [110] | (-)           | n.d.         | “Full moon” with Y-suture Opacities |
|        | P88S [34, 246] | (-) | n.d. | Zonular Pulverulent |
|        | P88Q [247] | (-)           | n.d.         | Lamellar Pulverulent |
| CT     | S276F [248, 249] | (-) | (-) | Nuclear Pulverulent |
|        | Cx50fs [250] | (-) | n.d. | Triangular |

(-) = Loss of function. (+) = Gain of function. n.d. = not determined

Location of mutations associated to diseases and their functional consequences on GJC and HCs
Taking advantage of the natural occurring mutations in Cxs and previous studies focuses in the effect of disease-associated mutations on the functional state of GJCs and HC, we looked for similarities and differences between Cx5s regarding the positions of mutations associated to the respective diseases and its functional consequences on GJCs and HC.

Tables summarize experimental results on GJCs and HC obtained for different Cxs and disease conditions. They show that independent of the disease and Cx, all mutations produce loss of function of the GJC, which can be partial or total. The decreased GJC activity can be consequence of reduced amount of channels in the appositional membranes or changes in the functional properties of single channels.

It has been well established that a loss of function of the GJCs elicited by Cx mutations is sufficient to develop pathology. However, it is not clear if the extent of the loss of function is related to the severity of the disease. An institutive reasoning is that there is a good positive correlation between the severity of the Cx-linked disease and the loss of function of the corresponding GJC. Unfortunately, the experimental data do not support this statement. On one side, positive correlation can be found when the analysis is restricted to some missense non-syndromic Cx26 mutations (V37I and A40G). While these genetic modifications induce GJCs with loss of function (A40G) and active channels with reduced permeability (V37I) [113], they produce a severe deafness phenotype and
a milder condition, respectively [4]. However, a clear correlation cannot be established when other mutations are analyzed, such as some Cx32 mutations associated to a mild to moderate (Null111-116) and moderate to severe (R220X) CMTX1 phenotypes. As expected, the permeability of these channels to different dye tracers decreases as the size of the probe increases [114]. However, unlike the channels containing the Null111-116 mutation, permeability of the R220X-Cx32 GJCs to small probes (neurobiotin) is not significantly different from that observed in wild type channels [115]. In the same region (TM1-ECL1) other mutations cause non-functional GJCs and HCs (eg. E48K, D47N, S50P) [110, 116–118]. In contrast, Cx50 W45S acts as a dominant negative when co-expressed with Cx50, reducing GJCs coupling between fibers cells [106]. The above evidences suggest that the disease mechanisms might be produced by subtle changes in GJCs permeability, which are impossible to detect by the common electrophysiological and dye coupling methodologies.

In order to know the location of mutations in the channel structure, we produced several molecular models of the different Cxs by homology modeling, taking the crystal structure of Cx26 GJC published by Maeda et al., (2009) as template [15]. Due to the lack of experimental structure for human Cx32, Cx43 and Cx50, we generated comparative structural models, using Modeller [119], based on the structure of human Cx26 as a template (pdb: 2ZW3) (Figs. 2 and 3). Missing residues of human Cx26 structure were inserted with Modeller. The backbone of the experimental Cx26 structure was fully conserved. Ten models were generated in each case and those with the lowest discrete optimized protein energy (DOPE) score were selected as the final models. Figure 1 shows the model of a Cx26 monomer in the context of the connexon as well the location of residues mutated in genetic deafness that produce loss of function GJCs. Clearly, although loss of function mutations can be located everywhere in the protomer, they are concentrated from the NT to the TM2 domains (Fig. 1), regions that line the pore and are critical for voltage gating, as we mentioned earlier [120]. Moreover, other mutations in the transmembrane regions seem to be located in protein-protein and protein-lipid interfaces (Fig. 1b, c). Those locations could be important for intra- or inter-protomer interactions [121], which might stabilize the channel or contribute GJCs channel assembly. For Cx32, the pattern for location of mutations that produced loss of function GJCs is very similar to that observed for Cx26 (Fig. 2b), suggesting strong similarities in the structural features between these two Cxs. For Cxs 43 and 50, mutations that produce loss of GJCs function are more restricted. The fact that they localize mostly from NT to ECL1 (Fig. 2c, d) confirms the importance of this region for the channel function in the whole Cx family. However, the ICL Cx43 also presents important amount of mutations producing loss of function GJCs (Table 3).

**Mutations affecting HCs function**

The HCs play important role in physiological and pathological conditions since they provide a route for paracrine/autocrine signaling between the cell and the extracellular environment [2, 122]. Hence, a plausible underlying mechanism for connexinopathies is the possibility that some disease condition arise upon HCs dysfunction. For example, aberrant gain of function HCs is associated to syndromic Cx26 mutations that lead to keratitis ichthyosis deafness syndrome (KID) [13, 123, 124]. For the other Cxs (Cx32, Cx43 and Cx50), very few cases have been reported making it difficult to establish a common mechanism of disease (Tables 1, 2, 3 and 4). Exceptions are some mutations in Cx32 (S85C and F235C), which induce aberrant gain of HC activity in CMTX1 [125, 126], which behaves similar to the KID-linked Cx26 mutations, i.e., causing a gain of function of the HCs [125] and a loss of function of the GJCs [127]. Although the S85C mutant induces abnormal HCs opening [128], this mutation has not been associated to any particular severe phenotype of CMTX1 [129].

Most of the mutations eliciting gain of HC function are clustered exclusively in the pore lining residues of the NT, TM1 and the ECL1. They also localize in TM2 to a lesser extent (Fig. 3). In the case of Cx26, several mutations related to severe clinical phenotypes of KID are located at the transition zone between TM1 and the ECL1, a domain involved in both voltage gating and the control of HCs by extracellular Ca$^{2+}$ [25]. Moreover, a cluster of syndromic mutations is found in the NT domain of the protein, which is involved in the fast gating of HCs [24, 130]. Nevertheless, a role of other regions on the regulation of HCs should be further considered. For example, the Cx32 mutation F235C, localized in the CT of the protein also induces HCs with gain of function [126].

The gain of HCs function has been also observed in Cx43 related connexinopathies, since mutations I31M (TM1), G138R (ICL) and G143S (ICL), all promotes gain of function (Table 3). As mentioned above, ICL is involved in regulation the fast V(j)-gating, which depend on the interaction with CT [28–32]. Moreover, Dobrowolski and co-workers (2008) [93] found an increased ATP-release in cultured cardiomyocytes from cardiac specific G138R-mutant mouse. Interestingly, the authors proposed that HCs with gain of function in G138R-mutated cardiomyocytes might be one of the causes of arrhythmias.
As expected, some mutations induce loss of function HCs (Table 1 and Fig. 3). For example, mutations related to non-syndromic sensorineural hearing loss generate non-functional HCs [113]. Indeed, there are some syndromic mutations that exhibit non-functional HCs that only become gain of function when are co-expressed with their wild type partner or under aberrant interaction with Cx43 [13, 131].

Finally, it should be considering that in normal tissues cells could express several Cxs isoforms raising the possibility of interaction among Cxs isoforms. Recent results obtained in Dr. Martinez’ lab [13] and Dr. White’s group [131] suggest that the interaction between the mutated Cx and the co-expressed Cxs forming heterotypic/heteromeric channels needs to be taking into account to explain the clinical phenotypes.
of connexinopathies. Thus, interaction of mutants with wild type Cxs might ameliorate or worsen the clinical phenotypes. This possibility might augment when mutations affect critical segment involved in oligomerization compatibility, giving rise to aberrant heteromeric HCs, which makes pathological condition and effective treatment complex. In this scenario, further studies attempting to explore the pathological mechanism of connexinopathies should consider to study Cxs in heteromeric rather than homomeric states, which more closely resembles native cellular conditions.

Fig. 3 Mutations affecting function of HCs. Models of single Cxs chains are represented as cartoons, and colored with a blue-green gradient from the N- to the CT region, for (a) Cx26, (b) Cx32, (c) Cx43 and (d) Cx50. Positions of loss of function mutations are colored as red and gain of function mutations as yellow. The figure was generated with PyMol and edited with Gimp.
Conclusions
Most mutations causing connexinopathies generates total or partial loss of GJCs function. However, it is unclear if the severity of disease correlates with the level of GJCs loss of function. Mutations associated with loss of function GJCs are distributed along the entire protein sequence with no clear pattern of clustering at any segment, which suggest that GJC functionality is very sensitive to minor changes in Cxs protein, and that subtle changes in GJC functionality are sufficient to cause diseases. Less in known about the effect of mutations associated to connexinopathies on the functional state of HCs. The clearest correlation between gain of function HCs and disease has been found in most types of syndromic deafness associated to Cx26, in particular in KID syndrome. For others Cxs, few mutations are associated to gain of HCs function, however, we can not discard that this condition may be underestimated because most studies in the past have been more focused in GJCs than HCs. Therefore, it is yet difficult to make a general statement that represent all Cxs associated to connexinopathies. Nevertheless, it is clear that all mutations eliciting gain of HCs function are clustered in pore-associated domains like the NT and the TM1/ECL1, which are critical regions for gating and regulation.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ADM, conceived the original idea. IEG, PP, and ADM co-wrote and co-edited the final version of the manuscript. AP, made the molecular models of Cxs subunits; OJ, PM, DRG, CFM, JGC, CSR, BIP, MAR and CG, co-designed and co-wrote the tables and contributed to the discussion. All authors read and approved the final manuscript.

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References
1. Laird DW. Syndromic and non-syndromic disease-linked Cx43 mutations. FEBS Lett. 2014;588(8):1339–48.
2. Sáez JC, Berthoud VM, Branes MC, Martínez AD, Beyer EC. Plasma membrane channels formed by connexins: their regulation and functions. Physiol Rev. 2003;83(4):1359–400.
3. Harris AL. Emerging issues of connexin channels: biophysics fills the gap. Q Rev Biophys. 2001;34(3):325–472.
4. Martínez AD, Acuña R, Figueroa V, Maripillán J, Nicholson B. Gap-junction channels dysfunction in deafness and hearing loss. Antioxid Redox Signal. 2009;11(2):309–22.
5. Delmar M, Makita N. Cardiac connexins, mutations and arrhythmias. Curr Opin Cardiol. 2012;27(3):236–41.
6. Beyer EC, Elbíhara L, Berthoud VM. Connexin mutants and cataracts. Front Pharmacol. 2013;4:43.
7. Laird DW, Castillo M, Kasprzak L. Gap junction turnover, intracellular trafficking, and phosphorylation of connexin43 in brefeldin A-treated rat mammary tumor cells. J Cell Biol. 1995;131(5):1193–203.
8. Lampé PD, Lau AF. Regulation of gap junctions by phosphorylation of connexins. Arch Biochem Biophys. 2000;384(2):205–15.
9. Ahmad S, Martin PE, Evans WH. Assembly of gap junction channels: mechanism, effects of calmodulin antagonists and identification of connexin oligomerization determinants. Eur J Biochem. 2001;268(16):4544–52.
10. Maza J, Das Sarma J, Koval M. Defining a minimal motif required to prevent connexin oligomerization in the endoplasmic reticulum. J Biol Chem. 2005;280(22):21115–21.
11. 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(6):879–88.
12. Martínez AD, Maripillán J, Acuña R, Minogue PJ, Berthoud VM, Beyer EC. Different domains are critical for oligomerization compatibility of different connexins. Biochem J. 2011;436(1):35–43.
13. García IE, Maripillán J, Jara O, Ceriani R, Palacios-Muñoza A, Ramachandran J, Olivero P, Perez-Acle T, González C, Sáez JC et al. Keratitis-Ichthyosis-Deafness Syndrome-Associated Cx26 Mutants Produce Nonfunctional Gap Junctions but Hyperactive Hemichannels When Co-Expressed With Wild Type Cx43. J Invest Dermatol. 2015;135(5):1338–47.
14. Fleishman SJ, Sabag AD, Ophir E, Avraham KB, Ben-Tal N. The structural context of disease-causing mutations in gap junctions. J Biol Chem. 2006;281(39):28958–63.
15. Maeda S, Nakagawa S, Suga M, Yamashita E, Oshima A, Fujiyoshi Y, Tsukihara T. Structure of the connexin 26 gap junction channel at 3.5 A resolution. Nature. 2009;458(7238):597–602.
16. Lagree V, Brunschwig K, Lopez P, Gilula NB, Richard G, Falk MM. Specific amino-acid residues in the N-terminus and TM3 implicated in channel function and oligomerization compatibility of connexin43. J Cell Sci. 2003;116(Pt 15):3189–201.
17. Unger VM, Kumar NM, Gilula NB, Yeager M. Three-dimensional structure of a recombinant gap junction membrane channel. Science. 1999;283(5405):1176–80.
18. Skerrett IM, Aronowit J, Shin JH, Cymes G, Kasperek E, Cao FL, Nicholson BJ. Identification of amino acid residues lining the pore of a gap junction channel. J Cell Biol. 2002;159(2):249–60.
19. Zhou XW, Pfahrl A, Werner R, Huddler A, Llanes A, Luebke A, Dahl G. Identification of a pore lining segment in gap junction hemichannels. Biophys J. 1997;72(5):1946–53.
20. Kronengold J, Trexler EB, Bukauskas FF, Bargiello TA, Verselis VK. Single-channel SCAM identifies pore-lining residues in the first extracellular loop and first transmembrane domains of Cx46 hemichannels. J Gen Physiol. 2003;122(4):389–405.
21. Tang Q, Dowd TL, Verselis VK, Bargiello TA. Conformational changes in a pore-forming region underlie voltage-dependent “loop gating” of an unaposed connexin hemichannel. J Gen Physiol. 2009;133(5):555–70.
22. Kwon T, Harris AL, Rossi A, Bargiello TA. Molecular dynamics simulations of the Cx26 hemichannel: evaluation of structural models with Brownian dynamics. J Gen Physiol. 2011;138(5):475–93.
23. Pumrick PE, Benjamin DC, Verselis VK, Bargiello TA, Dowd TL. Structure of the amino terminus of a gap junction protein. Arch Biochem Biophys. 2000;381(2):181–90.
24. Oshima A, Tani K, Hiroaki Y, Fujiyoshi Y, Sosinsky GE. Three-dimensional structure of a human connexin26 gap junction channel reveals a plug in the vestibule. Proc Natl Acad Sci U S A. 2007;104(24):10034–9.
in addition to deficient gap junction channels. J Membr Biol. 2007;219(1–3):9–17.

79. Cho KJ, Langlois S, Pan X, Shao Q, Laird DW. The potency of the fs260 connexin43 mutant to impair keratinocyte differentiation is distinct from other disease-linked connexin43 mutants. Biochem J. 2010;429(3):473–83.

80. Dobrowolski R, Sasse P, Schrickel JW, Watkins M, Kim JS, Rackauskas M, Troatz C, Gahemen T, Joosen J, et al. The conditional connexin43G138R mouse mutant represents a new model of hereditary ocudentalodigital dysplasia in humans. Hum Mol Genet. 2008;17(4):539–54.

81. Sever NS, Bruce AF, Dupont E, Rotchey S. Remodelling of gap junctions and connexin expression in diseased myocardium. Cardiovasc Res. 2008;80(1):19–19.

82. Garcia-Dorado D, Rodriguez-Sinovas A, Ruiz-Meana M. Gap junction-mediated spread of cell injury and death during myocardial ischemia-reperfusion. Cardiovasc Res. 2004;61(6):386–401.

83. van Kempen MJ, ten Velde I, Wessels AO, Oosthoek PW, Gros D, Jongsmi HJ, Moorman AF, Lamberts WH. Differential connexin distribution accommodates cardiac function in different species. Microsc Res Tech. 1995;35(4):240–36.

84. Huang QY, Xie LJ, Linak KL, Zhang C, Zhao XQ, Yang Z, Zhou GM, Wu YJ, Marquez-Rosado L, McElhinney DB, et al. Evaluating the role of connexin43 in congenital heart disease: Screening for mutations in patients with outflow tract anomalies and the analysis of knock-in mouse models. J Cardiovasc Dis Res. 2011;24(2):206–12.

85. Britz-Cunningham SH, Shah MM, Zuppan CW, Fletcher WH. Mutations of the Connexin43 GJ-junction gene in patients with heart malformations and defects of laterality. N Engl J Med. 1995;332(20):1233–9.

86. Thi bomba E, Xu J, Li Q, Li Q, Lam K, Veinot JP, Binnie DH, Jones DL, Kratovia RD, Lemery R, et al. Paradigm of genetic mosaicism and lone atrial fibrillation: physiological characterization of a connexin 43 deletion mutant identified from atrial tissue. Circulation. 2010;122(3):361–44.

87. TenBroek EM, Johnson R, Louis CF. Cell-to-cell communication in a differentiating ovin lens culture system. Invest Ophthalmol Vis Sci. 1994;35(1):215–28.

88. Dahlin R. Lens fibre cell differentiation - A link with apoptosis? Ophthalmic Res. 1999;31(3):163–83.

89. Dong P, Wang X, Niesman I, Wu Y, Benediti LE, Dunia I, Levy E, Gong X. Disruption of Gja8 (alpha connexin) in mice leads to microphthalmia associated with retardation of lens growth and lens fiber maturation. Development. 2002;129(1):167–74.

90. White TW, Brussone R, Goodenough DA, Paul DL, Mouse Cx50, a functional member of the connexin family of gap junction proteins, is the lens fiber protein MP70. Mol Biol Cell. 1992;3(7):711–20.

91. Minogue PJ, Tong JJ, Arora A, Russell-Eggitt I, Hunt DM, Moore AT, Eibhara L, Beyer EC, Berthoud VM. A mutant connexin50 with enhanced hemichannel function leads to cell death. Invest Ophthalmol Vis Sci. 2009;50(12):5837–45.

92. Xu X, Berthoud VM, Beyer EC, Berthoud VM. Functional role of the carboxyl terminal domain of human connexin 50 in gap junctional channels. J Membr Biol. 2002;186(2):101–12.

93. Tong JJ, Minogue PJ, Guo W, Chen TL, Beyer EC, Berthoud VM, Eibhara L. Different consequences of cataract-associated mutations at adjacent positions in the first extracellular boundary of connexin50. Am J Physiol Cell Physiol. 2011;300(1):C105–64.

94. Beyer EC, Berthoud VM. Connexin hemichannels in the lens. Front Physiol. 2014;5:20.

95. Paul DL, Eibhara L, Takemoto LO, Svenson KL, Goodenough DA. Connexin46, a novel lens gap junction protein, induces voltage-gated currents in nonjunctional plasma membrane of Xenopus oocytes. J Cell Biol. 1997;134(5):1154–6.

96. Jiang JD, Goodenough DA. Heteromeric connexins in lens gap junction channels. Proc Natl Acad Sci U S A. 1996;93(3):1287–91.

97. Rubin M, Villed D, Mhaske PV, White TW, Sinivas M. Functional effects of Cx30 mutations associated with congenital cataracts. Am J Physiol Cell Physiol. 2014;306(3):C212–20.

98. Gao J, Sun X, Martinez-Wittingham FJ, Gong X, White TW, Mathias RT. Connections between connexin46, calcium, and cataracts in the lens. J Gen Physiol. 2008;124(8):289–300.

99. Mathias RT, White TW, Gong X. Lens gap junctions in growth, differentiation, and homeostasis. Physiol Rev. 2010;90(1):179–206.

100. Jara O, Arca R, Garcia IE, Martipill J, Figueroa V, Saez JC, Araya-Secchi R, Lagos CF, Perez-Acile T, Berthoud VM, et al. Critical role of the first
transmembrane domain of Cx26 in regulating oligomerization and function. Mol Biol Cell. 2012;23(17):3299–311.

114. DeRosa AM, Xia CH, Gong X, White TW. The cataract-inducing S50P mutation in Cx50 dominantly alters the channel gating of wild-type lens connexins. J Cell Sci. 2007;120(Pt 23):4107–16.

115. Bicego M, Beltramelli M, Melchionda S, Carella M, Piazza V, Zelante L, Bukauskas FF, Arslan E, Cama E, Pantano S, et al. Pathogenetic role of the deafness-related M34T mutation of Cx26. Hum Mol Genet. 2006;15(17):2569–77.

116. Banks EA, Toloue MM, Shi Q, Zhou ZJ, Liu J, Nicholson BJ, Jiang JX. Connexin mutation that causes dominant congenital cataracts inhibits gap junctions, but not hemichannels, in a dominant negative manner. J Cell Sci. 2000;122(Pt 5):3378–88.

117. Arora A, Minogue PJ, Liu X, Addison PK, Russel-Eggitt I, Webster AR, Hunt DM, El Farra L, Beyer EC, Berchtold VM, et al. A novel connexin50 mutation associated with congenital nuclear pupillary cataracts. J Med Genet. 2008;45(3):155–60.

118. DeRosa AM, Mese G, Li L, Sellitto C, Brink PR, Gong X, White TW. The cataract-causing Cx50/S50P mutant inhibits Cx43 and intercellular communication in the lens epithelium. Exp Cell Res. 2009;315(6):1063–75.

119. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol. 1993;234(3):779–815.

120. Barjigaio TA, Tang Q, Oh S, Kwon T. Voltage-dependent conformational changes in connexin channels. Biochim Biophys Acta. 2012;1818(8):807–22.

121. Fleishman SJ, Ben Tal N. Progress in structure prediction of alpha-helical forms of connexin 32: evidence for loss of function as a pathogenic mechanism. Ann N Y Acad Sci. 2007;1127(1):337–45.

122. Retamal MA, Reyes EP, García LE, Pinto B, Martínez AD, González C. Diseases associated with leaky hemichannels. Front Cell Neurosci. 2015;9:267.

123. Lee JR, Derosa AM, White TW. Connexin mutations causing skin disease and deafness increase hemichannel activity and cell death when expressed in Xenopus oocytes. J Invest Dermatol. 2009;129(4):870–8.

124. Gerido DA, DeRosa AM, Richard G, White TW. Aberrant hemichannel properties of Cx26 mutations causing skin disease and deafness. Am J Physiol Cell Physiol. 2007;293(1):C337–45.

125. Abrams CK, Bennett MV, Verselis VK, Bargiello TA. Voltage opens unopposed Cx26 hemichannels at different levels of protein expression. Hum Genet. 2002;111(2):189–201.

126. Liang GS, de Miguel M, Gomez-Hernandez JM, Glass JD, Scherer SS, Mintz Garcia R. Identification of a high-resolution structure of Cx26. J Gen Physiol. 2010;136(1):47–62.

127. Abrams CK, Bennett MV, Verselis VK, Bargiello TA. Mutations in connexin 32: the molecular and biophysical bases for the X-linked form of Charcot-Marie-Tooth disease. Proc Natl Acad Sci U S A. 2002;99(6):3980–4.

128. Liang G, de Miguel M, Gomez-Hernandez JM, Glass JD, Scherer SS, Mintz Garcia R. Identification of a high-resolution structure of Cx26. J Gen Physiol. 2010;136(1):47–62.

129. Bakirtzis G, Choudhry R, Aasen T, Shore L, Brown K, Bryson S, Forrow S, Haack B, Schmalisch K, Palmada M, Bohner C, Kohlchmidt N, Keilmann A, Zechner U, Limberger A, Beckert S, Zennep HR, et al. Deficient membrane integration of the novel p. N14D-GJB2 mutant associated with non-syndromic hearing impairment. Hum Mutat. 2006;27(11):1158–9.

130. Choy SY, Lee KY, Kim HJ, Kim HK, Chang QG, Park HJ, Jeon CJ, Lin X, Bok J, Kim UK. Functional evaluation of GJB2 variants in nonsyndromic hearing loss. Mol Med. 2011;17:65–75.

131. DeRosa AM, Xia CH, Gong X, White TW. The cataract-inducing S50P mutation in Cx50 dominantly alters the channel gating of wild-type lens connexins. J Cell Sci. 2007;120(Pt 23):4107–16.

132. Liang GS, de Miguel M, Gomez-Hernandez JM, Glass JD, Scherer SS, Mintz Garcia R. Identification of a high-resolution structure of Cx26. J Gen Physiol. 2010;136(1):47–62.

133. Martin PE, Coleman SL, Casalotti SO, Forge A, Evans WH. Properties of connexin26 gap junctional proteins derived from mutations associated with non-syndromic hereditary deafness. Hum Mol Genet. 1998;7(13):2369–76.

134. Zhang Y, Tang W, Ahmad S, Sipp JA, Chen P, Lin X. Gap junction-mediated intercellular biochemical coupling in cochlear supporting cells is required for normal cochlear functions. Proc Natl Acad Sci U S A. 2005;102(42):15201–6.

135. Palmada M, Schmalisch K, Bohner M, Chu C, Nishizawa M, Fink D, Boccalini C, Aoyagi K, Inoue S, Kawamura Y. Connexin26 mutation associated with non-syndromic hearing loss. J Invest Dermatol. 2009;133(1):79–88.

136. Skreitter MI, Di WL, Kasperek EM, Kellsel DP, Nicholson BJ. Aberrant gating, but a normal expression pattern, underlies the recessive phenotype of the deafness mutant Connexin26M34T. FASEB J. 2004;18(7):860–2.

137. White TW, Deans NR, Kellsel DP, Paul DL. Connexin mutations in deafness. Nature. 1998;394(6694):630–1.

138. Brazzune R, Veronesi V, Gorner D, Bicego M, Duval N, Martin S, Pettic C, D’Andrea P, White TW. Loss-of-function and residual channel activity of connexin26 mutations associated with non-syndromic deafness. FEBS Lett. 2003;333(3–4):79–88.

139. Kenna MA, Wu BL, Cotanche DA, Korf BR, Rehm HL. Connexin 26 studies in patients with sensorineural hearing loss. Arch otolaryngol-head & neck Surg. 2001;127(10):1037–42.

140. Schmalisch K, Palmada M, Bohner C, Kohlchmidt N, Keilmann A, D’Andrea P, White TW. Loss-of-function and residual channel activity of connexin26 mutations associated with non-syndromic deafness. FEBS Lett. 2003;333(3–4):79–88.

141. Mani RS, Ganapathy A, Jalvi R, Addison PK, Russel-Eggitt I, Webster AR, Hunt DM. Functional evaluation of GJB2 variants in nonsyndromic hearing loss. Mol Med. 2011;17:65–75.

142. Martin PE, Coleman SL, Casalotti SO, Forge A, Evans WH. Properties of connexin26 gap junctional proteins derived from mutations associated with non-syndromic hereditary deafness. Hum Mol Genet. 1998;7(13):2369–76.

143. Zhang Y, Tang W, Ahmad S, Sipp JA, Chen P, Lin X. Gap junction-mediated intercellular biochemical coupling in cochlear supporting cells is required for normal cochlear functions. Proc Natl Acad Sci U S A. 2005;102(42):15201–6.

144. Sánchez HA, Verselis VK, Bargiello TA. Voltage opens unopposed gap junctional channels formed by a connexin 32 mutant associated with X-linked Charcot-Marie-Tooth disease. Proc Natl Acad Sci U S A. 2002;99(6):3980–4.

145. Sánchez HA, Verselis VK, Bargiello TA. Voltage opens unopposed gap junctional channels formed by a connexin 32 mutant associated with X-linked Charcot-Marie-Tooth disease. Proc Natl Acad Sci U S A. 2002;99(6):3980–4.

146. Sánchez HA, Verselis VK, Bargiello TA. Voltage opens unopposed gap junctional channels formed by a connexin 32 mutant associated with X-linked Charcot-Marie-Tooth disease. Proc Natl Acad Sci U S A. 2002;99(6):3980–4.

147. Bruzzone R, Veronesi V, Gorner D, Bicego M, Duval N, Martin S, Pettic C, D’Andrea P, White TW. Loss-of-function and residual channel activity of connexin26 mutations associated with non-syndromic deafness. FEBS Lett. 2003;333(3–4):79–88.

148. Mani RS, Ganapathy A, Jalvi R, Addison PK, Russel-Eggitt I, Webster AR, Hunt DM. Functional evaluation of GJB2 variants in nonsyndromic hearing loss. Mol Med. 2011;17:65–75.

149. Sánchez HA, Verselis VK, Bargiello TA. Voltage opens unopposed gap junctional channels formed by a connexin 32 mutant associated with X-linked Charcot-Marie-Tooth disease. Proc Natl Acad Sci U S A. 2002;99(6):3980–4.

150. Sánchez HA, Verselis VK, Bargiello TA. Voltage opens unopposed gap junctional channels formed by a connexin 32 mutant associated with X-linked Charcot-Marie-Tooth disease. Proc Natl Acad Sci U S A. 2002;99(6):3980–4.
156. Thomas T, Jordan K, Simek J, Shao Q, Jedezko C, Walton P, Laird DW. Mechanisms of Cx43 and Cx26 transport to the plasma membrane and gap junction regeneration. J Cell Sci. 2005;118(Pt 19):4451–62.

157. Thomas T, Telford D, Laird DW. Functional domain mapping and selective trans-domain effects exhibited by Cx26 disease-causing mutations. J Biol Chem. 2004;279(18):19157–68.

158. Thomas T, Aisen T, Hodgins M, Laird DW. Transport and function of Cx26 mutants involved in skin and deafness disorders. Cell Commun Adhes. 2003;10(4–6):353–8.

159. Rouan F, White TW, Brown N, Taylor AM, Lucke TW, Paul DL, Munro CS, Ulloa J, Hodgins MB, Richard G. Trans-dominant inhibition of connexin-43 by mutant connexin-26: implications for dominant connexin disorders affecting epidermal differentiation. J Cell Sci. 2001;114(Pt 11):2105–13.

160. Choi SY, Park HJ, Lee KY, Dinh EH, Chang Q, Ahmad S, Lee SH, Bok J, Lin X, Kim UK. Different functional consequences of two missense mutations in the GJB2 gene associated with non-syndromic hearing loss. Hum Mutat. 2009;30(7):E176–27.

161. Bruzzone R, Gomes D, Denoyelle E, Martino A, Monforti-Maggi L, Andere A, et al. Functional analysis of a dominant mutation of human connexin26 associated with nonsyndromic deafness. Cell Commun Adhes. 2001;8(4–6):425–31.

162. Melchinha S, Bicego M, Marciano E, Franco A, Morgutti M, Bortone G, Zcelante L, Carella M, DAndrea P. Functional characterization of a novel Cx26 (T55N) mutation associated to non-syndromic hearing loss. Biochem Biophys Res Commun. 2005;337(3):799–803.

163. Tinkin M, Arnaud D, Kx XJ, Delich MK, Xu XJ, Nance WE, Pandya A, W44 mutation in the connexin 26 gene associated with dominant non-syndromic deafness. Clin Genet. 2001;59(4):269–73.

164. Stong BC, Chang Q, Ahmad S, Lin X. A novel mechanism for connexin 26 mutation in the gene coding for Connexin 26 identified in a family with sensorineural hearing impairment. J Pediatr Otorhinolaryngol. 2008;72(5):693–9.

165. Carrasquillo MM, Zlotogora J, Barges S, Chakravarti A. Two different connexin 26 mutations in an inbred kindred segregating non–syndromic recessive deafness: implications for genetic studies in isolated populations. Hum Mol Genet. 1997;6(12):2163–72.

166. Kupka S, Braun S, Aberle S, Haack B, Ebauer M, Zeissler U, Zener HP, Blin N, Pfister M. Frequencies of GJB2 mutations in German control individuals and patients showing sporadic non-syndromic hearing impairment. Hum Mutat. 2002;20(1):77–8.

167. Wang HL, Chang WT, Li AH, Yeh TH, Wu CY, Chen MS, Hwang PC. Analysis of genetic testing for hereditary hearing loss. J Neurochem. 2003;84(4):735–42.

168. Ambrosi C, Boassa D, Pranskievich J, Smocik A, Oshima A, Xu J, Nicholson BJ, Soinsky GE. Analysis of four connexin26 mutation gap junctions and hemichannels reveals variation in hemaxer stability. Biophys J. 2010;98(9):1809–19.

169. Cyris K, Orzan E, Murgia A, Huygen PL, Moreno F, del Castillo I, Chamberlin GP, Azaiez H, Prasad S, Cucci RA, et al. A genotype–phenotype correlation for GJB2 (connexin 26) deafness. J Med Genet. 2004;41(3):147–54.

170. Meilh L, Hussain N, Mulkey DK, Dale N. Connexin26 hemichannels with a mutation that causes KID syndrome in humans lack sensitivity to CO2. Elife. 2014;3:e04249.

171. Marlin S, Garabedian EN, Roger G, Moatti L, Matha N, Lewin P, Petit C, Denoyelle F. Connexin 26 gene mutations in congenitally deaf children: pitfalls for genetic counseling. Arch Otolaryngol Head Neck Surg. 2001;127(8):927–33.

172. Man YK, Trowie C, Tattersall D, Thomas AC, Papakonstantinouopoulo A, Patel D, et al. A deafness–associated mutant human connexin 26 improves the epithelial barrier in vitro. J Memb Biol. 2007;218(1–3):29–37.

173. Bobbgy GW, Muller–Mytkos B, Horstmann RD. Connexin 26 R142S mutation associated with recessive nonsyndromic sensorineural deafness in Africa. N Engl J Med. 1998;338(8):548–50.

174. Xiao Z, Yang Z, Liu X, Xie D. Impaired membrane targeting and aberrant cellular localization of human Cx26 mutants associated with inherited recessive hearing loss. Acta Otolaryngol. 2011;131(1):59–66.

175. Bobbgy GW, Muller–Mytkos B, Horstmann RD. Connexin 26 R142S mutation associated with recessive nonsyndromic sensorineural deafness in Africa. N Engl J Med. 1998;338(8):548–50.

176. Rickard S, Kelsell DP, Sirimana T, Rajput K, MacArdle B, Bitner-Glindzicz M. Recurrent mutations in the deafness gene GJB2 (connexin 26) in British Asian families. J Med Genet. 2001;38(8):530–3.

177. Denoyelle F, Weil D, Maw MC, Lenoir P, Benini A, Pujol L, et al. A deafness–associated mutant human connexin 26 improves the epithelial barrier in vitro. J Memb Biol. 2007;218(1–3):29–37.

178. Chamberlin GP, Azaiez H, Prasad S, Cucci RA, et al. A genotype–phenotype correlation for GJB2 (connexin 26) deafness. J Med Genet. 2004;41(3):147–54.

179. Marlin S, Garabedian EN, Roger G, Moatti L, Matha N, Lewin P, Petit C, Denoyelle F. Connexin 26 gene mutations in congenitally deaf children: pitfalls for genetic counseling. Arch Otolaryngol Head Neck Surg. 2001;127(8):927–33.

180. Meilh L, Hussain N, Mulkey DK, Dale N. Connexin26 hemichannels with a mutation that causes KID syndrome in humans lack sensitivity to CO2. Elife. 2014;3:e04249.

181. Meilh L, Hussain N, Mulkey DK, Dale N. Connexin26 hemichannels with a mutation that causes KID syndrome in humans lack sensitivity to CO2. Elife. 2014;3:e04249.

182. Meilh L, Hussain N, Mulkey DK, Dale N. Connexin26 hemichannels with a mutation that causes KID syndrome in humans lack sensitivity to CO2. Elife. 2014;3:e04249.
196. Yilmaz A, Menesev S, Bayazit Y, Karamet R, Ergin V, Meneves A. Two novel missense mutations in the connexin 26 gene in Turkish patients with nonsyndromic hearing loss. Biochem Genet. 2010;48(3–4):248–56.

197. Deschenes SM, Walcott JL, Wexler TL, Scherer SS, Fischbeck KH. Altered trafficking of mutant connexin32. J. Neurosci. 1997;17(23): 9077–84.

198. Yoshimura T, Satake M, Ohnishi A, Tsutsumi Y, Fujikura Y. Mutations of GJA1 (connexin 43) associated with Charcot-Marie-Tooth disease. Hum Genet. 1995;96(3):486–91.

199. Frys JP, Van den Berghe H. Sex-linked recessive inheritance in Charcot-Marie-Tooth disease with partial clinical manifestations in female carriers. Hum Genet. 1980;55(2):413–5.

200. Gutierrez A, England JD, Sumner AJ, Ferer S, Warner LE, Lupski JR, Garcia CA. Unusual electrophysiological findings in X-linked dominant Charcot-Marie-Tooth disease. Muscle Nerve. 2000;23(2):182–8.

201. Senderek J, Hermans B, Bergmann C, Bajoouj B, Bajbouj M, Hungs M, Ramaekers VT, Quasthoff S, Karch D, Schroeder JM. X-linked dominant Charcot-Marie-Tooth neuropathy: clinical, electrophysiological, and morphological phenotype in four families with different connexin32 mutations. J. Neurol. 1999;167(2):190–1.

202. Martin PE, Mambetsaeva ET, Archer DA, George CH, Evans WH. Analysis of gap junction assembly using mutated connexins detected in Charcot-Marie-Tooth X-linked disease. J. Neurochem. 2000;74(2):711–20.

203. Wang HL, Chang WT, Yeh TH, Wu T, Chen MS, Wu CY. Functional analysis of connexin-32 mutants associated with X-linked dominant Charcot-Marie-Tooth disease. Neurobiol Dis. 2004;15(2):361–70.

204. Kleopa KA, Yum SW, Scherer SS. Cellular mechanisms of connexin32 mutations associated with CNS manifestations. J. Neurosci Res. 2002;68(S5): 522–34.

205. Yum SW, Kleopa KA, Shumais S, Scherer SS. Diverse trafficking abnormalities of connexin32 mutants causing CMTX. Neurobiol Dis. 2002;11(1):43–52.

206. Dubourg O, Tardieu S, Birouk N, Gouider R, Leger JM, Maisonobe T, Brice A, Delavelle J, Bouche P, LeGuern E, Roche M, de la Parra DR, Zenteno JC. Connexin43 mutations in X-linked dominant Charcot-Marie-Tooth disease. Mol Cell. 2002;11(1):43–52.

207. Shimaya J, Paznekas WA, Alevra X, Nicolaou P, Georgiou DM, Scherer SS, Fischbeck KH, Paul DL. A new GJA1 (connexin 43) mutation causing oculodentodigital dysplasia. Hum Mol Genet. 2000;11(1):43–52.

208. Ionasescu V, Ionasescu R, Searby C. Correlation between connexin 32 gene mutations and clinical phenotype in oculo-dento-digital dysplasia. J. Biol Chem. 2012;287(33):27784–91.

209. Yilmaz A, Mesnil M, Yamashita H. Connexin 32 mutations from X-linked Charcot-Marie-Tooth disease patients: functional defects and dominant-negative effects. Mol. Biol Cell. 1996;7(9):2106–16.

210. Abrams CK, Islam M, Mahmoud R, Kwon T, Bargiello TA, Freidin MM, Janssen EA, Kemp S, Hensels GW, Sie OG, de Die-Smulders CE, Hoogendijk JE, et al. Connexin32 gene mutations in X-linked dominant Charcot-Marie-Tooth disease. Hum Genet. 1997;99(4):501–5.

211. Van Norstrand DW, Asimaki A, Rubinos C, Srinivas M, Tester DJ, Saffitz JE, Duffy HS, Ackerman MJ. Connexin 43 mutations result in heterogeneous gap junction loss and sudden infant death. Circulation. 2012;125(3):247–54.

212. Van Norstrand DW, Asimaki A, Rubinos C, Dolmato娃 E, Srinivas M, Tester DJ, Saffitz JE, Duffy HS, Ackerman MJ. The connexin 43 A69S mutation causes retnin-dependent hypertension. J. Am Soc Nephrol. 2012;23(6):1031–40.

213. Kalcheva N, Qu J, Sandeep N, Garcia L, Zhang J, Wang Z, Lampe PD, Asimaki A, Rubinos C, Dolmato娃 E, Srinivas M, Tester DJ, Saffitz JE, Duffy HS, Ackerman MJ. The connexin 40 A69S mutation causes retnin-dependent hypertension. J. Am Soc Nephrol. 2012;23(6):1031–40.

214. Kalcheva N, Qu J, Sandeep N, Garcia L, Zhang J, Wang Z, Lampe PD, Asimaki A, Rubinos C, Dolmato娃 E, Srinivas M, Tester DJ, Saffitz JE, Duffy HS, Ackerman MJ. The connexin 40 A69S mutation causes retnin-dependent hypertension. J. Am Soc Nephrol. 2012;23(6):1031–40.

215. Kalcheva N, Qu J, Sandeep N, Garcia L, Zhang J, Wang Z, Lampe PD, Asimaki A, Rubinos C, Dolmato娃 E, Srinivas M, Tester DJ, Saffitz JE, Duffy HS, Ackerman MJ. The connexin 40 A69S mutation causes retnin-dependent hypertension. J. Am Soc Nephrol. 2012;23(6):1031–40.

216. Kalcheva N, Qu J, Sandeep N, Garcia L, Zhang J, Wang Z, Lampe PD, Asimaki A, Rubinos C, Dolmato娃 E, Srinivas M, Tester DJ, Saffitz JE, Duffy HS, Ackerman MJ. The connexin 40 A69S mutation causes retnin-dependent hypertension. J. Am Soc Nephrol. 2012;23(6):1031–40.
238. Zucker SN, Bancroft TA, Place DE, Des Soye B, Bagati A, Berenez R. A dominant negative Cx43 mutant differentially affects tumorigenic and invasive properties in human metastatic melanoma cells. J Cell Physiol. 2013;228(4):853–9.

239. van ES RJ, Wittebol-Post D, Beemer FA. Oculodentodigital dysplasia with mandibular retrognathism and absence of syndactyly: a case report with a novel mutation in the connexin 43 gene. Int J Oral Maxillofac Surg. 2007;36(9):858–60.

240. Gong Q, Qiao O, Lounsbery CS, Bai D, Laird DW. Functional characterization of a GJA1 frameshift mutation causing oculodentodigital dysplasia and palmpoplantar keratodermia. J Biol Chem. 2006;281(42):31801–11.

241. Dasgupta C, Martinez AM, Zuppan CW, Shah MM, Bailey LL, Fletcher WH. Identification of connxin43 (alpha1) gap junction gene mutations in patients with hypoplastic left heart syndrome by denaturing gradient gel electrophoresis (DGGE). Mutat Res. 2001;479(1–2):173–86.

242. Thomas BC, Minogue PJ, Vallunus V, Kanaporis G, Brink PR, Berthoud VM, Beyer EC. Cataracts are caused by alterations of a critical N-terminal positive charge in connexin50. Invest Ophthalmol Vis Sci. 2008;49(6):2549–56.

243. Zhu Y, Yu H, Wang W, Gong X, Yao K. Correction: A Novel GJA8 Mutation (p.V44A) Causing Autosomal Dominant Congenital Cataract. PLoS One. 2015;10:e0125949.

244. Vanita V, Singh JR, Singh D, Varon R, Sperling K. A novel mutation in GJA8 associated with jellyfish-like cataract in a family of Indian origin. Mol Vis. 2008;14:323–6.

245. Berry V, Mackay D, Khaliq S, Francis PJ, Hameed A, Mehdii SQ, Newbold RJ, Ionides A, Shiel A, et al. Connexin 50 mutation in a family with congenital "zonular nuclear" pulverulent cataract of Pakistani origin. Hum Genet. 1999;105(1–2):168–70.

246. Pal JD, Berthoud VM, Beyer EC, Mackay D, Shiel A, Ebihara L. Molecular mechanism underlying a Cx50-linked congenital cataract. Am J Phys. 1999;276(6 Pt 1):C1443–6.

247. Arora A, Minogue PJ, Liu X, Reddy MA, Ainsworth JR, Bhattacharya SS, Webster AR, Hunt DM, Ebihara L, Moore AT, et al. A novel GJA8 mutation is associated with autosomal dominant lamellar pulverulent cataract: further evidence for gap junction dysfunction in human cataract. J Med Genet. 2006;43(1):e2.

248. Liu Y, Qiao C, Wei T, Zheng F, Guo S, Chen Q, et al. Mutant connexin 50 (S276F) inhibits channel and hemichannel functions inducing cataract. J Genet. 2015;104(2):221–9.

249. Yan M, Xiong C, Ye SQ, Chen Y, Ke M, Zheng F, Zhou X. A novel connexin 50 (GJA8) mutation in a Chinese family with a dominant congenital pulverulent nuclear cataract. Mol Vis. 2008;14:418–24.

250. Minogue PJ, Beyer EC, Berthoud VM. A connexin50 mutant, Cx50fs, that causes cataracts is unstable, but is rescued by a proteasomal inhibitor. J Biol Chem. 2013;288(28):20427–34.