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Gap junctions in inherited human disorders of the central nervous system

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Abstract

CNS glia and neurons express connexins, the proteins that form gap junctions in vertebrates. We review the connexins expressed by oligodendrocytes and astrocytes, and discuss their proposed physiologic roles. Of the 21 members of the human connexin family, mutations in three are associated with significant central nervous system manifestations. For each, we review the phenotype and discuss possible mechanisms of disease. Mutations in *GJB1*, the gene for connexin 32 (Cx32) cause the second most common form of Charcot-Marie-Tooth disease (CMT1X). Though the only consistent phenotype in CMT1X patients is a peripheral demyelinating neuropathy, CNS signs and symptoms have been found in some patients with CMT1X. Recessive mutations in *GJC2*, the gene for Cx47, are one cause of Pelizaeus-Merzbacher-like disease (PMLD), which is characterized by nystagmus within the first 6 months of life, cerebellar ataxia by 4 years, and spasticity by 6 years of age. MRI imaging shows abnormal myelination. A different recessive *GJC2* mutation causes a form of hereditary spastic paraparesis, which is a milder phenotype than PMLD. Dominant mutations in *GJA1*, the gene for Cx43, cause oculodentodigital dysplasia (ODDD), a pleiotropic disorder characterized by oculo-facial abnormalities including microphthalmia, microcornia and hypoplastic nares, syndactyly of the fourth to fifth fingers and dental abnormalities. Neurologic manifestations, including spasticity and gait difficulties, are often but not universally seen. Recessive *GJA1* mutations cause Hallermann-Streiff syndrome, a disorder showing substantial overlap with ODDD.

Keywords

connexin; gap junction; CMT1X; PMLD1; SPG44; ODDD

1. Introduction

CNS glia and neurons express connexins, the proteins that form gap junctions (GJs) in vertebrates. (See Fig. 1 for nomenclature of gap junction channels and hemichannels.) To

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date 20 members of the connexin family have been identified in mice and 21 have been demonstrated in humans. (Reviewed by Rackauskas et al. [1].) Mutations in at least 10 of these connexin genes cause human disease, [2] including three connexins with significant CNS manifestations; these are the focus of this review. Here we emphasize the clinical phenotypes and current understanding of the pathogenesis of these genetic diseases, which will require a brief overview of connexin expression within CNS glial cells, reviewed in more detail elsewhere. [3, 4]

2. Connexins expressed by astrocytes and oligodendrocytes

2.1 Distribution and coupling

Astrocytes (As)¹ express Cx30 and Cx43, which can form homotypic (Cx43/Cx43 and Cx30/Cx30) but not heterotypic (Cx43/Cx30) GJs in a model system of transfected cells. [5] Anatomical approaches also provide evidence that A/A GJs are comprised of Cx43/Cx43 and Cx30/Cx30 homotypic channels. [6-9] Cx43-positive GJ plaques are found in both gray and white matter, whereas Cx30-positive GJ plaques are more prominent in gray matter. Although there are contradictory data, [10] some astrocytes may also express Cx26, [7, 9, 11, 12] which can also form homotypic as well as Cx26/Cx30 heterotypic channels. In mice, the deletion of the genes encoding Cx43 (*Gja1*) and Cx30 (*Gjb6*) results in the complete loss of A/A coupling. [13] Mutations in *GJB2* (encoding Cx26) and *GJB6* (encoding Cx30) are not associated with CNS abnormalities in humans or mice.

Oligodendrocytes (Os) express Cx29, Cx32, and Cx47. [9, 14-17] Cx29 is localized to the adaxonal membrane (apposing the axonal membrane) of CNS myelin sheaths, but does not form GJs. [11, 17-19] In transfected cells, Cx29 does not form functional channels, [20] but its human orthologue, Cx31.3, may form hemi-channels. [21] In rodents, Cx47 and Cx32 are found in GJ plaques that are localized on the cell bodies of oligodendrocytes, [17] and Cx32 is also localized within the myelin sheaths themselves, [15] although not as distinctly localized as in Schwann cell myelin sheaths [22]. Though O/O coupling was not seen in a number of brain regions by conventional electron microscopy (EM) or freeze replica immune labeling (FRIL), [8, 23] two groups have recently reported the functional O/O coupling in the mouse corpus callosum. [24, 25] The O/O coupling is mediated by Cx32/Cx32 and Cx47/Cx47 homotypic channels, as it is present in mice lacking Cx32 or Cx47, but is lost in mice that lack both Cx32 and Cx47. Furthermore, EM demonstrated that oligodendrocytes are directly joined by GJs. [24].

Electron microscopic studies in the 1980s provided anatomical evidence that oligodendrocytes were GJ coupled to astrocytes, but not to themselves [26, 27]. Our work in a model system of transfected cells shows that Cx43/Cx47 and Cx30/Cx32 (but not Cx43/Cx32 or Cx30/Cx47) form morphologic and functional GJs that have distinct electrophysiological properties, [5] but using a different approach, another group [28] showed that Cx30 and Cx47 can form functional channels. Anatomical studies in the CNS [7, 9, 13, 15] make a strong case that A/O GJs consist predominantly of Cx43/Cx47 and Cx30/Cx32 heterotypic channels (a role for Cx26/Cx32 channels remains to be excluded or proved). In the cerebral cortex, A/O coupling is lost in Cx47- (*Gjc2*), but not in Cx32- (*Gjb1*) null mice, indicating that Cx43/Cx47 channels (and not Cx30/Cx32 channels) are required for A/O coupling, at least in this region. [24]

¹Non-standard abbreviations. A-astrocyte O-oligodendrocyte GJ- gap junction KO-knockout(null) dKO-double knockout WMC:white matter changes

2.2 Roles of glial connexins in CNS glia

2.2.1 Lessons from mice with targeted ablation of one or more connexins—

The roles of connexins in CNS glia are now beginning to be explicated. Mice with targeted deletion of oligodendrocyte connexins Cx29, Cx32, or Cx47 and mice with targeted deletion of astrocyte connexins Cx30 and Cx43 have been examined. A thorough understanding of the phenotypes of these mice is a critical to understanding of the pathogenesis of disorders associated with mutations in these connexins. Cx32- (*Gjb1*-) null mice develop a demyelinating peripheral neuropathy after 3 months of age, [29, 30] but only subtle changes in CNS myelin thickness [31, 32] are seen. To date, no behavioral abnormalities have been described in this mouse. Similarly, Cx47- (*Gjc2*-) null mice show no behavioral abnormalities and only minimal and regionally limited abnormalities in some myelin sheaths. [14, 16] In contrast, mice lacking both Cx32 and Cx47 present with abnormal movements and seizures as well as striking CNS pathology including demyelinated and remyelinated axons, and enlarged extracellular space that separates the axon from its myelin sheath (See Fig. 2); these findings lead to the speculation that this vacuolization might be due to failure of spatial buffering of potassium with increased periaxonal accumulation in the double KO mouse. [16] This idea is supported by data showing that mice with targeted deletion of Kir4.1 [33], an inwardly rectifying K⁺ channel [34, 35], also develop vacuoles, [33] and that the null allele of Kir4.1 genetically interacts with the null alleles of Cx32 and Cx47. [36]

Though the original theory of spatial buffering of potassium referred only to the role of astrocytes (and presumably A/A coupling) in regulating the levels of extracellular potassium [37], work by Menichella et al. [36] supports the hypothesis that oligodendrocyte Cx32 and Cx47 are involved in spatial buffering of K⁺ during neuronal activity. Optic nerve vacuoles are reduced by suppressing activity of retinal ganglion cell (intraocular injection of tetrodotoxin); conversely, enhanced activity (intraocular injection of cholera toxin) causes increased vacuolization.

Although Cx29 (the mouse ortholog of human Cx31.3) is widely expressed in the CNS during development [38] and in the small myelinated fibers of mature brain and spinal cord [11, 17-19], no CNS physiological or pathological phenotype is seen in mice with targeted ablation of Cx29 [39] though peripheral auditory pathways are affected. [9, 40] Whether mutations in *GJF2*, [41] the gene encoding Cx31.3, cause hearing loss in humans needs to be confirmed.

Studies of mice with targeted deletion of Cx30 and Cx43, the two astrocyte connexins have also been revealing. Both Wallraff et al. [13] and Theis et al. [42] studied mice with astrocyte specific deletion of Cx43 alone and found approximately a 50% reduction in A/A coupling. Mice expressing no astrocyte Cx43 and no Cx30 in any cell (Cx43_{Astro}-Cx30 dKO) showed no A/A coupling and reduced hippocampal K⁺ buffering [13] as well as loss of metabolic coupling through astroglial networks. [43] Unlike the Cx32-Cx47 dKO mice, [16] these mice had no gross behavioral abnormalities (but see below in this section) or reduced life expectancy. [13, 44] These findings indicate that loss of O/O coupling has greater impact on CNS function than does loss of A/A coupling, since in both cases it is likely that all A/O coupling is eliminated. Theis et al. [42] found increased locomotor activity and an increase in the velocity of spreading depression in the astrocyte specific Cx43KO mouse. The authors suggest that astrocyte GJ communication may attenuate spreading depression by reducing extracellular K⁺ or glutamate. This hypothesis is supported by the recent work of Pannasch and colleagues [45] showing that astroglial gap junction coupling is required for clearance of extracellular glutamate generated by synaptic activity. Lutz et al. [44] found prominent white matter abnormalities in Cx43_{Astro}-Cx30 dKO mice that are similar to those noted in Cx32-Cx47 dKO and Kir4.1 KO mice (See Fig.

3). Grey matter involvement was restricted to astrocyte swelling in the CA1 region of the hippocampus. Behavioral testing showed decreased performance on testing of hippocampal spatial learning. Detailed behavioral analyses of this mouse as well as the Cx43Astro KO and Cx30 KO mice have been performed and are summarized in Table 1.

2.2.2 Physiologic roles of glial connexins—The best recognized role of connexins is to form GJs, which provide a pathway for electrical and/or metabolic coupling between cells. [46] In glial cells, GJ coupling is likely to underlie the formation of a glial syncytium. [47] Because glial gap junction coupling is a dynamic process regulated by intra- or extracellular signals (reviewed in Giaume et al. [48]) it has been suggested that coupled glia should be referred to as a network rather than a syncytium. One function of such a syncytium or network is propagation of Ca^{2+} waves (see [49] for more detail). As noted above (Section 2.1) Cx32 may also provide reflexive coupling between adjacent loops of non-compact myelin. [50] In the last decade, however, other roles are being described for connexins. GJs may be part of a larger complex that contains other junctional elements. Several connexins, including Cx43 and Cx47, have a PDZ-binding domain on their C-terminus, and bind to ZO-1, one of the defining members of PDZ family [19, 51-56] In *Gjc2*-null mice the association between Cx47 and ZO-1, ZO-1-associated nucleic acid binding proteins (ZONAB), and multi-PDZ domain protein 1 (MUPP1) is lost. [57] Instead of being co-localized with Cx47, ZONAB, a Y-box transcription factor, has increased cytoplasmic and nuclear localization. This redistribution could affect oligodendrocytes, as ZONAB can repress ErbB2, [58] a tyrosine kinase receptor implicated in oligodendrocyte development, differentiation and survival. [59-61] ZONAB also regulates a number of cell cycle regulatory genes. [62, 63] These findings suggest that the appropriate localization of Cx47 in the oligodendrocyte may be important for stabilization/organization of the oligodendrocyte membrane junctional complex, as well as for appropriate regulation of gene expression and control of the cell cycle. Other data suggest that connexins may have roles in regulation of cell growth [64-68] and resistance to both apoptotic and necrotic cell death [69] that are independent of formation of functional GJ channels. Recent work from one of our laboratories has shown that Schwann cell proliferation is regulated by Cx32 in a GJ-independent manner. [68]

Connexin hexamers can form functional membrane hemichannels that may mediate a variety of cellular processes, including ATP release and signaling [70-72], glutamate release [72, 73], glutathione release, [74] and responses to spinal cord injury [75]. Astrocyte Cx43 hemichannels are reportedly modulated in a number of pathogenic states including metabolic inhibition [76] and brain abscesses, [77] and may be upregulated by proinflammatory cytokines secreted from microglia. [78] Cannabinoids may antagonize the effect of proinflammatory cytokines; [79] this may be neuroprotective. [80] However, recent work suggests that at least in cultured astrocytes, pannexin 1 and not Cx43 is responsible for the membrane conductances and permeabilities ascribed to astrocytic hemichannels. [81] Hemichannel activities ascribed to Cx30, [82, 83] Cx47, [84] Cx32, [85-87] and Cx31.3 [21] have all been identified in cultured cells.

3. Disease manifestations of *GJB1* mutations

3.1 CMT1X clinical phenotypes MIM ID #302800

Charcot-Marie-Tooth disease (CMT) is the eponym for large number of inherited disorders affecting predominantly or exclusively the peripheral nerves. Symptoms are those of peripheral neuropathy and include weakness and sensory loss of the distal more than proximal extremities. The most common X-linked form of CMT, termed CMT1X, arises in patients with mutations in *GJB1*, the gene that encodes Cx32, which is expressed in both Schwann cells and oligodendrocytes and a number of other cell types. [88] In spite of its

widespread expression, a demyelinating peripheral neuropathy is the only consistent phenotype of the more than 400 mutations that have been reported to date. The CNS signs and symptoms that have been found in some patients with CMT1X are described below, along with their potential mechanisms.

CNS manifestations of CMT1X can be divided into three groups: 1) subclinical abnormalities of visual evoked responses (VERs) and auditory evoked responses (BAERs); 2) overt, generally mild fixed abnormalities on neurological examination and/or CNS imaging and which may or may not be accompanied by clinical manifestations; 3) severe transient CNS dysfunction accompanied by white matter abnormalities on MRI. See Fig. 4 for a schematic of mutations associated with these abnormalities. Although hearing loss has been associated with several mutations including R142Q [89], E186K [90] T191FS, T55R [91] and V38A [92] it remains to be shown that this is truly a phenotypic manifestation of these mutations.

3.1.1 CMT1X subclinical manifestations—Nicholson and colleagues [93, 94] were the first to report prolongation of BAERs in many but not all patients with CMT1X. A number of other investigators have confirmed and extended these finding, showing conduction slowing of VERs [89, 91, 95-101] as well as motor [102] and sensory evoked potentials (SEPs) [103] In a particularly noteworthy study Zambelis et al. [104] measured evoked potentials (blink responses, SEPs, and transcranial magnetic central motor conduction time) in seven affected family members (3 female and 4 male) carrying the Y154STOP mutation. All male and female patients had abnormalities in at least one of these tests; however, males tended to have abnormalities in multiple tests. [104] Nicholson and colleagues [94] reported that several mutations in CMT1X patients they evaluated were not associated with abnormalities of BAERs, including L239I [94] and Y151K, V181M, R183C (G Nicholson, personal communication). Interestingly, patients with complete absence of the coding sequence of Cx32 [90, 105] have no abnormalities of VERs or BAERs, suggesting that these CNS findings are due to gain of function.

3.1.2 Subtle clinical manifestations—A few mutations are associated with mild, static clinical abnormalities. For example, a number of patients have been reported with extensor plantar responses [91, 106-108]. In addition, fixed spasticity, hyperreflexia, dysarthria and ataxia have also been reported. [102, 107, 109, 110] A number of studies have reported MRI imaging abnormalities in CMT1X patients. In most cases, these imaging abnormalities are accompanied by clinical findings, [91, 102, 108, 109] while in at least two case no mention is made of accompanying clinical signs or symptoms [111, 112] Interestingly, two reports [102, 109] describe diffuse involvement of the corticospinal/corticobulbar tracts, suggesting that this tract may represent a selectively vulnerable region; the predominance of findings indicative of corticospinal dysfunction in patients with fixed abnormalities, and the predominance of corticospinal findings in patients with florid acute syndromes (see below) also suggest this possibility. A few additional reports describe patients with scattered white matter lesions. [91, 108, 111]

3.1.3 Florid syndromes—A growing number of CMT1X patients who experienced transient neurological dysfunction accompanied by transient white matter abnormalities on MRI have been reported (see Fig. 5). Findings typically include motor weakness and dysarthria [97, 113-119]. In addition, ataxia [113], respiratory distress [114], dysphagia [115, 117] and disorientation [119] have also been described. The duration of symptoms varies, but they usually resolve between a few hours and a few weeks. However, the MRI changes often lag, taking months to return to baseline. [97, 113, 115, 119, 120] Some of these episodes appear to occur without provocation, [97, 116] but many are associated with

stressors such as hyperventilation or exertion [97, 115, 120] re-acclimatization after return from high altitude above 8000 feet, [113] fever, [114, 119] or minor infections. [115, 117]

3.2 Pathophysiology of CNS phenotypes in CMT1X patients

The absence of clinical or even subclinical abnormalities of VERs or BAERs in patients with complete absence of the coding sequence of Cx32, [90, 105] and the absence of overt CNS manifestations in most patients with CMT1X and in the Cx32 KO mouse [29-31] suggest that Cx32 is not critical for CNS functioning. [30] Thus, the subclinical and overt CNS manifestations of CMT1X are unlikely to arise through simple loss of function of Cx32, suggesting that Cx32 mutants cause a gain of function. However, the nature of that gain of function is not known. One possibility is that Cx32 mutants interact heteromerically with Cx47 expressed in the same cells, thereby reducing the activity of Cx47. The additive effect would be greater than the loss of Cx32 activity alone, and sufficient to cause the subclinical symptoms or to predispose patients to the acute, florid syndromes described above, just as mice lacking expression of both Cx32 and Cx47 have a much more severe phenotype than mice lacking either connexin alone, as described in Section 2.2.1.

We and others have expressed some of these CMT1X_{CNS} mutants in heterologous cells, and examined their ability to form functional GJs. [86, 107, 121-128] Because many mutants associated with CNS phenotypes are localized intracellularly and do not form GJ plaques, it is tempting to speculate that abnormal subcellular localization predicts abnormal interaction with Cx47 and thereby predicts CNS manifestations. [124] (For example, among mutant showing abnormal subcellular localization [107, 124, 128, 129], R142W, R164W, and R164Q are associated with acute attacks as described in section 3.1.3 and A39V and L143P are associated with clinical and/or MRI findings as described in section 3.1.2.) However, other mutants associated with these same CNS phenotypes can form GJs [124, 127-129] (V139M is associated with acute attacks; V35M, P158A, N205S, R220x, show evoked potential abnormalities but no overt phenotype; and M93V and R183H are associated with clinical and/or MRI findings). Further, some mutants with abnormal subcellular localization have no clinical or subclinical CNS phenotype. [94]

As noted in section 3.1.3, the acute, reversible encephalopathy that occurs in CMT1X has often been associated with stressors such as an acute febrile illness or return to lower altitudes after adapting to higher altitudes. The precise physiological trigger(s) have not been identified, but pro-inflammatory cytokines (for systemic illnesses) and decreases in pH (associated with going from high to low altitude) are strong possibilities. We postulate that Cx32 mutants associated with these transient CNS events have trans-dominant-negative effects on Cx47, thereby reducing O/O and/or O/A coupling. Pro-inflammatory factors such as tumor necrosis factor- may directly inhibit glial GJs, [130] exacerbating already tenuous GJ coupling and leading to clinical manifestations. Returning to lower altitudes after adapting to higher altitudes decreases the pH of the cerebrospinal fluid; (CSF) [131] lower pH reduces GJ coupling. [132]. At 13 hours after return to normoxia only a small drop in CSF pH is seen (0.03 pH units) [131]; however longer time points were not studied and the full extent of this drop may be greater. In any case a small pH drop could conceivably affect already tenuous O/A and O/O coupling, since reducing the pH in patch electrodes from 7.4 to 7.2 has dramatic effects on Lucifer Yellow transfer from oligodendrocytes [24].

4. Disease manifestations of GJC2 mutations

4.1 Pelizaeus-Merzbacher-like disease 1 (PMLD1) MIM ID #608804

Pelizaeus-Merzbacher disease (PMD) is an X-linked disorder caused by mutations in *PLP*, the gene for proteolipoprotein (PLP), the major protein of CNS myelin [133]. Pelizaeus-Merzbacher-Like Disease is a group of diseases in patients with a PMD phenotype but

lacking *PLP* mutations. Patients typically develop nystagmus within the first 6 months of life, cerebellar ataxia by 4 years, and spasticity by 6 years of age [134-137]. In 2004 Uhlenberg and colleagues reported that mutations in *GJC2*, the gene encoding Cx47, were found in some PMLD patients [134]. *GJC2* mutations are found in only ~8% of PMLD patients. [138] (*GJC2* associated PMLD is termed PMLD1 or HLD2 (hereditary leukodystrophy type 2.) To date, 23 different CNS disease associated recessive mutations in the coding region of the gene for Cx47 have been identified. [134-139] All patients with PMLD have extensive white matter disease consistent with abnormal myelination on MRI; relative sparing of the corticospinal tracts has been reported (but is not particularly evident on the images shown in Fig. 7) [134-137] MRI spectroscopy studies in two patients showed normal or near normal choline, N-acetyl aspartate and creatine levels [137] (normal N-acetyl aspartate levels indicate lack of significant axonal damage). Figure 6 shows a schematic of Cx47 mutants associated with human disease.

4.2 Hereditary Spastic Paresis (SPG44) MIM ID #613206

Orthmann-Murphy et al. [140] described a large family with three affected members carrying a homozygous I33M mutation of *GJC2* (Fig. 6). One male patient (age 38) developed moderate walking difficulties, leg stiffness and dysarthria around age 31. His brother (age 36) had minimal motor difficulties since infancy but began to show progressive worsening of his gait and speech after age 20; he walks with a cane. A female cousin (age 55) was always considered dull, had difficulty walking and dysarthria in her teens and has been wheelchair bound since age 30; more recently she has developed urinary incontinence followed by retention. All three patients had spasticity, hyperreflexia, and intention tremor on exam and none had nystagmus. All heterozygous individuals in the family were normal. In summary, although only identified in one family thus far, SPG44 is a clinically much milder disease than is PMLD1 with correspondingly milder changes on the cerebral MRI (Fig. 8). Notably, some *PLP1* mutations can also present with a paraparesis phenotype (SPG2). [141] Thus, SPG44 and SPG2 are the only two known causes of hereditary spastic paraparesis associated with mutations in genes that are expressed primarily by oligodendrocytes, and both can also cause a more severe diffuse disorder of myelination. This is particularly interesting because the spastic paraplegia phenotype is thought to represent a length dependent axonopathy, and suggests that mutant forms of Cx47 may directly interfere with interactions between an oligodendrocyte and their associated axons. (The central motor axons to the lumbar segments are the longest central axons in the body and therefore, the most susceptible to length-dependent pathology.)

4.3 Hereditary lymphedema 1C MIM ID #613480

A recent report [142] identified six dominant mutations in Cx47 associated with hereditary lymphedema (Fig. 6). However, no neurological histories, examinations, or imaging were provided.

4.4 Pathogenesis of Cx47 associated diseases

One explanation, consistent with the recessive inheritance pattern and relatively uniform phenotype, is that PMLD1 arises due to loss of channel function of Cx47 in oligodendrocytes. The recent finding by Osaka and colleagues [143], that a mutation in the SOX10 binding site in the *GJC2* promoter causes a mild PMLD phenotype [144], intermediate between that of PMLD1 and SPG44, lends support to the idea that in humans, loss of function of Cx47 is pathogenic and that degree of loss of function may predict severity of phenotype. However, these findings do not rule out gain of function as a contributor to the more severe phenotypes. Data from our laboratories [140] suggest that PMLD1 mutations lead to complete loss of gap junctional coupling, while the SPG44 mutation causes radical alterations in gating, predicted to essentially eliminate Cx47-

mediated coupling; thus, it is unlikely that the milder phenotype of SPG44 results from retention of a small degree of Cx47 mediated coupling. The recent finding by Osaka and colleagues [143], that a mutation in the SOX10 binding site in the *GJC2* promoter causes a mild PMLD phenotype [144], intermediate between that of PMLD1 and SPG44, lends support to the idea that in humans, loss of function of Cx47 is pathogenic and that degree of loss of function may predict severity of phenotype. However, these findings do not rule out gain of function as a contributor to the more severe phenotypes. The work of Diekmann et al. [84][85] suggests that loss of hemichannel function is a feature of some Cx47 mutants, and may represent an additional component of loss of function of some or all of the Cx47 mutants. The finding that mice with targeted ablation of the gene for Cx47 show minimal [14] CNS pathology or phenotypic abnormalities is also inconsistent with a pure loss-of-function model for PMLD1; however it could simply reflect that mouse oligodendrocytes are less dependent on expression of Cx47 for normal function than are human oligodendrocytes. This notion is supported by the recent demonstration [145] that mice with targeted insertion of the Cx47M282T mutation (corresponding to the human PMLD1 causing Cx47M283T mutation) have a very mild phenotype compared to that seen in humans.

A second possibility is that both the PMLD1 and SPG44 mutants lead to a loss of channel function, but that the PMLD1 mutations lead to the loss of an additional non-channel based function. We found that each of three missense Cx47 mutants associated with PMLD1 accumulate in the endoplasmic reticulum, [146] while the SPG44 mutant I33M forms GJ plaques that are indistinguishable from WT Cx47 [140] (see Fig. 9). Our data [146] suggest that PMLD1 mutants may not fold and/or assemble properly. PLP mutants that cause the most severe phenotypes also accumulate in the ER, and activate the unfolded protein response (UPR), [147-149] a group of cellular signaling pathways that can lead to apoptosis. [150, 151] The three missense Cx47 mutants associated with PMLD1 did not activate CHOP, a downstream effector of the UPR [146] that is activated in PMD. [148] However, a more complete evaluation of the UPR pathway, particularly in oligodendrocytes, is needed before rejecting the hypothesis that the UPR is not involved in PMLD1. Finally, as discussed in section 2.2, the loss of junctional Cx47 leads to mislocalization of ZONAB to the nucleus, which could alter gene expression, and thus contribute to the greater severity of the PMLD1 phenotype.

5. Disease manifestations of *GJA1* mutations

5.1 Oculodentodigital dysplasia (ODDD) MIM ID #164200

ODDD is a pleiotropic autosomal dominant disorder seen in patients with mutations in *GJA1*, the gene for Cx43. [152]; these are depicted in Fig. 10. Although a number of patients with this disorder had already been described, [153] the definitive delineation of ODDD dates to the German language publication of “The Microphthalmos Syndrome” by Meyer-Schwickerath in 1957, [154] in which the term ‘dysplasia oculo-dento-digitalis’ was introduced. In 1963, Gorlin et al. [155] reviewed the known cases and defined the core features of syndrome of ODDD as oculo-facial abnormalities including microphthalmia, microcornia and hypoplastic nares, syndactyly of the fourth to fifth fingers and dental abnormalities such as hypoplastic enamel and microdontia. See supplementary tables 3 to 5 in Paznekas et al. 2009 [156] for detailed and comprehensive information on the general phenotypes of patients carrying specific mutations in Cx43. Neurologic manifestations are often but not universally seen, and may not become evident until the second or third decade. The most common are gait difficulties, [153, 157-169] due to either spasticity or ataxia, and urinary incontinence; [166-169] these typically present by the second decade. A wider range of neurologic symptoms have been reported, [153] including cognitive deficiencies, [157, 170] deafness, [171, 172] disorders of extraocular motility, [161, 168, 171] generalized

muscle weakness, and seizures. [152] MRI abnormalities include hypointensity of the deep grey matter, which may reflect iron deposition, and changes in the occipital and periventricular white matter [153, 164, 165]. (See Table 2 for further references to neuroimaging.) In some cases where CT imaging was used, the basal ganglia were calcified. [161, 173] Abnormalities in CNS imaging accompany overt CNS findings, but may also be seen in patients without neurological issues, [170] raising the possibility that many mutations reported as showing no neurologic phenotype may still cause subclinical alterations CNS changes. Along this line, an MRI imaging study [165] of a neurologically symptomatic mother and neurologically normal daughter with ODDD both showed identical changes in the white matter. The clinical and subclinical neurological findings in patients with ODDD are summarized in Table 2.

5.2 Hallermann-Streiff syndrome (HSS); MIM ID 234100

HSS is an autosomal recessive or sporadic syndrome that is at least sometimes associated with mutations in *GJA1*. The clinical phenotype shows substantial overlap with the ODDD, characterized by brachycephaly with frontal bossing, micrognathia, a beaked nose, microphthalmia, cataracts, dental anomalies, hypotrichosis, skin atrophy, and short stature [174-176] Mental retardation is present in some patients. [177] A homozygous R76H mutation was noted in a boy with HSS; [178, 179] his parents were heterozygous carriers of the mutation and were clinically normal. In another case of HSS, Pizzuti et al. [179] found no *GJA1* mutation in the open reading frame, but did not rule out a mutation in the promoter or untranslated region of *GJA1*. Reports of homozygous [180, 181] or compound heterozygous mutations [173] causing ODDD further demonstrate the overlap between HSS and ODDD. A few case reports of HSS had abnormal neuroimaging, but were not studied for *GJA1* mutations. Sigirici and colleagues [182] describe a four year old boy with the HSS phenotype, nystagmus, spasticity, optic atrophy and complete agenesis of the corpus callosum. Decreased cerebellar and medullary size on MRI has also been reported. [183] These reports should be viewed cautiously since, as noted above, at least one patient with a “full blown” HSS phenotype had no mutations in the *GJA1* coding region. [179]

5.3 Pathogenesis of Cx43 associated diseases

The genetics of ODDD and HSS indicate that ODDD is not the result of haploinsufficiency. The heterozygous parents of patients with the R33X [180] and R76S [179] mutations were normal. The mother of the severely affected girl with a compound G2frameshift and R101X mutations (both of which would likely cause loss of function) was heterozygous for the R101X mutation, and said to be have “mild microphthalmia and hypoplastic alae nasi” [173], although those features are difficult to appreciate in the image provided. In accord, heterozygous *Gja1* knockout mice show no overt phenotype in any organ system [184], whereas mice with dominant *Gja1* mutations have phenotypes that resemble those in ODDD (see below).

Many ODDD mutants have been expressed by transfection in heterologous cells (Table 2). These results bring up the following points:

1. Irrespective of known CNS involvement, most, but not all, ODDD mutants studied can form GJ plaques. [169, 185-191] (The number may be reduced for some mutants.) Thus, there is no apparent association between the targeting of the Cx43 mutant to gap junctions and the presence of a CNS phenotype.
2. Functional analyses show that ODDD mutants lead to partial or complete loss of intercellular GJ mediated coupling, and many have dominant-negative effects on wild type Cx43. [185, 188-190, 192] The degree of this dominant-negative effect varies, and it remains to be determined whether this correlates with the severity of

disease, including the presence of CNS manifestations. For example, L90V and V216L (“CNS mutants”) and R202H (a “non-CNS mutant”) reduce Cx43-mediated cell coupling to a similar degree. [185] In addition, it is possible that “trans-dominant-negative” effects of Cx43 mutants on Cx30 contribute to their clinical phenotypes.

3. Several mutants affect hemichannel properties: Y17S, G21R, A40V, F52dup, L90V and I130T all lack hemichannel function, I131M, G138R, G143S all show increased hemichannel activity, while the hemichannel activity of H94P is similar to that of WT. [188, 193] It is possible, but by no means proven, that hemi-channel activity contributes to the ODDD phenotype.
4. I131M, G138R, G143S, and H194P have reduced phosphorylation, a post-translational modification of Cx43 that is associated with increased coupling. [194] Because some (G138R and H194P) but not all (I131M and G143S) of these mutants are associated with neurological findings, the role of phosphorylated Cx43 in this regard remains unproved.
5. The accumulation of Cx43 mutants in intracellular organelles may have deleterious effects on the cells, possibly by activating the UPR as discussed for PMLD1 in Section 4.3. [147-149]
6. Reduced Cx43 in GJ plaques may have deleterious effects on localization of other proteins with which it normally interacts, as outlined in Section 2.2.2.

There are several mouse models of ODDD. Jrt mice are heterozygous for a dominant G60S mutation, and show many of the features of ODDD but have no overt CNS findings or brain MRI abnormalities at 52-60 weeks. [195] Immunohistochemistry and immunoblotting of spinal cord and cerebellum from Jrt mice show decreased staining and decreased levels of Cx43, but astrocytes were still well coupled by GJs, both in vitro and in vivo. [196] In transfected cells, G60S formed GJs that did not pass Lucifer Yellow, but a dominant-negative effect on wild type Cx43 could not be demonstrated [196]. Heterozygous mice with a targeted replacement of WT Cx43 by the ODDD I130T [197] or G138R [193] mutations have reduced embryonic viability, cardiac dysrhythmias, and reduced Cx43 levels in heart tissue, but the CNS was not investigated. Because none of the findings seen in the mouse models have been reported in heterozygous *Gjal* knockout mice [184], they are dominant effects.

In summary, the analysis of cellular and mouse models of ODDD demonstrate that ODDD mutants have several kinds of dominant effects. Of these, dominant-negative effects on wild type Cx43 are by far the best documented, but it remains to be shown that they are sufficient to account for all of the various attributes of ODDD. The striking phenotypic similarities between patients with ODDD (cause by gain of function mutations) and HSS (caused by loss of function mutations) makes a dominant-negative mechanism the most parsimonious explanation.

6. Conclusion

There are five CNS disorders associated with mutations in three connexin genes. Two disorders, PMLD1 and SPG44, are defined by their CNS manifestations and are associated with recessive *GJC2* mutations. Available evidence suggests loss of function of Cx47 is at least partially responsible for these disorders. The difference in severity between the patients with PMLD1 and SPG44 is not well understood, but may reflect either lesser degrees of loss of function for the mutation causing the milder SPG44 phenotype or a component of gain of function for the mutations causing the more severe PMLD1 phenotype. *GJB1* mutations cause CMT1X, which always manifests as a peripheral neuropathy, are often associated with

subclinical neurological findings, and are rarely associated with a severe but transient syndrome. Though the neuropathy appears related primarily to loss of function of Cx32 in the Schwann cell, CNS manifestations are more likely due to a toxic gain of function in oligodendrocytes. Dominant *GJA1* mutations cause ODDD, which is often associated with a CNS phenotype, and recessive *GJA1* mutations cause HSS, which strongly resembles ODDD. The genetics of HSS suggest that haploinsufficiency cannot explain the ODDD phenotype. However, a dominant negative effect on wild-type Cx43 has been demonstrated for a number of ODDD mutants; it remains to be demonstrated that such effects will provide an explanation for the full range of phenotypic manifestations of this disorder.

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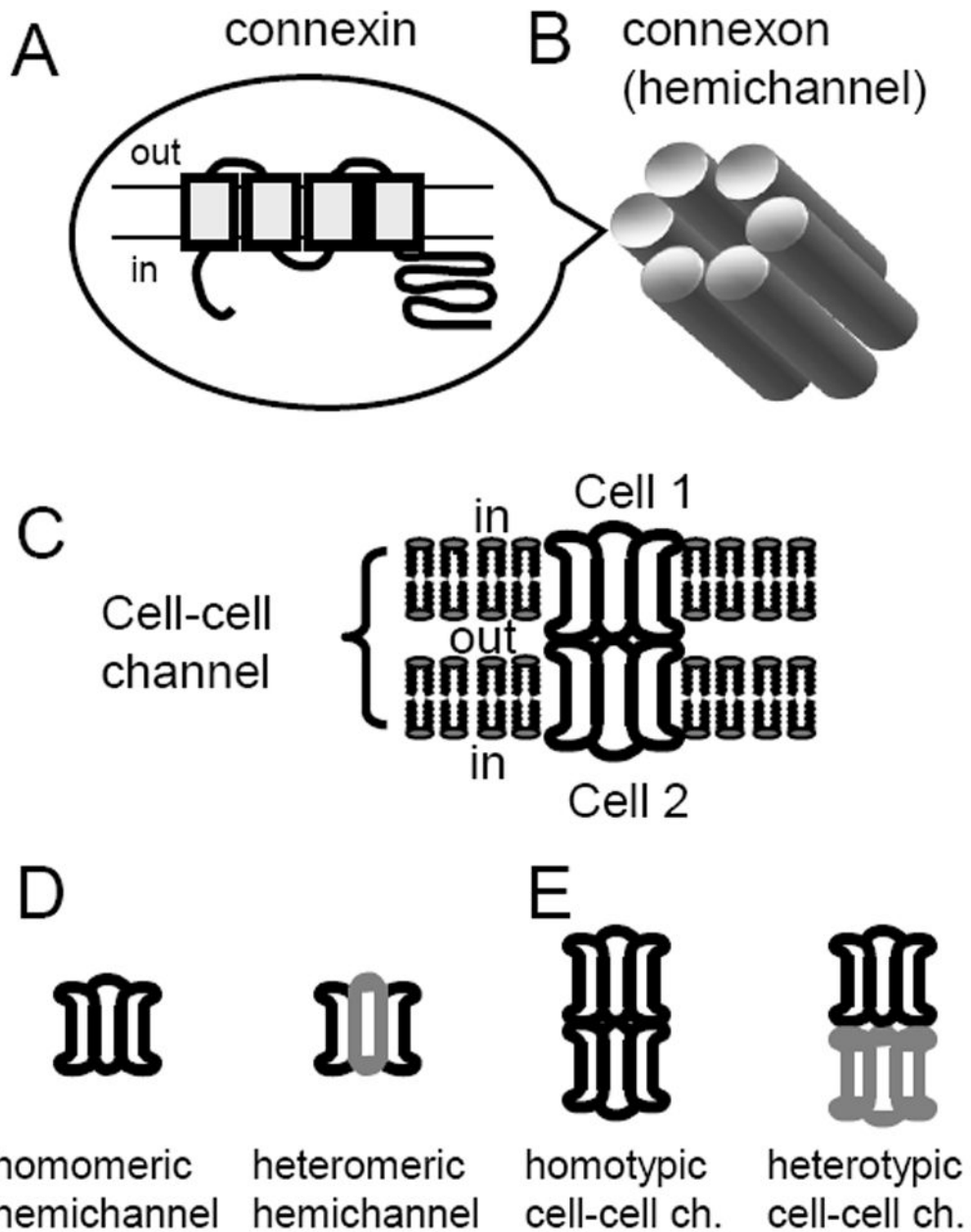
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Highlights

1. Astrocytes express Cx30 and Cx43 and Oligodendrocytes express Cx29, Cx32, and Cx47.
2. Mutations in *GJB1*, cause a form of Charcot-Marie-Tooth disease (CMT1X). Some patients have CNS signs and symptoms.
3. Recessive mutations in *GJC2*, the gene for Cx47, are one cause of Pelizaeus-Merzbacher-like disease.
4. A different recessive *GJC2* mutation causes a form of hereditary spastic paraparesis.
5. Dominant mutations in *GJA1*, cause oculodentodigital dysplasia (ODDD). Spasticity and gait difficulties may be seen.

**Figure 1.**

Connexin nomenclature A. Connexins are integral membrane proteins with four transmembrane domains and intracellular N and C termini. B. Each connexon or hemichannel is composed of six subunits called connexins. C. Cell-cell channels are formed by docking of two apposed connexons from apposed cells. D. If all six subunits are identical the connexon is termed homomeric. In cells expressing more than one type of connexin, different connexins may aggregate to form heteromeric connexons. E. Many members of the connexin family can form functional GJs by associating with a like connexon (a homotypic junction) or a different one (a heterotypic junction).

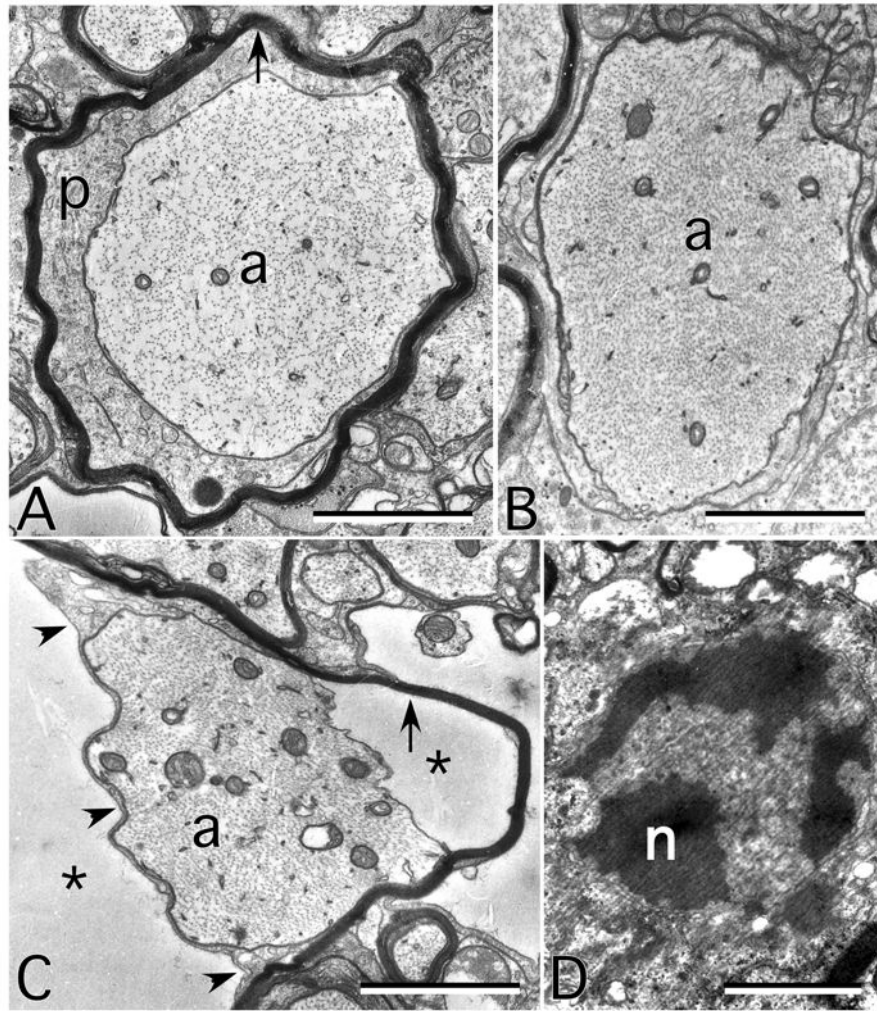


Figure 2. Ultrastructural findings in the CNS myelin of mice lacking expression of Cx32 and Cx47. All are electron micrographs of the ventral funiculus from P31 mice. A. An axon (a) is surrounded by a periaxonal cytoplasmic collar thin myelin sheath (arrow). B. A demyelinated axon. Note that neurofilament packing is tighter than in A. C. An axon still partially surrounded by the adaxonal membrane (arrowheads), but separated from its myelin sheath (arrow) by- extracellular space (asterisks). D. An apoptotic oligodendrocyte nucleus (n). Scale bar, 1 μ m. From Menichella et al. 2003, [16] used with permission of Society for Neuroscience.

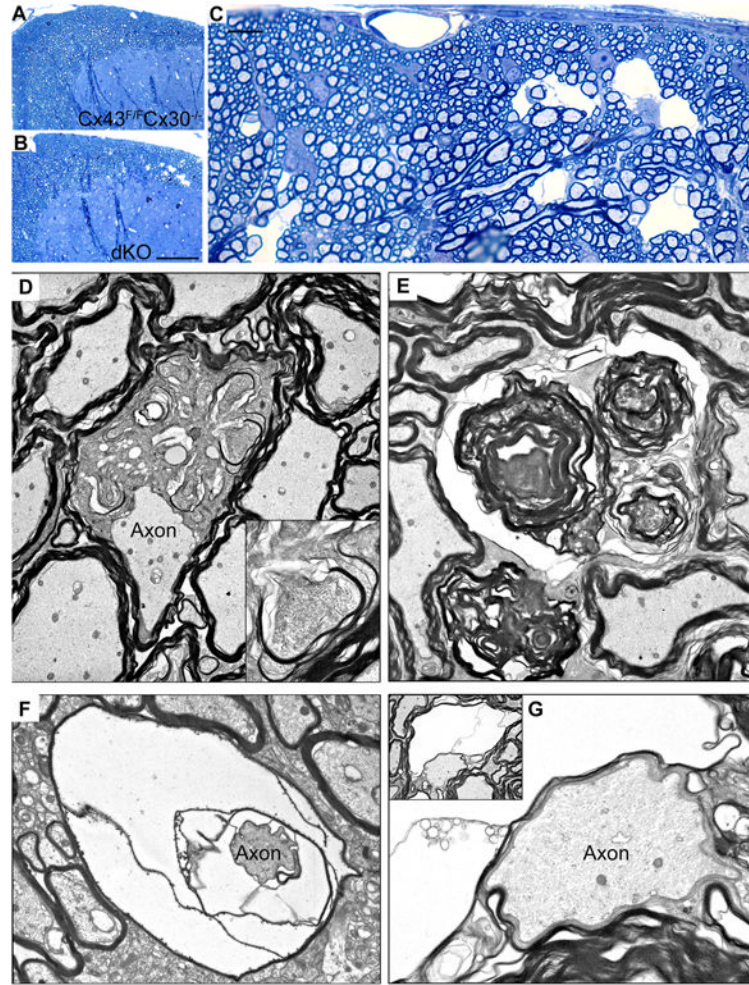


Figure 3. Pathologic findings in the CNS myelin of mice lacking expression of Cx30 globally and Cx43 in astrocytes (Cx30 Cx43_{astro} dKO). A–C. Toluidine blue stained 1 μ m epoxy sections of the dorsal cuneate of the lumbar spinal cord of non-Cre (*Cx43fl/fl Cx30^{-/-}*) (A) and Cx30 Cx43_{astro} dKO (B, C) mice. D, Electron micrograph showing enlarged oligodendrocyte cytoplasm interpolating between an apparently normal axon and its myelin sheath. Inset depicts ectopic expression of myelin in one of several locations within the oligodendrocyte cytoplasm. E, Myelin whorls, likely representing Wallerian axonal degeneration. F and G, Edema and splitting of myelin sheaths. Scale bar: (A, B) 100 μ m; (C) 10 μ m. Magnification: (D) 4000X, inset: 15,000X; (E) 9000X; (F) 8000X; (G) 13,000X, inset: 4000X. From Lutz et al. 2009 [44] used with permission of Society for Neuroscience.

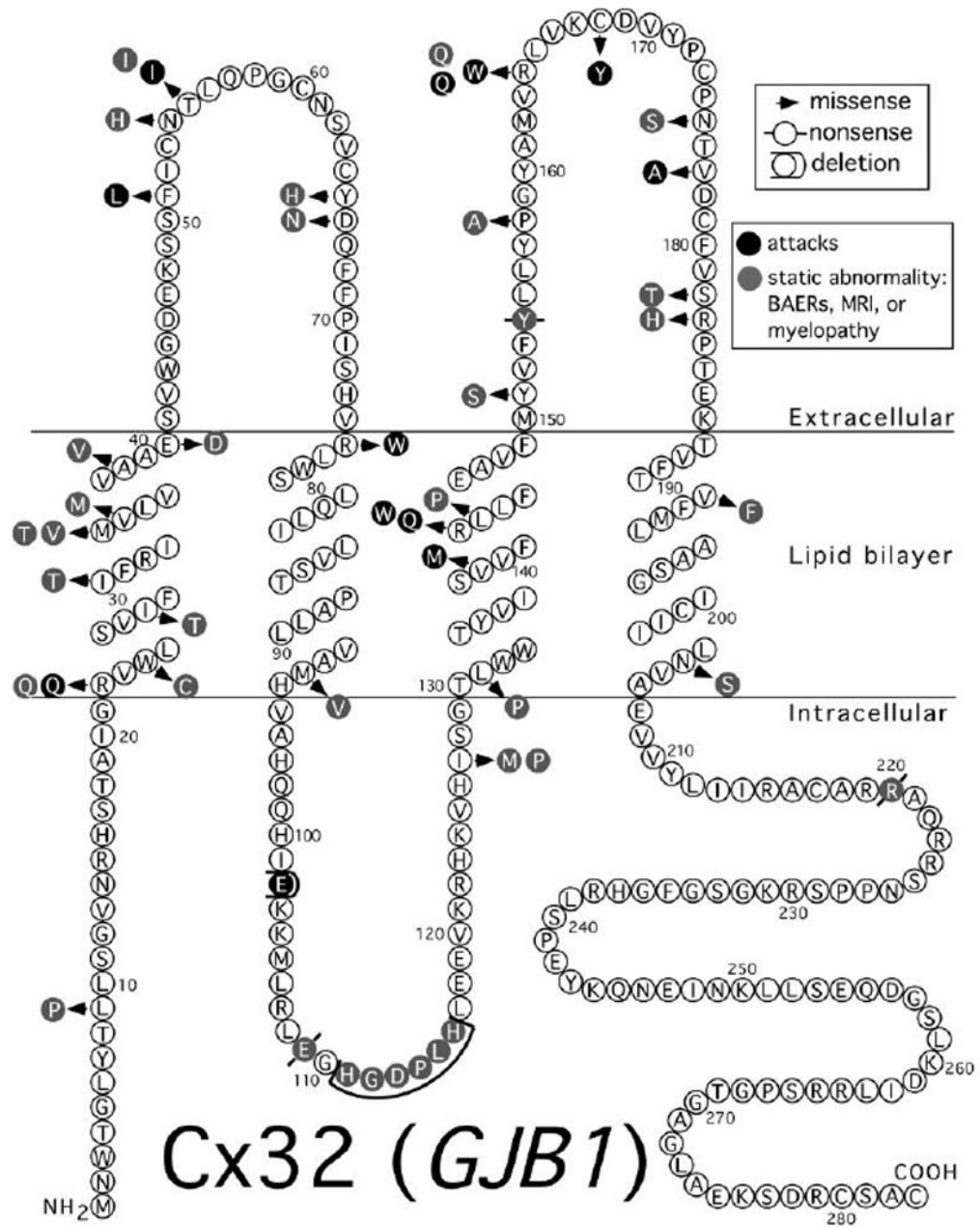


Figure 4. Mutations in Cx32 associated with CNS manifestations. Topology is as depicted in Yeager and Nicholson (1996) [198]

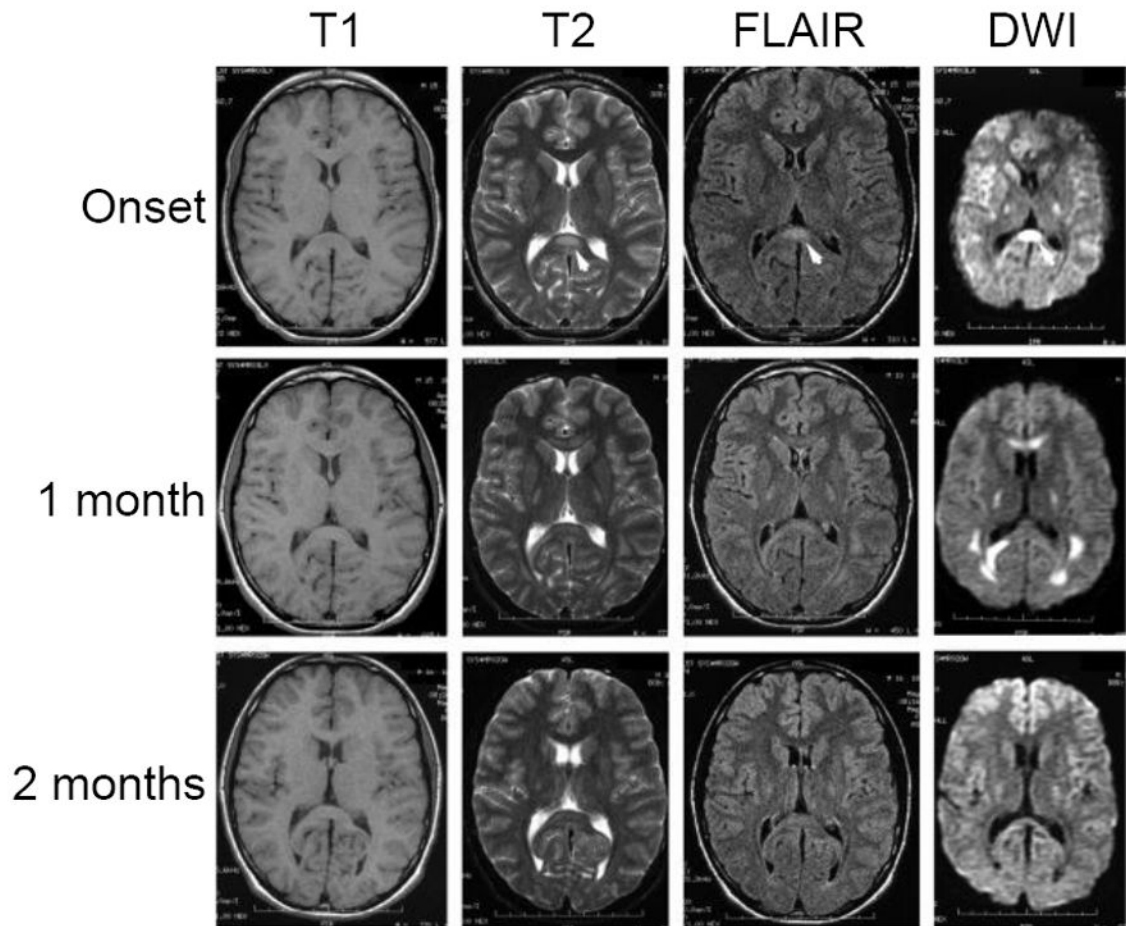


Figure 5.

MRI imaging of a CMT1X patient with an acute florid CNS presentation. T1-weighted, T2-weighted, fluid-attenuated inversion recovery (FLAIR), and diffusion-weighted magnetic resonance imaging (DWI) images during onset of an acute CNS syndrome and 1 and 2 months later show splenium of corpus callosum (*arrow in the T2, FLAIR and DWI images at onset*) and posterior limb of the internal capsule abnormalities at all three time points; signal intensities have almost returned to normal after 2 months. Modified from Paulson et al. (2003) [113], used with permission of Wiley-Davis.

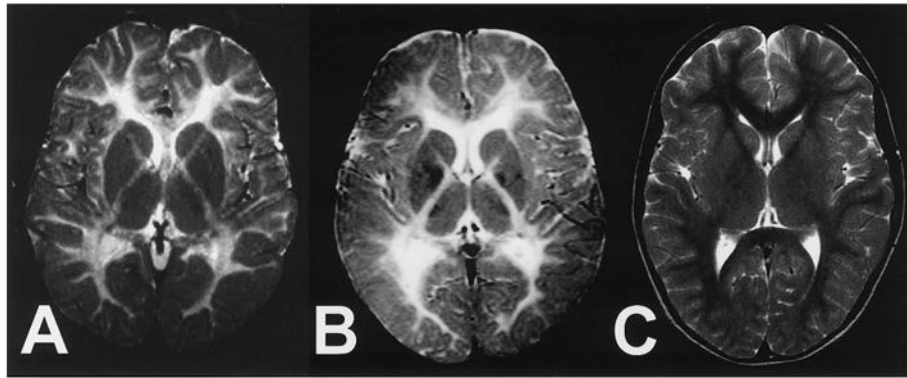


Figure 7. MRI imaging of patients with PMLD1. Axial T2-weighted magnetic resonance images of the brain at the level of the basal ganglia. A. Patient with PMLD at 6 years of age. B. Patient with PMD at 7 years of age. The patients in panels A and B show nearly identical patterns consistent with hypomyelination of central white matter, as indicated by diffusely enhanced signal intensity. C. Low signal of normal myelination in an unaffected child. From Uhlenberg et al. (2004) [134] used with permission of Elsevier.

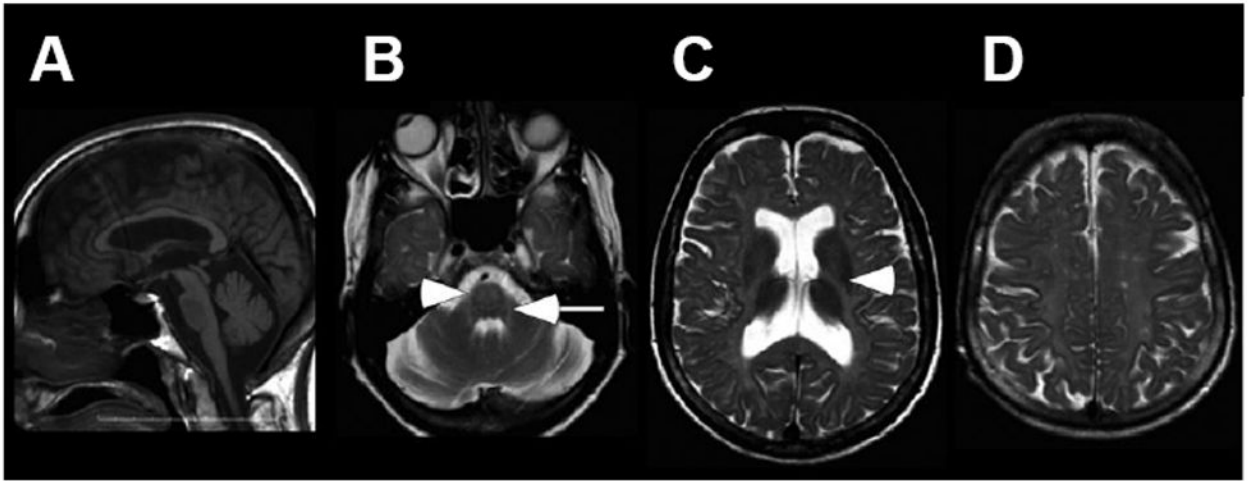


Figure 8.

MRI imaging of a patient with SPG44. A. Sagittal T1-weighted imaging shows diffuse thinning of the corpus callosum. B -D. Axial T2-weighted imaging shows symmetric hyperintensity in the region of the corticospinal/corticobulbar tracts at the level of the pons (B, arrows), and the posterior limb of internal capsule (C, arrowhead). In addition, there is diffuse hyperintensity in the subcortical, lobar and periventricular white matter (C and D), and enlarged ventricles (C) Modified from Orthmann-Murphy et al. (2009) [140] Used with Permission of Oxford University Press.

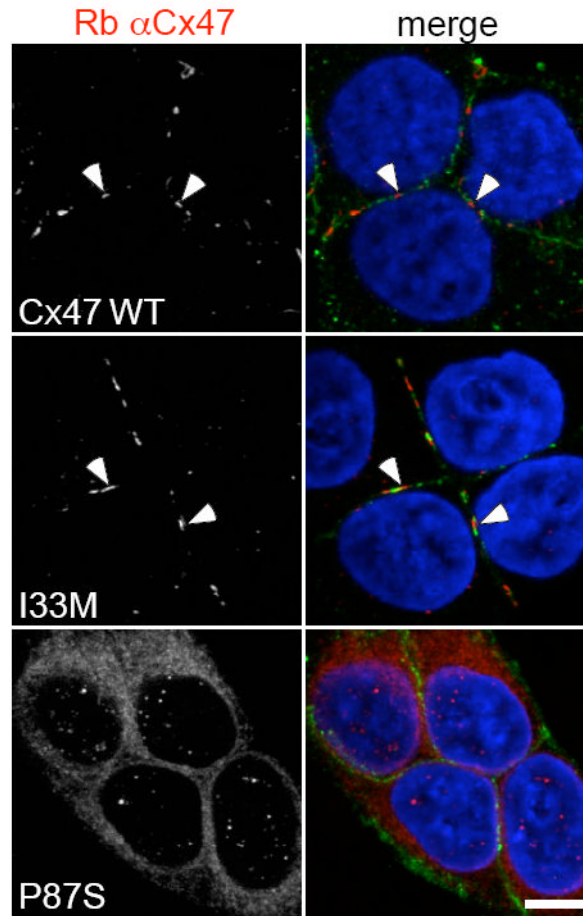


Figure 9.

Immunostaining for the I33M and P87S mutants. The SPG44 associated I33M mutant forms GJ plaques while the PMLD1 associated P87S mutant does not. These are confocal images of bulk-selected HeLa cells that express WT Cx47 or the indicated mutants, immunostained with a rabbit antiserum against human Cx47 (red) and a mouse monoclonal antibody against pan-cadherin (green), and counterstained with DAPI. The pan-cadherin staining at cell borders interdigitates with the cell surface staining of Cx47 in cells that express WT Cx47 (arrowheads) or I33M (arrowheads), but surrounds the staining of cells expressing the mutant P87S, which is localized in the endoplasmic reticulum. Scale bar: 10 μm . From Orthmann-Murphy et al. [140] Used with Permission of Oxford University Press.

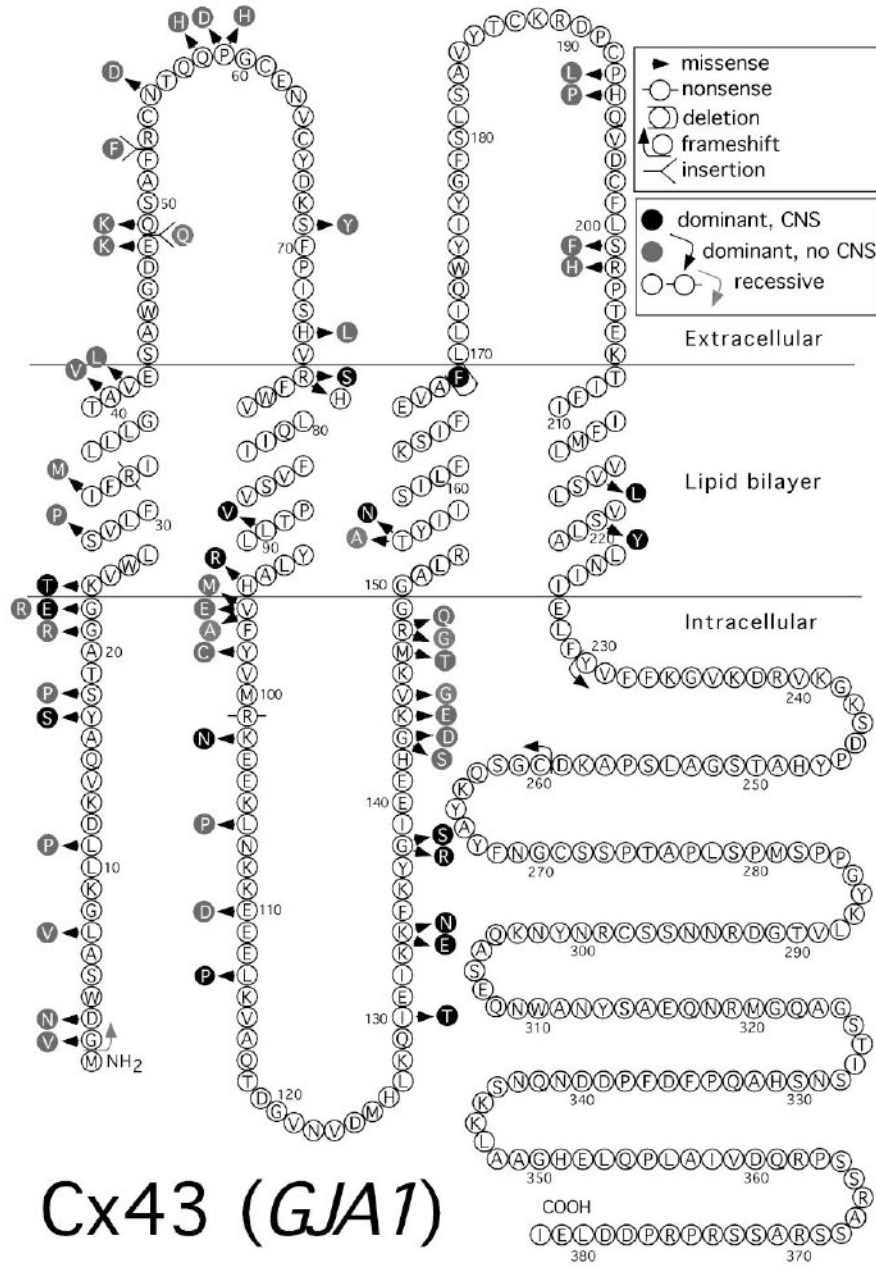


Figure 10. Mutations in Cx43 associated with human disease. Topology is as depicted in Yeager and Nicholson (1996) [198]

Table 1

Behavioral phenotypes of astrocyte connexin KO mice. NR not reported a-not analyzed day by day

phenotype Test	Cx43_{Astro} KO vs. WT [199]	Cx30 KO vs. WT [200]	Cx43_{Astro}-Cx30 vs. WT [44]
Rotarod	Reduced time on rod day 1.	<i>Trend</i> toward reduced time on rod.	Reduced time on rod. ^a
Balance beam	NR	NR	More falls, longer crossing time.
Spatial memory	NR	Reduced contact with displaced object.	Reduced contact with displaced object.
Visual memory	NR	NR	No difference.
Open field	Increased horizontal activity.	Reduced time in center and increased time in corners of field. Reduced rearings.	NR
Graded anxiety		No difference.	NR
Water maze	Steeper learning curve.	NR	NR
Plus maze	Slight increased entry into closed arms.	NR	NR

Table 2

Features of mutations in Cx43 associated with ODDD

Mutation	Functional assay (transfected cell lines unless noted)	Localization in mammalian cells (transfected cell lines unless noted)	Neurologic phenotype	References
Explicit statement of no neurologic manifestations				
SSC			9 YO "no mental retardation or brain wave abnormality." [201]	[201]
L11P			13 YO, de novo mutation. No neuro. findings. [202]	[202]
S18P		GJ-like plaques. (S. Scherer, unpublished)	No neuro. findings. [152]	[152] originally described in [203, 204];
V41A			11 YO, neuro. exam nl.	[205]
Q49K	Low homotypic coupling and minimal dominant negative effect on endogenous Cx43 mediated NRK cell coupling. [185]	GJ-like plaques. [185] (S. Scherer, unpublished)	No neuro. findings. [152]	[152] originally described in [206];
F52dup	No junctional homotypic coupling. [189] No dye transfer by scrape load or hemichannel activity by dye uptake. [188]	ER staining pattern [189] Very reduced puncta. [188] Normal localization (basolateral) in MDCK cells. [186] Endosomal. (S. Scherer, unpublished)	No neuro. findings. [152]	[152] originally described in [207];
P59H			No neuro. findings.	[208]
R76H			Affected child has homozygous mutation; parents are heterozygotes; no neuro. findings.	[209].
V96E			12 YO with de novo mutation in; no neuro. findings.	[157]
Y98C		GJ-like plaques. (S. Scherer, unpublished)	No neuro. findings. [152]	[152] originally described in [206, 210];
H194P	Normal hemichannel activity. No neurobiotin transfer [193]	GJ-like plaques and cytoplasmic. [187]	No syndactyly or spastic paraplegia. [156, 211]	[211]
R202H	No homotypic coupling. [189] Low coupling and dominant negative effect on endogenous Cx43 mediated NRK coupling. [185]	GJ-like plaques (S. Scherer, unpublished) No plaques, some overlap with ER marker. [189] Few plaques partial intracellular retention. [185]	No neuro. findings. [152]	[152, 212, 213]

Mutation	Functional assay (transfected cell lines unless noted)	Localization in mammalian cells (transfected cell lines unless noted)	Neurologic phenotype	References
C260fs	Decreased endogenous plaques when expressed in NRK cells; decreased coupling and dominant negative effect on WT Cx43 in transfected cells [192]	ER retained [192]	Neurologically normal [214]	[214]
Y230fs			No neuro. or eye problems. [156, 215]	[215]
A253V			Polymorphism	[152]
Neurologic manifestations not mentioned				
G2V			Nothing stated.	[216]
L7V			Nothing stated.	[156]
G22R			Nothing stated.	[156]
S27P			Nothing stated.	[212]
I31M			Nothing stated.	[212]
E48K			Nothing stated.	[217] [218]
Q49dup			Nothing stated.	[156]
N55D			Nothing stated.	[156]
Q58H			Nothing stated.	[156]
P59A			Nothing stated.	[156]
P59H			Nothing stated.	[219]
S69Y			Nothing stated.	[212]
H74L			Nothing stated.	[156]
V85M			Nothing stated.	[213]
V96M			Nothing stated.	[220]
E110D			Nothing stated.	[221]
I131M	Increased hemichannel activity. No neurobiotin transfer.[187]	GJ-like plaques and cytopasmic. [187]	Nothing stated.	[212]
G138D			Nothing stated.	[213]
G138S			Nothing stated.	[156, 213]
G143S	Increased hemichannel activity.	GJ-like plaques and cytopasmic. [187]	Nothing stated.	[212]

Mutation	Functional assay (transfected cell lines unless noted)	Localization in mammalian cells (transfected cell lines unless noted)	Neurologic phenotype	References
	No neurobiotin transfer. [193]			
G143D			Nothing stated.	[213] [156]
K144E			Nothing stated.	[156]
V145G			Nothing stated.	[156]
M147T			Sporadic case; nothing stated.	[221]
R148Q			Nothing stated.	[212] originally described in [222]
R148G			Nothing stated.	[156]
F169del			Nothing stated.	[221]
P193L			Nothing stated.	[156]
S201F			Nothing stated.	[156]
S201Y			Nothing stated.	[213]
Explicit description of neurologic manifestations				
G2fs			G2fs+R101x; severe CNS phenotype, psychomotor regression onset age 10, seizures; massive calcifications, hypomyelination, and atrophy on CT/MRI. [173]	[173]
D3N	Decreased coupling in patient fibroblasts. [169]	Partial localization in Golgi (but also some WT in golgi distribution) in patient fibroblasts. [169]	Neurogenic bladder, UMN. [169]	[156, 213] [169]
Y17S	No junctional coupling. [189] No dye transfer by scrape load or hemichannel activity by dye uptake. [188]	GJ-like plaques. (reduced nos.) [188] GJ-like plaques. (S. Scherer, unpublished)	“Neuro. deficits were prominent.” [206] UMN, ur. inc. [156]	[152] originally described in [206, 223]
G21R	No homotypic junctional coupling. [189] [190] No dye transfer by scrape load or hemichannel activity by dye uptake. [188] Dominant negative effect on endogenous 43 in NRK cells [190] and HeLa and N2a cells. [224]	GJ-like plaques. (reduced numbers) [189] [188, 190]	No neuro. symptoms but patient is a 2 YO with a sporadic mutation. [152] UMN. [156]	[152]
G22E		GJ-like plaques. (S. Scherer, unpublished)	“Neuro. Symptoms.” [152]	[152] originally described in [225]
K23T		GJ-like plaques. (S. Scherer, unpublished)	“Neuro. Symptoms.” [152] UMN, MRI WMC, tremor. [156]	[152] originally described in [155]

Mutation	Functional assay (transfected cell lines unless noted)	Localization in mammalian cells (transfected cell lines unless noted)	Neurologic phenotype	References
R33x			2 siblings homozygous for this mutation; speech delay, UMN, grossly abnormal grey/white matter differentiation on head CT. [180]	[180, 181]
A40V	No homotypic coupling. [189] No dye transfer by scrape load or hemichannel activity by dye uptake. [188]	GJ-like plaques. (reduced numbers) [188, 189] GJ-like plaques. (S. Scherer, unpublished)	No neuro. symptoms but patient is a 2 YO with a sporadic mutation. [152] gait difficulty, ur. urgency [156]	[152, 212, 221]
Q49P			Ur. inc.	[156]
R76S		GJ-like plaques. (S. Scherer, unpublished)	“Neurological deficits were prominent” [206] Epilepsy [152] MRI WMC [156]	[152] originally described in [226]
L90V	Coupling very low, single channel conductance normal. [189] Low junctional conductance and dominant negative effect on endogenous Cx43 mediated NRK coupling. [185] No dye transfer by scrape load or hemichannel activity by dye uptake. [188]	GJ-like plaques. (reduced nos.) [188, 189] Disrupted localization (partial apical) in MDCK cells. [186] GJ-like plaques. [185] GJ-like plaques. (S. Scherer, unpublished)	“Neuro. deficits were prominent” [206] Epilepsy. [152] Ur. inc. and spastic paraplegia (9/9 patients) [156, 227]	[152] originally described in [206, 227, 228]
H95R			Ur. inc. and spasticity [166] MRI WMC [156]	[166]
V96A			MRI WMC.	[156] originally described in [162]
R101x			Compound heterozygote G2fs +R101x; See G2fs above.	[173]
K102N		GJ-like plaques. (S. Scherer, unpublished)	Neurologic symptoms. [152] ur. and bowel inc. [156]	[152]
L106P			Spasticity, ur. inc.	[156]
L113P			Spastic paraparesis. [157] UMN, MRI WMC and low signal in grey matter c/w iron deposition.[153] Others note no neurologic findings [229]	[157, 212, 229] originally in [153]
I130T	Markedly decreased coupling; single channel size normal. [189, 191] No dye transfer by scrape load or hemichannel activity by dye uptake. [188]	GJ-like plaques. [191] GJ-like plaques (very reduced). [189]	Spastic para- or tetraparesis; epilepsy, ur Inc. MRI cerebellum/midbrain atrophy or hypoplasia [167]	[152, 167]

Mutation	Functional assay (transfected cell lines unless noted)	Localization in mammalian cells (transfected cell lines unless noted)	Neurologic phenotype	References
		GJ-like plaques (slightly reduced). [188]		
K134E	Markedly decreased or absent coupling; reduced single channel size (54 pS) [191] Reduced coupling. [189]	GJ-like plaques. [191] GJ-like plaques (very reduced). [189]	“Neurologic symptoms.” [152] UMN, MRI WMC. [156]	[152]
K134N	Reduced coupling.[189]	GJ-like plaques (very reduced). [189]	UMN. [156]	[212] probably described previously[230]
G138R	No homotypic,coupling, shifted G_j - V_j relation in hetrotypic with Cx43-EGFP. Increased hemichannel activity no neurobiotin transfer[193] [187] No homotypic coupling. [190, 191] Dominant negative effect on endogenous Cx43 in NRK cells [190] and HeLa and N2a cells. [224]	GJ-like plaques. [191] GJ-like plaques and cytoplasmic. [187]	4 generations 1 and 2: no neurologic findings. 3 and 4: spastic bladder paraparesis, square wave jerks and intention tremor presented 2 nd to 4 th decade. documented WMC on MRI [168] UMN, ur. inc., MRI WMC. [156]	[152] originally described in [168, 206]
T154A			Mild psychomotor delay and WMC on MRI scan. [231] Gait abnl, UMN, ur. inc., MRI WMC. [156]	[156, 231]
T154N			Spastic paraparesis, inc., tremor. [157]	[157]
V216L	Decreased coupling in patient fibroblasts. [169] No conductance and dominant negative effect on endogenous Cx43 mediated NRK coupling. [185]	Partial overlap with Golgi marker (but also some WT in Golgi distribution) in patient fibroblasts. [169] Few GJ-like plaques partial intracellular retention [185] GJ-like plaques. (S. Scherer, unpublished)	Spasticity, WMC on MRI.[172] Gastrointestinal hypomotility, neurogenic bladder, lost ambulation at age 54; hyperreflexia, spasticity. [169] UMN, MRI WMC ur. and bowel inc. [156]	[152, 169] originally described in [172, 206]
S220Y			Developmental and language disorder. [157]	[157]
No identified mutation				
?			Spasticity.	[158]
?			Severe spasticity.	[159]
?			Spastic tetraparesis.	[160]
?			Spastic paraparesis, nystagmus.	[161]
?			Spastic paraparesis and decreased signal c/w iron deposition in globus pallidus on MRI.	[232]

Mutation	Functional assay (transfected cell lines unless noted)	Localization in mammalian cells (transfected cell lines unless noted)	Neurologic phenotype	References
?			21 YO F spastic paraparesis MRI WMC and low signal in grey matter c/w iron deposition.	[164]
?			MRI diffuse WMC, Abnl low signal in globus pallidus, substantia nigra without calcification on CT c/w iron deposition.	[165]

WMC- white matter changes, typically increase signal on T2 weighted imaging. c/w-consistent with. GJ-gap junction. YO-years old. abnl-abnormal. nl-normal. h/o-history of. Gj-Vj – normalized conductance-voltage relation. UMN-spasticity/hyperreflexia. ur inc-urinary incontinence. neuro-neurological.