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X-linked Charcot-Marie-Tooth disease

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Abstract

The X-linked form of Charcot-Marie-Tooth disease (CMT1X) is the second most common form of hereditary motor and sensory neuropathy. The clinical phenotype is characterized by progressive muscle atrophy and weakness, areflexia, and variable sensory abnormalities; central nervous system manifestations occur, too. Affected males have moderate to severe symptoms, whereas heterozygous females are usually less affected. Neurophysiology shows intermediate slowing of conduction and distal axonal loss. Nerve biopsies show more prominent axonal degeneration than de/remyelination. More than 400 different mutations in *GJB1*, the gene that encodes the gap junction (GJ) protein connexin32 (Cx32), cause CMT1X. Many Cx32 mutants fail to form functional GJs, or form GJs with abnormal biophysical properties. Schwann cells and oligodendrocytes express Cx32, and the GJs formed by Cx32 play an important role in the homeostasis of myelinated axons. Animal models of CMT1X demonstrate that loss of Cx32 in myelinating Schwann cells causes a demyelinating neuropathy. An effective therapy remains to be developed.

Keywords

CMT; connexin32; gap junctions; myelin; neuropathy; oligodendrocytes; Schwann cells

Neuromuscular Manifestations of CMT1X

Shortly after Charcot, Marie, and Tooth published their descriptions of families with autosomal dominant inherited neuropathy that was later given their names (CMT), Herringham (1888) recognized a family in which males were selectively affected (well before Morgan's demonstration of X-linked inheritance in 1910). In the next 100 years, X-linked inherited neuropathy (CMT1X) was reported occasionally, and its existence was briefly questioned (Harding and Thomas, 1980), but has now emerged as the second most common form of CMT1 (Latour et al., 2006; Saporta et al., 2011).

Most males are clinically affected beginning at 10 years of age (Birouk et al., 1998; Shy et al., 2007). The initial symptoms include difficulty in running and frequently sprained ankles. The distal weakness progresses to involve the gastrocnemius and soleus muscles, even to the point where assistive devices are required for ambulation. Weakness, atrophy, and sensory loss also develop in the hands, particularly in thenar muscles. Muscle atrophy, particularly

of intrinsic hand muscles, positive sensory phenomena, and sensory loss may be more prominent in CMT1X than in CMT1A.

CMT1X is considered to be an X-linked dominant trait because it affects female carriers. Affected women usually have a later onset than men, and a milder version of the same phenotype at every age. Female carriers are less affected probably due to X-inactivation; only a fraction of their myelinating Schwann cells express the mutant *GJB1* allele (Scherer et al., 1998; Siskind et al., 2011). Women may even be asymptomatic, and a few kindreds have been reported to have “recessive” CMT1X. Even in these kindreds, however, at least some obligate carriers have electrophysiological evidence of peripheral neuropathy.

Men with CMT1X typically have “intermediate” slowing of nerve conduction velocities (NCV), and mildly prolonged distal motor and F-wave latencies. Forearm motor NCV are typically 30–40 m/s in affected males and 30–50 m/s in affected females (Nicholson and Nash, 1993; Birouk et al., 1998; Shy et al., 2007) – faster than in most CMT1 patients and slower than in most CMT2 patients. This intermediate slowing is characteristic of CMT1X and should raise the consideration of this diagnosis in an appropriate clinical setting. Compared with CMT1A, conduction slowing in CMT1X is less uniform among different nerves and dispersion is more pronounced (Tabaraud et al., 1999; Gutierrez et al., 2000). There is electrophysiologic evidence of distally accentuated axonal loss: the peroneal and tibial motor responses are frequently absent, the median and ulnar motor amplitudes are reduced, and EMG confirms the length-dependent loss of motor units.

Age-related loss of myelinated fibers, and in parallel an increasing number of regenerated axon clusters, are the most prominent pathological finding in nerve biopsies (Senderek et al., 1999; Hahn et al., 2001). Many myelin sheaths are inappropriately thin for the axonal diameter (suggesting chronic segmental demyelination and remyelination, or remyelination after axonal regeneration), although this is less prominent than in biopsies from other kinds of CMT1.

***GJB1* Mutations Cause CMT1X**

Since the first report of Bergoffen et al. (1993), more than 400 mutations in *GJB1* have been described, predicted to affect all regions of the connexin32 (Cx32) protein (Fig. 1). Only one of the reported amino acid changes is a polymorphism, indicating that all the affected residues are required for the normal function of Cx32. Many of the mutations have been reported more than once; some of these probably represent founder effects, whereas others may represent mutational “hot spots” in *GJB1*. In several CMT1X kindreds, the entire coding region of *GJB1* is deleted. In addition, a few mutations likely abolish the expression of Cx32 by affecting the promoter of the *GJB1* gene or the translation of Cx32 mRNA. Because different *GJB1* mutations, including a deletion, appear to cause similar degree of neuropathy, all *GJB1* mutations likely cause loss of function (Shy et al., 2007).

When expressed in *Xenopus* oocytes, many Cx32 mutants do not form functional channels; other mutants form functional channels with altered biophysical characteristics; two of these (S26L and M34T) maintain electrical coupling, but have reduced pore diameter such that they may prevent the diffusion of second messengers like inositol triphosphate (IP3), cyclic adenosine monophosphate (cAMP), and Ca^{2+} (Abrams et al., 2000). Mutants in the C-terminal domain form functional gap junctions (GJs), although compared with wild-type Cx32, some channels are less stable (Castro et al., 1999). The fact that several disease-related mutants (R15Q, H94Q, C217X, R238H, C280G, and S281 stop) form fully functional channels, underscores the limitations from these studies in relation to pathogenesis of CMT1X.

Expressing Cx32 mutants in mammalian cells reveals that intracellular trafficking of the mutant protein is often abnormal. Four patterns of Cx32 localization emerge (Yum et al., 2002): (1) no Cx32 is detected, even though its mRNA is expressed, (2) Cx32 appears to be retained in the endoplasmic reticulum, (3) Cx32 appears to be retained in the Golgi, and (4) GJ plaques on the cell surface are seen. Mutants that reach the cell membrane typically form functional GJs, although they may have abnormal properties (Abrams et al., 2000). The localization of mutants in mammalian cells can be reconciled to the functional studies in oocytes: mutants that form functional GJs in oocytes usually reach the cell membrane of transfected mammalian cells; mutants that do not form functional GJs in oocytes do not reach the cell membrane in mammalian cells.

Myelinating Schwann Cells Express Cx32

Connexins belong to a multigene family encoding 20 highly homologous proteins (Willecke et al., 2002). Connexins are predicted to have the same overall topology (Fig. 1). Six connexins form a hemichannel (or connexon), arranged around a central pore (Nakagawa et al., 2010). Two apposed hemichannels form a functional channel that provides a contiguous pathway between the adjacent cells or cell compartments. The channel diameter is too small to allow transfer of proteins and nucleic acids, but large enough to allow the diffusion of ions and other small molecules (<1000 Da).

Many cell types, including oligodendrocytes and Schwann cells, express Cx32. Despite this broad expression pattern, peripheral neuropathy is usually the sole clinical manifestation of *GJB1* mutations. The co-expression of other connexins may “protect” some tissues against the loss of Cx32. Oligodendrocytes, for example, also express Cx47, and the loss of both Cx32 and Cx47 is far more deleterious than the loss of either one alone (Menichella et al., 2003; Odermatt et al., 2003). Although myelinating Schwann cells in rodents express Cx29 (the human orthologue is Cx31.3), Cx29/Cx31.3 does not prevent the development of demyelinating neuropathy, perhaps because it does not form functional GJs (Ahn et al., 2008; Sargiannidou et al., 2008).

Cx32 is localized to non-compact myelin of incisures and paranodes (Bergoffen et al., 1993), where it likely forms these GJs between the layers of the Schwann cell myelin sheath (Balice-Gordon et al., 1998). A radial pathway formed by GJs at these locations would be up to 300-fold shorter than the circumferential pathway within the Schwann cell cytoplasm. It still remains to be shown that *GJB1* mutations disrupt this shortcut, as well as the exact role this pathway plays in the homeostasis of myelinated axons.

Animal Models of CMT1X

Mice with targeted deletion of the *Gjb1* gene develop a progressive, demyelinating peripheral neuropathy beginning at about 3 months of age (Anzini et al., 1997; Scherer et al., 1998). For unknown reasons, motor fibers are much more affected than sensory fibers, a feature not yet noted in CMT1X patients. Expressing the wild-type human Cx32 protein largely prevents demyelination in *Gjb1*-null mice (Scherer et al., 2005), confirming that the loss of Cx32 only in myelinating Schwann cells is sufficient to cause the demyelination seen in CMT1X.

To determine whether some Cx32 mutants have more than a simple loss of function, we generated transgenic mice expressing the 175 fs, R142W, C280G, and S281 stop mutations. No Cx32 protein could be detected and no peripheral neuropathy was noted in many lines of mice expressing the 175 fs transgene, even though transgenic/human mRNA was highly expressed in some lines (Abel et al., 1999). In contrast, the R142W mutation was retained in the perinuclear region and did not reach the incisures or paranodes, and did not rescue the

demyelinating neuropathy in *Gjb1*-null mice (Jeng et al., 2006). The C280G or S281 stop mutants were properly localized to incisures and paranodes, and appeared to prevent demyelination in *Gjb1*-null mice, indicating that these mutants may form functional channels in the myelin sheath (Huang et al., 2005). Thus, the functional abnormal attributes of the C280G and S281 stop mutants, and likely other mutants, remain to be elucidated.

CNS Manifestations of CMT1X

Many *GJB1* mutations appear to be associated with electrophysiological, clinical, and/or magnetic resonance imaging (MRI) findings of central nervous system (CNS) involvement (Nicholson and Corbett, 1996; Nicholson et al., 1998; Senderek et al., 1999). Furthermore, patients with R22Q, T55I, R75W, E102del, V139M, R142W, R164W, R164Q, C168Y, or V177A mutations have developed the striking picture of an acute, transient encephalopathy associated with MRI changes in CNS myelin, often “triggered” by travel to high altitudes, intense physical activity, or acute infections. Because these electrophysiological findings have not been found in patients with a deleted *GJB1* gene (Hahn et al., 2000), these mutants may cause a gain of abnormal function. However, two of these mutants (T55I and R75W) when expressed in oligodendrocytes had no apparent trans-dominant effect on expressed Cx47 (Sargiannidou et al., 2009). Elucidating the consequences of *GJB1* mutations both in peripheral nervous system and CNS is an important requirement for developing effective CMT1X treatments in the future, such as gene replacement strategies.

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Figure 1.

CMT1X mutants. This schematic shows the basic structure of Cx32, which has four transmembrane domains, one intracellular and two extracellular loops, as well as an amino- and a carboxy-terminal cytoplasmic tail. The *GJB1* mutations of the coding region are indicated, more than 400 altogether (<http://www.molgen.ua.ac.be/CMTMutations/Mutations/MutByGene.cfm>).