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REVIEW ARTICLE

Molecular Genetics of 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 molecularly designated form of hereditary motor and sensory neuropathy. The clinical phenotype is characterized by progressive distal muscle atrophy and weakness, areflexia, and variable sensory abnormalities. Affected males have moderate-to-severe symptoms, whereas heterozygous females are usually mildly affected or even asymptomatic. Several patients also have manifestations of central nervous system involvement or hearing impairment. Electrophysiological and pathological studies of peripheral nerves show evidence of demyelinating neuropathy with prominent axonal degeneration. A large number of mutations in the *GJB1* gene encoding the gap junction (GJ) protein connexin32 (Cx32) cause CMT1X. Cx32 is expressed by Schwann cells and oligodendrocytes, as well as by other tissues, and the GJ formed by Cx32 play an important role in the homeostasis of myelinated axons. The reported CMT1X mutations are diverse and affect both the promoter region as well as the coding region of *GJB1*. Many Cx32 mutants fail to form functional GJ, or form GJ with abnormal biophysical properties. Furthermore, Cx32 mutants are often retained intracellularly either in the endoplasmic reticulum or Golgi in which they could potentially have additional dominant-negative effects. Animal models of CMT1X demonstrate that loss of Cx32 in myelinating Schwann cells causes a demyelinating neuropathy. No definite phenotype–genotype correlation has yet been established for CMT1X and effective molecular based therapeutics for this disease, remain to be developed.

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Index Entries: CMT; neuropathy; X-linked; connexin32; gap junctions; Schwann cells; oligodendrocytes; myelin.

Introduction

Shortly after Charcot, Marie, and Tooth (CMT) published their descriptions of families with autosomal-dominant-inherited neuropathy that was later given their names, Herringham (1889) recognized a family in which males were selectively affected. He concluded that the affected men

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presented with a similar phenotype as the individuals described by Charcot, Marie, and Tooth. Although the women appeared to pass the trait of their fathers to their sons, they were themselves unaffected. Herringham's recognition that the inheritance of the disease was gender-linked is remarkable, as Mendel's discovery of autosomal inheritance would not be known until 1889 and Morgan's demonstration of X-linked inheritance would not appear until 1910.

In the century that followed Herringham's report, X-linked inherited neuropathy (CMT1X) was only reported in sporadic kindreds (Allan, 1939; Erwin, 1944; Woratz, 1964; Swift and Horowitz, 1969; Campeanu and Morariu, 1970; de Weerdt, 1978; Heimler et al., 1978; Fryns and Van den Berghe, 1980) and its prevalence was underestimated (Harding and Thomas, 1980b). However, subsequent investigations confirmed X-linked inheritance in many more families with inherited neuropathy (Iselius and Grimby, 1982; Phillips et al., 1985; Rozear et al., 1987; Hahn et al., 1990). With the advent of molecular genetics, mutation analysis of many Charcot-Marie-Tooth (CMT) families in different populations demonstrated that CMT1X is the second most common form of demyelinating CMT (after *PMP22* duplication), with a frequency of 7–11% among all CMT patients (Silander et al., 1998; Mersiyanova et al., 2000; Mostacciuolo et al., 2001; Boerkoel et al., 2002; Numakura et al., 2002).

Neuromuscular Manifestations of CMT1X

The clinical onset of CMT1X in affected males is between 5 and 20 yr of age (Hahn et al., 1990; Nicholson and Nash, 1993; Birouk et al., 1998; Hahn et al., 2000). The initial symptoms include difficulty in running and frequently sprained ankles; foot drop, and sensory loss in the legs develop later. Depending on the severity of the disease, the distal weakness may progress to involve the gastrocnemius and soleus muscles (Fig. 1), even to the point where assistive devices are required for ambulation. Weakness, atrophy, and sensory loss also develop in the hands, particularly in the thenar muscles (Fig. 1). These clinical manifestations are the result of a chronic, length-dependent axonal loss, and are nearly indistinguishable from those seen in patients with CMT1A or CMT1B. However, muscle atrophy, particularly of intrinsic hand muscles, positive sensory phenomena, and sensory loss may be more prominent in CMT1X patients. Neurological

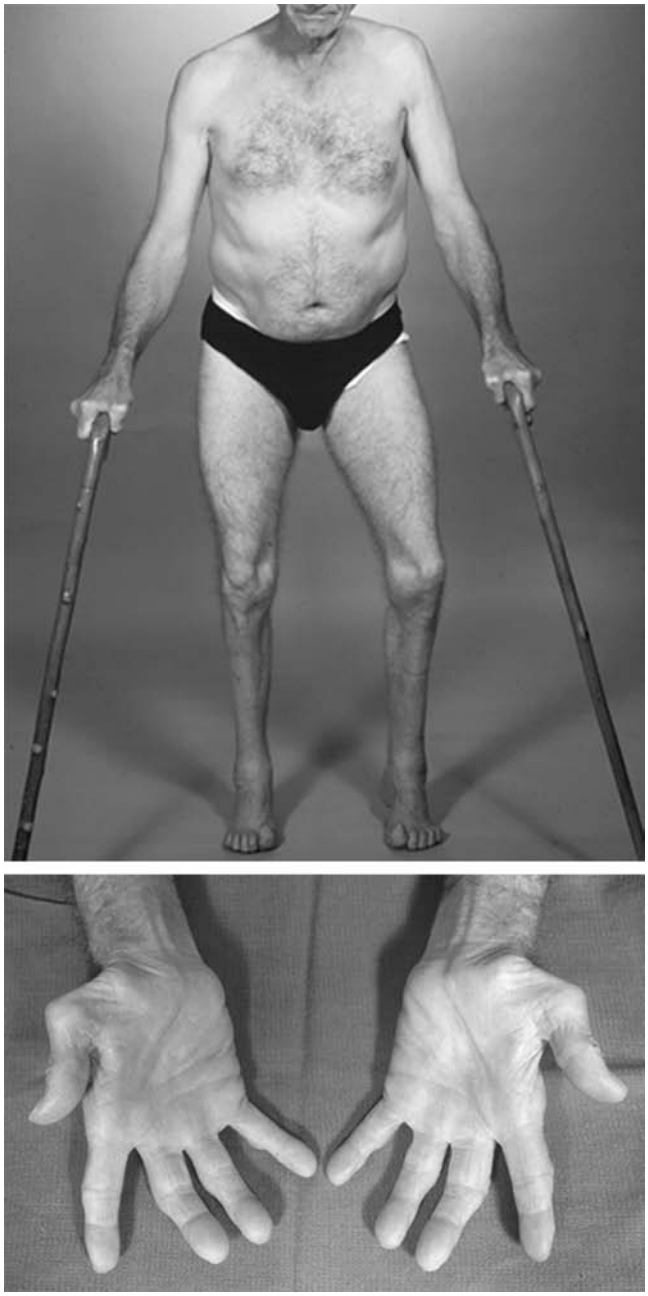

Fig. 1. Length-dependent weakness and atrophy in a 61-yr-old patient with the Y211stop mutation (patient IV.1; Hahn et al., 1990). Note the loss of bulk in the muscles below the knee and in the hands. The photographs were kindly supplied by Angelika Hahn, and used with permission of Oxford University Press.

examinations reveal weakness and atrophy, diminished to absent reflexes, and sensory impairment, all of which are length-dependent and worsen insidiously over time but to varying degrees in different patients. Pes cavus, varus deformities, and "hammer toes" are frequently present.

CMT1X is considered to be an X-linked dominant trait because it also affects female carriers. Affected women usually have a later onset than men, after the end of second decade, and a milder version of the same phenotype at every age. Female carriers are less affected probably because of X-inactivation; only a fraction of their myelinating Schwann cells express the mutant *GJB1* allele (Scherer et al., 1998). 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 (Niewiadomski and Kelly, 1996; Hahn et al., 1999). The neuromuscular manifestations of CMT1X, including their age-related progression and greater severity in males, are elegantly described by Hahn et al. (1990).

Electrophysiological Findings in CMT1X

Patients with CMT1X typically have "intermediate" slowing of nerve conduction velocities (NCV), and mildly prolonged distal motor and F-wave latencies. Forearm median or ulnar motor NCV are in the range of 30–40 m/s in affected males, and 30–50 m/s in affected females (Nicholson and Nash, 1993; Rouger et al., 1997; Birouk et al., 1998; Hahn et al., 1999; Senderek et al., 1999). These are 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 (Nicholson et al., 1998). 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). The overlap in motor NCVs in CMT1X and CMT2 has lead some to conclude that *GJB1* mutations cause CMT1X and CMT2, although this has been the subject of controversy (Hahn 1993; Timmerman et al., 1996; Birouk et al., 1998; Silander et al., 1998; Boerkoel et al., 2002). CMT1X would have been a more appropriate classification for these cases than CMT2, as there was no male-to-male transmission, and the motor NCVs of affected male patients showed intermediate slowing. Furthermore, the cutoff value of 38 m/s conduction velocity was proposed to distinguish axonal from demyelinating CMT in the premolecular era (Harding and Thomas, 1980a), at a time when CMT1X was underdiagnosed.

Consistent with the clinical finding of severe distal weakness and atrophy, CMT1X patients have electrophysiological evidence of distally accentuated axonal loss. The peroneal and tibial motor responses are frequently absent, the median and ulnar motor responses are reduced, and electromyography confirms the length-dependent loss of motor units. These abnormalities become more common with age and may contribute to the slowing of the motor conduction velocities (Rozear et al., 1987; Hahn et al., 1990, 1999; Nicholson and Nash, 1993; Rouger et al., 1997; Birouk et al., 1998; Senderek et al., 1999; Hattori et al., 2003). However, slowing of NCV is evident in presymptomatic or affected male children (Kuntzer et al., 2003; Vondracek et al., 2005), in keeping with the primary demyelinating nature of CMT1X neuropathy (Scherer et al., 1998).

Pathological Findings in CMT1X

Reported nerve biopsies from several patients with CMT1X appear similar (Rozear et al., 1987; Hahn et al., 1990, 1999; Nicholson and Nash, 1993; Birouk et al., 1998; Sander et al., 1998; Senderek et al., 1998, 1999; Tabaraud et al., 1999; Gutierrez et al., 2000; Vital et al., 2001), including those lacking the connexin32 (*Cx32*) gene (Hahn et al., 2000; Nakagawa et al., 2001). The most prominent finding is age-related loss of myelinated fibers, and in parallel, an increasing number of regenerated axon clusters (Fig. 2). 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 of CMT1A/B patients (Sander et al., 1998; Hahn et al., 2001; Vital et al., 2001; Hattori et al., 2003). Onion bulb-like structures were prominent in some cases (Rozear et al., 1987; Tabaraud et al., 1999; Gutierrez et al., 2000; Nakagawa et al., 2001), but are seldom well developed. Ultrastructural studies have shown enlargement and widening of the adaxonal Schwann cell cytoplasm (Senderek et al., 1999; Hahn et al.,

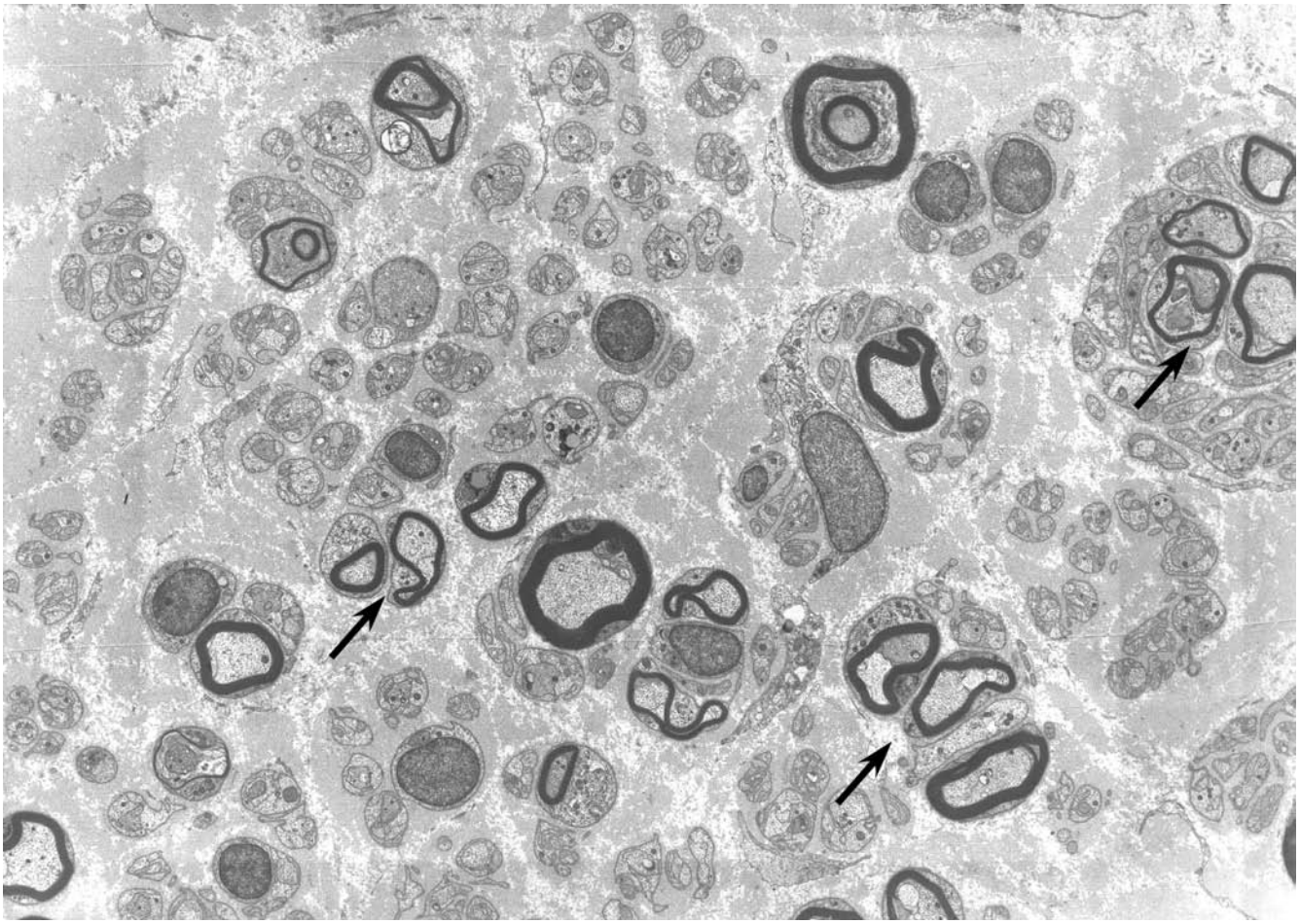

Fig. 2. Pathological findings in CMT1X. This is an electron micrograph of a biopsied peroneal sensory nerve from a 61-yr-old man with the Y211stop mutation (Hahn et al., 1990); the tissue was kindly supplied by Angelika Hahn. Clusters of regenerating axons with abnormally thin myelin sheaths are indicated by arrows. (Modified from Scherer and Kleopa, 2005, with permission from Elsevier.)

2001; Kuntzer et al., 2003) and increased packing density of neurofilaments (Hahn et al., 2001), features also noted in *Gjb1/cx32*-null mice (Anzini et al., 1997; Scherer et al., 1998). There are structural alterations in Schmidt-Lanterman incisures (Senderek et al., 1999), in which Cx32 is normally localized (Scherer et al., 1995). In *Gjb1/cx32*-null mice, macrophages may mediate some of the pathological changes (Kobsar et al., 2002) and immune deficiency ameliorates the severity of neuropathy (Kobsar et al., 2003). However, prominent inflammatory changes have not been reported in biopsied nerves, so it remains to be determined whether this is a clinically relevant mechanism in CMT1X patients.

GJB1 Mutations Cause CMT1X

The first linkage studies of CMT1X families excluded the distal short arm and the distal long arm of the X-chromosome (de Weerdt, 1978; Iselius and Grimby, 1982). Several kindreds were subsequently linked to the proximal long arm of the X-chromosome using restriction fragment length polymorphisms (Gal et al., 1985; Beckett et al., 1986; Fischbeck et al., 1986; Goonewardena et al., 1988; Ionasescu et al., 1988; Haites et al., 1989). Further recombination analyses in large CMT1X families refined the localization of the disease locus to an approx 1.5 Mb interval in Xq13.1 (Mostacciuolo

et al., 1991; Ionasescu et al., 1992; Bergoffen et al., 1993b; Fain et al., 1994; Le Guern et al., 1994), in which the three genes had been previously mapped, including *GJB1*. The three candidate genes were screened by Northern blot analysis of peripheral nerve. The assumption was that genes causing a demyelinating neuropathy should be expressed by myelinating Schwann cells and therefore should be detectable by Northern blot analysis as opposed to neuronal mRNA. Only the mRNA of Cx32 was present in peripheral nerve, indicating that this was the most likely CMT1X gene, and sequencing of *GJB1*/*Cx32* in eight families revealed seven different mutations (Bergoffen et al., 1993a). Subsequently, more than 270 different mutations affecting the open reading frame (ORF) have been reported, including missense (amino acid substitutions) and nonsense (premature stop codons) mutations, deletions, insertions, and frameshifts (listed in http://www.molgen.ua.ac.be/CMTMutations/), predicted to affect all regions of the Cx32 protein as shown schematically in Fig. 3. None of the reported amino acid changes have been determined to be polymorphisms, indicating that all of the affected residues are required for the normal function of Cx32 in myelinating Schwann cells. 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 at least three CMT1X kindreds, the entire coding region of *GJB1* is deleted.

Some CMT1X kindreds, including one family in the initial report (Bergoffen et al., 1993a), do not have a mutation in the ORF. In these families, mutations might affect the *GJB1* promoter, enhancers, the splice sites, or the untranslated portions of the mRNA. The *GJB1* gene (Fig. 4) contains three alternative promoters; the second exon contains the entire ORF (Neuhaus et al., 1995, 1996; Söhl et al., 1996, 2001). Cx32 transcripts in peripheral nerve are mainly initiated at the promoter termed P2, the one nearest the second exon. Transcripts in the liver, embryonic stem cells, oocytes, and pancreas, on the other hand, are initiated at the P1 and/or P3 promoters. Cx32 transcripts from the central nervous system (CNS) are initiated from both the P1 and P2 promoters. Thus, transcripts initiated at different promoters have divergent 5′-untranslated sequence, whereas the rest of the 5′-untranslated region (UTR), the coding region, and 3′-UTR are identical. The *GJB1* promoter contains binding sites for the EGR2 and SOX10 transcription factors, which are expressed in myelinating Schwann cells, and act synergistically to activate Cx32 expression (Kuhlbrodt et al., 1998; Warner et al., 1998).

Several sequence alterations in the noncoding region have been identified (Fig. 4). The –529 T > G and –527 G > C promoter mutations both alter a putative SOX10 binding site, and result in decreased expression in transient cotransfection assays (Ionasescu et al., 1996b; Bondurand et al., 2001; Houlden et al., 2004). The –459 C > T mutation in the 5′-UTR (Ionasescu et al., 1996b; Flagiello et al., 1998) abolishes an internal ribosome entry site that is essential for the translation of Cx32 mRNA (Hudder and Werner, 2000). A –713 G > A mutation in proximal P2 promoter was initially reported to cause CMT1X in a Taiwanese family and to impair the transcriptional activity of the Cx32 P2 promoter in vitro (Wang et al., 2000). However, the same alteration is a common polymorphism in a Caucasian population (Bergmann et al., 2001), and this alteration did not affect EGR2 and SOX10 function in transient cotransfection assays (Bondurand et al., 2001). Another nucleotide substitution (–458 G > A) within exon 1B was found in a family with CMT but did not segregate with the phenotype, suggesting an uncommon familial DNA variation without clinical significance (Bergmann et al., 2002). Taken together, these studies show that mutations outside of the *GJB1* ORF are rare, and allelic variants do not necessarily cause CMT1X.

Myelinating Schwann Cells Express Cx32

Gap junctions (GJ) are cell membrane channels found in most tissues, usually among adjacent cells, but as in the case of the myelin sheath also among different layers of the same cell (Bruzzone et al., 1996; White and Paul, 1999). Intercellular GJs have been postulated to be involved in a number of processes, including electrical conduction, metabolic cooperation, growth control, cellular differentiation, and pattern formation during development. Channels are made up of two apposed hemichannels (or connexons) that can provide a contiguous pathway among the adjacent cells or cell compartments. Each connexon is made up of a hexamer of connexin

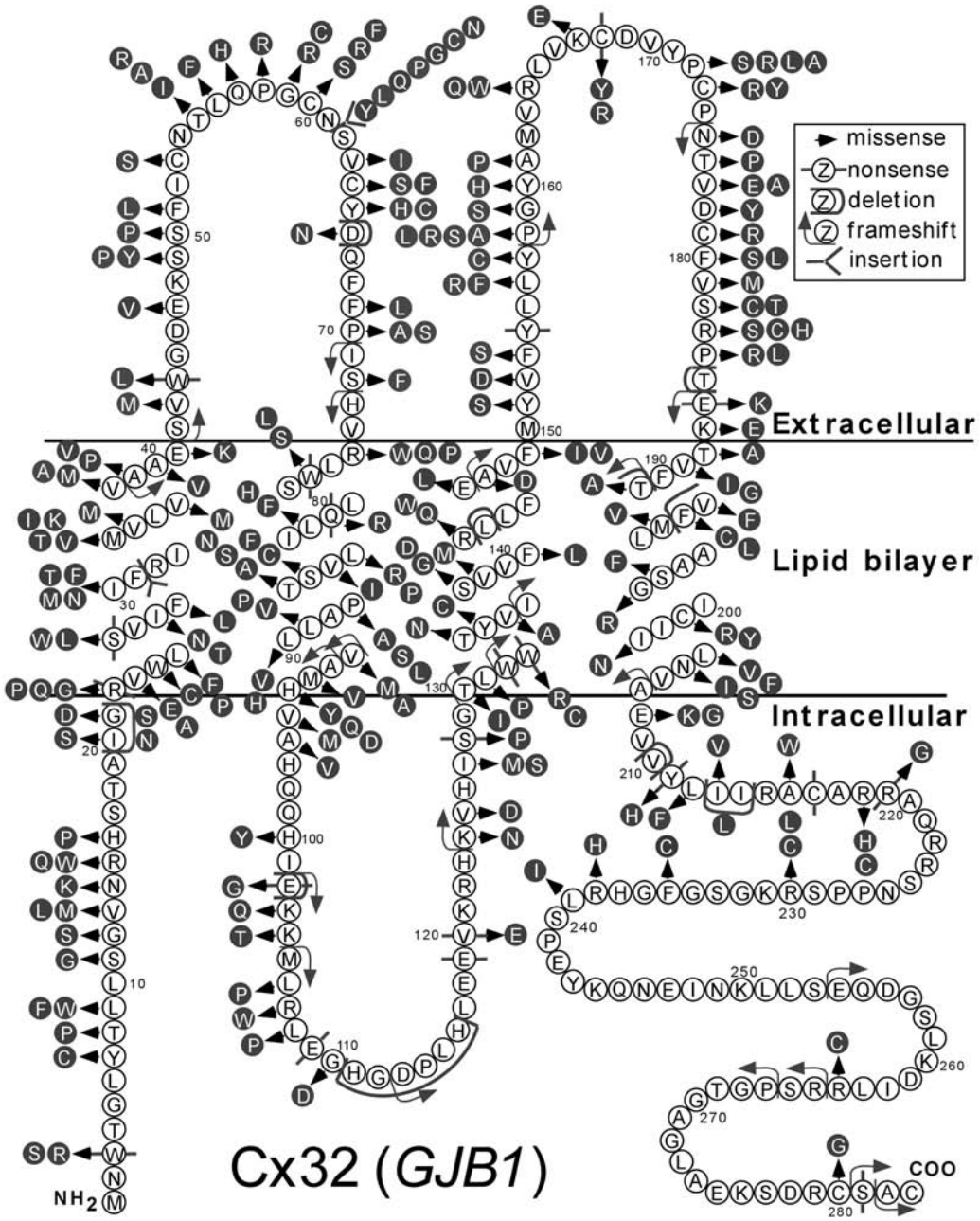

Fig. 3. Diagram of Cx32 showing the amino acid sequence and basic structure of this intrinsic membrane protein, including four transmembrane domains, one intracellular and two extracellular loops, as well as an amino- and a carboxy-terminal cytoplasmic tail. Reported *GJB1/Cx32* mutations in the coding region associated with CMT1X are indicated. (Modified from Kleopa and Scherer, 2002, with permission from Elsevier/WB Saunders Company.)

molecules arranged around a central pore. The channel diameter is 1.2 nm, 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).

Connexins belong to a multigene family of more than 20 proteins that form GJs in chordates (Willecke et al., 2002). Connexins are highly homologous, indicating that their structure and function were conserved as they evolved from a common

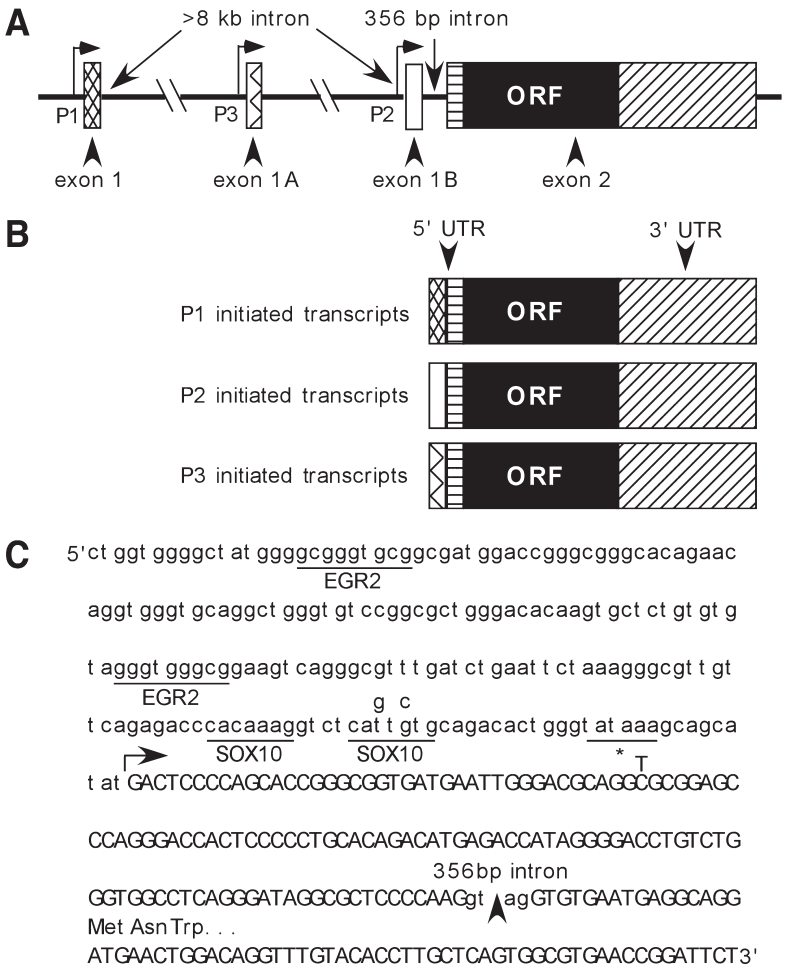

Fig. 4. The structure of the Cx32 gene and promoter. There are three alternative promoters, P1–P3 **(A)**, which give rise to three transcripts that differ only in part of their 5´-untranslated region (UTR); these are shown schematically **(B)**. The nucleotide sequence of the promoter, exon 1B, and the beginning of the ORF (exon 2) are depicted **(C)**. The locations of SOX10 and EGR2 binding sites, a TATAA box (asterisk), the start site of transcription (arrow), exon 1B (capitalized letters), the 356 bp intron, and exon 2 (capitalized letters) are indicated. The reported promoter mutations affecting the SOX10 binding site (–529 T > G and –527 G > C), as well the exon 1B mutation affecting the IRES region (–459 C > T) are shown. Bases are named according to their position from the ATG initiation codon. (Modified from Scherer and Paul, 2004, with permission from Elsevier.)

ancestral gene. In the most commonly used nomenclature, each connexin protein is named according to its predicted molecular mass (in kDa). Connexins are predicted to have the same overall topology (Fig. 3)—a cytoplasmic amino terminus, four transmembrane domains with α helix structure, one intracellular loop and two extracellular loops, and a cytoplasmic carboxy terminus (Bruzzone et al., 1996; Unger et al., 1999; White and Paul, 1999). The third transmembrane domain probably forms the central pore, with polar residues lining the wall of the pore. The intracellular loop and C-terminal domain are the most divergent parts of the connexins, and differences in their sizes account for the different molecular masses of the connexins (Willecke et al., 2002). The two extracellular loops regulate the connexon–connexon interactions, including heterotypic channel formation; each loop contains three cysteine residues that are conserved among all connexins and join the two loops via disulfide bonds.

Most connexins are expressed in more than one tissue and most tissues express more than one

connexin, raising the possibility that different connexins interact. Connexons may be made up of a single connexin (homomeric), or they may be heteromeric. Furthermore, homomeric connexons can couple with homomeric connexons made up of the same connexin (homotypic junctions), with connexons made up of a different connexin (heterotypic junctions), or even with different combinations of heteromeric connexons. Not all combinations of hemichannels, however, can form heterotypic junctions. The possibility of interactions among different connexins may relate to the pathogenesis of CMT1X since Cx32 mutants may have dominant-negative effects on other connexins expressed by myelinating cells (*see* "CNS Manifestations of CMT1X" section).

Cx32 was the first connexin to be cloned. It is highly conserved across mammalian species; the amino acid sequence of human Cx32 protein is 98% identical to those of the mouse and rat. Although Cx32 is most abundant in liver, it is also expressed by many cell types, including oligodendrocytes and perhaps some neurons, as well as by myelinating Schwann cells (Scherer et al., 1995; Chandross et al., 1996; Söhl et al., 1996; Ressot and Bruzzone 2000). Despite this broad expression pattern, peripheral neuropathy is usually the sole clinical manifestation of *GJB1* mutations. Why these other tissues are not affected is unclear. One reason may be the coexpression of one or more other connexins, which could "protect" against the loss of Cx32. Myelinating Schwann cells in rodents express Cx29 (Söhl et al., 2001; Altevogt et al., 2002), but this does not prevent the development of demyelinating neuropathy (Anzini et al., 1997; Scherer et al., 1998).

GJ-like structures were first observed by freeze-fracture electron microscopy at the incisures and paranodes (Schnapp and Mugnaini, 1978; Sandri et al., 1982, Tetzlaff, 1982). The localization of Cx32 in the same areas suggested that Cx32 forms these GJs among the layers of the Schwann cell myelin sheath (Bergoffen et al., 1993a). A radial pathway formed by GJs at these locations would be up to a 1000-fold shorter than the circumferential pathway within the Schwann cell cytoplasm (Scherer et al., 1995). Indeed, diffusion of 5,6-carboxyfluorescein, a fluorescent dye of low molecular mass (376 Da) across the incisures of the myelin sheath was documented by fluorescence microscopy following injection in the perinuclear region of living myelinating Schwann cells (Balice-Gordon et al., 1998). In contrast, injected large molecular mass fluorescent dyes did not reach the inner collar of cytoplasm and a pharmacological blocker of GJs prevented 5,6-carboxyfluorescein from reaching the inner collar of cytoplasm. These results demonstrated that there are functional GJs within incisures that mediate the diffusion of small molecules across the myelin sheath. Impairment of this radial pathway may damage myelinating Schwann cells and their axons, causing neuropathy. However, 5,6-carboxyfluorescein diffuses across the myelin sheath in *Gjb1/cx32*-null mice (Balice-Gordon et al., 1998) indicating that another GJ protein is present in the Schwann cell myelin sheath. This could be Cx29, which is also localized in incisures, except that Cx29 does not appear to form functional GJs in vitro (Altevogt et al., 2002).

Cellular and Molecular Effects of Cx32 Mutants

Different Cx32 mutants that cause CMT1X have been studied in heterologous cells (Abrams et al., 2000). When expressed in *Xenopus* oocytes, many mutants do not form functional channels, and some of these also exert dominant-negative effects on the normal Cx32 (Bruzzone et al., 1994), indicating the potential for such interactions with coexpressed connexins in CMT1X. Other mutants form functional channels with altered biophysical characteristics; two of these (S26L; M34T) maintain electrical coupling, but have reduced pore diameter such that may prevent the diffusion of second messengers like IP3, cAMP, and Ca^{2+} (Oh et al., 1997). These studies also showed that the position of the Cx32 mutation alone does not necessarily predict the molecular and functional consequences. The nature of the mutation may also be important, as the R15Q and H94Q mutants form normal functional channels, whereas R15W and H94Y do not (Abrams et al., 2001). Mutants in the C-terminal domain form functional GJs (Rabadan-Diehl et al., 1994; Castro et al., 1999), although compared to wild-type Cx32, some channels are less stable (Castro et al., 1999). F235C, another C-terminal mutant that is associated with a severe phenotype, has abnormal electrophysiological characteristics suggesting abnormal gain of function (Liang et al., 2005). The fact that several disease-related mutants (R15Q, H94Q, C217X, R238H, C280G, and S281X) form fully functional

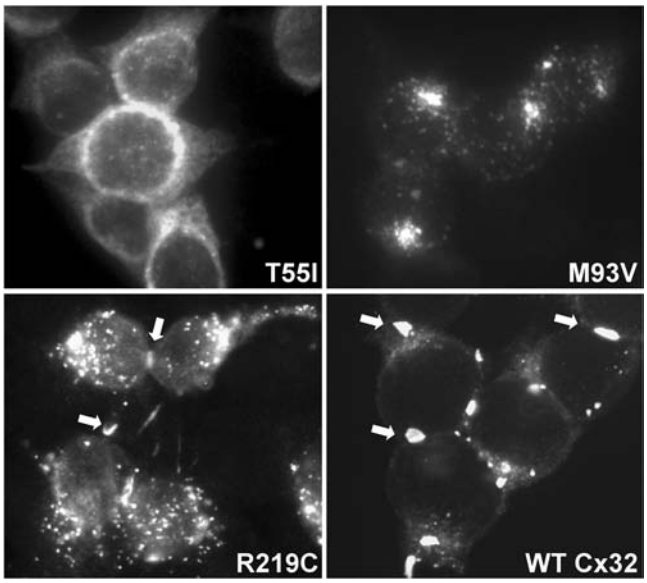

Fig. 5. Different patterns of cellular expression characteristic of Cx32 mutants. These are immunocytochemistry images of HeLa cells that have been permanently transfected to express the indicated mutants. T55I is localized to the endoplasmic reticulum; M93V is localized to the Golgi; R219C forms GJ-like plaques (arrowheads), similar to wild-type (WT) Cx32.

channels (Castro et al., 1999; Abrams et al., 2001), underscores the limitations from these studies in relation to pathogenesis of CMT1X.

Expression of Cx32 mutants in mammalian cells has led to the realization that trafficking is often abnormal (Fig. 5); this was not apparent when the same mutants were expressed in oocytes. 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 (ER).
3. Cx32 appears to be retained in the Golgi.
4. GJ-like plaques on the cell surface are seen.

The 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.

Expression studies of Cx32 mutants in oocytes and other cell types provide some tentative structure–function correlations (Abrams et al., 2000). Mutations affecting residues within the N-terminal domain have altered biophysical properties and may cause reversal of gating polarity by negative charge substitutions. This is in keeping with the role of this protein domain in the insertion of the nascent polypeptide chain into the ER, and along with the first transmembrane domain in the regulation of voltage gating. Shifted voltage gating has been also shown for several mutants affecting the first transmembrane domain. Substitution of a proline residue located at position 87 in the second transmembrane segment affects conformational changes associated with voltage gating. The nearby substitution S85C leads to formation of hemichannels with abnormally increased opening (Abrams et al., 2002). Mutating any of the six cysteines (to serines) in the two extracellular loops, which participate in interactions among apposed connexons, leads to a loss of functional channels. Mutations of the intracellular loop and C-terminal domain may affect pH gating (Castro et al., 1999). Two mutations that affect a consensus prenylation motif of Cx32 (C280G and S281X) abolish prenylation, a lipid modification (Huang et al., 2005).

Animal Models of CMT1X

Mice with targeted deletion of the *Gjb1/cx32* gene develop a progressive, demyelinating peripheral neuropathy beginning at about 3 mo of age (Anzini et al., 1997; Scherer et al., 1998). For unknown reasons, motor fibers are much more affected than sensory fibers; this feature has not been noted in CMT1X patients, but may be present. In heterozygous females only some myelinating Schwann cells express Cx32 (Scherer et al., 1998), owing to random X-chromosome inactivation. Heterozygous females have fewer demyelinated and remyelinated axons than age-matched *Gjb1/cx32*-null females or males (Scherer et al., 1998), in keeping with the clinical phenotype of affected women who are obligate carriers of CMT1X. Expression of wild-type

human Cx32 protein largely prevents demyelination in *Gjb1/cx32*-null mice (Scherer et al., 2005) confirming that the loss of Schwann cell autonomous expression of Cx32 is sufficient to account for demyelination in CMT1X.

To determine whether some Cx32 mutants have more than a simple loss of function, transgenic mice expressing the 175fs, R142W, C280G, and S281X mutations were generated. No Cx32 protein could be detected and no peripheral neuropathy was noted in 26 lines of mice expressing the 175fs transgene, even though transgenic/human mRNA was highly expressed in some lines (Abel et al., 1999). In contrast, mice expressing the R142W mutation developed a mild demyelinating neuropathy (Scherer et al., 1999). The mutant protein was retained in the perinuclear region and did not reach the incisures or paranodes, in which Cx32 is normally localized. Moreover, the presence of the mutant Cx32 reduced the level of the endogenous/mouse Cx32, indicating that R142W may have dominant-negative interactions with endogenous Cx32. In mice expressing the C280G or S281X mutations, the Cx32 mutants were properly localized to incisures and paranodes, and appeared to prevent demyelination in *Gjb1/cx32*-null mice, indicating that these mutants may form functional channels in the myelin sheath (Huang et al., 2005). The comparable localization of R142W, 175fs, C280G, and S281X mutants in myelinating Schwann cells and in transfected cells (Deschênes et al., 1997; Yum et al., 2002) indicates that altered synthesis or trafficking is the fundamental perturbation in most CMT1X mutants. Yet the abnormal attributes of many mutants, including C280G and S281stop, remain to be elucidated.

Genotype–Phenotype Correlations in CMT1X

Despite the large number of different mutations affecting every domain of the Cx32 protein (Fig. 3), the promoter and 5′-UTR (Fig. 4), the clinical severity caused by *GJB1* mutations appears to be relatively uniform in affected men, including those with a deleted gene, indicating that most mutants cause loss of function (Hahn et al., 2000; Dubourg et al., 2001; Nakagawa et al., 2001). This is in contrast to *PMP22*, *MPZ*, and *EGR2*, in which different mutations clearly cause phenotypes that differ in severity. Only two Cx32 mutations, the 265–273 deletion (Ionasescu et al., 1996a) and F235C (Lin et al., 1999), appear to cause severe neuropathy that also affects female patients and overlaps phenotypically with the Dejerine-Sottas syndrome. Whether unusually severe phenotypes in females result from skewed X-inactivation is unknown, but clinicians should be aware that *GJB1* mutations could rarely cause severe neuropathy in young children of either sex. Several other Cx32 mutations appear to cause more severe phenotypes, typically with early onset, including R22X, V38A, the complex allelic mutation [R22Q; V63I], V136A, 147fs, and C201R. How these mutations cause a more severe phenotype is unknown, but the mutant proteins probably have deleterious effect on myelinating Schwann cells that transcend a simple loss of function. Other Cx32 mutations may be associated with milder phenotypes—W3R, V63I, W77S, and T191A. It remains to be determined whether there is a genotype–phenotype correlation in CMT1X, as most of the kindreds that have been associated with either mild or severe phenotypes are small. Hahn and colleagues (Hahn et al., 1999) studied a large number of CMT1X patients and found that all mutations produced a similar phenotype, which varied among males even within the same family, suggesting that epigenetic factors modify the severity of disease. Genotype–phenotype correlations are being addressed in a "CMT database" lead by Michael Shy (mshy@cmb.biosci.wayne.edu) at Wayne State University in collaboration with Indiana University. Study of a larger number of patients with different mutations may provide more conclusive phenotype–genotype correlations.

CNS Manifestations of CMT1X

Many *GJB1* mutations appear to be associated with electrophysiological, clinical, and/or MRI findings of CNS involvement. Subclinical involvement is common. The latencies of brainstem auditory evoked responses are delayed in a high proportion of CMT1X patients, even in the absence of clinical symptoms (Nicholson and Corbett, 1996; Nicholson et al., 1998; Senderek et al., 1999), and central visual and motor pathways may also be affected (Bähr et al., 1999). Because these electrophysiological findings have not been found in

patients with a deleted *GJB1* gene (Hahn et al., 2000), they may represent a gain of function. Moreover, clinical manifestations (spasticity, extensor plantar responses and hyperactive reflexes) have been reported in patients with the A39V, T55I, M93V, R164Q, R183H, and T191fs mutations; the degree of these findings may even be masked by the peripheral neuropathy. More striking CNS findings have been reported in individual patients with duplication of amino acids 55–61 (cerebellar ataxia and dysarthria) (Kawakami et al., 2002) or the V63I mutation (mental retardation), but the relationship of these abnormalities to *GJB1* mutations is unproven. Acute, transient encephalopathy associated with MRI changes suggesting CNS myelin dysfunction have been described in patients with the T55I, R75W, E102del, R142W, R164W, and C168Y mutations. The acute deficits appear to have been triggered by travel to high altitudes (Paulson et al., 2002; Hanemann et al., 2003), intense physical activity (Hanemann et al., 2003; Taylor et al., 2003), or acute infection (Schelhaas et al., 2002; Hanemann et al., 2003).

Expression studies of these Cx32 mutants associated with clinical CNS involvement showed that they are retained intracellularly, which is not a unique attribute, as so are other mutations without CNS involvement (Kleopa et al., 2002). Perhaps these "CNS mutants" have dominant-negative effects on other connexins expressed by oligodendrocytes. Rodent oligodendrocytes express Cx32, Cx29, and Cx47; of these, Cx32 and Cx47 have partially overlapping distributions (Altevogt et al., 2002; Menichella et al., 2003; Odermatt et al., 2003; Kleopa et al., 2004). Furthermore, mice lacking both Cx32 and Cx47 (but not mice lacking either Cx32 or Cx47 alone) develop severe CNS dysmyelination (Altevogt et al., 2002; Menichella et al., 2003; Odermatt et al., 2003; Kleopa et al., 2004), suggesting that Cx47 and Cx32 have overlapping function in the CNS. Finally, recessive mutations in *GJA12*, the gene encoding human Cx47, cause a severe leukodystrophy, Pelizaeus-Merzbacher-like disease (Uhlenberg et al., 2004). Thus, dominant effects of Cx32 mutants on Cx47 could cause the CNS abnormalities in CMT1X patients. Diminished Cx47 expression could decrease GJ coupling between oligodendrocyte and astrocytes, as oligodendrocytes are coupled to astrocytes but not to themselves (Nagy and Rash, 2000; Nagy et al., 2003a, 2003b; Altevogt and Paul, 2004).

Hearing loss has been reported in CMT1X families with the V38A, T55R, V63I, R142Q, E186K, and T191fs mutations. In most cases, the onset of hearing loss was in early childhood, both in males and females. However, proof of causality is lacking for those mutations that do not cause hearing loss in other CMT1X families with the same mutation. Because mutations in other connexin genes (*GJB3*/*Cx31*, *GJB6*/*Cx30*, and especially *GJB2*/*Cx26*) are common causes of hereditary hearing loss (http://www.crg.es/deafness), the possibility that *GJB1* mutations cause hearing loss deserves more study. Because hearing loss is not reported in patients who have a *GJB1* deletion, it would represent a gain of function, the nature of which remains to be determined. Cx32 is expressed by the myelinating Schwann cells in the VIII nerve, but is not known to be co-expressed with either Cx26, Cx30, or Cx31, which are all expressed in the cochlea (Buniello et al., 2004).

Therapy and Medical Issues in CMT1X Patients

There are no known molecular-based treatments for CMT1X, although symptomatic therapies can improve quality of life as in other forms of CMT. It is recommended that patients with inherited neuropathies avoid vincristine, which has caused acute worsening of neuropathy in CMT1A patients. However, at least one CMT1X patient was treated with *cis*-platinum without noticeable clinical worsening (Cowie and Barrett, 2001). CMT1X patients with certain mutations may be predisposed to develop transient CNS manifestations that are triggered by factors such as travel and stay at high altitudes, intense physical activity, hypoxia, or fever. It seems appropriate to counsel these patients and even their at risk relatives that triggering factors are to be avoided when possible.

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