

ADHESION MOLECULES AND INHERITED DISEASES OF THE HUMAN NERVOUS SYSTEM*

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ABSTRACT

Mutations in the human genes for the adhesion molecules Po, L1, and merosin cause severe abnormalities in nervous system development. Po and merosin are required for normal myelination in the nervous system, and L1 is essential for development of major axon pathways such as the corticospinal tract and corpus callosum. While mutations that lead to a loss of the adhesive function of these molecules produce severe phenotypes, mutations that disrupt intracellular signals or intracellular interactions are also deleterious. Geneticists have found that more than one clinical syndrome can be caused by mutations in each of these adhesion molecules, confirming that these proteins are multifunctional. This review focuses on identifying common mechanisms by which mutations in adhesion molecules alter neural development.

Clinicians divide, geneticists unite.

Patrick Willems (Fransen et al 1997)

INTRODUCTION

The hereditary neurological disorders are a diverse group of syndromes that often share common features such as mental retardation, brain malformations,

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sensory disturbances, and muscle wasting or weakness. Neurologists have categorized these syndromes by using complex classification schemes based upon inheritance patterns, age of onset, manifestations (e.g. sensory vs motor vs autonomic), and pathological findings. Modern genetic analysis techniques coupled with molecular biological advances have provided new insight into many of these disorders. In several cases single genes have been found to be associated with two or more syndromes that had previously been considered distinct clinical entities. Identification of the gene responsible for a particular disease permits clinicians to diagnose affected individuals and counsel their families. It also establishes an exciting synergistic relationship in which clinical findings suggest lines of experimental investigation and results from basic science inquiries lead to a clearer understanding of disease pathophysiology.

Since 1992, dramatic advances have been made in understanding three adhesion molecules: merosin (also known as laminin-2), Po, and L1. Mutations affecting these proteins all cause inherited diseases in humans. In addition to affecting cellular adhesion, mutations in these molecules influence intracellular second-messenger signaling or cytoskeletal interactions, accounting for complex clinical findings. This review discusses the structure and function of these three adhesion molecules and relates this information to the clinical syndromes associated with mutations in the relevant genes.

MEROSIN AND CONGENITAL MUSCULAR DYSTROPHY

Structure of Merosin Heterotrimer

Merosin (laminin-2) is a substrate adhesion molecule originally found in basement membranes of the placenta, striated muscle, and Schwann cells (Leivo & Engvall 1988). It is related to laminin-1 ($\alpha 1$ - $\beta 1$ - $\gamma 1$) from the EHS sarcoma and s-laminin ($\alpha 1$ - $\beta 2$ - $\gamma 1$), which is found at the neuromuscular junction. Merosin is a heterotrimeric glycoprotein consisting of a heavy chain ($\alpha 2$) of 400 kDa and two light chains ($\beta 1$ and $\gamma 1$) of about 200 kDa each (Ehrig et al 1990). Homology between the $\alpha 1$ and $\alpha 2$ chains is 58.6% (Vuolteenaho et al 1994), and they are thought to have similar domain structures (Figure 1A). Domains VI, IVb, and IVa form globular structures, and domains V, IIIb, and IIIa (which contain cysteine-rich EGF-like elements) form rigid, rod-like structures. The three short arms of merosin are formed separately from N-terminal regions of the $\alpha 2$, $\beta 1$, and $\gamma 1$ chains. Domains I and II of the three chains participate in the formation of a triple-stranded coiled-coil structure (the long arm). The C-terminal G domain forms a large globular structure responsible for binding to α -dystroglycan (Sunada et al 1994). The short arm domains of laminin-1

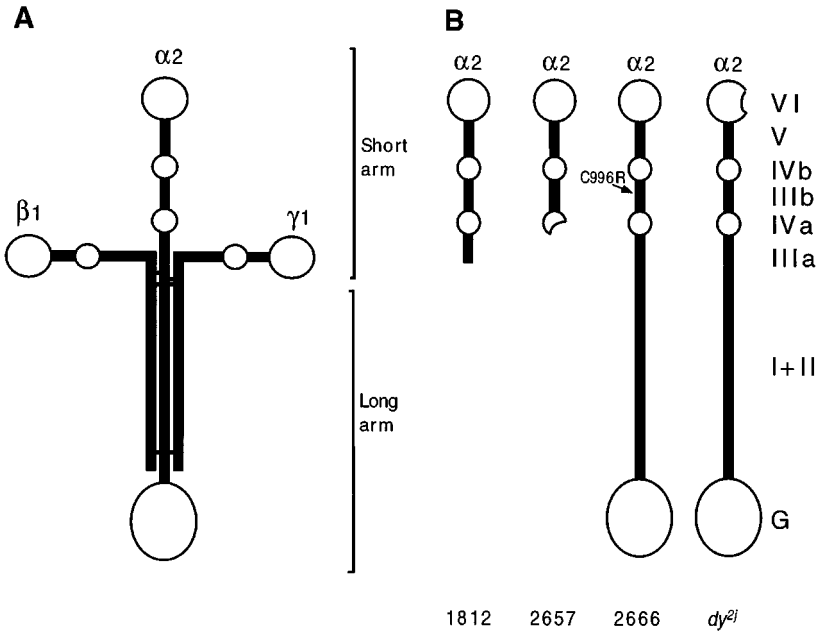


Figure 1 (A) Model of merosin showing the $\alpha 2$, $\beta 1$, and $\gamma 1$ chains. (B) Illustrations of the predicted products in three human mutations in the $\alpha 2$ chain (1812, 2657, and 2666) and the mouse dy^{2j} mutant. The different domains of the $\alpha 2$ chain are indicated at the right.

are involved in the self-aggregation process (Schittny & Yurchenco 1990) and interaction with extracellular matrix components, such as collagen type IV (Charonis et al 1985), and form a supramolecular structure in the basement membrane. The significant homology between the $\alpha 1$ and $\alpha 2$ chains, especially in the N-terminal domains, suggests that merosin has a similar structural organization.

Classification of Congenital Muscular Dystrophies

Congenital muscular dystrophies (CMDs) are characterized by severe dystrophic muscle wasting from birth or early infancy. In contrast, other inherited forms of muscular dystrophy, such as Duchenne muscular dystrophy, tend to present at later ages. There are four CMDs with autosomal-recessive inheritance: classical CMD, Fukuyama's CMD (FCMD), Walker-Warburg syndrome (WWS), and muscle-eye-brain disease (MED). Central nervous system (CNS) impairment is severe in FCMD, WWS, and MED but is mild in classical CMD.

In 1994 it was discovered that classical CMD could be subdivided into two categories, merosin-positive CMD (MP-CMD) and merosin-negative CMD (MN-CMD) (Tomé et al 1994), that have about the same prevalence in European countries (Dubowitz 1994). The defective gene for MN-CMD was mapped to chromosome 6q2 in the region of the $\alpha 2$ chain gene (LAMA2) (Hillaire et al 1994). FCMD patients also show a significant reduction in merosin expression (Hayashi et al 1993). However, this is considered to be a secondary effect, since the gene for FCMD is linked to chromosome 9q31–33 (Toda et al 1993).

MN-CMD has uniform clinicopathological features, including muscle weakness and hypotonia beginning in early infancy (more severe than in MP-CMD), arthrogryposis, minimal or absent mental retardation, brain white-matter lucency on computerized tomography/magnetic resonance imaging (CT/MRI), dysmyelinating peripheral neuropathy, and necrotic skeletal muscle fibers with basal lamina disruption and dense fibrosis. In contrast, MP-CMD rarely shows brain white-matter lucency (Philpot et al 1995), peripheral neuropathy (Shorer et al 1995), or muscle basal lamina disruption (Osari et al 1996). So far, several MN-CMD cases with abnormal formation of cerebral cortical gyri have been reported, suggesting disruption of neural migration (Sunada et al 1995b, Pini et al 1996).

Merosin and Muscular Dystrophy

Both laminin-1 and merosin appear as early as eight weeks gestation in human skeletal muscle development (Sewry et al 1995b). In the normal adult, merosin becomes predominant in the basal lamina of skeletal muscle, while the $\alpha 1$ chain is restricted to the neuromuscular junction and blood vessels (Sanes et al 1990). Perhaps the most important function of merosin in skeletal muscle is to provide mechanical reinforcement to the sarcolemma by linking the extracellular matrix and subsarcolemmal cytoskeleton.

The binding of merosin to the sarcolemma is mediated by the sarcolemmal protein complex, termed dystrophin-glycoprotein complex, which consists of dystrophin, dystrophin-associated proteins (DAPs), and dystrophin-associated glycoproteins (DAGs) (Figure 2). The G domain of merosin binds to α -dystroglycan (156DAG) outside the muscle cell, which in turn binds to β -dystroglycan (43DAG), a transmembrane protein. At least three other transmembrane proteins associated with the dystroglycan complex have been identified: α -sarcoglycan (50DAG, Adhalin), β -sarcoglycan (43DAG), and γ -sarcoglycan (35DAG). Finally, dystrophin binds the DAG complex through its C-terminal domain and actin-filament through its N-terminal domain. The syntrophin triplet (59DAP) also associates with the C-terminal domain of dystrophin but does not exhibit any binding activity to DAGs. Thus, the dystrophin-glycoprotein complex functions as a merosin receptor, linking the extracellular

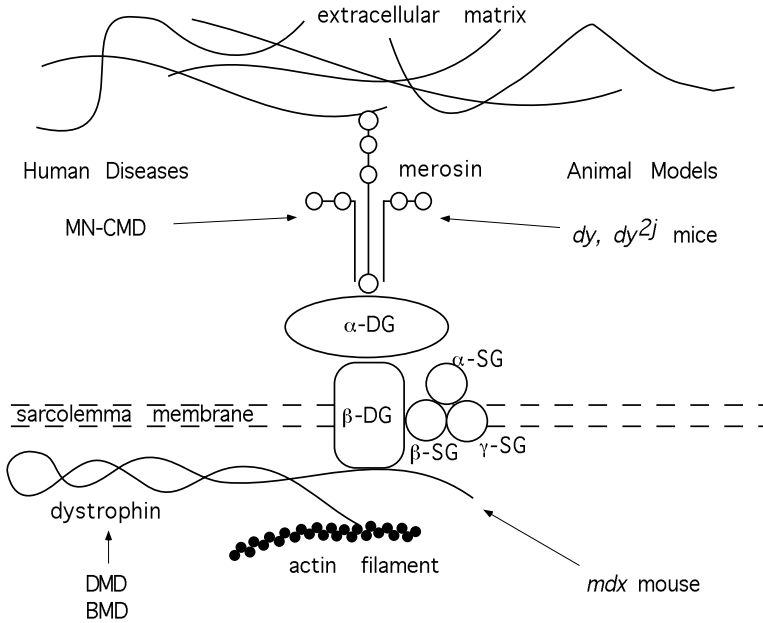


Figure 2 Model of the interactions between the cytoskeleton, the dystrophin-glycoprotein complex, merosin, and the extracellular matrix. The site of defects in human diseases (merosin-negative congenital muscular dystrophy, MN-CMD; Duchenne muscular dystrophy, DMD; Becker muscular dystrophy, BMD) and mouse mutations (*dy*, *dy^{2j}*, and *mdx*) are also shown.

matrix and subsarcolemmal cytoskeleton (Campbell 1995, Worton 1995). It is postulated that this prevents sarcolemmal disruption induced by forces that occur with muscle contraction (Petrof et al 1993).

The dystrophin-glycoprotein complex was originally thought to serve a purely structural function, forming a transmembrane linkage, until Yang et al (1995) demonstrated that β -dystroglycan interacts directly with Grb2, an adapter protein involved in signal transduction and cytoskeletal organization. This interaction raises the possibility that the dystrophin-glycoprotein complex is a signaling receptor for merosin that maintains muscle cell survival. It may also help to explain why apoptosis (Matsuda et al 1995, Sandri et al 1995, Tidball et al 1995), which requires specific signals, precedes necrotic change in the muscles of *mdx* (dystrophin-deficient) mice that lack all the components of the dystrophin-glycoprotein complex (Ohlendieck & Campbell 1991).

Skeletal muscles from MN-CMD patients showed overexpression of the laminin $\alpha 1$ (Sewry et al 1995a). Why does laminin-1 not compensate for the lack of

merosin, considering that both laminin-1 and merosin bind to α -dystroglycan? In vitro experiments showed that laminin-1 promotes myoblast proliferation; both laminin-1 and merosin promote myoblast fusion and myotube formation synergistically; and merosin is specifically required for stabilizing myotubes and preventing apoptosis (Vachon et al 1996).

The ability of merosin to prevent apoptosis in an in vitro system correlates well with the pathogenesis of MN-CMD. However, the cell surface receptors and signaling pathways that are responsible for the different responses to merosin and laminin-1 at specific stages of myogenesis remain unknown. One possibility is the involvement of integrin receptors, which have been shown to play an important role in myogenesis (Sastry et al 1996). In mammalian skeletal muscle, the $\alpha 7 \beta 1$ integrin is regarded as the primary laminin-binding receptor (Song et al 1993, Echtermeyer et al 1996). Variant splice forms of its two subunits are expressed differentially during myogenesis; the $\alpha 7 B \beta 1 A$ integrin found in proliferating myoblasts is replaced by the $\alpha 7 A \beta 1 D$ and $\alpha 7 B \beta 1 D$ isoforms upon induction of differentiation (Collo et al 1993). This switch of integrin isoform expression may be responsible for the stage-specific functions of laminin-1 and merosin during myogenesis. Another possibility is that α -dystroglycan acts with merosin to prevent apoptosis.

Merosin and Peripheral Nerves

Merosin, produced by Schwann cells, is the predominant laminin isoform present in the endoneurial basement membrane of developing and mature peripheral nerves (Jaakkola et al 1993). Merosin promotes Schwann cell migration (Anton et al 1994) and neurite outgrowth in vitro (Engvall et al 1992) and nerve regeneration in vivo (Anton et al 1994). The two major laminin isoforms, laminin-1 and merosin, exhibit similar functional properties in the nervous system mediated mostly by the $\beta 1$ integrin subfamily. However, these two isoforms display differential selectivity for $\beta 1$ integrins in some cell types. For instance, neurite outgrowth from dorsal root ganglion neurons is mediated predominantly by $\alpha 1 \beta 1$ as well as $\alpha 3 \beta 1$ on a laminin-1 substrate, and by $\alpha 3 \beta 1$ on a merosin substrate (Tomaselli et al 1993). In addition, K562 cells expressing $\alpha 3 A \beta 1$ adhere to merosin but not to laminin-1 (Delwel et al 1994). It is likely that the selectivity of different integrin receptors for merosin vs laminin-1 has significant functional consequences.

Merosin is implicated in peripheral myelinogenesis, since both individuals with MN-CMD and merosin-deficient (*dy*) mice exhibit peripheral dysmyelination in addition to muscular dystrophy (Sunada et al 1994, Tomé et al 1994). During development, Schwann cells migrate along bundles of axons, proliferate, and synthesize a basal lamina consisting of laminins, fibronectin, type IV collagen, and other components. The $\beta 1$ integrins (predominantly $\alpha 6 \beta 1$ and to a lesser extent $\alpha 1 \beta 1$) present on undifferentiated Schwann cells mediate

Schwann cell/basal lamina interactions which are essential for myelinogenesis (Fernandezvalle et al 1994). As Schwann cells differentiate to engulf an axon and initiate spiral formation, they express increasing amounts of $\alpha 6 \beta 4$ integrins on their abaxonal surface, apposing the basal lamina (Einheber et al 1993).

Developmental changes in the expression of laminin isoforms and integrins in human fetal peripheral nerves have shown that by 11 weeks of gestation, Schwann cells express $\beta 4$ integrins and are surrounded by continuous basal laminae (Jaakkola et al 1993). Merosin is present in the basal laminae as early as 11 weeks and continues to be expressed until adulthood. In contrast, laminin-1 is not detectable in the basal laminae until 35 weeks, when most axons are already myelinated. These findings strongly suggest that merosin rather than laminin-1 is important in Schwann cell/basal lamina interactions, especially at early developmental stages.

In the peripheral nervous system (PNS), laminin-1, merosin, and agrin in the endoneurial basal lamina bind to a 120-kDa isoform of α -dystroglycan expressed by Schwann cells, which in turn links with β -dystroglycan (Yamada et al 1996). However, the function of dystroglycans in peripheral nerves remains to be determined.

Merosin and the CNS

Extracellular matrix components, including laminins, influence developing neural cells, mediating cell attachment, migration, survival, and axonal elongation. Although laminin-1 and merosin have very similar structures, several *in vitro* experiments have indicated that their effects can be quantitatively or qualitatively different. Both laminin-1 and merosin promote neuronal cell migration from embryonic olfactory epithelium via $\alpha 6 \beta 1$ integrins (Calof & Lander 1991). Merosin, however, promotes more rapid cell migration than laminin. Although retinal ganglion cells lose their ability to extend neurites on laminin-1 as they mature, owing to a developmental loss of $\alpha 6$ integrins, they retain the ability to respond to merosin (Cohen & Johnson 1991, de Curtis et al 1991). The differences in cell migration and axon growth rates are probably due to the involvement of a $\beta 1$ integrin other than $\alpha 6 \beta 1$ (Calof et al 1994).

Another merosin-binding protein, 120-kDa α -dystroglycan (cranin), colocalizes with β -dystroglycan and dystrophin in various areas of the brain and retina (Górecki et al 1994). Dystrophin and at least three dystrophin isoforms have been shown to interact with β -dystroglycan (Jung et al 1995). These molecules would form dystrophin-glycoprotein complexes, producing a functional receptor complex for merosin. Presently, little is known about the function of dystrophin-glycoprotein complex in the brain, although humans with mutations affecting this complex have CNS symptoms.

The merosin $\alpha 2$ chain is expressed along the optic pathway as retinal ganglion cell axons extend into the brain, while laminin-1 expression is much more

restricted, being found only in pial membranes (Morissette & Carbonetto 1995). The ability of merosin to promote axon growth along with its expression in developing axon pathways suggests that it plays a significant role in assisting axons to reach their targets.

Merosin also is implicated in CNS myelinogenesis because hypomyelination has been observed in *dy* mice (Tsuji & Matsushita 1985), and neuroradiological abnormalities suggestive of dysmyelination have been observed in MN-CMD (Sunada et al 1995a,b, Yamashita et al 1996). Differentiating oligodendrocytes in culture express several classes of integrins that could serve as merosin receptors (Milner & French-Constant 1994). Unlike Schwann cells, oligodendrocytes do not express merosin and do not secrete a basal lamina. However, oligodendrocytes and their precursors bind to merosin, and oligodendrocyte precursors migrate rapidly on merosin substrates (Payne et al 1996). Since merosin is found in developing optic nerve, perhaps oligodendrocytes migrate into axon bundles using merosin as a substrate. However, expression of merosin $\alpha 2$ chain in the brain has been confirmed only in the optic pathway, hippocampus, and capillary basement membranes so far (Morissette & Carbonetto 1995). Further characterization of merosin expression in the developing CNS is needed to understand the role it plays in oligodendrocyte differentiation.

Mutations in the Merosin $\alpha 2$ Gene

Three mutations have been discovered in the human $\alpha 2$ gene and one in mice (Figure 1B). In one family (1812), a mutation near exon 31 disrupts splicing, leading to a premature stop codon near the border of domains IIIa and II. In family 2657, a point mutation produces a stop codon in domain IVa. In a third family, a point mutation (causing C996R) would result in abnormal disulfide cross-linking and folding in the short arm of the $\alpha 2$ chain (Nissinen et al 1996). In *dy*^{2J} mice the mutation results in a 57-amino acid deletion (residues 34–90) and a substitution of Q91E in domain VI of the short arm (Helbling-Leclerc et al 1995, Sunada et al 1995a).

The two mutations in the families 1812 and 2657 lead to truncated $\alpha 2$ chains that would result in a complete lack of a merosin heterotrimer. In contrast, the mutations in family 2666 and *dy*^{2J} mice should allow the mutated $\alpha 2$ chains to be incorporated into a heterotrimer, as the short arm does not participate in the trimer formation. This correlates well with the immunohistochemical findings showing reduced but not absent staining for the $\alpha 2$ chain in the 2666 family and *dy*^{2J} mice (Sunada et al 1995a, Nissinen et al 1996). The identification of two different mutations that alter the structure of the short arm domain of $\alpha 2$ and cause MN-CMD despite continued expression of merosin, albeit at reduced levels, points to a critical function for the short arm domain. The laminin-1

short arm is thought to be involved in self-aggregation and interactions with extracellular matrix components in the basement membrane. Merosin is likely to function in a similar fashion.

At present, a diagnosis of MP-CMD and MN-CMD is usually made based on $\alpha 2$ chain expression, as assessed by immunohistochemistry and/or immunoblotting. This may lead to misdiagnosis, since a mutant merosin molecule could be immunoreactive but biologically inactive, as seen in the case of family 2666. Merosin expression could also be reduced secondarily to some other condition, as seen in FCMD. So genetic linkage studies must be done to confirm suspected cases of MN-CMD.

Summary

Merosin has numerous binding partners in the extracellular matrix and on cell surfaces. It is no surprise that mutations disrupting the expression or structure of merosin have widespread effects in muscle, the PNS, and the CNS. While the major effects of merosin mutations are observed in muscle, the disruption of myelination in the PNS and CNS and the abnormal brain development are also important. Determining exactly which merosin interactions—such as binding to integrins, α -dystroglycan, or ECM components—are responsible for alterations in nervous system development remains a major area for exploration. Merosin expression can be down-regulated secondarily to mutations in other genes, and this loss of merosin expression may contribute to the clinical condition in other syndromes.

Po AND PERIPHERAL NEUROPATHIES

Structure and Function of Po

Po is a small intrinsic membrane glycoprotein that comprises the major structural protein (40–80%) of peripheral myelin (Greenfield et al 1973). It is one of the simplest immunoglobulin (Ig)-related proteins, consisting of a single V-type Ig domain linked to a basic cytoplasmic domain via a single pass transmembrane region (Lemke & Axel 1985). The gene for Po is located in the q22–23 region of chromosome 1 and is approximately 7 kb long. It consists of six exons with no alternative splicing (Hayasaka et al 1993c). The extracellular domain measures 124 amino acids, with a 26-amino acid transmembrane portion and a cytoplasmic segment of 69 amino acids. There is a high degree of amino acid conservation between species, with roughly 80% homology between rat, chick, and cow, and 97% homology between human and rat. Mature Po is approximately 28–30 kDa, about 6% of which is accounted for by glycosylation (Lemke & Axel 1985, D'Urso et al 1990) with the carbohydrate attachment

at a single extracellular site, Asp 93. The molecule also undergoes acylation, sulfation, and phosphorylation. Acylation occurs at Cys 153, just inside the cytoplasmic domain (Bizzozero et al 1994). Phosphorylation takes place at cytoplasmic serines 181, 204, and 214 (Suzuki et al 1990).

Po appears on myelinating Schwann cells in the PNS of mammals (Greenfield et al 1973, Poduslo 1984). Po expression is markedly up-regulated with axonal contact and drastically down-regulated by loss of contact (Trapp et al 1988). Po is implicated in myelin compaction, with each specific domain playing different but essential roles (Lemke & Axel 1985). The extracellular region of Po localizes to the intraperiod line of the myelin sheath, where the extracellular surfaces of the Schwann cells meet and produce compaction through homophilic interactions. Further compaction occurs at the major dense line of the myelin sheath, where the cytoplasmic Schwann cell surfaces oppose, implicating the cytoplasmic domain of the molecule in the compaction process (Ding & Brunden 1994). Limited experimental evidence implicates a role for Po in neurite outgrowth, presumably through a heterophilic binding partner on neurons (Schneider-Schaulies et al 1990). Further evidence also suggests a role for Po in signal transduction that may regulate or direct the production of myelin by the Schwann cell.

Crystalline ultrastructural analysis has helped define molecular interactions in myelin. Po homotetramers form within the plasma membrane, interacting with tetramers on adjacent membrane surfaces (Shapiro et al 1996). Outwardly projecting tryptophan residues at the apex of the projecting molecule may also interact directly with the lipids in the opposing membrane (Wells et al 1993).

The homophilic adhesive properties of Po were discovered in 1990, when it was found that cells transfected with Po aggregated (D'Urso et al 1990, Filbin et al 1990). Cell aggregation was inhibited by Po antibodies or solubilized Po extracellular portion added to culture media (Schneider-Schaulies et al 1990). Glycosylation of Po significantly increases cellular adhesion (Filbin & Tennekoon 1991). Interestingly, loss of axonal contact from Schwann cells alters carbohydrate expression, and both homophilic partners must be glycosylated for adhesion to take place (Filbin & Tennekoon 1993). Elimination of the Cys21-Cys98 disulfide bond, the only such bond in the Ig domain, abolishes aggregation of cells transfected with Po (Zhang & Filbin 1994).

Alterations of the cytoplasmic domain of Po also affect the adhesive properties of transfected cells. Cells with deletions of either the terminal 52 or 59 intracellular amino acids fail to aggregate because the cytoplasmic domain is crucial in the formation of functional homotetramers (Wong & Filbin 1996). When cytoplasmic truncated Po was expressed in cells in equivalent amounts with normal Po molecules, cell aggregation did not occur. Also, Po did not cluster normally in the presence of truncated Po, indicating disruption of normal

protein interactions by the aberrant protein. Alterations in the cytoplasmic domain could have additional consequences beyond influencing adhesion of the extracellular domain. During myelin compaction, adhesion of the cytoplasmic membrane faces are believed to occur through a heterophilic interaction taking place via electrostatic interactions between the basically charged cytoplasmic region of Po and negatively charged lipid headgroups within the opposing membrane (Ding & Brunden 1994).

Charcot-Marie-Tooth and Dejerine-Sottas Diseases

Charcot-Marie-Tooth disease (CMT) is the most common genetically transmitted peripheral neuropathy, occurring in roughly 1 in 2500 members of the population (Lebo et al 1993). Dejerine-Sottas syndrome (DS) is a much rarer and disabling type of hereditary sensory motor neuropathy (HSMN). The classification of CMT and DS is extremely complicated because of variable genetic and clinical presentations (Chance & Fischbeck 1994). CMT and DS are encompassed in the schemata of HSMNs shown in Table 1. CMT1 are hypertrophic demyelinating forms of CMT, with little axonal loss. In contrast, CMT2 has a significant loss of large myelinated axons. Genetic mapping studies revealed that CMT1 and DS are both associated with two identical genetic loci. CMT1A and DSA map to chromosome 17p11.2, the site of peripheral myelin protein 22. CMT1B and DSB are associated with 1q22–23, the site of Po protein. Both CMT1 and CMT2 are inherited in an autosomal dominant (AD) fashion. DS has been thought to be inherited in an autosomal recessive (AR) fashion, although new evidence suggests AD action for some forms. Additional uncommon genetic variants of CMT have also been localized to chromosome 8q, the X chromosome (CMTX, associated with mutations in the gap junction protein connexin32), and other autosomal and X-linked loci.

Clinical Features of CMT1B and DS

The clinical presentation of CMT1B varies considerably, even within affected families. Onset is typically delayed until the first or second decade of life, differing from patients with DS who present very early in infancy. It is very rare for symptoms to appear after age 30. The classical peroneal muscle atrophy gives the so-called “inverted champagne bottle” or “stork-leg” appearance to the leg. Skeletal anomalies are common, including pes cavus (hollow foot) and hammer toes. Upper limbs can be involved late in the disease course. Deep tendon reflexes are affected: Ankle jerks are almost invariably absent. Hypertrophic enlarged nerves can often be palpated subcutaneously. Sensory involvement tends to be minor (usually decreased vibratory or joint position sense). Patients usually present with gait difficulties or progressive foot deformities. Motor conduction velocities are delayed to about 40 m/s. Pathological features of

Table 1 Features of the major hereditary sensory motor neuropathies^a

Disease	Frequency	Inheritance/ chromosomal localization	Age of onset	Main clinical/ electrophysiologic features	Pathology
<u>HSMN I</u>					
CMT1A ^b	70%	AD 17p11.2	First to second decade	Distal leg weakness and atrophy Minor sensory problems: loss of JPS/vibratory sense, dysesthesias	Segmental demyelination and remyelination producing areas of hypertrophy ("onion bulbs")
CMT1B ^b	8%	1q22-23		Alteration of DTRs Palpable hypertrophic nerves Marked decreased NCV (<38-40 m/sec)	Minor loss of large myelinated fibers, mild axonal atrophy
<u>HSMN II</u>					
CMT2 (axonal form)	20%	AD	Second to fifth decade	Normal NCV	Atrophy and significantly decreased number of large myelinated axons
<u>HSMN III</u>					
DS A	Rare	AD and AR 17p11.2	Infancy (<1 year)	Delayed developmental milestones Distal followed by proximal muscle atrophy	Hypomyelination Myelinated axon loss
DS B	Rare	1q22-23		Stocking/glove sensory loss Loss of DTRs Enlarged palpable nerves Severely slow NCV (<12 m/s)	Some onion bulb formation

^aAbbreviations: HSMN: hereditary sensory motor neuropathy; CMT: Charcot-Marie-Tooth disease; DS: Dejerine-Sottas syndrome; AD: autosomal dominant; AR: autosomal recessive; NCV: nerve conduction velocity; JPS: joint position sense; DTR: deep tendon reflex.

^bDenervating hypertrophic forms.

CMT1B are hypertrophic “onion bulb” nerves with regions of demyelination and remyelination. There is some loss of large myelinated fibers and associated decreased axonal caliber and number of neurofilaments in those remaining. Although CMT is a progressive disease, rate of deterioration from CMT1B tends to be slow, and patients will often have a normal life expectancy.

In contrast to CMT, DS is a rare disease that afflicts patients very early in childhood and is severely disabling, resulting in delayed motor development and premature death. Progressive proximal and distal weakness and total loss of reflexes are typical. A stocking/glove sensory loss is common. Enlarged (palpable) hypertrophic nerves with conduction speeds <12 m/s are usual features. Microscopically, there is decreased myelination and myelinated axon loss.

Correlation of Mutations with Biology of Po

The physiological role of Po in vivo has been explored by eliminating the Po gene using homologous recombination (Giese et al 1992, Martini et al 1995). By four weeks, $Po^{-/-}$ animals demonstrated clasping of the hindlimbs when lifted, weak/jerky movements of the hindlimbs when walking, clumsy swimming, and tremors. With aging, these deficits increased, but no animal actually became paralyzed. Painful, dysesthetic sensory phenomenon is suggested by self-mutilatory behavior. By adulthood, limbs appear atrophied. Histological analysis of 9-week-old mice revealed multiple abnormalities in myelination varying from loosely compacted myelin with widened interperiod lines and undulating major dense lines, to uncompact whorls of Schwann cell membrane (the majority of fibers), to axons surrounded incompletely by Schwann cells, to axons surrounded by Schwann cells in various degrees of degeneration (even bare) or, in the worst cases, fibers with degenerating axons (rare, 2–5% of units). Axons of reduced caliber were rarely seen in association with endoneurial macrophages. This suggests the presence of not only hypomyelination, but also degeneration of Schwann cells, remyelination, and axonal degeneration, as seen in patients with DS and CMT. The number of fibers showing loosely compacted myelin decreased over time with a concomitant increase in nonmyelinating Schwann cells and increased onion bulb formation.

Heterozygous $Po^{+/-}$ mice exhibit normal myelination until 4 weeks of age. By 4 months, motor unit myelin sheaths are thinner than normal and paranodes exhibited anomalies with myelin ovoids pressing into axonal lumens. At one year, increasing alteration in myelin-to-axon ratios are visible along with thinner compacted sheaths and onion bulb formation. Some axonal atrophy was noted.

Electrophysiological examination of nerves in homozygous mice showed marked conduction delay, polyphasia, and increased latencies. At early ages, heterozygous mice demonstrated no nerve conduction anomalies, but with

Table 2 Classification of Po mutations

Class 1 ^a	Class 2 ^b	Class 3 ^c
Gly(138)Arg ^d	Ser(34)Cys ^d	Gly(74)fs ^g
Leu(145)fs ^d	Arg(69)Cys ^d	Tyr(125)stop ^g
Gln(186)stop ^e	Trp(72)Cys ^f	Tyr(152)stop ^g
Ala(192)fs ^d	18 other mutations ^g	
Val(203)fs ^f		

^aAlterations in the transmembrane cytoplasmic domain that should remain in the membrane.

^bMutations in single amino acids in the extracellular domain.

^cMutations that would give a product unlikely to remain in the membrane.

^dDS.

^eCongenital hypomyelination.

^fSevere CMT.

^gCMT.

aging, increased distal latencies and a reduction in conduction velocity became detectable.

Within a year of the report that abnormal expression of Po caused neuropathy in mice (Giese et al 1992), several reports documented Po mutations in patients with CMT1B and DS (Hayasaka et al 1993a–d, Himoro et al 1993, Kulkens et al 1993). More recently, a mutation has been discovered in a likely case of congenital hypomyelination that exhibited a severe phenotype similar to DS (Warner et al 1996). Thus far, 29 mutations in Po (Table 2) have been described in association with a HSMN (Warner et al 1996).

Precise genetic analysis of both parents and children with mutations in Po has led to a new understanding of the clinical severity and inheritance of these diseases. Heterozygous loss-of-function mutations, which reduce the total amount of normal Po proteins, result in CMT phenotype with AD inheritance. In contrast, dominant negative mutations that disrupt the function of normal Po proteins result in the DS phenotype with AD. DS was previously believed to be inherited in an AR fashion, but of the seven Po mutations with DS phenotype, five mutations occur in a heterozygous (AD) state. The two mutations that produce DS in the homozygous (AR) state [Phe(35)del and Gly(74)fs] produce the CMT phenotype in the heterozygous (AD) state. This is similar to the situation in mice where the *Po*^{-/-} mice have a severe phenotype while the *Po*^{-/+} mice exhibit milder problems later in life.

We have categorized Po mutations into three classes that correlate well with the severity of the diseases (Table 2). Class 1 mutations are those that alter the transmembrane or cytoplasmic domains yet remain in the plasma membrane. Gln(186)stop, Ala(192)fs, and Val(203)fs, which alter only the cytoplasmic tail, and Leu(145)fs, which possesses an abnormal but longer cytoplasmic sequence than normal Po, are likely to be integrated into the membrane. All of

these mutations result in severe phenotypes (severe CMT, DS, or congenital hypomyelination) by dominant negative effects; these results are consistent with *in vitro* studies showing that Po cytoplasmic domain truncations disrupt adhesion mediated by normal Po (Wong & Filbin 1996).

Class 2 mutations involve alterations in the extracellular region, excluding truncations. These almost always lead to CMT expressed in a heterozygous state. In one family, homozygous expression yields DS syndrome (Ikegami et al 1996). This mimics the situation in the Po knockout mice. It suggests that late-onset problems in myelination result from inadequate expression of functional Po in the heterozygous state and that the absence of Po when two copies of the mutant gene are present prevent normal myelination. There are three exceptions, where point mutations produce DS or severe CMT in the heterozygous state. All three are mutations that result in the substitution of a cysteine for the original amino acid [Ser(34)Cys, Arg(69)Cys, Trp(72)Cys]. It has been suggested that these abnormal cysteines can result in the production of abnormal disulfide bonds at outwardly projecting thiols and that these bonds could either form large aggregates of abnormal protein or abnormal:normal protein, interfering with normal myelin compaction and leading to the more severe phenotype.

The class 3 mutations, defined as mutations that lead to an extracellular truncation, usually result in the CMT phenotype. However, if expressed in the homozygous state, DS can result (Warner et al 1996). Class 3 mutations probably result in decreased expression of Po rather than a dominant negative phenotype. The class 3 mutation is similar to the bulk of the class 2 mutations, where the mutant Po does not interfere with the function of the normal Po that is expressed in the heterozygous condition. This suggests that products from class 3 mutations, even if they are secreted, do not disturb the function of normal Po.

Summary

Significant information has been gleaned from the studies of humans with mutations of Po. First, these patients represent relatively few of all the cases of CMT and DS, confirming the previously recognized genetic heterogeneity of these syndromes, and perhaps underscoring the potential for reclassification of the disorders based on their pathophysiology/etiology/molecular basis rather than their clinical phenotype. Second, there is excellent correlation between the effect of human mutations and *in vitro* studies that use transfected cell lines. For example, in both cases truncation of the cytoplasmic domains of Po produces a dominant negative effect, thereby disrupting adhesion and producing the more severe DS phenotype, but only if the Po remains integrated in the plasma membrane. In contrast, most point mutations of the extracellular domain of Po cause CMT, indicating that adhesion of the normal Po also expressed by the Schwann

cells is not disrupted. In order for an extracellular mutation to produce a dominant negative effect, the mutation must produce a large alteration in structure, such as an abnormal cysteine bridge.

However, the human mutations also raise many more questions than they answer. When one considers the potential mechanisms by which dysfunction of Po could alter myelination in a mature individual but not in early development, much more complex situations must be considered. Do these mutations lead to altered half-lives of Po in the membrane, decreasing the stability of myelin? What accounts for the "onion bulb" pattern of demyelination and remyelination? A targeted molecular and cellular approach to studying these questions based on effects seen in patients is clearly called for.

THE L1 CELL ADHESION MOLECULE AND CRASH SYNDROME

Structure and Function of L1

L1 is a member of the Ig superfamily of cell adhesion molecules (CAMs) (Brummendorf & Rathjen 1994). It is a transmembrane glycoprotein (see Figure 3) of approximately 200 kDa with six Ig-like domains followed by five fibronectin type III domains, a single pass transmembrane region, and a cytoplasmic domain (Moos et al 1988). L1 has a high degree of conservation in mammals, with 80–95% amino acid identity in each of the extracellular regions, and complete homology of the cytoplasmic domain (Hlavin & Lemmon 1991). Related molecules have been found in birds (NgCAM), fish (e587), and *Drosophila* (neuroglian).

The L1 gene is located on the X chromosome in both mice and humans, and maps to Xq28 in humans. It is composed of 28 exons, two of which are spliced alternatively. These two, exon 2 and exon 27, are expressed in neurons but not in other L1-expressing cells such as Schwann cells, melanocytes, or lymphocytes (Takeda et al 1996). Exon 27 codes for four amino acids, Arg-Ser-Leu-Glu (RSLE), in the cytoplasmic domain (Miura et al 1991).

During CNS development, L1 is expressed on the surface of long axons and growth cones. It continues to be expressed in the adult on unmyelinated axons. L1 is also expressed on some migrating neurons. Consequently, it is well positioned to influence axon guidance and cell migration. L1 purified from brain or expressed in fibroblasts serves as an excellent substrate for neurite outgrowth (Lagenaur & Lemmon 1987), while antibodies to L1 disrupt fascicle formation in vitro (Stallcup & Beasley 1985) and in vivo. Antibodies to L1 were found to disrupt migration of neurons in vitro (Lindner et al 1983, Barami et al 1994), and L1 expressed in fibroblasts can promote migration of neurons.

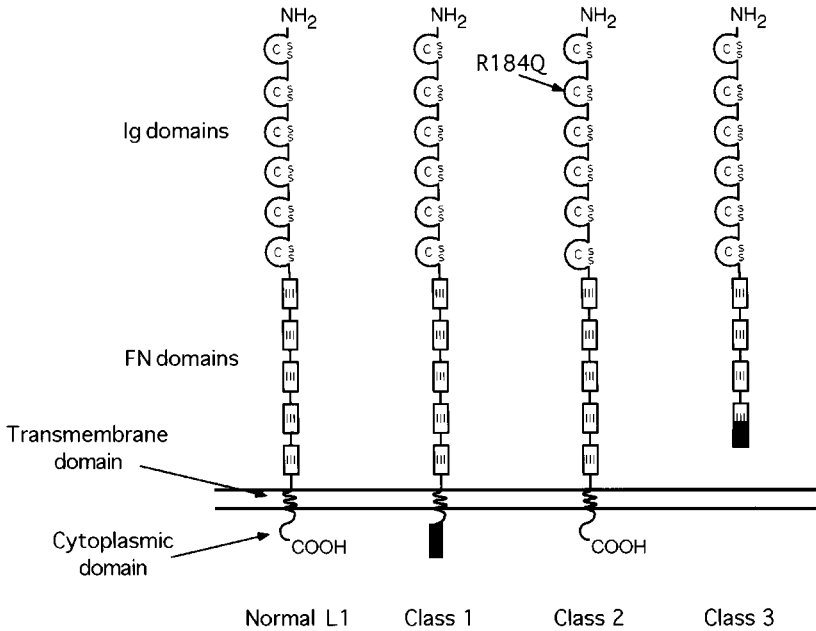


Figure 3 Structures of L1 and examples of the three classes of L1 mutations observed in humans are shown. Class 1 mutations alter the cytoplasmic domain of L1. Class 2 mutations have point mutations in the extracellular domain of L1. Class 3 mutations result in a stop codon that prevents L1 from being expressed on the cell surface.

L1 binds homophilically to L1 (Grumet & Edelman 1988), and this is likely its most common mode of action, given the widespread distribution of L1 on axons. The site of homophilic binding is thought to be in the second Ig domain (Zhao & Siu 1995). L1 binds heterophilically with the Ig superfamily adhesion molecules, axonin-1/TAG-1 (Rader et al 1996), F3/F11/contactin (Brummendorf et al 1993), and DM1-GRASP (DeBernardo & Chang 1996). Recent studies show that there is a *cis* association of axonin-1 and L1 that takes place in the plane of the membrane and that this plays a crucial role in L1-mediated neurite growth (Stoeckli et al 1996). L1 can also bind to the extracellular matrix component phosphacan (Milev et al 1994). This large variety of potential binding partners, combined with both *trans*- and *cis*-binding, suggests that L1 has varying functions at different times and locations during development.

While the adhesive function of L1 was recognized almost immediately, it was subsequently found that antibodies to L1 influence intracellular second

messengers such as inositol phosphates, Ca^{2+} , and pH in neural cells (Schuch et al 1989). Several lines of investigation have implicated kinases as being crucial in the initiation and regulation of these signal cascades. Doherty, Walsh, and colleagues have published an extensive series of experiments (e.g. Doherty & Walsh 1996) showing that the FGF receptor, a receptor tyrosine kinase, directly influences L1-mediated neurite outgrowth and may bind to L1 in a *cis*-fashion. The nonreceptor tyrosine kinase src has been implicated as a downstream regulator of neurite growth in experiments in which neurons from src knockout mice show impaired neurite growth on L1 substrates but not on NCAM (Ignelzi et al 1994). Two serine kinases, casein kinase II and p90rsk, can phosphorylate the L1 cytoplasmic domain and immunoprecipitate with L1 from developing brain (Wong et al 1996). Complicating matters further, the capacity of a neuron to exhibit an L1-dependent calcium response can be both transient and developmentally regulated (Goldman et al 1996).

After the signaling function of L1 was identified, it was found that the cytoplasmic domain of L1 binds to ankyrin (Davis & Bennett 1994). Ankyrin is a structural protein found on the inner surface of the plasma membrane that has binding sites for integral membrane proteins and the cytoskeleton. If L1 is expressed in B28 glioma cells, the L1 clusters in long linear arrays that colocalize with actin fibers (Dahlin-Huppe et al 1997). Disruption of the actin fibers with cytochalasin causes the L1 linear arrays to become disorganized. The ability of L1 to interact with the actin cytoskeleton is dependent on a highly conserved sequence in the L1 family of molecules that is next to the inner surface of the plasma membrane. This region is immediately adjacent to the serine phosphorylated by p90rsk (S^{1152}). If a peptide spanning this region is loaded into neurons, their ability to send out neurites on L1 is inhibited (Wong et al 1996). If a peptide where S^{1152} has been converted to an alanine is used instead, neurite outgrowth is not inhibited, suggesting phosphorylation of S^{1152} is important in this process. Regulation of the cytoskeletal association with L1 by kinases or phosphatases could regulate growth cone behavior, such as the rapid alteration in shape as growth cones grow from laminin onto L1 (Burden-Gulley & Lemmon 1996). It is also interesting that humans with mutations in Rsk-2, a p90rsk isoform, have agenesis of the corpus callosum (Trivier et al 1996), raising the possibility that the p90rsk interaction with L1 plays a crucial function in callosal development.

CRASH Syndrome: X-Linked Hydrocephalus, MASA Syndrome, and X-Linked Spastic Paraplegia

X-linked hydrocephalus (XLH) was first described by Bickers & Adams (1949). Edwards (1961) reported four additional families and defined the syndrome of

sex-linked hydrocephalus as hydrocephalus, mental deficiency, spastic paraplegia, and flexion deformity of the thumbs. XLH is a relatively rare condition, affecting about 1 in 20,000 live births. MASA syndrome—an acronym for mental retardation, aphasia, shuffling gait, and adducted thumbs—was first reported by Bianchine & Lewis (1974). Hereditary spastic paraplegia (HSP) has been associated with several different genetic loci, and one family has been found to have a mutation in the L1 gene (Jouet et al 1994). Since XLH, MASA syndrome, and one form of HSP have been attributed to mutations in the L1 gene (and their clinical signs overlap), a new terminology is warranted. Different investigators have proposed “HSAS/MASA spectrum” (Schrander-Stumpel 1995), “L1 syndrome” (Jouet & Kenwrick 1995), and “CRASH syndrome” (Fransen et al 1995), with CRASH syndrome being an acronym for corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia, and hydrocephalus. We prefer “CRASH syndrome” because it summarizes the major characteristic symptoms associated with mutations in L1.

Clinical, Pathological, and Radiological Manifestations of CRASH Syndrome

Early reports attributed X-linked hydrocephalus to aqueductal stenosis (Edwards 1961). It has been postulated that mechanical deformation of the brain stem secondary to communicating hydrocephalus produces an aqueductal narrowing (Landrieu et al 1979). Subsequent pathological and radiological studies have failed to find evidence of aqueductal stenosis in a number of cases, and total occlusion is extremely rare (Renier et al 1982, Yamasaki et al 1995). The degree and progression of ventricular dilatation is variable within and between families. Some patients have progressive hydrocephalus, which produces raised intracranial pressure and huge macrocephaly, and require ventriculo-peritoneal (VP) shunting. Other patients present with arrested hydrocephalus that results in ventricular dilatation and slight macrocephaly but require no shunting procedure. Still other patients demonstrate normocephaly with enlarged ventricles. Most of these patients were diagnosed as MASA patients, particularly before recent advances in brain imaging. In these individuals, the increased ventricular size may be due to loss of white matter. It seems plausible that some of the variability seen in diagnosing XLH vs MASA syndrome was due to different definitions of hydrocephalus used by different clinicians.

Mental Retardation

Mental retardation is the most common symptom among individuals with L1 mutations, including patients without hydrocephalus. The mental retardation

is often profound, resulting in IQs below 50. In patients with severe hydrocephalus, shunting does not improve mental function, even when treated immediately after birth. This is in sharp contrast to patients with hydrocephalus associated with myelomeningocele, who often have normal or above normal intelligence. Therefore, it is clear that L1-associated mental retardation is unlikely the result of hydrocephalus but is probably due to abnormal CNS development.

Shuffling Gait/Spasticity and Absence of Corticospinal Tract

Spastic paraplegia, hyper-reflexia, or shuffling gait are observed in all cases of CRASH syndrome. This is independent of the presence of hydrocephalus or the degree of mental retardation. In all 25 cases of XLH in which neuropathological studies of the medulla have been performed, the pyramids are absent or diminished in size (Yamasaki et al 1995). This suggests that abnormal development of the corticospinal tract is a characteristic finding in CRASH syndrome that accounts for the gait disturbances or paraplegia associated with this syndrome.

Adducted Thumbs

This malformation is highly associated with L1 mutations. Surgical exploration indicates hypoplasia of the extensor pollicis (EP) muscles (Yeatman 1984). Electromyographic analyses and nerve conduction studies indicated lower motor neuron dysfunction but only of the motoneurons innervating the EP muscles (Landrieu et al 1979). This sign is not directly correlated with hydrocephalus or impaired cortical function. Rather it appears to be a specific developmental defect—perhaps loss of fasciculation of axons growing to the EP muscles.

Other Findings

Agenesis of the corpus callosum (ACC) is commonly reported in CRASH syndrome, although it is by no means unique to this syndrome. ACC is associated with more than 50 different disorders (Jeret et al 1987). Thalamic fusion is observed by autopsy and by MRI in XLH. The cerebellum is often abnormal, with hypoplasia of the anterior vermis. Abnormalities in the development of the corpus callosum, pyramids, thalamus, colliculi, and anterior vermis all point to a defect associated with midline development, perhaps involving abnormal cell migration. Hirshsprung's disease, in which there is failure of colonic neural migration, is associated with some cases of CRASH syndrome (Kaplan 1983).

Correlation of L1 Mutations with Pathological Findings and L1 Biology

Since 1991, when the cDNA for human L1 was cloned (Hlavin & Lemmon 1991) and an L1 mutation was reported in an XLH family (Rosenthal et al

1992), 75 different mutations have been reported in 80 families. An up-to-date list can be obtained from the L1 Home Page maintained by Willems and associates (Van Camp et al 1996). The mutations can be classified into three structural categories, detailed below (see Figure 3).

The first class of mutation affects the cytoplasmic domain of L1 and includes a missense mutation, a nonsense mutation, frameshifts, duplications, and deletions. This type of mutation would be expected to disrupt L1 signaling and associations with the cytoskeleton. However, mutations in the cytoplasmic domain would not necessarily affect the adhesivity of L1, since it has been shown that deleting most of the L1 cytoplasmic domain does not significantly alter L1-mediated adhesion (Wong et al 1995).

The second class consists of missense point mutations in the extracellular domain. Based on molecular modeling studies, the mutations cluster in a few regions where they alter conformation, disrupt domain structure, or affect surface properties of L1 (Batemann et al 1996). Mutations in this class might prevent either L1 homophilic (Zhao & Siu 1996) or heterophilic binding, or perhaps both simultaneously. This type of mutation might also disrupt the *cis*-interactions that are important in L1-mediated neurite growth. Finally, these mutations might prevent a change in conformation of L1 that is required for the production of intracellular signals initiated by L1 binding to its various ligands.

Class 3 mutations include those that result in a premature stop codon in the extracellular domain. This class of mutation would be expected to lose all normal function of L1, including homophilic and heterophilic binding and also any signaling events mediated by the L1 cytoplasmic domain.

We found a strong correlation between the severity of the disease and the class of mutation (Yamasaki et al 1997) (Figure 4). Individuals with class 3 mutations (extracellular truncations) have the worst outcome, being less likely to survive the first year after birth and more likely to have severe hydrocephalus and severe mental retardation. Class 2 mutations have more severe consequences than class 1 mutations. All three classes of mutations have similarly high incidences of adducted thumbs.

Since class 1 mutations produce abnormal axon growth but not severe hydrocephalus, it is likely that loss of L1-mediated signaling or interactions with the cytoskeleton may be a crucial factor in axon guidance in some projection systems. Class 2 mutations also produce spasticity associated with abnormal corticospinal tract development, indicating mutations at numerous locations in the extracellular domain of L1 probably compromise its ability to transduce extracellular binding to ligands into the intracellular events required for proper axon guidance. Clearly, class 3 mutations would prevent any L1-mediated

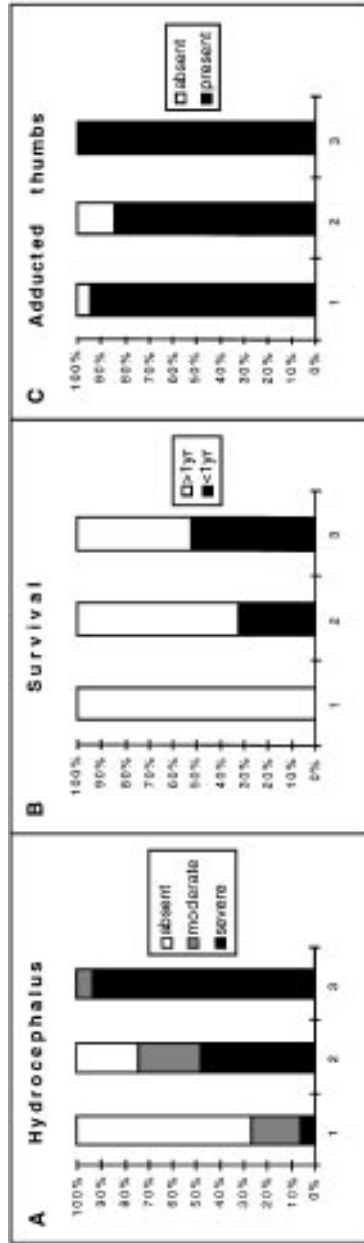


Figure 4 CRASH syndromes associated with different classes of L1 mutations. (A) Severe hydrocephalus is much more common in association with class 3 mutations than with class 1 mutations. (B) Death before the age of 1 year is much more common in association with class 3 mutations than with class 1 mutations. (C) Adducted thumbs are almost always present in association with all three classes of L1 mutations.

signals necessary for axon growth or guidance. Enlargement of the ventricles is associated with all three classes of mutations. To some degree, this may represent hydrocephalus ex vacuo, where the increase in ventricle volume results from loss of white or gray matter.

Class 3 mutations are more likely to cause severe hydrocephalus than class 1 or 2 mutations. Class 2 mutations can be subdivided into two groups based on whether the mutation alters only surface features of structural domains or causes major changes in domain structure (Batemann et al 1996). Surface changes rarely (28%) lead to severe hydrocephalus, while major structural changes are very likely (78%) to cause severe hydrocephalus. So it is plausible that loss of L1-mediated adhesion in class 2 and 3 mutations is responsible, directly or indirectly, for severe hydrocephalus. L1 has been implicated in migration of immature neurons away from the ventricular proliferate zone in birds (Barami et al 1994). If there is a loss of L1-mediated adhesion, perhaps immature neurons have difficulty emigrating from some proliferative zones, leading to abnormalities such as fused thalami, flat quadrageminal plates, and failure of cells to reach target zones such as the anterior vermis and neocortex. However, most brain regions do not exhibit obvious disorganization similar to that found in other disorders affecting cell migration, so neuronal cell death following the failure to establish correct axon-target relationships may also be involved in CRASH syndrome.

Summary

There are good correlations between the laboratory studies on L1 and findings in humans with mutations that alter L1 expression. Mutations that eliminate L1 expression are most likely to cause severe consequences. Mutations that disrupt the cytoplasmic domain are the least severe but clearly alter the development of important axon tracts. However, it remains unclear why L1 mutations cause other brain abnormalities. Are they due to disruptions in cell migration, axon guidance, or some unknown function of L1? Rapid advances in clarifying these problems may be difficult, since the first attempt at producing an L1 knockout mouse failed to result in abnormalities that paralleled those seen in humans (Hynes 1996).

CONCLUSION

Perhaps the most interesting conclusion that comes from comparing clinical syndromes due to mutations affecting merosin, Po, or L1 is that intracellular processes have a significant role in producing the phenotypes. Loss of merosin expression leads to apoptotic cell death in muscle cells, implying altered

intracellular signals. Mutations in the cytoplasmic domain of Po lead to DS with AD inheritance, probably resulting from disruption of the formation of functional homotetramers that mediate adhesion. If the cytoplasmic domain of L1 is altered, some major axon pathways are eliminated, implicating this domain in axon guidance. This certainly does not suggest that the adhesive functions of these molecules are of no consequence. In all three cases mutations that eliminate expression of functional adhesion molecules have severe consequences.

The identification of adhesion molecules associated with specific human syndromes has followed a pattern often observed with other human-inherited diseases; multiple clinical syndromes can be attributed to different types of mutations in single genes, for example MASA syndrome vs XLH or CMT vs DS. Furthermore, similar symptoms can result from mutations in different genes that code for interacting proteins, as is the case with merosin and dystrophin, or L1 and Rsk-2. While our understanding of the function of these adhesion molecules has been greatly increased by studying related human diseases, there remain large gaps in our knowledge that will be closed only by careful study of appropriate animal models.

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