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New Domains of Neural Cell–Adhesion Molecule L1 Implicated in X-Linked Hydrocephalus and MASA Syndrome

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Summary

The neural cell–adhesion molecule L1 is involved in intercellular recognition and neuronal migration in the CNS. Recently, we have shown that mutations in the gene encoding L1 are responsible for three related disorders; X-linked hydrocephalus, MASA (mental retardation, aphasia, shuffling gait, and adducted thumbs) syndrome, and spastic paraplegia type I (SPG1). These three disorders represent a clinical spectrum that varies not only between families but sometimes also within families. To date, 14 independent L1 mutations have been reported and shown to be disease causing. Here we report nine novel L1 mutations in X-linked hydrocephalus and MASA-syndrome families, including the first examples of mutations affecting the fibronectin type III domains of the molecule. They are discussed in relation both to phenotypes and to the insights that they provide into L1 function.

Introduction

We have demonstrated that X-linked hydrocephalus and two related neurological disorders, MASA (mental retardation, aphasia, shuffling gait, and adducted thumbs) syndrome and spastic paraplegia type 1 (SPG1), are caused by heterogeneous mutations in the gene encoding the neural cell–adhesion molecule L1 (Rosenthal et al. 1992; Jouet et al. 1993*b*, 1994; Jouet and Kenwrick 1995). X-linked hydrocephalus is the most common form of congenital hereditary hydrocephalus. Excessive accumulation of cerebrospinal fluid results in high-pressure hydrocephalus, which is often associated with narrowing of the aqueduct of Sylvius. A variety of other brain malformations have been reported, including agenesis of the corpus callosum, fusion of thalami, and hypoplasia of the corticospinal tract. Hydrocephalus develops in utero or during the neonatal period and is lethal if untreated. Survivors all suffer from mental retardation, developmental delay, and spastic paraparesis and can present with unusual flexion and adduction of the thumbs (Bickers and Adams 1949; Edwards et al. 1961; Halliday et al. 1986; Willems et al. 1987).

The clinical profiles described for individuals with MASA syndrome and SPG1 overlap with those observed for X-linked hydrocephalus, although the lack of congenital high-pressure hydrocephalus results in a longer life expectancy (Bianchine and Lewis 1974; Gareis and Mason 1984; Kenwrick et al. 1986; Winter et al. 1989; Schrander-Stumpel et al. 1990). For all three disorders, wide variation is observed between the clinical signs of patients from different families, as well as between patients belonging to the same family. Indeed, families have been described with cases of both MASA syndrome and X-linked hydrocephalus (Fryns et al. 1991).

The L1 cell–adhesion molecule implicated in these disorders is a multidomain cell-surface glycoprotein expressed primarily on the axons of postmitotic neurones (for reviews, see Rutishauser 1989; Grumet 1991; Sonderegger and Rathjen 1992). Results from in vitro assays and antibody-perturbation experiments have shown that L1 is involved in neuronal migration and neurite extension and that it may also have a role in the myelination of neurones in the peripheral nervous system. Although the activity of L1 is known to be mediated by homophilic and heterophilic interactions at the cell surface, little is known with regard to the functional domains of the protein (Lemmon et al. 1989; Kuhn et al. 1991). The discovery that L1 mutations are responsible for X-linked hydrocephalus, MASA syndrome, and SPG1 not only confirms its vital role in neuronal development but also highlights regions of the protein critical for correct function. A description of the full spectrum of mutations that can give rise to these disorders will provide invaluable information regarding the function of this developmentally important molecule.

To date, 14 different L1 mutations have been de-

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scribed in as many families, including 9 with X-linked hydrocephalus, 4 with MASA syndrome, and 1 with SPG1 (Rosenthal et al. 1992; Jouet et al. 1993*b*, 1994; Van Camp et al. 1993; Coucke et al. 1994; Fransen et al. 1994; Vits et al. 1994; Jouet and Kenwrick 1995). Here, we report nine novel mutations from nine families affected by X-linked hydrocephalus, MASA syndrome, or mental retardation/clasped-thumb syndrome. These mutations are discussed in relation both to the phenotype of the patients and to the information that they provide regarding the critical regions of the L1 protein.

Patients, Material, and Methods

Patient Samples

These studies were conducted with permission from the Cambridge Ethical Committee. Families H1 and H3 have been described by Jouet et al. (1993*a*), who observed linkage of the disorder with Xq28 markers. H29 was originally described, by Yeatman (1984), as a pedigree with mental retardation/clasped-thumb syndrome. Linkage analysis indicates Xq28 linkage in this family (N. Carpenter, unpublished data). Families H5, H7, H19, H38, H39, and H44 have not been previously described.

Mutation Detection by L1 cDNA Analysis

PCR amplification.—Epstein Barr virus–transformed lymphocytes were used as a source of cDNA, as described by Rosenthal et al. (1992). In brief, poly A+ RNA was obtained, reverse-transcribed, and used as a template for PCR amplification of the L1 gene. A nested-PCR approach was adopted, since L1 mRNA levels in B cells proved to be low. First-round PCRs were designed to amplify the L1 cDNA (3.7 kb) in three reactions producing overlapping fragments, on the basis of the sequence published by Hlavin and Lemmon (1991). The overlapping fragments were produced by using primer pairs A15–A18 (2.58 kb long), A93–A92 (2.48 kb long) and A19–A103 (1.98 kb long), going from a 5′ to a 3′ direction (table 1). Their respective overlaps were 1.3 kb between the first and second fragments and 1.9 kb between the second and third fragments. The first and third fragments also overlapped, but only by 220 bp. Each reaction was carried out in 30 μl and contained 15 pmol of each primer, 0.25 mM dNTPs, 10 mM Tris-HCl pH 8.8 at 25°C, 50 mM KCl, 0.001% gelatine, 0.5% Tween 20, 0.1% Triton x100, 2 mM $MgCl_2$, and 2 units of *Taq* polymerase (native *Taq* polymerase; Cetus). The PCR conditions applied were the same for all three pairs of primers: 95°C for 1 min followed by 35 cycles of 95°C for 30 sec, 55°C for 1 min and 72°C for 3 min. This was followed by 5 min at 72°C. No specific L1 product was seen following the first round of PCR. An aliquot of each first reaction was diluted 1:25 and 1 μl aliquots used as templates for nested reactions. The reactions were set in a 25 μl volume containing 10 pmol of each primer, 0.2 μM dNTPs, 10 mM Tris-HCl pH 8.8 at 25°C, 50 mM KCl, 0.001% gelatine., 0.5% Tween 20, and 0.1% Triton x100, 2 mM $MgCl_2$, and 1 unit of *Taq* polymerase (Promega).

Automated sequencing.—Amplified PCR products were gel-purified and recovered by using Magic PCR preparations (Promega). Automated fluorescent sequencing was performed on 200–300 ng of double-stranded product by using a *Taq* Dye Deoxy Terminator Cycle Sequencing Kit (ABI) according to the manufacturer's recommendations. The sequenced fragments were purified on Sephadex G-50 (Rosenthal and Charnock-Jones 1992), lyophilized, and resuspended in formamide loading buffer. They were run on a 6% acrylamide/7 M urea/1 × Tris-borate EDTA sequencing gel.

L1 gene analysis by SSCP and heteroduplex analysis.—SSCP analysis was performed as described by Jouet et al. (1994). For heteroduplex analysis, ~250 ng of mutant and ~250 ng of wild-type PCR fragments were mixed in a final volume of 10 μl. The mixture was denatured by heating to 94°C for 4 min, followed by cooling down to room temperature over 2 h to ensure maximum annealing. The reaction was mixed 1:1 with standard formamide loading dye, and 5 μl were run on the gel, which corresponded to ⩾50 ng of each product. Heteroduplex gels were 1 mm thick and silver-stained as described elsewhere (Jouet et al. 1994).

Direct radioactive cycle sequencing.—The detection of a band shift or of a heteroduplex prompted the sequencing of the double-stranded PCR fragment with the fmol sequencing system kit (Promega). Either the sense or antisense primer originally used to amplify the fragment was 5′ end–labeled and used in the sequencing reaction.

Single-nucleotide-primer extension (SNuPE).—When a mutation did not create a novel recognition site for a restriction enzyme, mutation detection was carried out by SNuPE analysis. This one-base primer-extension assay requires the designing of a specific primer with its 3′ end located just before the mutated base. This method was originally described by Kuppuswamy et al. (1991), and our adapted method has been described by us elsewhere (Jouet et al. 1994). SNuPE primers sequences were as follows: SX4 (5′ ATGGGACATGGCGGTGC 3′), SX18 (5′GCCCTGGCAGGAGCAGATT 3′), SX8 (5′ GGCCAGGCAGCGGTACT 3′), and SX21 (5′ CGCCGTTGTGGCTGAGT 3′).

Results

Nine families were screened for mutations in the L1 gene by using either direct cDNA sequencing or SSCP in conjunction with heteroduplex analysis. All families show a clear X-linked pattern of inheritance. Six families

Table 1

L1 cDNA primers

cDNA Primer	5′ Sequence 3′	Position	Annealing Temperature (°C)
A15	ATGGTCGTGGCGCTGCGGTACG	1–22	55
L7	CAAAGCAGCGGTAGATGCCCTG	346–325	
L8	GTACCAGTCGCCCCACTCTGG	258–278	59
L9	CCGTTCTGGCCCATCGTCAC	572–553	
L10	CAGAGCCTCTCCGGATCTAC	491–510	59
L11	GCACTCCAGGACCAATGGCTG	792–772	
L12	GCATGATTGACAGGAAGCCGC	701–721	59
L13	ATGGCTCTGGGGCTTGTGCAG	1026–1006	
L14	CCGAGAACTCACTGGGCAGTG	941–961	59
L15	ACGTAGATGTAGGCATTGGC	1262–1243	
L16	CTGATCCTGAGCAACGTGCAGC	1165–1186	56
A11	AGGGTCCCATTGGCATAGGG	1445–1426	
A2	TGGGACAACAGTGCTTCAGG	1392–1411	56
A9	CTACTCTCCACCACATCCAG	1808–1789	
A4	AGTGACAAGTACTTCATAGA	1696–1715	56
L17	CAGACTGTACCATTTTTCAG	1995–1976	
L18	CTGGAGTCCTGCAGAAGACC	1902–1921	56
A7	CTTCCACGTGATGACCATAT	2208–2189	
A6	TCTGAGACTGTGGTCACACC	2110–2129	56
A5	CCAGCACGGCACTTGAGTTG	2491–2472	
A8	TCACTATCGGGCTACTCTGGAG	2405–2425	55
L25	GGGTGCTGAAGGTGAACTCG	2740–2721	
A10	TCCTACCACCTGGAGGTG	2668–2685	53
L19	GTCAGGTTGTGTGTCCGAAG	2942–2923	
L20	CTACGTGCTCTCCTACCACC	2850–2869	59
A1	TGAACAAGATATGGAACCT	3154–3136	
A12	GATTTTGGCAACATCTCAGC	3055–3074	59
L5	GAAGCCAGCAGGAGGGAGCCTCAC	3351–3328	
L21	CTGACTACGAGATCCACTTG	3251–3270	53
A92	GTACTCGCCGAAGGTCTCATC	3528–3508	
L22	GTGAAGGATAAGGAGGACACC	3457–3477	53
L23	TCCACTATTCTAGGGCCACG	3778–3759	
A18	TGCTGTGCTTCCTCTGACTG	2587–2568	
A19	GTCCAGGCCGTCAACAGCCAG	2362–2382	
A93	GACTGCCAAGTCCAGGGCAGGCC	1057–1079	
A103[a]	GGAAGGACAGGGGTACAAAC	4670–4651	

NOTE.—Primers are arranged in pairs, as used. Sequences for primers A18, A19, A93, and A103, used only in the first PCR, are also shown.

[a] Lies in the 3′ UTR.

(H1, H3, H5, H7, H19, and H44) were described as X-linked hydrocephalus, one (H29) as mental retardation/clasped-thumb syndrome, one (H38) as MASA syndrome, and one (H39) as both MASA syndrome and X-linked hydrocephalus. Clinical details of affected patients from these families are reported in table 2. All nucleotide and amino acid changes are described in terms of the nomenclature used in Hlavin and Lemmon (1991), with the first base of the translation initiator methionine denoted as nucleotide 1.

Table 2

Clinical Details

	H1	H3	H5	H7	H19	H29	H38	H39	H44
Progressive hydrocephalus	2/4	2/2	2/2	1/2	3/3	0/6	2/2	2/3	2/2
Enlarged ventricles	4/4	2/2	2/2	2/2	3/3	0/1[a]	2/2	3/3	2/2
Mental retardation	+	+	+	+	+	+	+	+	+
Spastic paraplegia	+	+	+	+	+	+	+	+	+
Death before 1 year	1/4	1/2	1/2	0/2	2/3	0/6	0/2	0/3	1/2
Aphasia	?	+	?	+	?	+	+	+	?
Pyramidal tract malformation[b]	+	?	?	?	?	?	?	?	+
Adducted thumbs	?	+	+	−	+	+	+	+	+

NOTE.—Where the information was available, the proportion of affected cases with each disorder is given. + = present; − = absent; and ? = information not available.

[a] Only one case examined.

[b] Evidence from postmortem or MRI scans.

Mutation Detection by cDNA Sequencing Analysis

Two novel missense mutations were detected in two unrelated X-linked hydrocephalus families by using direct sequencing of L1 cDNA PCR fragments (fig. 1). In family H5, a G-to-T change at nucleotide 2302 was detected, which causes a valine-to-phenylalanine substitution at residue 768 (Val768Phe). Mutation detection in relatives and controls was carried out by SNuPE analysis using primer SX18. This mutation was not found in 55 independent X chromosomes, and therefore it does not correspond to a common polymorphism.

In family H7, a G-to-A base change was identified at position 361, resulting in a missense mutation where Gly121 is replaced by serine. Mutation detection was also performed, by using SNuPE analysis with primer SX4. Both affected brothers were found to harbor this mutation, as was their carrier mother. This mutation was not found in 51 chromosomes from healthy controls tested and therefore does not correspond to a common polymorphism either.

It is interesting that sequencing H7 L1 cDNA also detected two other base changes (data not shown). One was a silent mutation, and the other caused the replacement of an isoleucine by a threonine. However, neither of these mutations was present in the patient's genomic DNA. The patient sequenced simultaneously with this one was also analyzed at the genomic level for these two mutations; and he did not harbor them either. We conclude that these two spurious mutations correspond to an artifact of reverse-transcription PCR.

In both family H5 and family H7, a single generation is affected by X-linked hydrocephalus, and the mothers of the affected boys are carriers of the mutations described (fig. 1). Our mutation analysis enabled us to confirm that they indeed suffer from X-linked hydrocephalus and not from a rarer autosomal recessive form of the disease. Accurate genetic counseling is now available for these two families. During sequencing, evidence for alternative splicing of L1 RNA in B cells was obtained, since neither exon 2 (15 bp) nor exon 27 (12 bp) was found in B-cell RNA from control or patient cells (described in Jouet et al., in press; genomic organization provided courtesy of A. Rosenthal, EMBL database HSNCAMX).

Mutation Detection by SSCP and Heteroduplex Analysis of the L1 Gene

Seven mutations were identified by SSCP and heteroduplex silver-stain analysis combined with direct double-stranded sequencing (fig. 2). In family H1, an SSCP shift in exon 24 was caused by an A-to-G transition at position 3209, resulting in a Tyr1070Cys substitution. This mutation creates a novel recognition site for the enzyme *Bso*FI, and segregation of this mutation with the disease in the family was confirmed (fig. 2). In family H3, the SSCP shift detected in exon 1 was caused by a G-to-C transversion at position 26, which converts Trp9 to a serine. The mutation destroys an *Hae*III site; and segregation of the mutation with disease in this family was also proved by restriction-digest analysis.

No SSCP shift was observed for family H38. However, a heteroduplex was detected in exon 8. Sequencing revealed that it was due to a G-to-A transition causing Glu309Lys. This mutation was analyzed in the family by SNuPE using primer SX8, and segregation with the disease was demonstrated.

In family H39, where male patients from different generations are clearly affected by either MASA syndrome or X-linked hydrocephalus, the SSCP shift present in exon 21 was due to a C-to-T missense mutation at position 2822, causing Pro941Leu. Segregation of the mutation with the disease in this family was demonstrated by SNuPE analysis using primer SX21.

In family H44, an obligate carrier mother with famil-

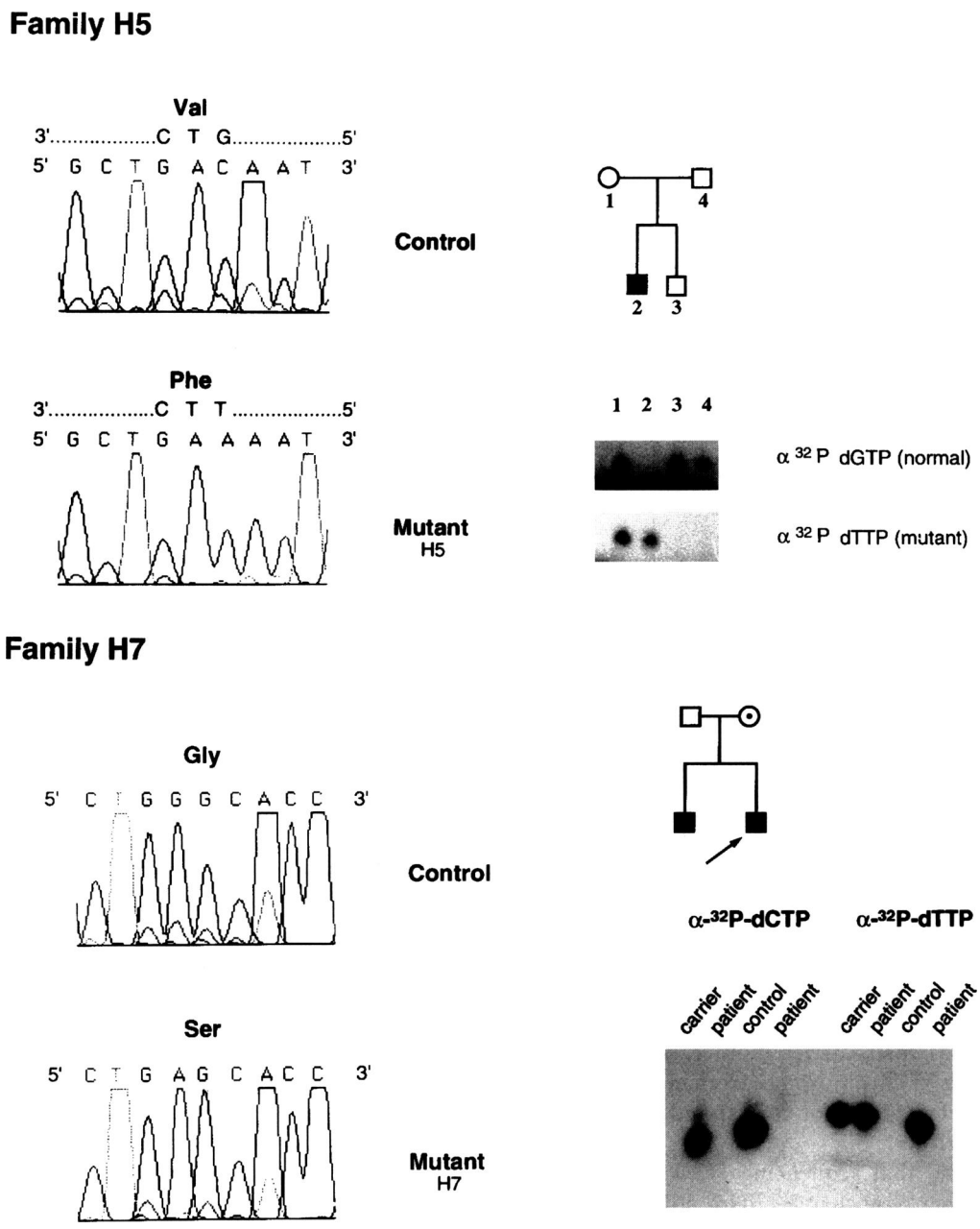


Figure 1 Mutation analysis by automated cDNA sequencing. Mutations in Families H5 and H7 were detected by using primers A5 and L8, respectively. Right-hand panels show segregation of the mutation in the families as assayed by SNuPE analysis with the primers SX18 and SX4, respectively. Only family members for whom DNA samples were available are shown. Mutation analysis was conducted by using DNA from the subject indicated by the arrow.

ial antecedents for X-linked hydrocephalus was analyzed for a possible L1 mutation. The SSCP shift identified in exon 14 was caused by a nonsense C-to-T mutation at base 1756, resulting in Gln586STOP.

Two mutations affect the recognition of splicing signals in the L1 gene. In family H19, where a single case of hydrocephalus was available for analysis, a base change at the intron 10 donor splice site (a +4 to t) produced an SSCP shift (fig. 2). This mutation was found to affect recognition of the 5′ splice site and to result in skipping of exon 10, as shown by the sequencing of the patient's corresponding cDNA fragment (fig. 3). This would result in the in frame deletion of 48 amino acids from the native L1 protein.

In family H29, the SSCP shift was caused by a point mutation in the acceptor site (g-12 to a) in intron 26 (fig. 2). This mutation creates a novel recognition site for the restriction enzyme *Mva*I, and segregation of the

mutation through the family was checked by restriction-digest analysis (fig. 2). One patient (patient 2 in our pedigree) was said to be mentally retarded, but his Xq28 haplotype was not consistent with inheritance of an L1 mutation (N. Carpenter, unpublished data). Our analysis shows that he does not harbor the L1 mutation found in all other affected members of the family, and therefore his mental retardation must have a different etiology. cDNA analysis of the region surrounding the mutation indicated the presence of a slightly larger mRNA species, in addition to the normal cDNA in B cells from an affected member of this family (data not shown). Although this result was consistent with a population of mRNA molecules with an out-of-frame 10-base insertion, insufficient fragment could be obtained for sequencing. This result indicates that mental retardation/clasped-thumb syndrome belongs to the same clinical spectrum as MASA syndrome and X-linked hydrocephalus. All of the mutations described above were not found in >50 control X chromosomes. The positions of the mutations reported here, with respect to L1 protein structure, are shown in figure 4.

Discussion

The nine novel mutations reported here include six missense mutations, one nonsense mutation, and two mutations affecting mRNA splicing. When these are considered along with the 14 mutations previously reported, several general points can be made. To date, no common mutations have been found. Each family has a unique mutation; the mutations are distributed across the L1 gene and are varied in type. The most common are missense mutations (12), although splicing (5), deletion (3), and nonsense changes (2), as well as a duplication (1), have been described.

L1 is a cell-adhesion molecule found on the surface of developing neural cells that plays a role in neuronal cell adhesion and migration in the developing brain. As a member of the immunoglobulin superfamily, it contains extracellular Ig and fibronectin (FnIII) domains and an intracellular cytoplasmic domain. L1 interacts homophilically or heterophilically with other neural cell–adhesion molecules or components of the extracellular matrix. Apart from L1 itself, several ligands have been identified for the L1 protein, including Ig family member axonin-1/TAG-1 and the proteoglycan neurocan (Kuhn et al. 1991; Friedlander et al. 1994). The specificity of these interactions has not been elucidated yet, except that neurite extension in response to L1:L1 binding is mediated by interaction between L1 molecules on the neuronal cell surface and members of the fibroblast growth-factor receptor (FGFR) family. Stimulation of the tyrosine kinase function of these receptors results in a rise in intracellular calcium and stimulates neurite outgrowth (Williams et al. 1994). Although the cytoplasmic domain is known to bind to the cytoskeletal protein ankyrin (Davis and Bennett 1994), little is known about the function of the individual extracellular domains. Therefore, it is not possible to predict the specific consequences of an L1 mutation on neural development. However, it is of interest to determine whether correlations exist between the types of L1 defect and the resulting clinical picture.

The mutation in family H44 is the third mutation that we have described that would truncate the protein prior to the transmembrane domain, eliminating cell-surface expression of L1 (H6 and H24; Jouet et al. 1994). In all three families, severe hydrocephalus results, and the mutations are associated with early mortality.

Krawczak et al. (1992) estimate that 15% of all point mutations causing human genetic diseases result in mRNA splicing defects, and, to date, 5/23 L1 mutations fall into this category. Two novel splicing L1 mutations are reported here. In one case (H19), mutation of an absolutely conserved base within a donor splice site results in exon skipping. In family H29, the mutation creates a novel upstream acceptor site. Utilization of this new site may be facilitated because it is closer to the polypyrimidine tract than is the normal acceptor site (Reed 1989). In both of these families, as for the two previously described splicing mutations, production of normal mRNA is also apparent in cDNA studies on B-cell lines. If these results can be extrapolated to the developing brain, they indicate that these mutations act in a dominant-negative fashion at the cellular level, although they remain recessive in heterozygous females. Many models can be proposed to explain how this is mediated, including selective interaction with some ligands, dilution of downstream signal transduction, and creation of novel interactions. In the case of family H29, this dominant effect must involve cytoplasmic interactions, since all extracellular domains remain intact. Interestingly, the H29 mutation would truncate the protein immediately prior to an alternatively spliced, highly conserved motif "RSLE" (exon 27) that is only present in the CNS.

Six novel missense mutations are described here, bringing the reported total to 12. A summary of missense mutations reported here and elsewhere is included in figure 4. Some clustering is apparent for missense mutations in the region containing Ig domains, and, prior to this report, only Ig-region missense mutations had been described for the extracellular region of L1. A prominent role for this region is supported by studies indicating that both L1-mediated neurite extension and adhesion require Ig domains (Appel et al. 1993). Here, for the first time, we describe mutations affecting three different fibronectin type III domains, mutations that result in phenotypes comparable to those caused by more distal mutations, indicating that these domains are also critical for L1 function in brain development.

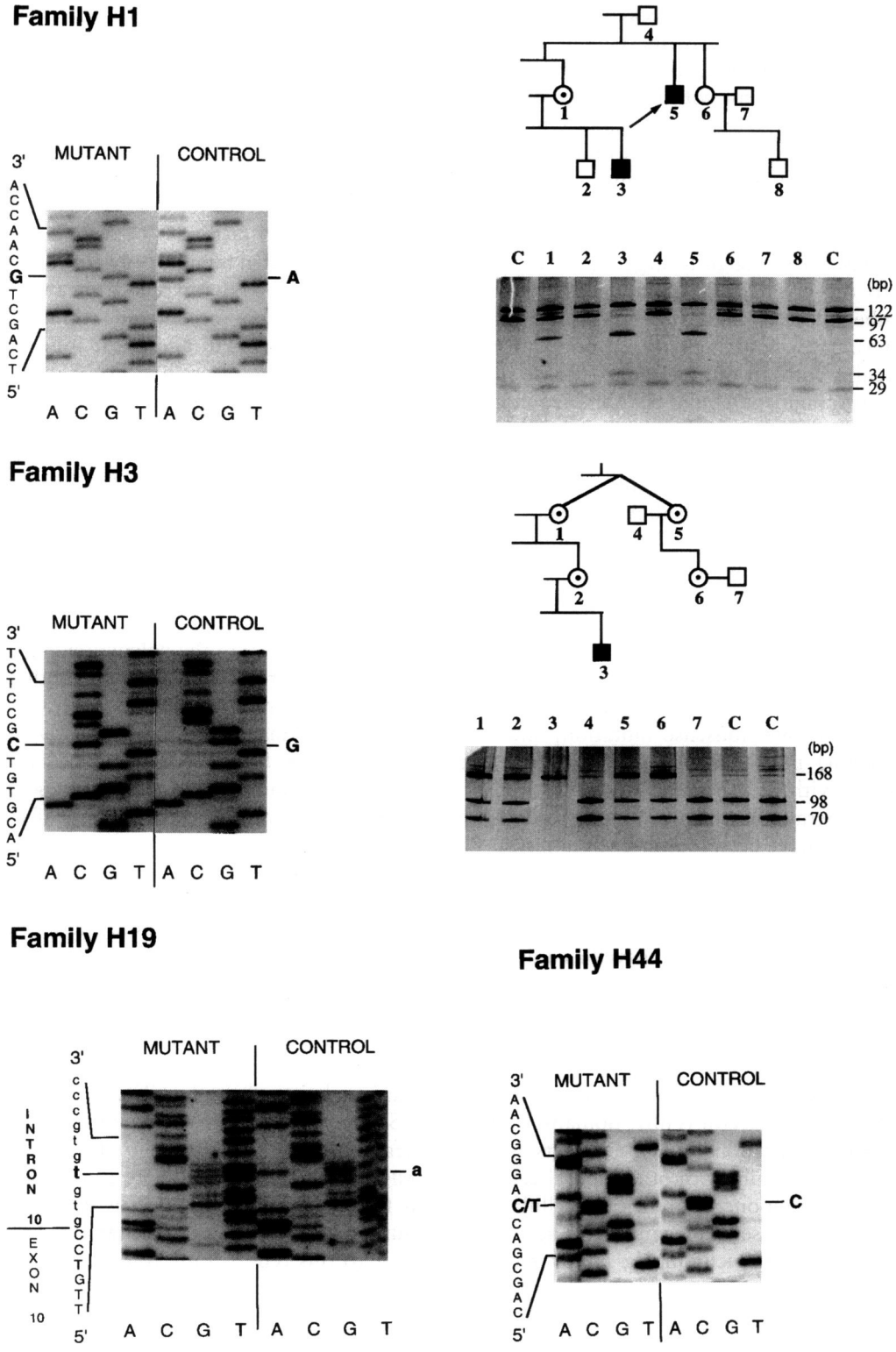
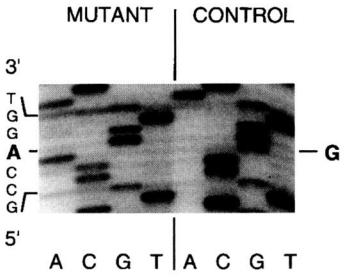
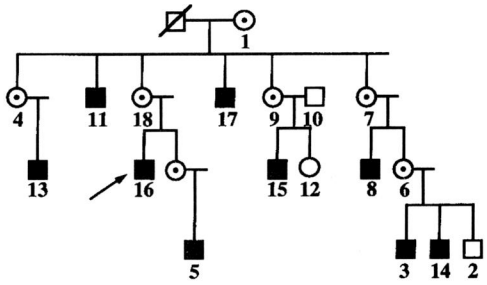
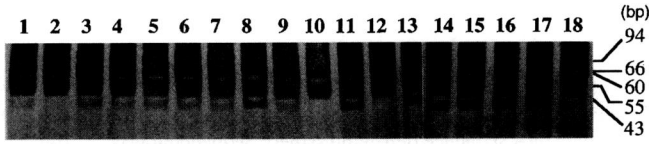


Figure 2 Mutation analysis following detection by SSCP/heteroduplex analysis. The mutation in family H1 was sequenced with primer G52, H3 with G1, H38 with G10, H39 with G35, H44 with G21, H19 with G14, and H29 with G44 (primers are described in Jouet et al. 1994). For families H38 and H39, segregation of the mutation in the families was carried out by SNuPE with the primers SX8 and SX21, respectively. For families H1, H3, and H29, restriction-enzyme digestion was employed. In family H1, creation of a site for

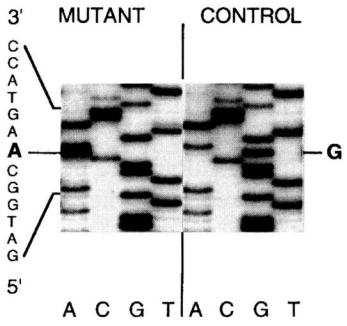
Family H29

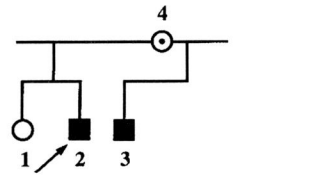


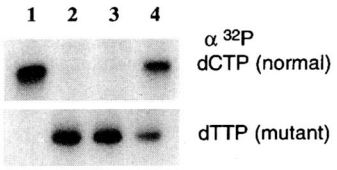




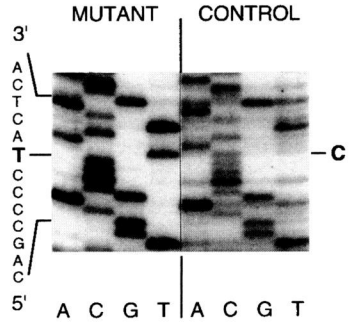
Family H38

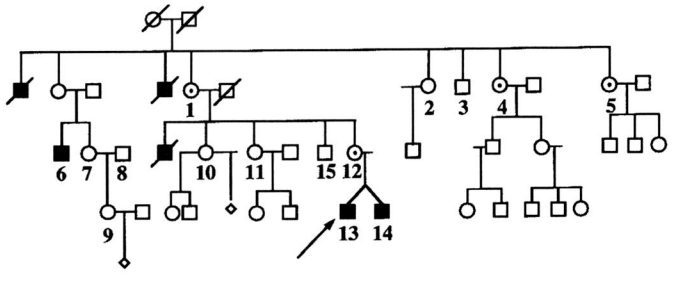


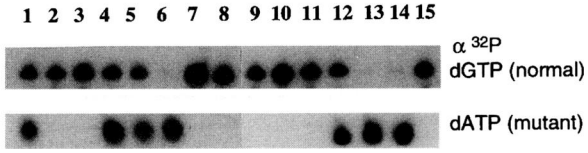




Family H39







*Bso*FI by the mutation results in cleavage of a 97-bp band into fragments of 63 and 34 bp. For family H3, cleavage of a 168-bp band into 98 and 70 bp by *Hae*III indicates the presence of the mutation; and, for family H29, creation of a mutant *Mva*I site results in cutting of a 60-bp fragment into 43- and 17-bp products. Only family members for whom DNA samples were available are shown. Mutation analysis was conducted by using DNA from the subject indicated by the arrow.

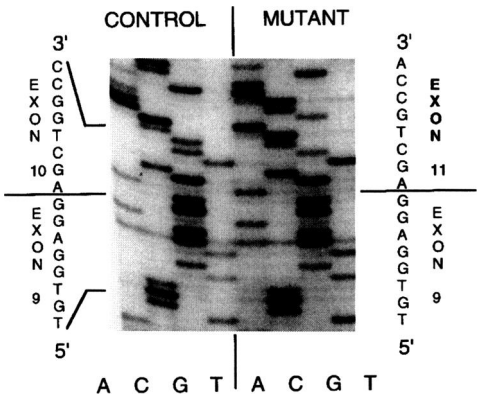


Figure 3 Sequence analysis of L1 cDNA from the proband of family H19, showing deletion of exon 10 (right-hand panel), compared with control cDNA sequence (left-hand panel). Sequencing was carried out by using primer G14 (Jouet et al. 1994) on a PCR fragment generated by using primers A93 and A11 (table 1).

Patients with missense mutations in the extracellular domains vary widely in phenotype, and the severity of their disorder is not correlated with mutation of particular domains. Conservation of amino acids affected by missense mutations, both with respect to L1-like molecules in other species and between related domains within L1, is presented in table 3 (Moos et al. 1988; Bieber et al. 1989; Burgoon et al. 1991; Hlavin and Lemmon 1991; Miura et al. 1991). Interestingly, four (H2, H7, H18, and H6) of five mutations capable of giving rise to congenital high-pressure hydrocephalus affect very conserved residues. Mutations affecting residues that are highly conserved between related domains (e.g., mutations C264Y, G121S, R184Q, and G452R) are likely to disrupt the structural integrity of individual domains. Those affecting less conserved residues (mutations D598N, H210Q, and E309K) are more likely to affect surface-recognition properties or quaternary structure. The latter class of mutations are particularly valuable in that they highlight functionally important residues that would not have been obvious through conservation comparisons.

The clinical variation within families demonstrates the complexity of the relationship between genotype and phenotype in this set of disorders. For example, in families such as H7 and H39, some affected males present with typical X-linked hydrocephalus whereas others present with MASA syndrome, although they are all affected by the same L1 mutation. Thus, epistatic interactions moderate expression of an L1 mutation. It is clear that the presentation of these disorders is so wide-ranging that assigning them to categories such as X-linked hydrocephalus or MASA syndrome is somewhat arbitrary. Thanks to the progress made in the fields of neurosurgery, obstetrics, and genetic counseling, neonates affected by an L1 mutation and expressing a severe X-linked hydrocephalus phenotype are now more likely to survive and are more likely to fit the description of a milder phenotype as they grow older (this is the case in family H38, where two affected boys with progressive

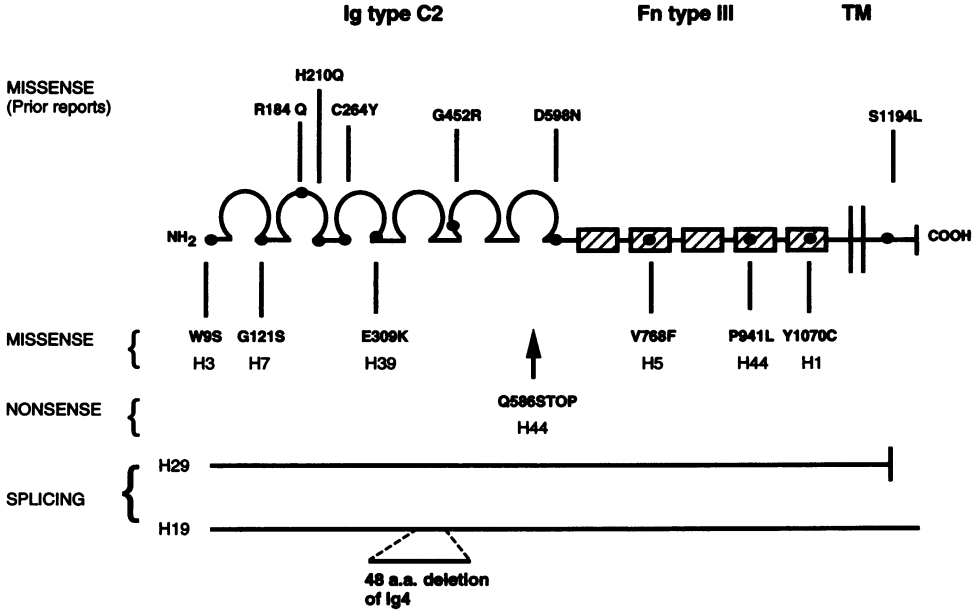


Figure 4 Schematic representation of the L1 protein. The bottom part of the diagram shows all nine mutations reported here. The upper part shows previously reported missense mutations (Jouet et al. 1993*b*, 1994; Fransen et al. 1994; Vits et al. 1994), for comparison. Missense (*black dots*), nonsense (*arrow*), and splicing (*black lines*) are indicated.

Table 3

Conservation of Residues Affected by Missense Mutations

Family	Mutation	Mouse	Rat	Chicken	Fly	Domain	Conserved Position in Related Domains[a]
H3	Trp9Ser	+	−	−	−	preIg	
H2	Cys264Tyr	+	+	+	+	Ig 3	6/6
H7	Gly121Ser	+	+	+	+	Ig 1	4/6
H18	Arg184Glu	+	+	+	+	Ig 2	4/6
H6	Gly452Arg	+	+	+	+	Ig 5	3/6
MASA5	Asp598Asn	+	+	+	+	Ig 6	0/6
H12	His210Glu	−	−	+	−	Ig 2	0/6
H38	Glu309Lys	+	+	+	−	Ig 3	0/6
H5	Val768Phe	+	+	+	−	Fn 2	2/5
H39	Pro941Leu	+	+	−	+	Fn 4	0/5
H1	Tyr1070Cys	+	+	−	+	Fn 5	?
MASA	Ser1194Leu	+	+	+	+	Cytoplasmic	

NOTE.—Amino acid residues affected by mutations are marked as identical (+) or not identical (−) in the same position in L1-like molecules in different species. Mouse (L1), rat (NILE), chicken (NgCAM), and *Drosophila* (neuroglian) comparisons are made. Mutations from present study (families H3, H7, H38, H5, H39, and H1) and from previous studies (families H2, H18, H6, MASA5, H12, and MASA) are summarized.

[a] Proportion of times this residue is conserved in other like domains of the human L1 protein.

hydrocephalus were diagnosed late, at ages 20 and 22 years, as suffering from MASA syndrome, their hydrocephalus having arrested without surgical intervention). In view of this, X-linked hydrocephalus and MASA syndrome can be considered as variable manifestations of the same disorder, which could be more appropriately named "L1 syndrome." Now that it is clear that L1 defects are responsible for this group of disorders, the crucial questions left to answer are precisely what role L1 plays in neural development and how its biological function is disrupted in disease states.

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