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L1-associated diseases: clinical geneticists divide, molecular geneticists unite

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The neuronal cell adhesion molecule L1 (L1CAM) is a transmembrane glycoprotein belonging to the immunoglobulin superfamily and is essential in the development of the nervous system. It is mainly expressed on neurons and Schwann cells, and plays a key role in axon outgrowth and pathfinding through interactions with various extracellular ligands and intracellular second messenger systems. Mutations in L1 are responsible for a wide spectrum of neurologic abnormalities and mental retardation. This spectrum includes X-linked hydrocephalus, MASA syndrome, X-linked complicated spastic paraplegia type 1 and X-linked agenesis of the corpus callosum. These four diseases were initially described as distinct clinical entities with an overlapping clinical spectrum, but can now be lumped into one syndrome caused by mutations in the L1 gene. The main clinical features of this spectrum are Corpus callosum hypoplasia, mental Retardation, Adducted thumbs, Spastic paraplegia and Hydrocephalus, which has led to the acronym CRASH syndrome.

INTRODUCTION

In 1949 Bickers and Adams described a British family with several male sibs that died at birth from congenital hydrocephalus (1). Post-mortem examination showed structural brain malformations with narrowing of the aqueduct of Sylvius. As the aqueduct stenosis was assumed to be the cause of hydrocephalus, the syndrome became referred to as Hydrocephalus due to Stenosis of the Acqueduct of Sylvius (HSAS).

The clinical and pathological features of this recessive X-linked condition have been extensively reviewed (2–5). The incidence is generally estimated to be around 1 in 30 000 male births (6). In general, patients with X-linked hydrocephalus show a broad spectrum of clinical and neurological abnormalities. The severity of these abnormalities is highly variable, sometimes even within the same family (2,7). The only obligate feature of HSAS is mental retardation with IQs usually between 20 and 50. Most patients also have spasticity of the lower limbs, which is most likely caused by hypoplasia of the corticospinal tract (5). Adducted thumbs (or clasped thumbs) are also found in many cases. Other manifestations of HSAS include a variety of structural brain malformations, among which agenesis or dysgenesis of the corpus callosum or the septum pellucidum.

MUTATIONS IN L1 ARE RESPONSIBLE FOR HSAS

In 1990 we found close linkage of HSAS to polymorphic markers located on the distal part of the long arm of the X-chromosome, in band Xq28 (8). Further mapping studies refined the disease locus to a region of 2 Mb, between DXS52 and F8C (9–11). At that time, few genes were known in the Xq28 region. The most interesting candidate gene was the gene encoding L1 (L1 Cell Adhesion Molecule, also referred to as L1CAM), since the L1 protein was known to be involved in the development of the brain (12). In 1992, aberrant splicing of the L1 mRNA was reported in a HSAS patient, suggesting that L1 is the HSAS gene (13). Definite proof for the involvement of the L1 gene in HSAS was obtained by the finding of two additional L1 mutations in HSAS families: a 1.3 kb duplication near the 3′ end of the L1 open reading frame (14) and a missense mutation disrupting a disulfide bridge (15).

L1-ASSOCIATED DISEASES

HSAS shows considerable clinical overlap with a number of X-linked conditions, including MASA syndrome (Mental Retardation, Aphasia, Shuffling gait, Adducted thumbs), complicated Spastic Paraplegia type 1 (SP-1), Agenesis of Corpus Callosum (ACC) and Mental Retardation with Clasped Thumbs (MR-CT) (16–19). Linkage analysis had assigned the disease loci for MASA syndrome (20), SP-1 (17) and MR-CT (19) to Xq28, the L1 region. Several of these phenotypes occur in a single family (21–23). These data led to the hypothesis that these conditions represent pleiotropic effects of mutations in a single gene (9), which was confirmed when L1 mutations were found in all these conditions (24–27). This proved that HSAS, MASA, SP-1, ACC and MR-CT are not separate conditions, but rather represent overlapping clinical spectra due to mutations in the L1 gene. Since a separation between the different L1-associated diseases no longer made sense, and most typical symptoms include Corpus callosum agenesis, Retardation, Adducted

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thumbs, Shuffling gait and Hydrocephalus, the acronym CRASH syndrome was proposed to refer to the clinical spectrum of diseases caused by an L1 mutation (28).

Additional conditions that might be caused by L1 mutations are MRX3 and PH. MRX3 is a form of aspecific mental retardation linked to Xq28 (29,30). So far, no evidence for L1 mutations has been reported. Periventricular Heterotopia (PH), which was recently mapped to Xq28 (31), is caused by a migration defect during cerebral cortical development, and affected patients suffer from several types of epilepsy. L1 was suggested as a candidate gene, but no evidence of L1 mutations has yet been found (C.A.Walsh, personal communication).

CRASH syndrome covers a very wide and variable clinical spectrum, that is limited to mental retardation alone in some patients. Hence, the possibility of an L1 mutation should be considered in mentally retarded males without additional symptoms. Therefore, mutations in L1 might be a more frequent cause of mental retardation than can be derived from the literature.

THE L1 CELL ADHESION MOLECULE: A NEURONAL CELL ADHESION MOLECULE INVOLVED IN NERVOUS SYSTEM DEVELOPMENT

Structural features

L1 is a transmembrane glycoprotein belonging to the immunoglobulin superfamily (IgSF). In human, the mature protein has 1256 amino acids with an extracellular part consisting of six Ig-like domains and five fibronectin type III-like domains, a single-pass transmembrane domain and a short cytoplasmic C-terminal tail (32,33). L1 homologues have been discovered in: rat (NILE), mouse (l1), *Drosophila* (Neuroglian), Manduca (Neuroglian), chicken (NgCAM), zebrafish (L1.1- and L1.2-CAM), *Fugu* and goldfish [reviewed in (34)]. The domain structure of L1 is characteristic for a subgroup of the IgSF which is now referred to as the L1 subfamily of adhesion molecules (35). It comprises a number of evolutionary related neural cell adhesion molecules, all implicated in the development of the nervous system, including L1 (and its homologues in other species), Bravo/NrCAM, neurofascin/ABGP and CHL1 (34,36). The Ig domains of L1 were originally described as belonging to the C2 set of Ig-like domains. However, comparison with other proteins revealed that the Ig domains of L1 make a close match to the sequence profile from a novel structural set of the IgSF, referred to as the I set (37,38).

In humans, the gene encoding L1 has 28 exons. An alternative splicing variant lacking exons 2 and 27 has been described (39,40). This isoform seems to be restricted to non-neural cells, although the functional significance remains to be elucidated (41). The alternatively spliced exon 27, located in the cytoplasmic domain, is conserved among many vertebrate members of the L1 family, which underlines its functional importance (34).

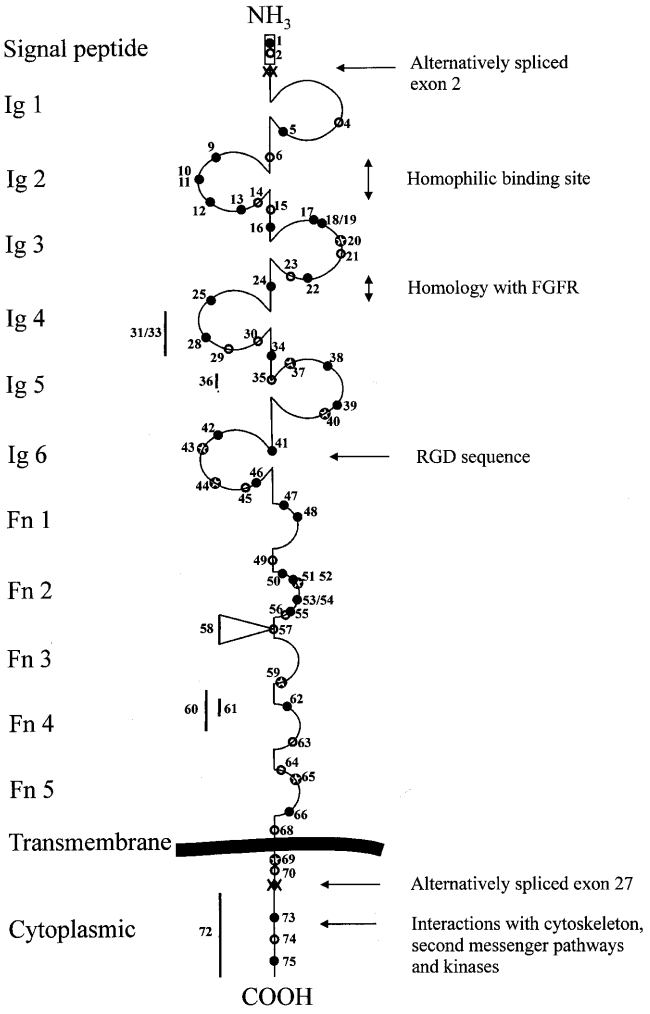

Figure 1. The L1 protein and mutations in CRASH syndrome. The signal peptide, the six Ig domains, five fibronectin domains, the transmembrane and the cytoplasmic domain are indicated on the left. The functional sites and alternatively spliced exons are denoted on the right. Missense point mutations are denoted by a filled dot (•). Deletions which do not alter the reading frame are represented by a line parallel with the molecule. Mutations causing a frameshift are schematically represented by an open dot (○). In-frame nonsense mutations are denoted by an asterisk (✪) The intron mutations which are very likely to cause alternative splicing, but that are not yet fully characterized on the mRNA level, are not shown. The numbers of the mutations are the same as in Table 1.

L1 functions

Cell adhesion molecules play critical roles in mediating the interaction between a cell and its environment [reviewed in (42)]. L1 expression was originally thought to be limited to the nervous system, particularly on outgrowing axons and growth cones of postmitotic nerve cells in the central (CNS) and peripheral (PNS) nervous system, and on Schwann cells in the PNS. During nervous system development, L1 plays a role in adhesion between neurons and between neurons and Schwann cells (12,43,44), in myelination (45), in axon outgrowth and pathfinding (46,47), axon fasciculation (48,49), growth cone morphology (50,51) and neuronal migration (52,53). In addition, L1 is involved in regeneration of damaged nerve tissue (54), and has been implicated in long-term memory formation (55) and the establishment of long-term potentiation in the hippocampus (56).

Recently, non-neural L1 expression of an alternatively spliced L1 lacking exons 2 and 27 has been described in the male urogenital tract (57), in the intestinal crypt cells (58) and in cells of hematopoetic origin (59). The function of L1 in these tissues, however, is unclear.

Table 1. Overview of L1 mutations in CRASH syndrome[a]

Number	Exon/intron	Domain	cDNA change	Type of mutation	Protein change	Reference mutation	Reference patient
1	E 1	s.p.	G26→C	missense	W9S	89	11
2	E 1	s.p.	52 ins C	insertion 1 bp	FS 18	90	
3	I 1	s.p.	76+1 g→t	splice site	?	91	
4	I 4	Ig 1	400+5 g→a	splice site	FS 108	92	7
5	E 4	Ig 1	G361→A	missense	G121S	89	
6	E 5	Ig 2	404 CC→A	complex	FS 135	Antwerp, unpublished	
7	I 5	Ig 2	523+26 del 31	splice site	?	Antwerp, unpublished	
8	I 5	Ig 2	524–2 a→c	splice site	?	Antwerp, unpublished	
9	E 6	Ig 2	T536→G	missense	I179S	93	22
10	E 6	Ig 2	C550→T	missense	R184W	94	
11	E 6	Ig 2	G551→A	missense	R184Q	25	95
12	E 6	Ig 2	A581→G	missense	Y194C	90	
13	E 6	Ig 2	C630→G	missense	H210Q	24,25	23
14	E 6	Ig 2	644 del G	deletion 1 bp	FS 215	94	3
15	E 7	Ig 3	712 ins 4	insertion 4 bp	FS 239	94	
16	E 7	Ig 3	C719→T	missense	P240L	90	
17	E 7	Ig 3	G791→A	missense	C264Y	15	11
18	E 7	Ig 3	G803→A	missense	G268D	Antwerp, unpublished	
19	I 7	Ig 3	807–6g→a	splice site	FS 268	96	
20	E 8	Ig 3	G828→A	nonsense	W276X	90	
21	E 8	Ig 3	841 del 5	deletion 5 bp	FS 281	97	
22	E 8	Ig 3	G925→A	missense	E309K	89	
	E 8	Ig 3	G925→A	missense	E309K	Antwerp, unpublished	19
23	E 8	Ig 3	955 del G	deletion 1 bp	FS 319	90	
24	E 9	Ig 3	C998→G	missense	P333R	Sue Forrest, pers.comm.	
25	E 9	Ig 4	G1108→A	missense	G370R	93	98
26	I 9	Ig 4	1124–1 g→a	splice site	?	Antwerp, unpublished	
27	I 9	Ig 4	1124–1 g→c	splice site	?	Antwerp, unpublished	
28	E 10	Ig 4	T1172→C	missense	L391P	Antwerp, unpublished	
29	E 10	Ig 4	1198 del A	deletion 1 bp	FS 400	94	
30	E 10	Ig 4	1248 del T	deletion 1 bp	FS 416	94	99
31	I 10	Ig 4	?	splice site	ΔE10	100	
32	I 10	Ig 4	1267+1 g→a	splice site	?	94	
	I 10	Ig 4	1267+1 g→a	splice site	?	Antwerp, unpublished	
33	I 10	Ig 4	1267+4 a→t	splice site	ΔE10	89	
34	E 11	Ig 5	C1277→A	missense	A426D	Antwerp, unpublished	
35	E 11	Ig 5	1296 del 4	deletion 4 bp	FS 433	Antwerp, unpublished	
36	E 11	Ig 5	1316 del 15	in-frame deletion	439 del 5 AA	96	
37	E 11	Ig 5	C1318→T	nonsense	Q440X	88	
38	E 11	Ig 5	G1354→A	missense	G452R	25	
39	E 12	Ig 5	T1445→C	missense	L482P	88	
40	E 12	Ig 5	C1453→T	nonsense	R485X	25	
41	E 13	Ig 6	1576 del 3	in-frame deletion	ΔS526	88	
42	E 13	Ig 6	T1624→C	missense	S542P	88	
43	E 13	Ig 6	C1672→T	nonsense	R558X	96	
44	E 14	Ig 6	C1756→T	nonsense	Q586X	89	
45	E 14	Ig 6	1780 del A	deletion 1 bp	FS 594	94	
46	E 14	Ig 6	G1792→A	missense	D598N	24	20
47	E 15	Fn 1	G1895→C	missense	R632P	27	18
48	E 16	Fn 1	A1963→G	missense	K655E	101	

Table 1. *continued*

Number	Exon/intron	Domain	cDNA change	Type of mutation	Protein change	Reference mutation	Reference patient
49	E 17	Fn 2	2153 del C	deletion 1 bp	FS718	Antwerp, unpublished	
50	E 18	Fn 2	T2222→C	missense	M741T	88	
51	E 18	Fn 2	G2254→A	missense	V752M	88	
52	E 18	Fn 2	G2262→A	nonsense	W754X	96	
53	E 18	Fn 2	G2302→A	missense	V768I	88	
54	E 18	Fn 2	G2302→T	missense	V768F	89	
55	E 18	Fn 2	A2351→G	missense	Y784C	96	
56	E 18	Fn 2	A2374→GG	complex	FS 791	96	
57	E 18	Fn 2	2430 del CT	deletion 2 bp	FS 810	Antwerp, unpublished	
58	I 18	Fn 2	2432–19 a→c	splice site	811 ins 23 AA[b]	13	11
					ΔE 19[b]		
59	E 20	Fn 3	C2701→T	nonsense	R901X	96	
60	E 21	Fn 4	?	splice site	ΔE 21	100	
61	E 21	Fn 4	2805 del 39	in-frame deletion	936 del 13 AA	96	
62	E 21	Fn 4	C2822→T	missense	P941L	89	
63	E 22	Fn 4	2885 del G	deletion 1 bp	FS 962	25	
	E 22	Fn 4	2885 del G	deletion 1 bp	FS 962	Antwerp, unpublished	
64	E 23	Fn 5	3088 del A	deletion 1 bp	FS 1030	85	87
65	E 23	Fn 5	C3124→T	nonsense	Q1042X	88	
66	E 24	Fn 5	A3209→G	missense	Y1070C	89	11
67	I 24	?	3322+2 t→c	splice site	?	96	
68	E 25	Fn 5	3323 del G	deletion 1 bp	FS 1108	Antwerp, unpublished	
69	E 25	Cytopl.	3453 del 4	nonsense	Y1151X	Antwerp, unpublished	
	E 25	Cytopl.	3453 del 4	nonsense	Y1151X	Antwerp, unpublished	
70	E 26	Cytopl.	3489 del TG	deletion 2 bp	FS 1164	25	17
71	I 26	Cytopl.	3531–12 g→a	splice site	?	89	102
72	I 27→28	Cytopl.	3543 del 2 kb	large deletion	del 1181→end	24	103
73	E 28	Cytopl.	C3581→T	missense	S1194L	26	21
74	E 28	Cytopl.	3543 dpl 125	large duplication	FS 1223	14	2
75	E 28	Cytopl.	T3685→C	missense	Y1229H	Antwerp, unpublished	

[a]An updated table of L1 mutations is available electronically at the L1CAM mutation homepage (http://hgins.uia.ac.be/dnalab/l1/)
[b]mRNA studies indicated the presence of two aberrantly spliced mRNA species, besides the normally spliced mRNA.
s.p., signal peptide; FS, Frameshift; bp, basepair; Cytopl., Cytoplasmic domain.

Binding partners of L1

As most IgSF members, L1 protein has homophilic interactions with an L1 protein on the membrane of an adjacent cell (60). The homophilic binding site has been mapped to the second Ig domain (Fig. 1) (61). Heterophilic interactions have been described with cell adhesion molecules belonging to the IgSF, including axonin-1/TAG-1 (49,62), F3/F11 (63) and DM-GRASP (64). Furthermore, L1 interacts with the neuronal chondroitin sulfate proteoglycans neurocan and phosphacan (65,66). Apart from interactions with other cells (*trans*-interactions), *cis*-interactions have been reported with NCAM (67) and the heat-stable antigen nectadrin (HSA, murine CD24) (68,69). Recently, evidence has emerged that L1 is involved in integrin-mediated cell–cell and cell–matrix interactions, both in neuronal cells, tumor cells and cells of haematopoetic lineage [reviewed in (70)]. Human $\alpha_v\beta_3$ integrin (vitronectin receptor) (71,72) and murine fibronectin receptor $\alpha_5\beta_1$ (VLA-5) (73) bind to L1 via the RGD sequence in the sixth Ig domain of L1, and may induce haptotactic cell migration and activation of immune responses.

Signal transduction

Several signal transducing pathways are involved in passing extracellular interactions of L1 to the intracellular machinery of the cell. Antibody-induced stimulation of L1 leads to changes in intracellular pH, Ca^{2+} and inositol phosphates, depending on the cell type (74,75). Doherty and asssociates postulated that L1 signal transduction occurs via *cis*-activation of the Fibroblast Growth Factor Receptor (FGFR) through an amino acid motif shared between FGFR and L1 (76–78). Maness and co-workers obtained evidence that the non-receptor tyrosine kinase $pp60^{c\text{-}src}$ is part of the L1 signaling cascade, as L1-mediated neurite outgrowth is diminished in src-minus neurons (79). A number of kinase activities, co-precipitating with L1 immunoprecipitates, phosphorylate the cytoplasmic domain at distinct Ser residues.

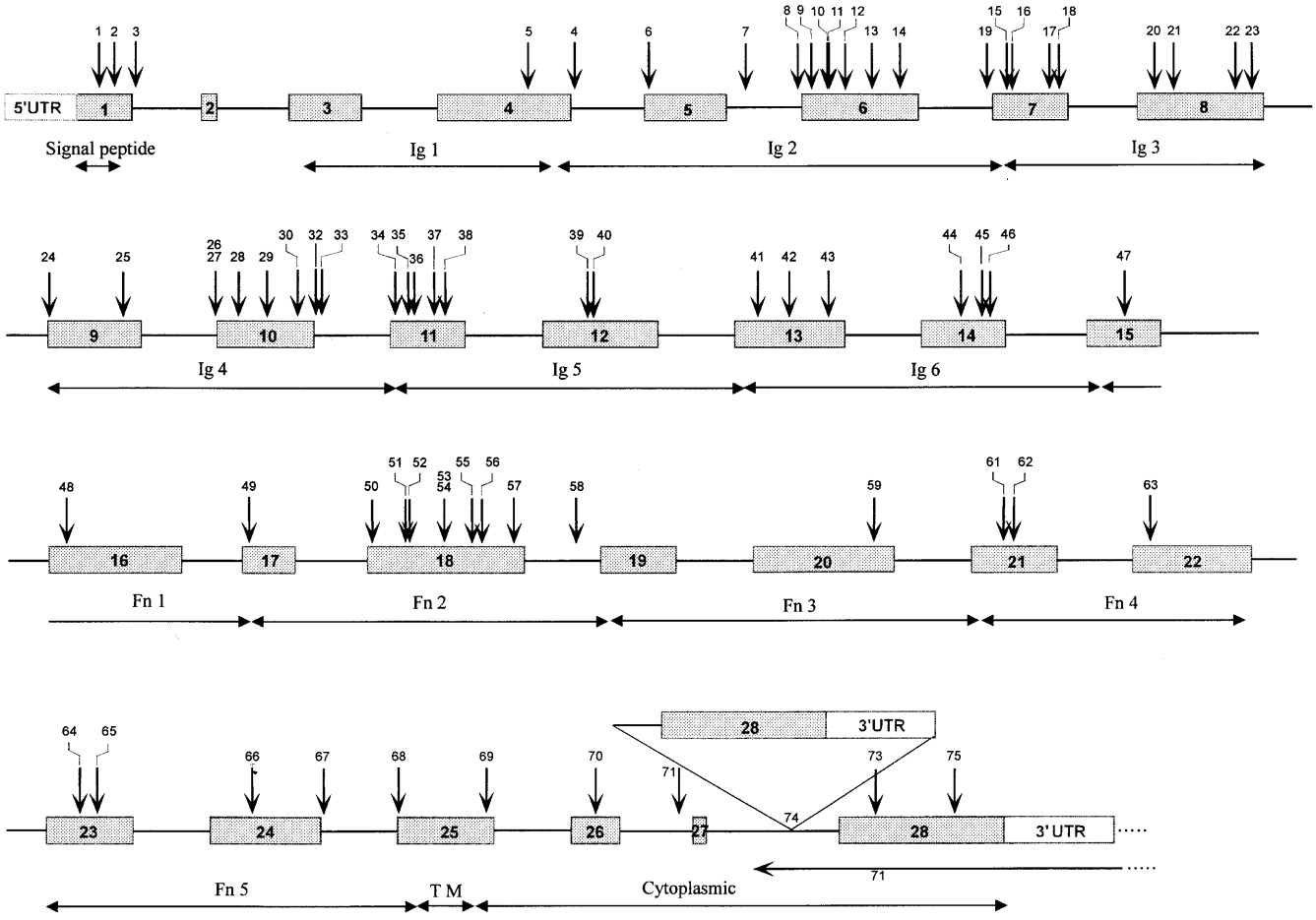

Figure 2. Position of the various mutations dispersed over the L1 gene. Exons are drawn as grey boxes, introns as lines. The exons but not the introns are drawn to scale. The position of the different domains with regard to the genomic structure is outlined. The mutations, numbered according to Table 1, are indicated by vertical arrows.

Phosphorylation may be a way for modulation of L1 signal transducing activity (49,74,80,81). The L1 cytoplasmic domain is anchored to the actin cytoskeleton via an interaction with ankyrin, a shared feature between many neuronal cell adhesion molecules (69,82,83).

L1 MUTATIONS: AN UPDATE

Up to now, 75 different L1 mutations have been described in 79 different CRASH families. These mutations are summarized in Table 1, and depicted in Figures 1 and 2. Most L1 mutations are private mutations that occur in only one family. Frequently occurring L1 mutations have not been found, and only four mutations were reported in two independent families: (mutations 22, 32, 63 and 69). The mutations are dispersed throughout the entire L1 gene and have been found in each domain. No indication for mutation hot spots has been found, although the regions around exons 6, 10, 11 and 18 are relatively rich in mutations. The mutational spectrum includes two gross rearrangements, 14 splice site mutations (including one branch point signal mutation), 30 missense mutations, nine nonsense mutations, three in-frame deletions and 17 mutations leading to a frameshift (13 small deletions, two small insertions and two complex mutations). Only two of the mutations (mutations 72 and 74) are detectable by Southern blot analysis. Mutation screening of the L1 gene has been greatly facilitated by the availability of the complete genomic sequence of L1 (EMBL accession no. Z29373) (84).

As the list of L1 mutations is still growing, we constructed the L1 mutation Web Page (http://hgins.uia.ac.be/dnalab/l1/), a WWW page with information about L1, including a continuously updated overview of all L1 mutations (85).

Two cases of somatic and germline mosaicism have been reported [mutations 47 (27) and 64 (86)]. The occurrence of germline mosaicism is a potential caveat in the genetic counseling of families with CRASH syndrome.

GENETIC HETEROGENEITY

Up to now, no conclusive evidence for genetic heterogeneity of X-linked hydrocephalus (HSAS) has been found although many families have been described. Nevertheless, two families with HSAS were reported in which the L1 gene was excluded as the disease gene by linkage analysis. In the first family linkage to the L1 region in Xq28 was initially excluded (87), but afterwards an L1 mutation was identified in this family (85). Screening of the

entire family for the presence of the mutation revealed that the mutation had occurred *de novo* in a female, who was a somatic and germline mosaic for the mutation. This mosaicism had led to a discordant segregation of the Xq28 markers and the disease. Also in a small German HSAS family (Family 12) comprising an affected male and his affected nephew, linkage to Xq28 has been excluded by us and others (9,88), based upon the observation that one apparently normal brother had inherited the same Xq28 haplotype as the two patients. Recently, an L1 amino acid substitution (V768I) was identified in both the two patients and the apparently normal brother (88). The authors concluded that the V768I substitution is a rare non-pathogenic polymorphism, and not the disease-causing mutation, and that HSAS is caused by a gene other than L1 in this family. In our opinion, however, it cannot be excluded that the V768I substitution in L1 is the disease-causing mutation in this family for two reasons. First, non-pathogenic amino acid substitutions have never been found in L1. Second, mildly affected CRASH patients have been reported previously (3,23) and the apparently healthy sib might be mildly affected, as he refused any clinical and IQ testing. In conclusion, convincing evidence for genetic heterogeneity of X-linked hydrocephalus has yet to be presented.

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