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Short communication

Exon 2 of the gene for neural cell adhesion molecule L1 is alternatively spliced in B cells

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Abstract

L1CAM is a neural cell adhesion molecule expressed mainly on neurones' cell surface and plays an important role in the developing fetal brain. Recently, we have shown that mutations in the gene encoding L1CAM are responsible for three related neurological disorders including the most common form of inherited hydrocephalus. During our genetic analysis, we have discovered that L1CAM is also expressed on the surface of B cells but that the messenger RNA in this tissue is different to that in brain through alternative splicing of the L1 gene. This indicates that this region of the L1 molecule has a distinct role in brain cells compared to B lymphocytes and confirms its importance in brain development.

Keywords: L1CAM; Hydrocephalus; Splicing; Alternative mRNA; Exon 2

The human cell adhesion molecule L1 belongs to the large immunoglobulin superfamily of adhesion molecules. It is involved in neuronal cell adhesion, migration and neurite extension and thus plays an important role in the developing nervous system [1,10].

Recently, we and others have shown that mutations in the L1 gene are responsible for X-linked hydrocephalus, MASA syndrome and spastic paraplegia type I [3–5,9,11,12]. The main characteristics of all three disorders are: mental retardation, brain abnormalities, dysphasia, visual defects and spastic paraparesis.

During our analysis of the L1 gene, Epstein Barr virus (EBV)-transformed B cells were used as a source of L1 mRNA. Polyadenylated RNA was reverse transcribed and sections of L1 cDNA were amplified by performing nested PCRs with specific L1 primers.

Sequence analysis of the 16 fragments representing the 3.7 kb cDNA revealed that a 15 base pair region close to the 5′ end was missing compared with the cDNA sequence in human brain [2]. Comparison with the genomic structure of the gene reveals that this 15 base pair region corresponds to exon 2: a mini exon coding for 5 amino acids YEGHH (Fig. 1A, upper panel, A.R. unpublished data). To show that this alternative transcript, missing exon 2, is not an artefact of B cell immortalisation, untransformed lymphocytes were also used as a source of mRNA to produce L1 cDNA. The same alternative message was found (see Fig. 1B, left panel).

Mouse L1 brain cDNA has been sequenced and elucidation of some of its genomic structure reveals that the exonic arrangement is conserved in mouse and human [6,7]. To examine alternative splicing in mouse lymphoid cells, L1-specific primers were designed and mRNA isolated from mouse spleen, rich in B cells, was reverse transcribed and used as a template for nested PCR amplification of the region surrounding exon 2. Sequence analysis showed that exon 2 is also spliced out in the mouse spleen L1 mRNA (Fig. 1B, right panel).

In both human and mouse this exon splicing event would substitute the first amino acid encoded by exon 3, a valine, by a leucine, as well as causing the absence of the 5 amino acids encoded by exon 2.

These experiments show that the L1 gene has an alternative mRNA in B cells where exon 2 is skipped and that this is the message that prevails in circulating blood cells. This novel alternative splicing event is only the second reported to occur in the L1 gene. Exon 27,

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a 12 base pair exon located in the 3′ region of the gene, encoding four amino acids (RSLE), is spliced out in L1 mRNA in the peripheral nervous system [8]. During the course of our experiments, we found that exon 27 is also spliced out in human B cells. The small number of identified isoforms for L1 produced by alternative splicing contrasts with NCAM, a related cell adhesion molecule, which has over 20 differentially processed transcripts [13].

It is interesting to note that the sequence at the 5′ end of exon 3 is 73% identical to the sequence of exon 2. This points to the fact that exon 2 may have evolved from exon 3 through a duplication event.

L1 is a cell surface glycoprotein expressed primarily on neuronal cells. Proceeding from the amino terminus it consists of 6 immunoglobulin type C2 (IgC2) domains, 5 fibronectin type 3 domains, a short transmembrane section and a cytoplasmic tail. The region encoded by exon 2 precedes the region containing the immunoglobulin domains in the L1 protein. The role of this region in cell adhesion and general L1-mediated cellular interactions is not known, although, our results suggest that this region may have distinct roles in B cells compared to neural cells.

Recently, we have found one novel mutation affecting this region of the L1 molecule in a patient with

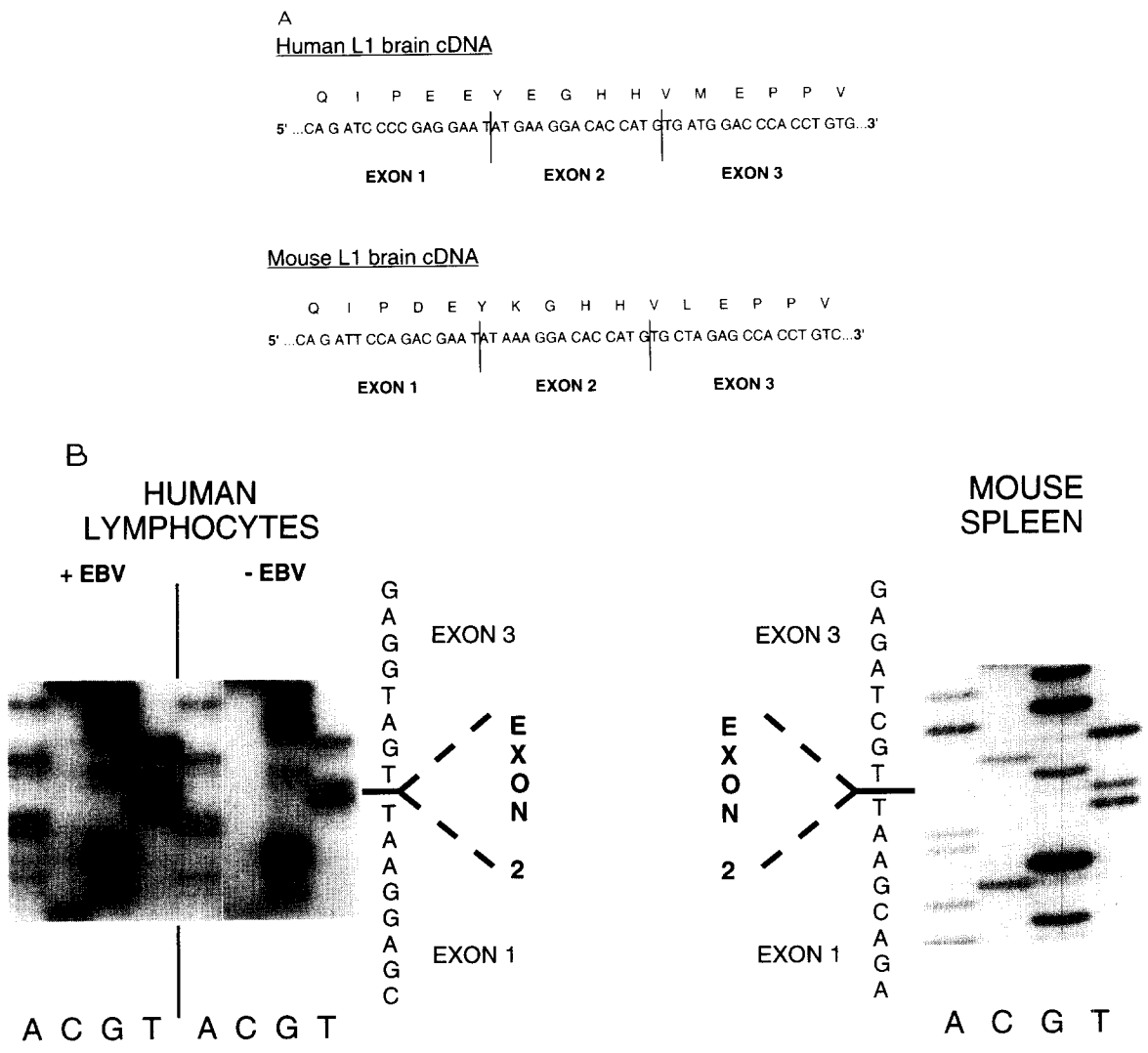

Fig. 1. A: human and mouse L1 cDNA sequences after Hlavin and Lemmon [2] and Moos et al. [7] showing exon 2 sequences. B: sequences surrounding exon 2 of L1 cDNA in human B cells and mouse spleen. Left panel: primers used for the first PCR on human lymphocytes cDNA were H1 (5′ ATGGTCGTGGCGCTGCGGTACG 3′) and H2 (5′ TGCTGTGCTTCCTCTGACTG 3′) annealing at 55° C. An aliquot of the first reaction was taken and added to a final dilution of 1:125 in the nested PCR using primers H1 and H3 (5′ CAAAGCAGCGGTAGATGCCCTG 3′) annnealing at 58° C. Sequencing was performed using primer H1 and the f-mole© kit from Promega. Right panel: similar reactions were carried out on mouse spleen cDNA using primers M1 (5′ ATGGTCGTGATGCTGCGGTACGTG 3′) and M2 (5′ GAAGGAGCCA-GAATAGGGTGCCTC 3′) annealing at 67° C and M3 (5′ GTACGTGTGGCCTCTCCTCC 3′) and M4 (5′ TGCCTCATGCACCACTACACCC 3′) annealing at 62° C for the nested reaction. Sequencing was performed by the same method using primer M3.

X-linked hydrocephalus [3]. This mutation is a transversion at the first and most conserved base of intron 1 donor splice site and would be expected to result in the skipping of exon 2 since the 5′ splice site would no longer be recognised. This L1 gene defect causes sufficient disruption of the L1 molecule to give rise to disease state and serves to confirm its importance in brain development.

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References

[1] Grumet, M., Cell adhesion molecules and their subgroups in the nervous system, *Current Op. Neurobiol.*, 1 (1991) 370–376.
[2] Hlavin, M.L. and Lemmon, V., Molecular structure and functional testing of human L1CAM, *Genomics*, 11 (1991) 416–423.
[3] Jouet, M. and Kenwrick, S., Gene analysis of L1 neural cell adhesion molecule in prenatal diagnosis of hydrocephalus, *Lancet*, in press.
[4] Jouet, M., Rosenthal, A., Armstrong, G., MacFarlane, J., Stevenson, R. and Paterson, J., X-linked spastic paraplegia (SPG1), MASA syndrome and X-linked hydrocephalus result from mutations in the L1 gene, *Nature Genet.*, 7 (1994) 402–407.
[5] Jouet, M., Rosenthal, A., MacFarlane, J., Donnai, D. and Kenwrick, S., A missense mutation confirms the L1 defect in X-linked hydrocephalus (HSAS), *Nature Genet.*, 4 (1993) 331.
[6] Kohl, A., Giese, K.P., Mohajeri, M.H., Montag, D., Moos, M. and Schachner, M., Analysis of promoter activity and 5′ genomic structure of the neural cell adhesion molecule L1, *J. Neurosci. Res.*, 32 (1992) 167–177.
[7] Moos, M., Tacke, R., Scherer, H., Teplow, D., Früh, K. and Schachner, M., Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin, *Nature*, 334 (1988) 701–703.
[8] Reid, R.A. and Hemperley, J.J., Variants of human L1 cell adhesion molecule arise through alternate splicing of RNA, *J. Mol. Neurosci.*, 3 (1992) 127–135.
[9] Rosenthal, A., Jouet, M. and Kenwrick, S., Aberrant splicing of L1CAM mRNA associated with X-linked hydrocephalus, *Nature Genet.*, 2 (1992) 107–112.
[10] Sonderegger, P. and Rathjen, F.G., Regulation of axonal growth in the vertebrate nervous system by interactions between glycoproteins belonging to two subgroups of the immunoglobulin superfamily, *J. Cell Biol.*, 119 (1992) 1387–1394.
[11] Van Camp, G., Vits, L., Coucke, P., Lyonnet, S., Schrander-Stumpel, C. and Darby, J., A duplication in the L1CAM gene associated with X-linked hydrocephalus, *Nature Genet.*, 4 (1993) 421–425.
[12] Vits, L., van Camp, G., Coucke, P., Fransen, E., De Boulle, K.D. and Reyniers, E., MASA syndrome is due to mutations in the neural cell adhesion gene *L1CAM*, *Nature Genet.*, 7 (1994) 408–413.
[13] Walsh, F.S. and Doherty, P., Structure and function of the gene for neural cell adhesion molecule, *Seminars Neurosci.*, 3 (1991) 271–284.