Mutations in L1-CAM in two families with X linked complicated spastic paraplegia, MASA syndrome, and HSAS

Juan Carlos Ruiz, Harry Cuppens, Eric Legius, Jean-Pierre Fryns, Thomas Glover, Peter Marynen, Jean-Jacques Cassiman

Abstract
The suggestion that the three X linked syndromes X linked spastic paraplegia (MIM 312900), MASA syndrome (MIM 303550), and X linked hydrocephalus owing to stenosis of the aqueduct of Sylvius (MIM 307000) are variable clinical manifestations of mutations at the same locus at Xq28 was confirmed by the finding of mutations in the L1-CAM gene in the three syndromes. Recently, two families in which different subjects showed a clearly different phenotype within the same family of the three X linked syndromes were described. A reverse transcription PCR assay was developed for the analysis of the L1-CAM cDNA in two of the members of these families. RNA isolated from EBV transformed cell lines and a colon carcinoma derived cell line was used as a starting material. The L1-CAM cDNA of two male patients from each family was sequenced. We report two new mutations in the L1-CAM gene in these two families showing that the three different phenotypes observed in different generations within the same family are variable phenotypic expressions of the same mutation.

Materials and methods
Blood samples for DNA extraction were obtained from affected and normal subjects from two previously reported families.8 Family 1 (fig 1) was reported by Fryns et al8 as a three generation family which suggested that X linked spastic paraplegia, MASA syndrome, and X linked hydrocephalus owing to congenital stenosis of the aqueduct of Sylvius are variable clinical manifestations of mutations at the same locus at Xq28. The second family (fig 2) was reported by Kaeppernick et al.9 In this family similar variable manifestations of clinical symptoms were observed, varying from hydrocephalus to MASA syndrome in affected members, while two obligate carrier females had mild mental retardation and adducted thumbs. In addition, EBV transfected cell lines were available from at least two affected people in each family. These cell lines were used for RNA extraction using the RNeasy Kit (TEL-TEST Inc, Texas, USA) following the recommendations of the manufacturer. A human colon carcinoma cell line SW480 (ATCC CCL 228) was used as a control since the expression of L1-CAM in this cell line has been documented.10 RNA (2 µg) was reverse transcribed with M-MLV reverse transcriptase (BRL) according to the recommendations of the manufacturer. The cDNA was then amplified in four overlapping fragments by a hemi-
nested PCR. Primers used for amplification were elaborated using the published L1-CAM cDNA sequence\textsuperscript{11} and are shown in table 1A. The amplification was performed in 100 μl solution containing 16.6 mmol/l (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 67 mmol/l Tris-HCl pH 8.8, 10 mmol/l β-mercaptoethanol, 6-7 μmol/l EDTA, 0.17 μg BSA/10μl, 1 mmol/l MgCl\textsubscript{2}, 0.2 mmol/dNTP, 10% DMSO, 2-5 U Taq DNA polymerase, and 20 μl of cDNA reaction. For fragments A and B, 30 cycles of amplification were performed with one minute denaturation at 95°C, one minute annealing at 55°C, and an extension step at 72°C for three minutes. For fragments C and D, 40 cycles were performed with an annealing temperature of 60°C. All the amplifications included an initial denaturing step of five minutes at 95°C and a final extension time of 10 minutes at 72°C. All PCR products were sequenced using a solid phase approach (Dynal\textsuperscript{6}, Norway and Pharmacia, Auto Read\textsuperscript{TM} Sequencing Kit, Sweden) with an internal FITC labelled oligonucleotide primer. When ambiguities were present in the sequence, the complementary strand was also sequenced.

All DNA sequences were numbered according to the published sequence (GEN-BANK: M77640); the amino acid numbering starts at the first methionine.

To identify the mutation in other subjects a dot blot assay was performed using an end labelled oligonucleotide containing the mutated sequence or the wild type sequence as a probe (table 1B). PCR products containing exons 4, 5, and 6 for mutation 11798, and PCR products containing exons 8, 9, and 10 for mutation G370R (table 2) were used in the dot blot procedure. After hybridisation with the labelled probe, a stringent wash was performed in 2 × SSC/0-5% SDS at 55°C for 10 minutes.

**Results**

In all the subjects analysed and in the control cell line several differences from the published sequence of the L1-CAM gene were detected.\textsuperscript{12} A 15 bp deletion was found at coding position 97 of the cDNA compared to the published sequence and a 12 bp deletion at bp 3551. The latter has already been described as an alternative splicing form of L1-CAM.\textsuperscript{12} The deletion at bp 97 might therefore also be the result of an alternative splicing event in white blood cells. At nucleotide position 875 a T to
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Table 2  Primers used to amplify genomic DNA

<table>
<thead>
<tr>
<th>Family 1 (exons 4, 5, and 6)</th>
<th>Primer sequence</th>
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<tbody>
<tr>
<td>I179S</td>
<td>5'-GTCCACTTCAAACCCAAG-3'</td>
</tr>
<tr>
<td>PG1.5</td>
<td>5'-GCCGTCGACAGATGTCT-3'</td>
</tr>
<tr>
<td>PG1.3</td>
<td>5'-GCCGTCGACAGATGTCT-3'</td>
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</tbody>
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<table>
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<tr>
<th>Family 2 (exons 8, 9, and 10)</th>
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</thead>
<tbody>
<tr>
<td>G370R</td>
</tr>
<tr>
<td>PG2.5</td>
</tr>
<tr>
<td>PG2.3</td>
</tr>
</tbody>
</table>

Figure 3  Sequence of part of exon 6 of L1-CAM gene showing the T to G mutation at bp position 556 (I179S) (indicated by an arrow) in the family reported by Prym et al. (A) Control sequence SW480, (B) mutated sequence.

Figure 4  Sequence of part of exon 9 of L1-CAM gene showing the G to A mutation at bp position 1128 (G370R) (indicated by an arrow in the family reported by Kasperek et al.) (A) Control sequence SW480, (B) mutated sequence.

C transition was observed, which at the protein level would be a silent mutation, and an insertion of a cytosine at nucleotide position 3806 within the 3' untranslated region of the L1-CAM transcript was detected. Dot blot analyses of genomic DNA of the other members of the family showed that the mutation segregated in all female carriers (II-3, III-1, III-9, IV-5, IV-6, and IV-8) as determined previously by linkage analysis, and all affected males (II-5, spastic gait and borderline intelligence; III-3, spastic paresis, mentally retarded, and adducted thumbs; III-8 spastic paresis without involvement of the thumbs).

A different point mutation was found in the two patients of family 2 (fig 2), a G to A transversion at nucleotide position 1128 of the L1-CAM gene sequence, resulting in the change of glycine into arginine at position 370 of the amino acid sequence (G370R) (fig 4).

In family 2 the mutation G370R was detected by dot blot analysis of genomic DNA in the two partially expressing females (samples 6 and 30), in all obligate female carriers (samples 10, 13, 20, 26, and 51), in the females predicted to be carriers by linkage analysis (samples 45, 53, 54, and 62), and all the affected males (samples 16, 17, 23, 24, 31, 33, 40, 41, 60, 79, and 80). No G370R mutation was found in the normal males (patients 12, 14, 19, 25, 27, 35, 42, 26, 47, 55, and 84) and non-carriers as determined previously by linkage analysis (patients 15, 28, 44, 48, 49, 52, 61, 63, 64, 65, 68, and 77). Neither I179S nor G370R mutations were present in 100 X chromosomes from normal controls including two samples which had been completely sequenced and used as negative controls.

Discussion

L1-CAM belongs to the immunoglobulin superfamily of proteins. These proteins can be structurally divided into three segments: (1) an extracellular portion consisting of six immunoglobulin-like (Ig) domains and five fibronectin III (Fn) domains; (2) a transmembrane domain, and (3) a cytoplasmic domain.

We present here two new mutations in the L1-CAM gene in affected subjects from two different families: an I179S mutation located within the loop of the first Ig-like domain of L1-CAM and a G370R mutation located within the fourth Ig-like domain. Dot blot analyses showed that neither of the mutations were polymorphisms found in the general population and confirmed that they segregated with the clinical syndrome. Moreover, hybridisation to both the mutated oligonucleotide and the wild type oligonucleotide showed the presence of one affected and one normal allele in all carrier females.

It is interesting to note that these mutations were observed in affected subjects from two families where we observed the three syndromes, complicated spastic paraplegia, MASA syndrome, and X linked hydrocephalus owing to stenosis of the aqueduct of Sylvius as apparent variable expression of mutations at the same locus at Xq28.8. All the reports published so far identified mutations in the L1-CAM gene in families with one of the three phenotypes. These data further illustrate the
fact that the three clinical entities are phenotypic variants of the same genetic defect and not separate entities, as originally suggested by Straussberg et al.16

The finding of a single point mutation in each of the members of the same family with variable clinical phenotypes suggests that the phenotypic expression of the L1-CAM gene is influenced by other genetic or environmental factors. This observation may then explain the absence of a definitive phenotype-genotype correlation in these X linked syndromes.

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