

# Five novel mutations in the L1CAM gene in families with X linked hydrocephalus

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## Abstract

**Five novel mutations have been identified in the gene encoding L1CAM, a neural cell adhesion protein, in families with X linked hydrocephalus (XHC). Interestingly, all five mutations are in the evolutionarily highly conserved Ig-like domains of the protein. The two frameshift mutations (52insC and 955delG) and the nonsense mutation (Trp276Ter) most probably result in functional null alleles and complete absence of L1CAM at the cell surface. The two missense mutations (Tyr194Cys and Pro240Leu) may considerably alter the structure of the L1CAM protein. These data provide convincing evidence that XHC is genetically extremely heterogeneous.**

(*J Med Genet* 1996;33:103-106)

Key words: L1CAM gene; X linked hydrocephalus.

Hydrocephalus (HC) in combination with stenosis of the aqueduct of Sylvius is an X linked recessive condition (XHC, McKusick No 307000) with a frequency of approximately 1 in 30 000 male births. Additional major features of the disorder are mental retardation, spastic paraparesis, and adducted thumbs. The gene for XHC has been mapped genetically to Xq28 between DXS52 and F8C.<sup>1,2</sup> Rosenthal *et al*<sup>3</sup> first reported that XHC was caused by a mutation of the gene encoding L1CAM, a neural cell adhesion molecule. The L1CAM gene consists of 28 exons defining a transcript of 3.8 kb. To date, 12 different mutations have been identified in patients with XHC.<sup>4-9</sup> Several recent reports have confirmed that mutations of the L1CAM gene are also responsible for both X linked spastic paraplegia (SPG1) and MASA syndrome (mental retardation, aphasia, shuffling gait, and adducted thumbs).<sup>5,6,9,10</sup> The correlation between the different mutations and the different phenotypes is not yet fully understood. Therefore, it is important to characterise additional mutants associated with either of the possible phenotypes. Here we present our results on the identification and characterisation of five novel mutations in the L1CAM gene in patients with XHC.

## Materials and methods

Genomic DNA samples used in this study were isolated from peripheral blood according to standard protocols. Amplification of the exons of the L1CAM gene was performed by

the polymerase chain reaction (PCR) using the oligonucleotide primers described previously.<sup>5,9</sup> Single strand conformation polymorphism (SSCP) and heteroduplex analyses were performed as described elsewhere.<sup>11</sup> Electrophoresis for SSCP was performed on polyacrylamide gels (6-10%) with or without glycerol in 1 × TBE (Tris-borate-EDTA buffer). PCR products showing an aberrant SSCP pattern were subjected to direct sequencing.<sup>11</sup> The sequence changes identified were confirmed by a second independent assay, usually restriction digestion.

## Results and discussion

The pedigree of patient HC8 is shown in fig 1. The index case was born with a large HC that made the implantation of a shunt necessary. Adducted thumbs and poor mental development suggested XHC. At the age of 6 years, nuclear magnetic resonance tomography suggested a complex brain malformation with agenesis of the corpus callosum and fusion of the thalamus. A maternal uncle of HC8 (III.1) was born with hydrocephalus and cleft lip and palate. A shunt operation was not performed. The boy was severely handicapped, both physically and mentally, could not walk unaided, and died at the age of 12 years. A single nucleotide insertion (52insC, numbering of nucleotides and amino acids as given in ref 12) was found in exon 1 of the L1CAM gene of patient HC8 (fig 2A). This mutation predicts a shift in the reading frame with the inclusion of nine amino acids unrelated to the L1CAM protein, and a premature termination of translation at codon 26. SSCP analysis showed the same band shift both in the patient and his mother but not in the maternal grandmother (II.2) (fig 3). The finding that II.2 does not carry the mutation is unexpected as her son had hydrocephalus. Assuming that the HC of III.1 resulted from the same mutation as that of the index case, II.2 must be a germline mosaic. Alternatively, the hydrocephalus of III.1 may be secondary to the developmental defect(s) responsible for the cleft lip and palate. This latter assumption is supported by the results of a segregation analysis. It has been shown that HC8 inherited the paternal allele of his mother at the DXS52 locus (fig 1), known to be closely linked to the XHC locus, suggesting that the L1CAM mutation detected occurred in his maternal grandfather during spermatogenesis. This family therefore clearly shows that in cases with HC caution is neces-

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Received 11 July 1995  
Revised version accepted for publication 4 October 1995

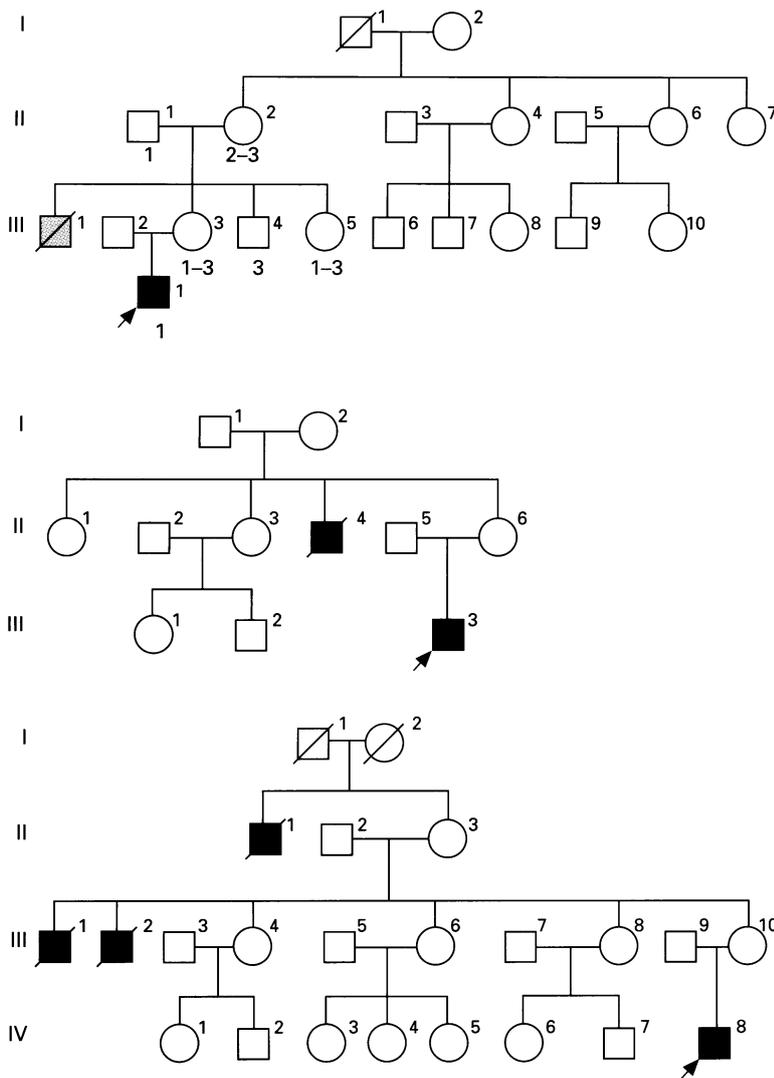


Figure 1 Pedigrees of patients HC8, HC10, and HC7 (from top to bottom). In the first pedigree, numbers below symbols show the alleles of the polymorphism at DXS52.

sary in interpreting anamnestic data and thereby determining the mode of inheritance.

HC14 corresponds to a 22 week fetus presenting with severe hydrocephalus on routine ultrasound examination. The pregnancy was terminated and necropsy showed stenosis of the aqueduct. A subsequent pregnancy of the mother was terminated in the second trimester because of hydrocephalus in the fetus seen by ultrasonography. In HC14, a single base pair deletion (955delG) was detected in exon 8 of the L1CAM gene (fig 2B). This mutation predicts a shift in the reading frame with the inclusion of 70 amino acids unrelated to the protein before a premature stop codon appears. The mother was shown to be heterozygous for the mutation by SSCP analysis (results not shown). As the family history was unremarkable, and apart from HC no other feature typical of XHC was seen at necropsy, determination of the pattern of inheritance and hence genetic counselling of the mother and her at risk relatives was difficult. Our finding shows that DNA analysis may be helpful and should be increasingly considered as a tool for differential diagnosis in such families.

The pedigree of patient HC10 is shown in fig 1. Both II·4 and III·3 presented with typical features of XHC. II·4 died two days after birth, while III·3 is now 9 years old. A G to A transition was detected at nucleotide position 828 in exon 8 of the index case which alters codon 276 (TGG for Trp) to a premature stop codon (TGA, Trp276Ter, fig 2C). The sequence change can be detected by restriction digestion analysis as a *Bbv*I and a *Fnu*4HI site is destroyed by the mutation. We have shown that the mutation is inherited with the XHC phenotype in the patient's family (results not shown).

In patient HC19, profound hydrocephalus internus was diagnosed immediately after birth. The oldest son of the mother was also born with HC. After rapid progression of the disease, that child died at the age of 3 months. The second pregnancy of the mother ended with a stillbirth of a male child. An A to G substitution was detected at nucleotide position 581 in exon 6 of the L1CAM gene in patient HC19 predicting the replacement of tyrosine 194 by cysteine (Tyr194Cys, fig 2D). SSCP analysis showed that, as expected, the mother was heterozygous for the mutation (results not shown).

Patient HC7 belongs to a large family (fig 1). The three affected males in generations II and III died between 5 and 8 months of age. In the index case, HC was diagnosed by ultrasound examination at the age of 7 months. Although a ventriculoperitoneal shunt was implanted, the development of the child was very poor. Of the associated clinical features, adducted thumbs, short stature (3rd to 10th centile), spasticity, severe mental retardation, and moderate deafness were present. A substitution of C by T at nucleotide position 719 in exon 7 of the L1CAM gene has been identified which predicts the exchange of proline 240 by leucine (Pro240Leu, fig 2E). The C-T transition creates a new restriction site for *Alu*I and abolishes a *Bst*UI site present at this position in the wild type DNA. Either of the above assays allows a simple and rapid detection of the mutation. Restriction digestion analysis showed that the mutation cosegregated with the disease phenotype in the family of HC7 (results not shown).

None of the five mutations described above was found on 60 X chromosomes of unaffected controls.

L1CAM is a large multifunctional protein involved in cell adhesion, neuronal migration, fasciculation, and neurite outgrowth.<sup>12-15</sup> Structurally, L1CAM belongs to the immunoglobulin (Ig) super family of cell adhesion molecules having a motif of six repeating Ig-C2 domains followed by five fibronectin type III domains.<sup>16</sup> It has been suggested that L1CAM plays an essential role in cell adhesion and interaction both with other cells and the extracellular matrix. The Ig-like domains are highly conserved through evolution and thought to be of primary importance in the function of L1CAM. All five mutations described in this paper are in the Ig-like domains. The two frameshift mutations as well as the nonsense mutation should lead to a premature stop of protein translation and should probably

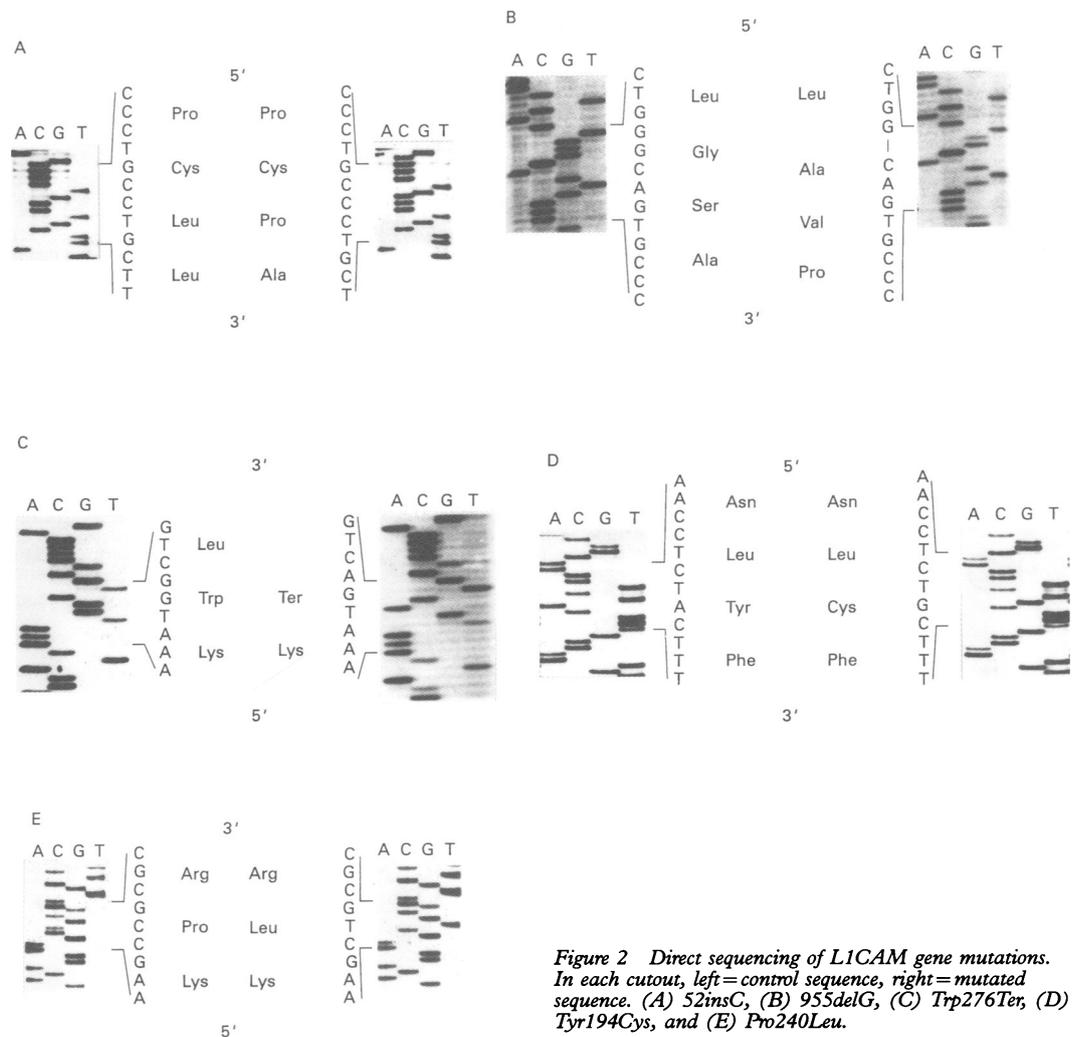


Figure 2 Direct sequencing of L1CAM gene mutations. In each cutout, left = control sequence, right = mutated sequence. (A) 52insC, (B) 955delG, (C) Trp276Ter, (D) Tyr194Cys, and (E) Pro240Leu.

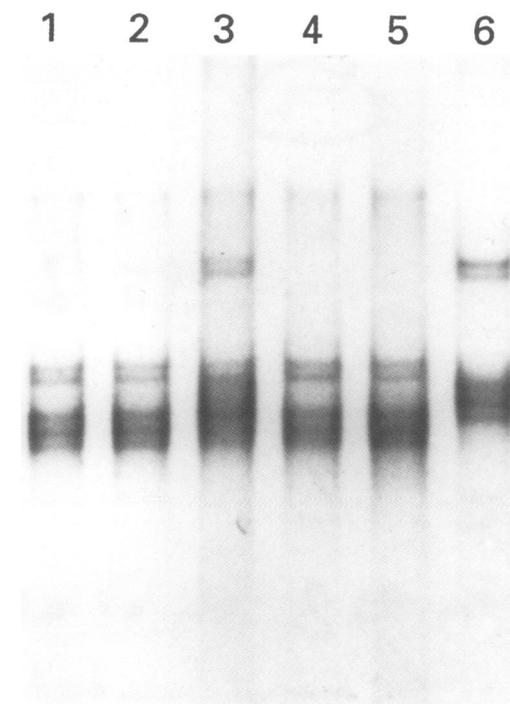


Figure 3 Band shifts seen in single strand conformation polymorphism analysis caused by the mutation 52insC in the L1CAM gene of patient HC8 and his family members. Lanes 1-6: II-1, II-2, III-3, III-4, III-5, and IV-1 (HC8), respectively.

result in functional null alleles and complete absence of L1CAM at the cell surface. The two missense mutations may considerably alter the structure of the protein. Tyrosine 194 is located in the second Ig domain of the molecule close to cysteine 209, which forms a disulphide bridge with cysteine 158. The introduction of an additional cysteine at position 194 may interfere with the formation of the S-S bond between residues 158 and 209 and thereby disturb the proper "Ig specific" folding in this part of the L1CAM molecule. In patient HC7, the substitution of leucine for proline occurs in the third Ig domain. Proline is a hydrophobic amino acid. Owing to its limited rotational ability, proline is frequently found in bends and turns in proteins. As leucine is a hydrophilic residue, its inclusion may considerably alter the protein's secondary structure.

In conclusion, a total of 12 different mutations have been reported to date in the L1CAM gene in patients with XHC. These data, together with the five novel mutations presented in this communication, provide convincing evidence that XHC is genetically extremely heterogeneous.

This study was financially supported by the Deutsche Forschungsgemeinschaft (DFG). S-MG is recipient of a fellowship of the Deutscher Akademischer Austauschdienst (DAAD). Thanks are due to Ms Heike Wedemann for her continuous

support in this project. This article is based in part on the doctoral studies by S-MG and AV in the faculties of medicine and biology, respectively, University of Hamburg.

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