# A missense mutation confirms the L1 defect in X-linked hydrocephalus (HSAS)

Sir — We present data confirming that mutations at the L1 locus are responsible for the most common inherited form of congenital hydrocephalus, HSAS. HSAS males often die neonatally and those that survive are always physically and mentally disabled[1]. Last year in *Nature Genetics*, we described aberrant processing of L1 mRNA in affected individuals of a single HSAS family[2]. Translation of the two novel mRNAs

would yield abnormal L1 polypeptides with either an insertion or premature termination. A mutation at a potential branch point signal in an intron of the L1 gene was found to segregate with the disease in this family and could give rise to the abnormal mRNA processing by influencing the choice of 3' splice acceptor sites. These results provided the first strong evidence that the L1 locus corresponds to the HSAS gene.

To confirm this hypothesis, we have obtained the entire L1 coding sequence from a patient with severe HSAS (CV542) and compared this with that of a healthy male control (JS). A G to A transition was observed in the patient at nucleotide position 791 of the cDNA sequence. This mutation creates an *RsaI* site allowing easy detection in relatives and controls. It segregates fully with the disease in the extended pedigree (H2: Fig. 1*a*) and does not correspond to a common polymorphism.

L1 is a cell surface glycoprotein with a multidomain structure. The G to A mutation results in a Cys264Tyr substitution in the third immunoglobulin type C2 domain of the mature protein (Fig. 1*b*). This would eliminate the potential for disulphide bridge formation and have a profound effect on L1 secondary structure. Although the function of C2 domains in the L1 protein has not been elucidated, studies on the closely related neural cell adhesion molecule, NCAM, indicate that they are involved in both homophilic and heterophilic interactions with the extracellular environment and influence the signal transduction events that control axonal growth[3]. The importance of Cys264 is also reflected in the conservation of this residue in analogous proteins in rat[4], mouse[5], chicken[6] and *Drosophila*[7]. The disruptive nature of this missense

mutation and its segregation with the disease through the affected family confirms our proposal that mutations in the L1 gene cause HSAS.

A wide variation in clinical signs has been observed for HSAS and as neither of the two mutations that we have reported have been found in at least six other unrelated HSAS families, a spectrum of mutations will likely emerge. Determining how disruption of L1 causes the clinical phenotype will require a thorough understanding of L1 function. L1 expressed on postmitotic developing neurones and the Schwann cells of the peripheral nervous system is an important mediator of cell migration fasciculation and nerve regeneration[3]. Identification of HSAS mutations will highlight individual regions of the L1 protein that are essential for correct function and provide valuable insight into the role of L1 in development.

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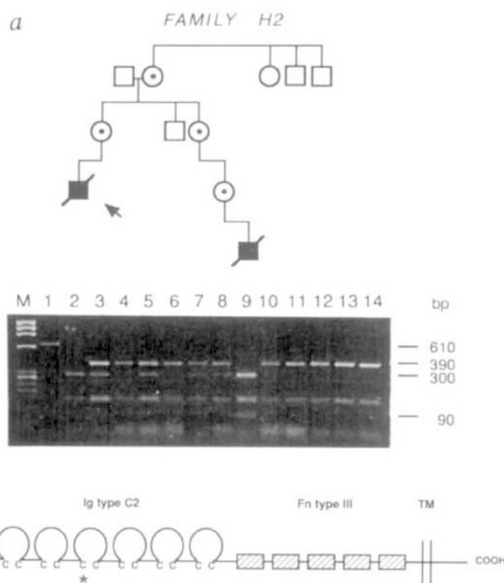**Dian Donnai**
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Fig. 1 *a*, Segregation of a G to A mutation in HSAS family H2 (ref. 8) as assayed by *RsaI* digestion of a 610 bp genomic PCR product. The mutation was detected by sequencing 16 overlapping PCR products covering the L1 coding region[9]. Primer sequences are available on request. CV542 is indicated by an arrow. Lanes: M, HaeIII-digested ΦX174 DNA; 1, undigested DNA; 2–12, digested PCR fragments from family H2; 13–14, digested DNA from healthy male controls. We are grateful to Malcolm Taylor and Karen Temple for providing cell lines and blood samples. *b*, Domain structure of the L1 protein showing disulphide bridges between adjacent cysteine residues in the immunoglobulin type C2 (Ig type C2) regions, fibronectin type II (Fn type III), transmembrane (TM) and cytoplasmic domains. Residue 264 is marked with an asterisk.

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