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Brief Clinical Report

A NOVEL MUTATION IN L1CAM GENE IN A JAPANESE PATIENT WITH X-LINKED HYDROCEPHALUS

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Summary L1CAM is a member of the immunoglobulin gene superfamily of neural adhesion molecule. Abnormality of the L1CAM gene is associated with X-linked recessive form of congenital hydrocephalus (HSAS; hydrocephalus due to congenital stenosis of aqueduct of Sylvius) and some allelic disorders. Four new patients with congenital hydrocephalus consistent with the X-linked type were described. One of them had a novel mutation in the L1CAM gene.

Key Words L1CAM, X-linked hydrocephalus, HSAS syndrome

INTRODUCTION

X-linked hydrocephalus, HSAS (hydrocephalus due to congenital stenosis of aqueduct of Sylvius; McKusick 307000) is the most common type of the hereditary forms of hydrocephalus. Severe mental retardation, spastic tetraplegia and bilateral adducted thumbs are characteristic manifestations. Neuroradiological findings are distinct from other forms of hydrocephalus. By linkage analysis, the locus for X-linked hydrocephalus was mapped to Xq28 (Willems *et al.*, 1990). Further molecular studies revealed that abnormalities in the L1CAM gene, a member of the immunoglobulin gene superfamily of neural adhesion molecule is the basic

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defect of this syndrome (Rosenthal *et al.*, 1992). L1CAM plays an important role in the neuronal migration, adhesion, neurite outgrowth, fasciculation and myelination (Hlavin and Lemmon, 1991). Four new Japanese patients with X-linked hydrocephalus are described. One of them had a novel mutation in the L1CAM gene.

CLINICAL REPORT

Case 1. This 8-year-old boy (Fig. 1, III-1) was the first child of non-consanguinous healthy Japanese parents. The maternal uncle (II-3) died in the neonatal period due to congenital hydrocephalus. After birth, the diagnosis of congenital

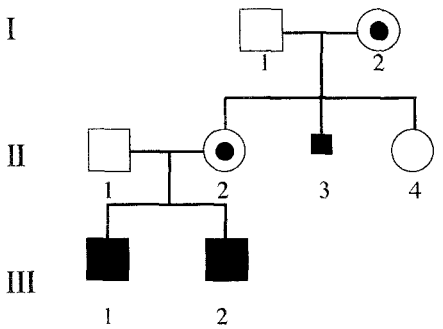

Fig. 1. Pedigree of cases 1 and 2.

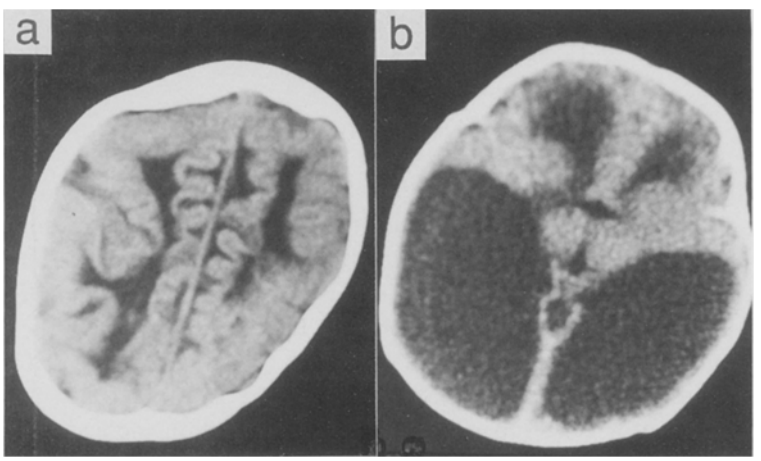

Fig. 2. (a) Neuroradiological studies of case 1 revealed enlarged lateral ventricles, irregular ventricular wall, and hypoplastic white matter. (b) CT scan before shunting of case 2 in the neonatal period. Severe dilatation of the lateral ventricle was noted.

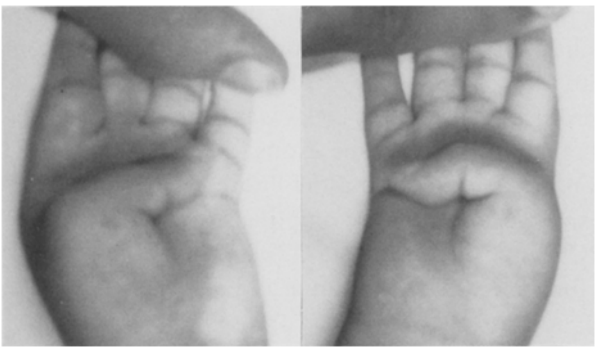

Fig. 3. Bilateral adducted thumbs in case 4.

hydrocephalus was made. Ventriculo-peritoneal shunting was performed. Physical examinations revealed nystagmus, moderate spasticity in extremities, bilateral adducted thumbs and flexion contracture of fingers. His development was severely retarded. He could not control his head or pursuit objects. Neuroradiological studies revealed enlarged lateral ventricles, irregular ventricular wall, and hypoplastic white matter (Fig. 2a).

Case 2. This boy is the younger brother of case 1 (III-2). He was 3 years old. Prenatal diagnosis of hydrocephalus was made by ultrasonography. At birth, severe hydrocephalus was noted (Fig. 2b). His clinical course was similar to that of case 1. He also had bilateral adducted thumbs and spastic quadriplegia.

Case 3. This 5-year-old boy had congenital hydrocephalus which progressed after birth. His brother was stillborn and also had congenital hydrocephalus. He showed severe mental retardation, spastic quadriplegia, and bilateral adducted thumbs. Neuroradiological studies revealed marked enlargement of the lateral ventricle.

Case 4. This 9-year-old boy had no family history of hydrocephalus. Ultrasonography revealed fetal hydrocephalus. Ventriculo-peritoneal shunting was performed after birth. He had severe mental retardation, spastic quadriplegia, and bilateral adducted thumbs (Fig. 3). Ocular pursuit and response to sound were not evident. Neuroradiological examinations revealed markedly enlarged ventricles, irregular ventricular wall, hypoplastic white matter, agenesis of corpus callosum and agenesis of septum pellucidum.

MATERIALS AND METHODS

Blood samples from the patients were obtained with informed consent. DNA was extracted by the phenol/chroloform method. PCR was performed in 100 μl water comprising 85 μl water, 10 μl 10$\times$PCR buffer, 1 μl 20 mM dNTP mix, 50 pmol of sense and anti-sense primers, 1 μl of the genomic DNA and 5 U of *Taq*

polymerase. The primer sequences flanking the 28 exons of the L1CAM gene and PCR conditions have been reported by Jouet *et al.* (1994). The amplified product was inserted into the pT7Blue T-vector plasmid (Novagen) according to the manufacturer's protocol. The cloned DNA was subjected to fluorescence-based dye primer sequencing analysis. For some exons, direct sequencing of PCR products was performed on both strands using the fluorescent dideoxy terminator method. In this report, exons 21 and 22 were amplified simultaneously with primers G35 and G38. The PCR conditions were 95°C for 2 min followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 1 min and 72°C for 4 min.

RESULTS

In case 4, sequence analysis of exon 22 of the L1CAM gene revealed a 1 bp deletion from 3000 to 3002 (Fig. 4). The *Apa*I site was lost by the deletion (data not shown). This deletion is in the fibronectin type III domain of the L1CAM molecule and results in a frameshift and a premature stop codon. Translation of this mRNA will create a truncated protein without any transmembrane and cytoplasmic regions. Proper functions of L1CAM will be lost without membrane binding.

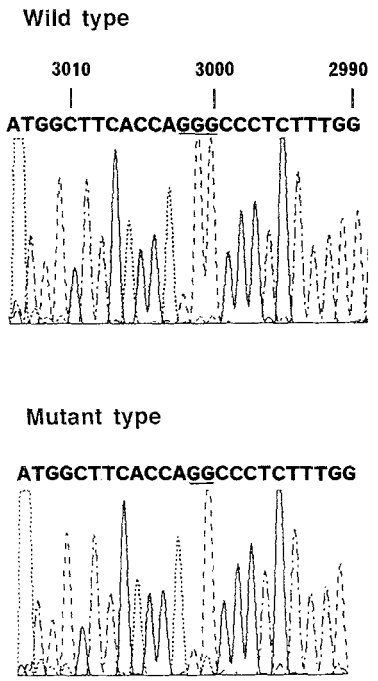

Fig. 4. Sequence analysis of L1CAM gene in case 4. Single base deletion in exon 22 was found. Numbers of base pairs are according to Hlavin and Lemmon (1991).

Although the 28 exons of the L1CAM gene were sequenced, cases 1, 2, 3 did not have a mutation in the coding region and exon-intron junctions.

DISCUSSION

We found a novel mutation in a sporadic patient whose clinical and neuroradiological manifestations were consistent with X-linked hydrocephalus. L1CAM is a cell surface protein with 6 immunoglobulin type C2 domains and 5 fibronectin type III domains followed by a transmembrane segment and intracellular domain. Rosenthal *et al.* (1992) found novel L1CAM mRNA species in cells from affected members of an HSAS family containing deletions and insertions produced by the utilization of alternative 3′ splice sites. Jouet *et al.* (1993) described a missense mutation in the L1CAM gene which resulted in a Cys264Tyr substitution in the third immunoglobulin type 2 domain of the mature protein. Van Camp *et al.* (1993) conducted a mutation analysis of L1CAM in 25 HSAS families and identified a 1.3 kb genomic duplication which cosegregated with HSAS and significantly changed the intracellular domain of the L1CAM gene. Truncated L1CAM protein due to frameshift and early stop codon are associated with severe congenital hydrocephalus (Kenwrick *et al.*, 1996). Mutations in the L1CAM gene have been found in MASA syndrome (mental retardation, aphasia, shuffling gait and adducted thumbs) and X-linked spastic paraplegia (Jouet *et al.*, 1994; Vits *et al.*, 1994; Fransen *et al.*, 1994). The three disorders are allelic with overlapping profile of clinical manifestations and intrafamilial heterogeneity (Schrander-Stumpel *et al.*, 1995). The acronym, CRASH syndrome (corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia and hydrocephalus), was suggested (Fransen *et al.*, 1995). Reports on new mutations in the L1CAM gene are accumulating (Coucke *et al.*, 1994; Fransen *et al.*, 1994; Jouet *et al.*, 1995; Ruiz *et al.*, 1995).

Not all patients with X-linked hydrocephalus have a mutant L1CAM gene. Cases 1, 2, 3 did not have a mutation. Although at least 30 cases with definite or probable X-linked hydrocephalus have been reported in Japan (Yamazaki *et al.*, 1995), there has been only one report on the mutant L1CAM gene (Takechi *et al.*, 1996). Mutations outside of the coding region cannot be excluded. This syndrome may have genetic heterogeneity. Another locus for X-linked hydrocephalus has been found by linkage analysis (Strain *et al.*, 1994).

Yamazaki *et al.* (1995) reported that X-linked hydrocephalus is not a disease of simple ventriculomegaly due to aqueduct stenosis alone but involves other complicated nervous system anomalies. A genesis of corpus callosum or septum pellucidum, fusion of the thalamic fornices, colliculi and corpora quadrigemina, irregular ventricular wall, white matter dysgenesis, and absence or hypoplasia of the corticospinal tract are common findings. L1CAM is expressed at high levels during corticospinal tract development. In HSAS syndrome, the aqueduct stenosis

is produced secondarily by compression of the dilated ventricle (Landrieu *et al.*, 1979; Yamazaki *et al.*, 1995). The acronym, HSAS, is not appropriate.

An L1CAM abnormality exists in certain cases of congenital hydrocephalus in Japan. Molecular analysis of L1CAM will help definitive diagnosis of X-linked hydrocephalus in sporadic cases. Further studies on the relationship between genotype and phenotype will help to clarify the role of the L1CAM molecule. Additionally, detection of mutations may allow prenatal diagnosis of X-linked hydrocephalus as reported by Jouet and Kenwrick (1995).

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