

L1(CAM) Mutation in Males with Mental Retardation

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KEY WORDS Mental Retardation; L1(CAM) mutation; CRASH.

ABSTRACT L1 CAM (L1 cell adhesion molecule) plays a key role in the development of nervous system. Recent studies have shown the evidence for the involvement of L1 (CAM) mutations in X-linked mental retardation syndromes. No such studies were undertaken from India and hence, an attempt was made to detect L1 (CAM) mutations in mental retardation. Eight patients from different families were selected to know the molecular basis of mental retardation in these patients. Genomic DNA from all the patients and controls was analyzed by polymerase chain reaction. PCR analysis was carried out using specific primers to screen for L1 (CAM) mutations. Among the eight patients, in two patients mutations in exon-4 and intron-4 junction were detected.

INTRODUCTION

L1 (CAM) (L1 cell adhesion molecule) is a transmembrane glycoprotein belonging to the immunoglobulin superfamily. The molecular weight of L1 (CAM) protein is about 200 kDa and containing various domains, that include six immunoglobulins, five fibronectin type III domains in the extra cellular part, transmembrane segment and a cytoplasmic domain. L1 gene is present on the long arm of telomere region of X-chromosome. The L1 gene spans about 16 kb and consists of 28 exons. It encodes a polypeptide consisting of 1256 amino acids.

L1 (CAM) plays an important role in the development of nervous system and in mediating the interaction between cell and environment. L1 homologous have been identified in rat (NILE), mouse, drosophila, chicken, zebrafish, etc. (Fransen et al. 1997).

Willems et al. (1992) from Belgium have suggested that L1 as a possible candidate gene for X-linked mental retardation syndromes or CRASH. Rosenthal et al. (1992) from UK, Van camp et al. (1993) and Coucke et al. (1994) from

Belgium provided evidence for the involvement of mutations in the neural cell adhesion molecule L1 in Hydrocephalus (HSAS, MIM 307000), MASA syndrome (MIM 303350), Spastic paraparesis (SP1, MIM 312900) and Corpus callosum agenesis (ACC MIM 3041000). Recently from U.S.A Needham et al. (2001) and Du et al. (1998) observed mutations in L1 (CAM) gene in X-linked mental retardation syndromes. Weller and Gartner (2001) from Germany reported L1 (CAM) mutations in X-linked Hydrocephalus. Sztirha et al. (2001) detected a mutation in the L1 (CAM) gene in a CRASH syndrome from United Arab Emirates.

In India, 2-3% of population are afflicted with mental retardation. In more than 50% of the cases, the cause for mental retardation could not be established. Hence, an attempt was made to see whether or not involved L1 (CAM) are involved in the causation of mutations cause mental retardation in our population.

MATERIALS AND METHODS

Eight children from different families in the age group of 6 months-15 years afflicted with mental retardation were selected for the present study. The patients were clinically examined and information on age, sex, physical features, pedigree, health status and intelligence quotient was recorded in a standard questionnaire.

Ten ml blood was collected from each patient and control samples. Genomic DNA was isolated according to the protocol of Maniatis et al. (1982) and quantified using spectrophotometer.

Polymerase Chain Reaction (PCR) was carried out with the following forward and reverse primers to amplify the region at the exon 4— intron 4 junction:

F- 5' GGC TCA TGG CCG AGG GTT C-3'
R- 5' AGG GGA GAA GCT GGG GTG G-3'

The PCR reaction contained 200 ng of genomic DNA, 2mm dNTP's, 50 pmol of each primer, 1 U Taq Polymerase, 10XPCR Taq buffer (10mM Tris (pH: 8.3), 50mM KCL, 15mM MgCl₂

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and 0.01% Gelatin) in a reaction volume of 50 μ l. PCR amplification was carried out for 35 cycles. Each cycle consisting of the following denaturation for 30 sec at 95°C, annealing at 64°C for 30 sec and extension step at 72°C for 30 sec. This was followed by final extension step at 72°C for 7mts. The PCR product was purified and digested with 2U of Taq I restriction enzyme at 37°C overnight. The digested PCR product was then electrophoresed on 2% Agarose gel and stained with ethidium bromide.

RESULTS AND DISCUSSION

Polymerase chain reaction was carried out with the genomic DNA isolated from all the eight patients with mental retardation along with controls using the primers described in materials and methods. The amplified PCR products were purified and digested with Taq I restriction enzyme. After Taq I, digestion PCR product of 139 bp was seen in the control samples and in six out of eight patients with mental retardation (Fig. 1).

In the remaining two patients the Taq I digested PCR product showed 157 bp band instead of 139 bp band (Fig. 1). The result clearly indicates the absence of Taq I enzyme site in these two patients. The absence of Taq I enzyme

site in these cases may be due to a mutation in this region of the gene.

On clinical examination, the proband TA who is two years old male presented with mild microcephaly and delayed milestones. Another proband who is 11 months old male presented with hypotonia and delayed milestones. Both the patients were the products of healthy non-consanguineous parents and full term normal pregnancy.

L1 (CAM) plays an important role in the development of brain and nervous system. Any damage or mutation to L1 might result in the brain damage resulting in neurological abnormalities including mental retardation. The results clearly showed that a mutation has occurred in L1 gene at exon 4–intron 4 junction in two children with mental retardation from different families. So far 142 mutations from 153 unrelated families were reported in the world literature (L1 Home page www.uia.ac.be/dnalab/l1/). Almost all the mutations are private mutations that occur only in one family. The mutation we have reported here was similar to that of Coucke et al. (1994) and Saugier-Verber et al. (1998). Studies are in progress in order to delineate the type of mutation and to detect the carriers in the family of these cases.

The mutation that observed in the present

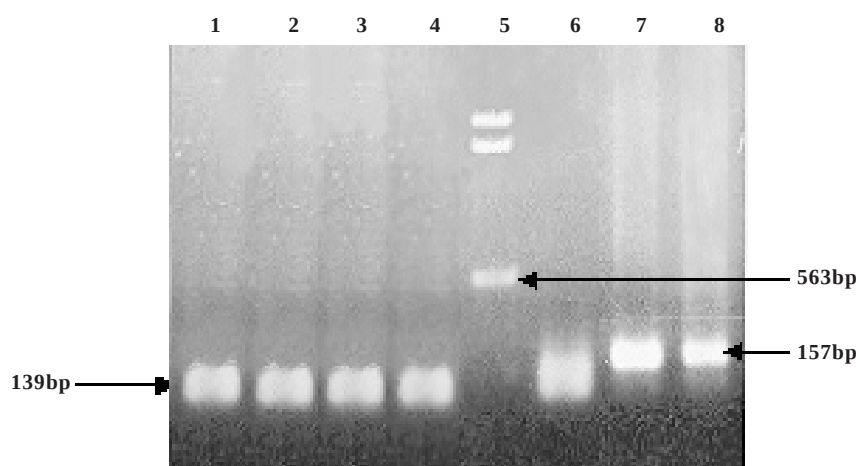


Fig. 1. PCR analysis of patients with mental retardation

Lanes 1-4: Mental retardation patients
 Lane 5 : λ Hind III Marker
 Lane 6 : Control sample
 Lanes 7 & 8 : Mental retardation patients (Mutation observed – 157bp)

study in these two patients is on the extracellular part of the protein. Fransen et al. (1998) found that mutations in the extracellular part of L1 leads to truncation or absence of L1 expression which causes a severe phenotype. In the present study as children are very young, it is difficult to know the severity of the cases.

The mutations so far detected in L1 (CAM) gene are deletions, insertions, missense, nonsense, splice site etc. Vits et al. (1994) detected large deletion of 2 kb. Small base pair deletions like 31 bp, 15 bp, 14 bp, 5 bp, 4 bp, 3 bp, 2 bp and 1 bp (Fransen et al. 1997; Farlane et al. 1997; Finckh et al. 2000) missense mutations (Graf et al. 2000) and nonsense mutations (Gu et al. 1996) were also reported.

X-linked mental retardation syndromes or CRASH syndrome covers a wide range of clinical spectrum where there is an associated additional symptoms like Hydrocephalus, shuffling gait along with mental retardation. It is limited to mental retardation alone in some patients. So, mutations in L1 might be a more frequent cause of mental retardation (Fransen et al. 1997).

In view of the above observation, it is worthwhile to screen L1 gene for mutations in all the cases of idiopathic mental retardation. Detection of mutations will be very helpful for prenatal diagnosis in the high risk families and to impart genetic counseling to the parents and families to reduce genetic morbidity in the population.

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