Detection of L1 (CAM) mutations in X-linked mental retardation: A study from Andhra Pradesh, India

Department of Environmental Toxicology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, Andhra Pradesh, India

**BACKGROUND:** Mental health is an essential ingredient in the quality of life. Recent studies carried out in countries like Germany, USA, France, England and Belgium have provided evidence for the involvement of L1 (CAM) mutations in various X-linked mental retardation syndromes. L1 CAM is a neural cell adhesion molecule belonging to the superfamily of the immunoglobulins and is critical for proper CNS development in humans.

**AIM:** This study was aimed to screen idiopathic mental retardation cases for L1 CAM mutations.

**MATERIALS AND METHODS:** In this study, we screened 15 cases with mental retardation. Genomic DNA from the patients and control subjects was analyzed by polymerase chain reaction using specific primers.

**RESULTS:** In 2 out of 15 patients, mutation was detected between exon 26 and 27.

**CONCLUSION:** It is worthwhile to screen idiopathic mental retardation cases for L1 CAM mutations to reduce genetic morbidity in the population by offering genetic counseling and prenatal diagnosis.

**Key words:** L1 CAM mutation, MASA, X-linked mental retardation syndromes

Mental retardation is the condition of developmental disability with multiple etiologies, including genetic and nongenetic causes. Among the genetic factors, chromosomal anomalies and metabolic disorders were found to be causative factors in 50% of the cases with mental retardation. However in the remaining 50% of cases, the cause is not established (idiopathic). Studies conducted in the field of molecular genetics and developmental neurobiology revealed that some types of brain malformations and mental retardation are due to mutations in L1 gene. Cell adhesion molecule (L1CAM), a protein coded by L1 gene, plays a key role in the development of nervous system. The gene encoding L1 is located on the X chromosome and mapped in the region Xq 28. L1CAM is a transmembrane glycoprotein belonging to the immunoglobulin superfamily and is an important determinant of histogenesis in the nervous system. L1 is a part of a variety of important processes, including neuronal migration, neurite growth and fasciculation, myelination and synaptic plasticity.

L1 protein has a molecular mass of 200 KD, with 138 KD due to polypeptide and the rest due to carbohydrate. It is composed of various domains - including a cytoplasmic domain, which is highly conserved among various species; a transmembrane domain, composed of 23 hydrophobic residues; five fibronectin-III-like domains; and six immunoglobulin type C2 like domains, each containing one disulfide bridge.

The extracellular part of L1 interacts with its surroundings through homophilic binding with other L1 molecules and through heterophillic interactions with other neural cell adhesion molecules, such as axonin-1 (TAG-1) and phosphocan. The cytoplasmic tail of L1 interacts with ankyrin, a protein involved in the binding of the cytoskeleton to the cell membrane. Glycosyl phosphotidyl inositol carries this attachment of the L1 CAM. Willems et al. from Belgium have suggested that L1 is a possible candidate gene for X-linked mental retardation syndromes. However, no studies on mutational analysis of L1 CAM gene were undertaken from India. Hence an attempt was made for the first time by Swarna et al. from our institute, and it provided evidence for the involvement of L1CAM mutations in mental retardation in India. Thus a pilot study to understand the mutational frequency in hotspot region (exon 26-27) of L1 gene in children with mental...
retardation was taken up.

Materials and Methods

Patients suspected for mental retardation from different hospitals and clinics were referred to the Institute of Genetics and Hospital for Genetic Diseases, Hyderabad, for confirmation. Karyotyping and biochemical analysis for aminoacidopathies, carbohydrate metabolism were carried out, and cases with unknown etiology (idiopathic) were only included for molecular studies. Fifteen children in the age group of 6 months to 15 years were selected for the study. For comparison, an equal number of healthy and normal children in the same age group and socioeconomic status were selected as controls.

The patients and the healthy subjects were clinically examined and information on their age, sex, physical features, pedigree, health status, etc., was recorded in a standard questionnaire.

Psychological assessment was performed using standard I.Q. assessment tests such as Seguin Form Board, Raven’s progress in matrices and Bharatraj’s developmental screening test. Patients whose I.Q. was found to be in the range of 55-69 were considered as mildly retarded, according to the classification of American Association on Mental Retardation (AAMR, 1992).[8]

Genomic DNA was isolated from peripheral blood of patients and control subjects according to the protocol of Maniatis et al.[9] and quantified using spectrophotometer (Sanyo-SP 55).

Polymerase chain reaction was carried out with the following forward and reverse primers to amplify the region from exon-26 to exon-27:

F: 5’ TCA GGC TGG GGC GGG AGA AGA AG 3’
R: 5’ CTA TAG GGA GAC CTT GCT GTT GG 3’

Amplification of DNA was carried out as described by Jouet et al.[10] The PCR reaction contained 200 ng of genomic DNA, 10 mM dNTPs, 50 pmol of both primers and 1.5 units of Taq polymerase, 10X PCR Taq buffer (10 mM Tris [pH: 8.3], 50 mM KCl, 15 mM MgCl₂ and 0.01% gelatin) in a reaction volume of 50 µl.

Polymerase chain reaction was performed with 35 cycles, each cycle consisting of incubation at 95°C for 1 min, 67°C for 1 min and 72°C for 2 min. Prior to cycles, initial denaturation at 95°C for 5 min and final extension at 72°C for 7 min were carried out. PCR products were analyzed on 2% agarose gel stained with ethidium bromide.

Results

Amplification of DNA of 15 patients and 15 control subjects was carried out using specific primers as described under the heading 'Material and methods.' The PCR results on DNA samples of 13 patients showed an amplification of 275bp. However, the DNA of 2 patients did not show amplification in the 275bp band, indicating a change in the nucleotide sequence of the primer region of exon-26 and exon-27 or deletion of DNA segment in this region [Figure 1].

On clinical examination, it was noticed that both the patients were normal in appearance, without any dysmorphic features, and their psychological assessment revealed that they were with mild mental retardation.

Discussion

The present study revealed that a mutation has occurred in L1 gene at exon 26-27 in 2 out of the 15 patients. Needham et al.[11] observed cytoplasmic domain

![Figure 1: PCR analysis of L1 (CAM) mutations](image-url)
mutations of the L1 (CAM) in X-linked mental retardation. Swarna et al.\textsuperscript{[13]} from this institute, reported a deletion in region between nucleotides 13,773 (intron 25) and 14,158 (intron 27) of the L1 gene in one case of idiopathic mental retardation. Exon 26-27, analyzed in this study, revealed a code for cytoplasmic domain of L1 CAM containing a highly conserved region capable of binding to the cytoskeletal protein ankyrin, a process that is most important in cell-cell adhesion, and hence it can be considered as mutation-rich or hotspot region.

There is a close correlation between genotype and phenotype. The patients in our study have shown only milder phenotypes with I.Q. between 50 and 69 and with less mental retardation features. While some earlier studies reported milder phenotypes, some other studies reported severe mental retardation. Fransen et al.\textsuperscript{[13]} observed that mutations occurring in the extracellular part, leading to truncation or absence of L1, cause a severe phenotype while mutations in the cytoplasmic domain of L1 give rise to a milder phenotype. Extracellular missense mutations affecting amino acids situated on the surface of a domain cause a milder phenotype than those affecting amino acids that are buried in the domain.

L1 gene is a single copy gene of 28 exons and spans 16kb. The open reading frame has 3825bp and encodes a protein of 1,275 amino acids. The exons 3-14 encode the 6-immunoglobulin domains with 2 exons for each domain; exons 15-24 encode the 5 fibronectin domains; exon 25, the transmembrane domain; and exon 26-28, the cytoplasmic domain. The 100 or so amino acid stretch of the cytoplasmic domain is highly conserved in all species, and its functional importance is demonstrated by the fact that even a single amino acid deletion or addition in this region can cause neurological disease in man.

Kancmura et al.\textsuperscript{[14]} have reported a case with MASA syndrome (Mental retardation, Aphasia, Shuffling gait, Adducted thumbs) and proved that the abnormal development of axon tracts resulting in the corpus callosum hypoplasia and adducted thumbs appears to be caused by malfunction of the cytoplasmic domain of L1 CAM. It is possible that mutations in the cytoplasmic domain disturb the transduction of extracellular signals into the cell via an unknown signal pathway. Alternatively, it is possible that these mutations lead to impaired binding with the intracellular cytoskeleton.\textsuperscript{[15]}

The mutations so far detected in L1 CAM gene are deletions, insertions, missense, nonsense, splice site, etc. Vits et al.\textsuperscript{[16]} detected large deletion of 2kb. Small base pair deletions like 31bp, 15bp, 14bp, 3bp, 2bp and 1bp\textsuperscript{[17-19]}, missense mutations\textsuperscript{[20]} and nonsense mutations\textsuperscript{[21]} were also reported.

**Conclusion**

In the present study, a deletion was observed in two cases with idiopathic mental retardation in exon 26-27. Hence it is worthwhile to screen for L1CAM mutations in cases of idiopathic mental retardation. Detection of mutations will be very helpful for prenatal diagnosis and to impart genetic counseling to the parents and families who are at risk.

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L1 (CAM) mutations in mental retardation


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Announcement

An International conference on “Emerging Trends in Haematology and Immunohaematology” is being organized to celebrate the Golden Jubilee of Institute of Immunohaematology from 31st January to 3rd February 2007 at Mumbai.

Further details will be available shortly at our website : www.iihicmr.org.