DETECTION OF L1 CAM MUTATION IN A MALE CHILD WITH MENTAL RETARDATION

M. Swarna, M. Sujatha, P. Usha Rani and P.P. Reddy

Institute of Genetics and Hospital for Genetic Diseases, Begumpet, Hyderabad-500 016, A.P., India

ABSTRACT

Recent studies have presented evidence for the involvement of L1CAM gene mutations in various X-linked mental retardation syndromes. The neural cell adhesion molecule, L1CAM is a transmembrane protein belonging to the super family of the immunoglobulins that play a key role in embryonic development of the nervous system and is involved in memory and learning. No studies were carried out from India on L1 CAM gene in X-linked mental retardation syndromes. Hence, an investigation was taken up to delineate the role of L1CAM gene in mental retardation.

Two families (Family I and Family II) having only two members affected with mental retardation in each family were studied for mutations in L1CAM gene. In family II, the younger sibling showed deletion involving region between the nucleotide 13,773 (intron 25) and 14,158 (intron 27) region. The mutation what we observed in younger sibling of the family II is a novel mutation which was not hitherto reported in the world literature

KEY WORDS

X-linked mental retardation syndromes, CRASH, L1 CAM mutation.

INTRODUCTION

Extensive studies carried out to delineate the cause for mental retardation have indicated that genetic and non-genetic factors are responsible for mental retardation. Of the genetic factors, chromosomal anomalies (1) and metabolic disorders (2) were found to be causative factors for mental retardation. However, in almost all the studies carried out, in majority of the cases cause for mental retardation could not be established. Efforts have been made in recent years to find out the cause of mental retardation utilizing molecular and cytogenetic techniques. Willems *et al.* (3) have suggested that L1 as a possible candidate gene for X-linked mental retardation syndromes.

L1 is a transmembrane glycoprotein, which belongs to the superfamily of the immunoglobulins. It plays an essential role in the development of nervous system and in mediating the interaction between a cell and its environment. The protein has 1256 amino acids with an extra cellular part consisting of six Ig-like domains and five fibronectin type III-like domains, a single pass transmembrane domain and a short cytoplasmic c-terminal region. L1 homologues have also been discovered in rat (NILE) and mouse (4) and drosophila, chicken, etc (5).

The gene encoding L1 has 28 exons and is present on the long arm of telomere region of X-chromosome. The innovative studies carried out from Belgium, United Kingdom and U.S.A(6-13) clearly provided evidence for the involvement of mutations in the neural cell adhesion molecule L1 in X-linked mental retardation syndromes or CRASH (Corpus Callosum Hypoplasia, Retardation, Aphasia, Spastic paraplegia, Hydrocephalus). Recently, Weller and Gartner (14) from Germany reported L1CAM mutations in X-linked hydrocephalus. From USA, Needham *et al.* (15) observed cytoplasmic

Author for correspondence :

Prof. P.P. Reddy,
Director,
Institute of Genetics &
Hospital for Genetic Diseases,
Begumpet, Hyderabad - 500 016, INDIA

E-mail : reddypp@rediffmail.com

domain mutations of the L1CAM in X-linked mental retardation. Sztriha et al (16) detected a mutation in the L1CAM gene in a CRASH syndrome from United Arab Emirates. However, no studies on mutational analysis of L1CAM gene were undertaken from India. Hence, an attempt was made for the first time, to study the L1CAM mutations in mental retardation from the state of Andhra Pradesh. We have randomly selected certain regions of L1CAM gene and designed different sets of primers to screen the mutations/deletions in the mentally retarded children. Initially we screened the patients' DNA with different sets of primers in the region of exon 4 of L1CAM. No mutation/deletion was detected in this region in the mentally retarded children analyzed. Later, we used primers for intron 25 and intron 27 regions to screen the same DNA samples. The preliminary study revealed the detection of a novel mutation in a family.

MATERIALS AND METHODS

Two families were referred to the Institute of Genetics from the National Institute for the Mentally Handicapped, Secunderabad for genetic evaluation of X-linked mental retardation and genetic counseling. In these two families, two male siblings were affected with mental retardation. These two families do not have any normal offsprings. The affected and the parents were clinically examined and information on age, sex, physical features, pedigree, health status, intelligence quotient, etc. were recorded in a standard questionnaire. On clinical examination, after ruling out other types of X-linked mental retardation, a provisional diagnosis of L1 mutation was suspected. So genomic DNA was isolated from the whole blood of siblings and parents of the family using standard protocol (17) and quantified using spectrophotometer. Healthy and normal school children in the same age group belonging to the same socio-economic status formed the control group. Chemicals for DNA isolation were obtained from Qualigens Company, Mumbai.

Polymerase Chain Reaction (PCR) was carried out by the modified method of Vits *et al.* (11) for which primers, proteinase K, 100 bp DNA ladder, Taq enzyme, dNTP's were obtained from Bangalore Genei Pvt. Ltd, Bangalore. PCR reaction was made using 200 ng of genomic DNA to amplify the region between the nucleotide position 13,871 (exon 26) and 14,158 (intron 27) [F1-R1] and 13,773 (intron 25) to 14,048 (intron 27) [F2-R2]:

F1 - 5'- ATG AGA CCT TCG GCG AGT ACA G-3'

R1 - 5'- GTG TTG GCC TCT CCC TGG AAT GA-3'

F2 - 5'- TCA GGC TGG GGC GGG AGA AGA AG-3'

R2 - 5'- TAT AG G GA G AC C TT G AT G TT G G-3'

PCR with primer set F1-R1 was performed with 35 cycles, each cycle consisting of denaturation at 95°C for 30 sec, annealing at 64°C for 30 sec and polymerization at 72°C for 1 minute. Prior to cycles, initial denaturation at 95°C for 5 minutes and after completing the cycles final extension at 72°C for 5 minutes was carried out. PCR with primer set F2-R2 was also performed using the same conditions except annealing condition at 66°C for 30 sec. PCR products were analyzed on 1.5% agarose gel and stained with ethidium bromide.

RESULTS

The pedigree of family I is given in Fig. 1. It shows that the two affected siblings were born to consanguineous parents. There is no other family member with mental retardation. The first proband aged 5 years was severely retarded. On clinical examination, he showed elongated facies, clasped thumbs with spastic paraplegia. The second affected proband was a one year old with a narrow forehead, without any facial dysmorphism and was moderately retarded.

The pedigree of family II is given in Fig. 2. The two affected males were products of non-consanguineous parents. The elder male was aged 13 years. On examination, he had microcephaly, prominent jaw, narrow forehead and spasticity of all limbs and was severely retarded.

The second affected was 11 years old with microcephaly, a prominent jaw, narrow forehead and spasticity of all four limbs. He was moderately retarded.

PCR was performed using the DNA of the siblings and parents of two families with the primer sets of F1-R1 and F2-R2. PCR products of 287 bp and 275 bp were obtained from the control sample (school children) as expected. Similar results were obtained with the DNA of siblings and parents of the family I.

However, in the family II the DNA from parents and older sibling gave 287 bp and 275 bp bands respectively with the same set of primers similar to control but the younger sibling (11 yrs old) did not show the 287 bp (Fig. 3) and 275 bp band (Fig. 4).

Fig. 1. Pedigree of Family I

Fig. 2. Pedigree of Family II

DISCUSSION

L1CAM mediates a network of cellular reactions that might lead to morphogenesis of the nervous system. L1CAM is expressed both in the brain and in the peripheral nervous system and functions as a membrane bound protein with an extra and an intra cellular cytoplasmic domain linked by a transmembrane domain. Hence, any damage to L1 might cause adverse effects such as neurological abnormalities and mental retardation.

In the present study, we observed a deletion involving region between nucleotides 13,773 (intron 25) and 14,158 (intron 27) region of L1 gene. The mutation at this region was not reported till today in mental retardation or X-linked syndromes. So far 142 mutations from 153 unrelated families were reported in literature (L1 Home page www.uia.ac.be/dnalab/1/). The mutations, which are found till date, are private mutations that have never been observed in more than one family. The mutations

Fig. 3. PCR analysis using the DNA of Family I and II with primers (F1 and R1)

- Lane 1-4, 7-8 : DNA of Family I and II
- Lane 5, 11 & 12 : Control samples
- Lane 6 : 100 bp DNA ladder
- Lane 9 : DNA of younger sib of Family II
- Lane 10 : DNA of older sib of Family II

Figure 4: PCR analysis using the DNA of Family I sibs and Family II with primers F2 and R2

- Lane 1 : DNA of Father (Family II)
- Lane 2 : DNA of Mother (Family II)
- Lane 3 : Older sib (Family II)
- Lane 4 : Younger sib (Family II)
- Lane 5 : 100 bp DNA ladder
- Lanes 6-7 : DNA of Family I siblings
- Lane 8 : Control sample

are dispersed throughout the L1 gene i.e. functional protein domain.

Mutations in L1 cause case variable spectrum of clinical anomalies. A close relationship has been observed between the disease phenotype and the position of L1 mutation. Variability of clinical spectrum and numerous L1 mutations have also been described.

The patient in our study has shown only milder phenotype. Fransen *et al.* (18) observed that mutations occurring in the extra-cellular part leading to truncation or absence of L1 cause severe phenotype; mutations in the cytoplasmic domain of L1 give rise to milder phenotype. Extra-cellular missense mutations affecting amino acids situated on the surface of a domain cause a milder phenotype than those which are buried in the domain.

In the present study we observed deletion involving region in mental retardation. Vits *et al.* (11) reported 2kb deletion in the 3' region of L1CAM gene. Fransen *et al.* (19) observed 31 bp deletion in intron 5 region while 15bp and 14 bp deletions were observed by MacFarlane *et al.* (20) and Graf *et al.* (21) respectively. Small base pair like 4 bp, 3 bp, 2 bp and 1 bp deletions (4, 18, 22) missense mutations (4) nonsense mutations (13) and splice site mutations (23) were also reported in mental retardation.

In the present study, a deletion involving region was observed in a case with idiopathic mental retardation. Thus it is worthwhile to analyse the entire L1CAM gene for mutations in the other case. Screening of L1CAM gene in cases with mental retardation is helpful to identify mutations and to offer genetic counseling.

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