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Research article

A truncation mutation in the *L1CAM* gene in a child with hydrocephalus

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Abstract: Hydrocephalus is a neurodevelopmental, X-linked recessive disorder caused by mutations in the *L1CAM* gene. The *L1CAM* gene encodes for L1CAM protein which is essential for the nervous system development including adhesion between neurons, Myelination, Synaptogenesis etc. Herein, the present study has reported mutations in L1 syndrome patient with Hydrocephalus and Adducted thumb. Genomic DNA was extracted from patients whole blood (n = 18). The 11 exons of the *L1CAM* gene were amplified using specific PCR primers. The sequenced data was analysed and the pathogenicity of the mutation was predicted using the various bioinformatics programs: PROVEAN, PolyPhen2, and MUpro. The results revealed that the proband described here had nonsense mutation G1120 \rightarrow T at position 1120 in exon 9 which is in extracellular immunoglobulin domain (Ig4) of the *L1CAM* gene. This nonsense mutation is found to be truncated with a deleterious effect on developing brain of the child, and this is the first report of this novel mutation in patient with X-linked Hydrocephalus in India.

Keywords: L1CAM; hydrocephalus; adducted thumb; X-linked recessive; nonsense mutation

1. Introduction

X-linked hydrocephalus (XLH) is the most common form of inherited hydrocephalus and is associated with severe neurological deficits and premature death [1] affecting about 1 in 25,000 live births [2]. X-linked hydrocephalus is a clinical subtype of L1 syndrome and this subtype represents the severe end of the L1 syndrome spectrum [3]. The most common phenotypes of L1 syndrome condition

are mental retardation, aphasia, and adducted thumb [2]. In severe condition the L1 syndrome patients presented with additional clinical features including hydrocephalus, shuffling gait, spastic paraplegia and hirschsprung's disease in very rare cases [3,9].

X-linked hydrocephalus is a result of defect in L1CAM protein which is involved in diverse processes at different stages during development of the nervous system, including cell-cell adhesion, neurite growth, neural migration, axonal fasciculation, myelination, and synaptic plasticity [4–6]. The L1CAM gene encoding L1CAM protein is located on the X chromosome at q28 position and it is expressed predominantly in neural cells [7]. The *L1CAM* gene is 24,657 bp in length, consisting of 28 exons (NM_000425.5) encode for 1,257 amino acids [8].

L1 syndrome patients are evidenced with wide varieties of mutations in the *L1CAM* gene [10], and all of them have been listed in *L1CAM* mutation database [11]. Many recent studies have reported several truncated mutations in L1 syndrome condition [12–15] and most of them are private mutations specific to individual family [8,16–18]. The present study aimed to investigate pathogenic variants in a child diagnosed with hydrocephalus. Herein, a novel mutation in exon 9 of L1CAM gene is reported and it has not been listed yet in the *L1CAM* mutation database. To the best of our knowledge this is the first study reporting the novel mutation in Indian cohorts [18,19].

2. Materials and methods

2.1. Clinical report

The proband was diagnosed for hydrocephalus in neonatal condition. He had a bilateral adducted thumb along with the birth weight of 3,200 g (Figure 1). His neurosonographic findings showed obstructive hydrocephalus with dilation of lateral ventricles and third ventricle suggestive of Aqueductal stenosis (Figure 2). His developmental milestones were delayed because of the hydrocephalus and at the age of 10 months the proband was severely affected both mentally and physically. The proband belongs to the family with consanguineous parents and the family has history of having an affected male in the previous generation. The proband had a healthy female sibling.



Figure 1. Patient showing Macrocephalic condition with an adducted thumb (arrow).



Figure 2. Neurosonographic image showing dilation of lateral ventricles and third ventricle.

2.2. Patient

The present case was referred by Paediatric neurologists, Department of Paediatrics, SIMS-Shivamogga Institute of Medical Sciences, Shivamogga, Karnataka, India. The proband was the second child of healthy, consanguineous parents. After birth, bilateral adducted thumbs, and flexion contractures of the fingers were noted. His typical L1 syndrome features prompted us to screen the mutations in the *L1CAM* gene. The family history was positive for neurological abnormalities like Mental retardation and spasticity (II:5 in Figure 3).

2.3. Clinical examination and sample collection

A standard questionnaire was used to collect the family history of the patients along with the clinical parameters related to the L1 syndrome and patient consent was also obtained by the family. The affected members of the family and the parents were clinically examined and information of age, sex, physical features, pedigree, health status, intelligence quotient, etc., was recorded. Blood sample of the patient was collected by venipuncture of the patient arm to a K3-EDTA tube and stored at 4 $^{\circ}$ C until further analysis.

2.4. DNA isolation

Genomic DNA was isolated from the whole blood using Qiagen Human blood DNA isolation kit [20]. Isolated DNA was quantified by Bio spectrophotometer.

2.5. Amplification of DNA

DNA was amplified by Polymerase chain reaction using primers designed on different exon and intron regions of the *L1CAM* gene (Table 1). Primers were designed on the *L1CAM* GenBank sequence (Accession num: NM_000425.5) by using NCBI Primer-Blast [21] (Table 1).

Name	Sequence $(5' \rightarrow 3')$	Exon/Intron	Amplicon size (bp)
F14	5'ATGCAGACACATACGGGGA3'	Exon 1	623
R14	5'AGTCAGGGTCCGGAACAG3'		
F9	5'CTGCCTAGCACCATCTGGAC3'	Exon 5,6	672
R9	5'GAGGGACTCGACACTCCA3'		
F10	5'TGGAGTGTCGAGTCCCTCA3'	Exon 7–9	877
R10	5'ATTGAGCTGCGTTGAGGC3'		
F11	5'ATGCGTACTATGTCACCG3'	Exon 9,10	905
R11	5'ATTCCAGGCCTAACTGACAG3'		
F12	5'TGGCAGCTGATGTGTCCTCG3'	Exon 11	765
R12	5'GCATAGGGGAAGAAGCGTTCG3'		
F13	5'ATGACCTGGGTGTCTGTTGC3'	Exon 18	458
R13	5'GAAAGGCGCACACCAATGC3'		
F1	5'ATGAGACCTTCGGCGAGTACAG3'	Exon 26 to intron 27	289
R1	5'GTGTTGGCCTCTCCCTGAAATGA3'		

Table 1. Human *L1CAM*-specific primers used in the study.

The PCR reaction contains 200 ng of genomic DNA, 10 μ M of each primer and PCR master mix (TAKARA) and the final reaction volume was set to 25 μ l. PCR amplification was carried out for 35 cycles, each cycle consisting of denaturation at 94 °C for 10 seconds, annealing at 57 °C for 30 seconds, and extension at 72 °C for 1 minute. Initial denaturation was carried out at 94 °C for 5 minutes and the final extension was carried out at 72 °C for 5 minutes. The amplified DNA product was confirmed on 2% agarose gel, with a 100 bp DNA ladder.

2.6. Sanger sequencing

The PCR amplified product was sequenced using Applied Biosystems genetic analysis systems series 3500 at Eurofins Analytical Services India Private Limited, Bengaluru. The sequencing was employed in both forward and reverse directions of each template and the sequence data was analysed by using the "BioEdit" tool [22].

3. Results

The pedigree of the family is presented in Figure 3, and it is revealed that the affected boy born to consanguineous parents (II:3 Father and II:4 Mother). According to family history, an individual (pedigree II 5) who was a maternal uncle of the proband was found to have suffered from mental retardation and walking disability and died at the age of 12 years.



Figure 3. Pedigree of the family. II:4 indicates the carrier mother, II:5 indicates the deceased uncle and III:1 is the proband in this study.

PCR amplification was performed using the DNA of the proband with the primer sets F10 & R10 designed specific to region between exon 7 to 9 (Table 1). The primer pairs were designed using the genomic sequence for *L1CAM* (NM_000425.5) and the PCR product of 877 bp was obtained (Figure 4).



Figure 4. PCR profile of the amplified DNA of the patient (B10), control sample (Contr), L-100 bp Ladder.

The sequencing result of the participated members has revealed that the father II:3 have wild type variant (Figure 5A), whereas the mother II:4 was a carrier with a mutated variant (Figure 5B). Mutation analysis revealed that III:1 has inherited the mutated variant from his carrier mother denoted as G1120 \rightarrow T (Figure 5C). The G1120 \rightarrow T is a transversion of G (Guanine) to T (Thymine) at position 1120 which results a STOP codon in the exon 9 of the *L1CAM* gene. This nonsense variant yielded truncated L1 protein (E374X) at Immunoglobulin four domain (Ig4) in the extracellular portion (Figure 6). This nonsense variant (G1120 \rightarrow T) was not found in any control individuals and was identified to be deleterious as shown by PROVEAN (-3.192), probably damaging shown by PolyPhen2 (0.999) and decrease stability shown by MUpro (0.971).



Figure 5. DNA sequence analysis of exon 9 of the *L1CAM* gene. (A) Chromatogram shows the wild type sequence in healthy father; (B) Chromatogram shows mutation in carrier mother; (C) chromatogram shows the novel nonsense mutation $G1120 \rightarrow T$ identified in hydrocephalus patient.



Figure 6. Structure of L1 protein with six immunoglobulin (Ig) domains, five fibronectin (Fn) type 3 domains, a short transmembrane region, and a cytoplasmic domain. Transversion of G (Guanine) to T (Thymine) at position 1120 (G1120 \rightarrow T) in the Ig 4 extracellular domain (black arrow) has resulted the conversion of Glutamic acid to STOP codon at position 374 (E374X).

4. Discussion

The X-linked hydrocephalus is one of the major disorders among spectrum of L1 syndrome conditions including mental retardation, aphasia, spasticity, and adducted thumbs and shows an X-linked recessive mode of inheritance [3,23]. Many studies have revealed a correlation between the numbers of clinical characteristics, the number of affected family members and the chance of finding mutation. And also reported that there is a genotype-phenotype correlation between the severity of the disease and the type of *L1CAM* variant [2,3,24].

L1CAM is the transmembrane glycoprotein with six Ig-like domains followed by five fibronectin type III domains, a single-pass transmembrane region, and a cytoplasmic domain (Figure 6). The heterogeneity in phenotype and variety of *L1CAM* mutations are characteristics of the L1 syndrome. *L1CAM* mutations are classified into 4 distinct classes: Class I includes mutations in the extracellular region of L1 protein lead to truncation or absence of the protein. Class II includes missense mutations resulting in an amino acid substitution in the extracellular region of L1. Class III includes mutations in

the cytoplasmic domain. Class IV comprising extracellular mutations resulting in aberrant splicing of pre mRNA [24,25]. Because of the class I mutations the L1 protein would not remain integrated into the cell membrane and therefore loss of all normal functions. This leads to development of severe phenotypes like Agenesis of the corpus callosum, adducted thumbs, spastic paraplegia and aphasia. The Yamasaki et al. [25], and Fransen et al. [24], pointed out a striking correlation between the mutation class and severity of the phenotypes.

The proband included in this study was belonged to a family with one affected male in generation II of the pedigree (Figure 3), the II-5 is a maternal uncle presented with mental retardation and walking disability, which are typical features of the L1 syndrome.

Patient carried a mutation showing G \rightarrow T transversion at position 1120 (G1120 \rightarrow T) of exon 9 of the *L1CAM* gene (Figure 5C) creating stop codon. Exon 9 encodes the fourth immunoglobulin domain (Ig4) and this nonsense mutation led to a change of glutamic acid to STOP codon (GAG \rightarrow TAG) in position 374 (E374X) in the extracellular portion (Figure 6). This mutation has truncated the L1 protein because of the stop codon, leading to the disruption in the interaction between L1 molecules, and resulted in abnormal development of the brain. Previous studies have suggested that the mutations in this region manifest with variable clinical symptoms from Hydrocephalus to MASA syndrome [2,10,26,27]. The function of the glutamic acid is to activate the protein or binding sites and it acts as an important neurotransmitter, and very important for brain development [28]. Based on the classifications suggested by Yamasaki et al. [25], and Fransen et al. [24], this extracellular truncated mutation E374X represents class I mutations.

5. Conclusions

The present investigation has resulted in the identification of a novel nonsense pathogenic variant E374X in *L1CAM* gene in a proband belonging to a family presented with consanguineous marriage. The proband's mother clearly shows the heterogeneity of the pathogenic variant G1120 \rightarrow T. This novel nonsense mutation has truncated the L1CAM protein at extracellular portion at Ig4 domain. Since the identification of L1 syndrome patients and L1CAM mutations in Indian cohorts is very less, the testing for carrier status and prenatal molecular diagnosis for high-risk pregnancies are recommended to prevent the recurrence of the identified pathogenic variants. Identification of the novel mutations in the *L1CAM* gene helps to understand the pathogenesis of the L1 syndrome.

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Conflict of interest

The authors declare no conflict of interest for this study.

References

- 1. Bickers D, Adams R (1949) Hereditary stenosis of the aqueduct of Sylvius as a cause of congenital hydrocephalus. *Brain* 72: 246–262.
- 2. Finckh U, Schroder J, Ressler B, et al. (2000) Spectrum and detection rate of *L1CAM* mutations in isolated and familial cases with clinically suspected L1-disease. *Am J Med Genet* 92: 40–46.
- 3. Fransen E, Camp GV, Vits L, et al. (1997) L1-associated diseases: clinical genetics divide, molecular genetics unite. *Hum Mol Genet* 6: 1625–1632.
- 4. Samatov TR, Wicklein D, Tonevitsky AG (2016) L1CAM: Cell adhesion and more. *Prog Histochem Cytochem* 51: 25–32.
- 5. Kamiguchi H, Hlavin ML, Lemmon V (1998) Role of L1 in Neural Development: What the Knockouts Tell Us. *Mol Cell Neurosci* 12: 48–55.
- 6. Moos M, Tacke R, Scherer H, et al. (1988) Neural Adhesion Molecule L1 as a Member of the Immunoglobulin Superfamily with Binding Domains Similar to Fibronectin. *Nature* 334: 701–703.
- 7. Mikulak J, Negrini S, Klajn A, et al. (2012) Dual REST-dependence of L1CAM: from gene expression to alternative splicing governed by Nova2 in neural cells. *J Neurochem* 120: 699–709.
- 8. Bertolin C, Boaretto F, Barbon G, et al. (2010) Novel mutations in the *L1CAM* gene support the complexity of L1 syndrome. *J Neurosci* 294: 124–126.
- 9. Okamoto N, Del Maestro R, Valero R, et al. (2004) Hydrocephalus and Hirschsprung's disease with a mutation of L1CAM. *J Hum Genet* 49: 334–337.
- 10. Vos YJ, De Walle HE, Bos KK, et al. (2010) Genotype-phenotype correlations in L1 syndrome: a guide for genetic counseling and mutation analysis. *J Med Genet* 47: 169–175.
- 11. L1CAM mutation database. Available from: http://www.l1cammutationdatabase.info/default.aspx.
- 12. Kanemura Y, Takuma Y, Kamiguchi H, et al. (2005) First case of *L1CAM* gene mutation identified in MASA syndrome in Asia. *Congenital Anomalies* 45: 67–69.
- 13. Marin R, Ley-Martos M, Gutierrez G, et al. (2015) Three cases with L1 syndrome and two novel mutations in the *L1CAM* gene. *Eur J Pediatr* 174: 1541–1544.
- 14. Ochando I, Vidal V, Gascon J, et al. (2015) Prenatal diagnosis of X-linked hydrocephalus in a family with a novel mutation in *L1CAM* gene. *J Obstet Gynaecol* 36: 403–405.
- 15. Silan F, Ozdemir I, Lissens W (2005) A novel *L1CAM* mutation with L1 spectrum disorders. *Prenat Diagn* 25: 57–59.
- 16. Camp GV, Vits L, Coucke P, et al. (1993) A Duplication in the *L1CAM* Gene Associated with X-Linked Hydrocephalus. *Nat Genet* 4: 421–425.
- 17. Fransen E, Lemmon V, Camp VG, et al. (1995) CRASH syndrome: clinical spectrum of corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraparesis and hydrocephalus due to mutations in one single gene, L1. *Eur J Hum Genet* 3: 273–284.
- 18. Swarna M, Sujatha M, Usha Rani P, et al. (2004) Detection of L1 CAM mutation in a male child with Mental retardation. *Indian J Clin Biochem* 19: 163–167.
- 19. Jharna P, Hemabindu L, Siva Prasad S, et al. (2006) Detection of L1 (CAM) mutations in X-linked mental retardation: A study from Andhra Pradesh, India. *Indian J Hum Genet* 12: 82–85.
- 20. QIAamp DNA Mini Blood Mini Handbook. Available from: https://www.qiagen.com/us/resources/resourcedetail?id=62a200d6-faf4-469b-b50f-2b59cf738962&lang=en.

- 21. NCBI Primer-Blast. Available from: https://www.ncbi.nlm.nih.gov/tools/primer-blast/.
- 22. BioEdit tool. Available from: http://www.mbio.ncsu.edu/BioEdit/bioedit.html.
- 23. Rosenthal A, Jouet M, Kenwrick S (1992) Aberrant splicing of neural cell adhesion molecule L1 mRNA in a family with X-linked hydrocephalus. *Nat Genet* 2: 107–112.
- 24. Fransen E, Camp GV, Hooge RD, et al. (1998) Genotype-phenotype correlation in L1 associated diseases. *J Med Genet* 35: 399–404.
- 25. Yamasaki M, Thompson P, Lemmon V (1997) CRASH syndrome: mutations in *L1CAM* correlate with severity of the disease. *Neuropediatrics* 28: 175–178.
- 26. De Angelis E, Watkins A, Schafer M, et al. (2002) Disease-Associated Mutations in L1CAM Interfere with Ligand Interactions and Cell-Surface Expression. *Hum Mol Genet* 11: 1–12.
- 27. Kaepernick L, Legius E, Higgins J, et al. (1994) Clinical aspects of the MASA syndrome in a large family, including expressing females. *Clin Genet* 45: 181–185.
- 28. Betts MJ, Russell RB (2003) Amino acid properties and consequences of substitutions. *Bioinf Genet* 14: 289–316.



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