A Heterozygous *STXBP1* Gene *de novo* Mutation in an Iranian Child With Epileptic Encephalopathy: Case Report

Masoud Heidari[1], Morteza Soleyman-Nejad[2], Mohammad Hossein Taskhiri[2,3], Alireza Isazadeh[4], Manzar Bolhassani[2], Javad Shahpouri[5], Mansour Heidari[2,6], Nahid Sadighi[7]

[1] *Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran*

[2] *Ariagene Medical Genetics Laboratory, Qom, Iran*

[3] *Department of Molecular Biology, Islamic Azad University of Qom, Qom, Iran*

[4] *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[5] *Pediatric Clinical Research of Development Center, Qom University of Medical Sciences, Qom, Iran*

[6] *Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[7] *Advanced Diagnostic and Interventional Radiology Research Center (ADIR), Tehran University of Medical Sciences, Tehran, Iran*

Received: 28 Jan. 2019; Accepted: 28 Jul. 2019

Abstract- The Syntaxin Binding Protein 1 (*STXBP1*) plays an important role in regulating neurotransmitter release and synaptic vesicle fusion through binding to syntaxin-1A (*STX1A*) and changing its conformation. In this study, we identified a de novo mutation (c.C1162T: p.R388X) in exon 14 of the *STXBP1* gene causing an epileptic encephalopathy, early infantile, non-epileptic movement, and unclassified infantile spasms disorders in a 5-year-old boy by whole-exome sequencing. The segregation of this genetic variant was examined in the patient as well as in his parents. We found the R388X in heterozygous state in the proband but not in his parents. This genetic change could be due to de nova mutation or germlinemosaicism.

Acta MedIran 2019;57(8):518-521.

Keywords: Syntaxin binding protein 1 (*STXBP1*) gene; Mutation; Epileptic encephalopathy

Introduction

Encephalopathy with epilepsy is a condition characterized by recurrent seizures (epilepsy), abnormal brain function (encephalopathy), and intellectual disability. The signs and symptoms of this condition typically begin in infancy but can first appear later in childhood or early adulthood. In many affected individuals, seizures stop in early childhood, with other neurological problems continuing throughout life. However, some people with *STXBP1* encephalopathy with epilepsy have seizures that persist. The prevalence of *STXBP1* encephalopathy with epilepsy is unknown. At least 200 individuals with this condition have been described in the medical literature (1,2).

The *STXBP1* gene contains 20 exons, is located on chromosome 9 (9q34.11), which encodes *STXBP1* protein. This *STXBP1* protein plays an important role in regulating neurotransmitter release and synaptic vesicle fusion through binding to syntaxin-1A (*STX1A*) and changing its conformation (3). Assessing the role of *STXBP1* mutations in Dravet syndrome (4), spasticity, and childhood-onset ataxia (5) has shown a progressive and extensive disease phenotype. The extensive phenotype of *STXBP1* encephalopathy is maybe due to the involvement of *STXBP1* in the synaptic release of neurotransmitters, which reduced production of *STXBP1's* protein product, syntaxin-binding protein 1 and syntaxin-1 with a heterozygous mutation (6).

Here, we report a 5-year-old boy who referred to diagnostic evaluation of speech regression, intractable epilepsy, resting tremor, episodic ataxia, following a period of apparently normal early development.

Methods and Materials

Patients and sample collection

In the present study, a 5-year-old Iranian male,

Corresponding Author: M. Heidari*, N. Sadighi**
* Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 88953005, Fax: +98 21 88953005, E-mail address: mheidari@sina.tums.ac.ir
** Advanced Diagnostic and Interventional Radiology Research Center (ADIR), Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 61192501, Fax: +98 21 88953005, Email address: sadighii@yahoo.com

referred to Ariagene Medical Genetics Laboratory (Qom-Iran), was investigated in February 2019. In this family ,the proband was the offspring of consanguineous marriage. Also, the 100 healthy age and ethnically matched subjects were selected as healthy controls. To exclude the epidemiological bias, the healthy controls were selected from the population of Qom-Iran, which were unrelated genetically, and matched age and ethnically. According to the ethical standards of the Declaration of Helsinki, the patient and his parents, and all healthy controls were informed about the study and informed consent was signed. The study was performed with the approval of the Institutional Review Board (IRB) and informed consent was obtained from a patient, or authorized representative/guardian, and controls before genetic testing. The pedigree of the patient was drawn (Cyrillic 2.1 software) to determine the inheritance pattern of the disease.

Genomic DNA extraction and whole-exomesequencing (WES)

In the present study, five mL peripheral blood sample was drawn from the patient, his parent, and all healthy controls. Genomic DNA extraction was performed using a DNA purification kit (Roche, Switzerland) according to the manufacturer's instructions as described previously (7). The quantity of extracted genomic DNA samples was evaluated using a nanodrop instrument (Thermo Fisher Scientific, USA) and the OD 260/280 ratio between 1.7-1.9 was selected for molecular study. Also, the quality of extracted genomic DNA samples was evaluated using electrophoresis on 1% agarose gel. The extracted genomic DNA samples with desirable quantity and quality were selected and stored at -20° C until molecular analysis. Whole Exome Sequencing (WES) was employed to enrich all genomic coding regions and some important other genomic regions. Next-generation sequencing was conducted to sequence close to 100 million reads on Illumina Sequencer (Illumina, San Diego, CA, USA). In general, the test platform examined >95% of the targeted with a sensitivity of above 99%. In this point mutations and micro-insertion/deletions and duplication (<20bp) can be simultaneously detected. Bioinformatics analysis of the sequencing results was performed using international databases. Genetic variants such as point mutations and indels were identified using SAMtools and annotated by ANNOVAR software. A candidate gene was considered a variant that fulfilled the following criteria: (i) missense, nonsense, frameshift, and splice-site variants, (ii) absent or rare (frequency below 1%) in the two databases (dbSNP, 1000 G), and (iii) homozygous variants in the patient.

Confirmation and validation of WES results by sangersequencing

The target exon containing mutation of *STXBP1* gene was typically amplified using 10 pmole of primer, 0.2 U Taq DNA polymerase (Roche, Mannheim, Germany), 200 µM of each dNTPs, 0.67 µl of 50 mM $MgCl_2$, 60 ng DNA and 2.5 µl of PCR buffer in 25 µl of PCR reactions. The PCR conditions included an initial denaturation step for 3 min at 95° C, 30 sec at 95° C, 30 sec at 60° C with a 1° C at 72° C for 35 cycles, and finally 10 min at 72° C. The PCR products were separated on 2% agarose gels and visualized GelGreen® stained. Subsequently, to confirm the identified mutation, the PCR products were subjected to direct sequencing. Then, the PCR products were sequenced on an ABI 3130 automated sequencer (Applied Biosystems, Forster City, CA, USA). Sequence data searches were performed in non-redundant nucleic and protein databases BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Case Report

The proband presented to the Ariagene Medical Genetics Laboratory (Qom-Iran) at the age of 5 years for evaluation of developmental delay and seizures. He was a male who was born at 38 weeks of pregnancy to a 23-year-old mother by cesarean section. The electroencephalogram showed seizures in the left temporal lobe. Also, he was suffering episodic ataxia since infancy. His epilepsy became refractory to medical treatment despite multiple antiepileptic therapies. He showed normal audiology and normal echocardiogram evaluation and without gastrointestinal, renal or growth. Pedigree of three-generation was normal development and health. His parents were healthy, full, and recognized consanguinity.

The examination of the clinical laboratory, such as glycan analysis guanidinoacetate analysis, vitamin E, pyruvate, urine creatine, very-long-chain fatty acids, phenylalanine, urine oligosaccharide, white cell enzymes, alpha-fetoprotein, and serum lactate was all normal. The next-generation sequencing of the mitochondrial DNA genome in skeletal muscle revealed no pathogenic mutation and polymorphism. Also, an analysis of his karyotype revealed no chromosomal alterations or mutation.

The genomic DNA of whole blood from the proband and his parents was extracted using a DNA purification kit (Roche, Switzerland), and sequenced using Illumina Sequencer (Illumina, San Diego, CA, USA). A heterozygous nonsense mutation (c.C1162T, p.R388X) in exon 14 of the *STXBP1* gene was identified, which was not inherited from his father or mother. Sanger sequencing was used to confirmation of parental health for this mutation. Therefore, the identified mutation of *STXBP1* gene in the proband occurred de novo (Figure 1). This mutation was not observed in either the ESP6500 public or 1000genome databases and predicted to be pathogenic by LRT, PolyPhen-2, SIFT and Mutation Taster algorithms.

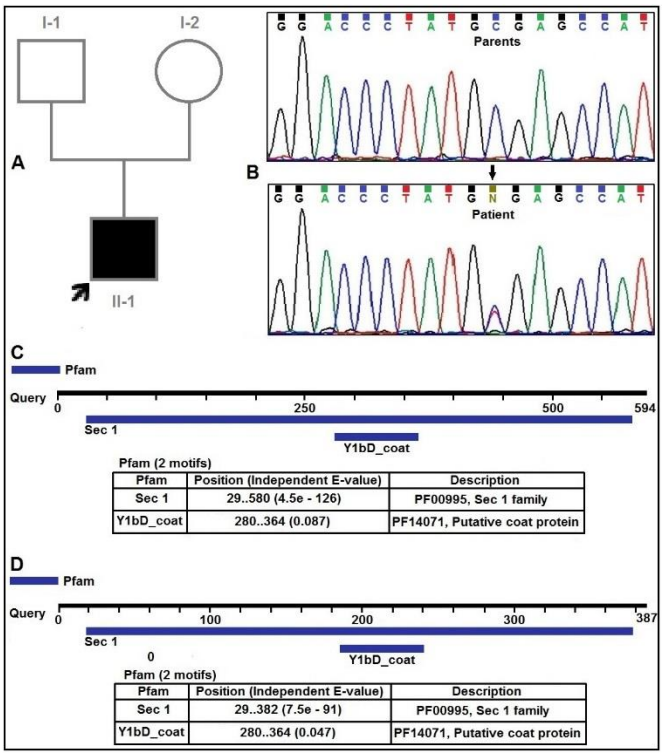

Figure 1. Pedigree analysis and molecular evaluation of a patient with *STXBP1* gene mutation. (A) Family pedigree indicating STXBP1 mutation status and phenotype. (B) Sanger sequencing of the *STXBP1* gene. (C) In the normal form, the number of amino acids 594. (D) In the form of mutants due to mutation, the number of amino acids decreased by 387

In-Silico study

We subjected the identified variants in *STXBP1* protein to two different bioinformatics tools, including SIFT and PolyPhen-2, to investigate whether these variants have any biological effect on *STXBP1* protein. Based on SIFT findings, genetic variants scoring tolerance index (TI) of ≤0.05 are considered intolerant. PolyPhen-2 results predicted can be classified into three categories, probably damaging, possibly damaging and benign genetic changes.

Discussion

In the present study, we report a de novo nonsense mutation in exon 14 of the *STXBP1* gene, which was associated with vary widely neurodevelopmental disorders in a 5-year-old boy from Iranian families. The identified de novo mutation (c.C1162T, p.R388X) was found in the patient in heterozygosis form, which his mother and father were negative for the mentioned mutation. This mutation is located in the domain 3 *STXBP1* protein, which interacts with domain 1 and creates a central cavity to provide a surface for binding of syntaxin-1 (8). The c.C1162T, p.R388X mutation causes to truncate *STXBP1* protein toward the middle, as a result, removed a substantial portion of the domain 1, which interacted with syntaxin-1 (domains 1/3) (8). This mutation was a previously reported pathogenic heterozygous stop gain mutation, which leads to encoding a shorter protein. The proband with the mentioned mutation showed early developmental delay and onset seizures, which was a phenotype that showed in other previously reported cases (9).

In a study by Hamdan *et al.*, (2009) reported the

same mutation (c.C1162T, p.R388X) with overlapping features as our proband (10). This reported patient is a 15 years old French-Canadian female. The progression of epilepsy in this reported patient is similar in onset to our study. These both reported patients have ataxia findings and intellectual disabilities, which are common in this disease (10).

This mutation (c.C1162T, p.R388X) eliminates the Arginine codon in mRNA at exon 14. At the protein, this mutation leads to complete loss of domain 2 and part of domain 3b in STXBP1 protein. The domains 1 and 3a form the central cavity providing the binding surface for syntaxin-1. Therefore, the identified mutation should not affect STXBP1 binding to STX1A (7). The literature describes mutations in the same functional domain as STXBP1, which points to the pathogenicity of this domain (11,12).

In general, the association between various mutations *STXBP1* gene and their pathological implications remain unknown. Therefore, additional studies are required for identifying the cellular function of the *STXBP1*.

Acknowledgments

We would like to thank MS Zahra Karimi and MS Zahra Shiri for their kind cooperations in *STXBP1* mutational analysis. Also, the authors would like to thank the patient and the family members who have participated and collaborated with this study.

References

1. Saitsu H, Kato M, Mizuguchi T, Hamada K, Osaka H, Tohyama J, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nat genet 2008;40:782-8.
2. Barcia G, Chemaly N, Gobin S, Milh M, Van Bogaert P, Barnerias C, Kaminska A, Dulac O, Desguerre I, Cormier V, Boddaert N. Early epileptic encephalopathies associated with STXBP1 mutations: could we better delineate the phenotype? Eur J Med Genet 2014;57:15-20.
3. Gerber SH, Rah JC, Min SW, Liu X, de Wit H, DulubovaI, Meyer AC, Rizo J, Arancillo M, Hammer RE, Verhage M. Conformational switch of syntaxin-1 controls synaptic vesicle fusion. Science 2008;321:1507-10.
4. Carvill GL, Weckhuysen S, McMahon JM, Hartmann C, Møller RS, Hjalgrim H, Cook J, Geraghty E, O'Roak BJ, Petrou S, Clarke A. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. Neurology 2014;82:1245-53.
5. Deprez L, Weckhuysen S, Holmgren P, Suls A, Van Dyck T, Goossens D, Del-Favero J, Jansen A, Verhaert K, Lagae L, Jordanova A. Clinical spectrum of early-onset epileptic encephalopathies associated with STXBP1 mutations. Neurology 2010;75:1159-65.
6. Patzke C, Han Y, Covy J, Yi F, Maxeiner S, Wernig M, Südhof TC. Analysis of conditional heterozygous STXBP1 mutations in human neurons. J Clin Invest 2015;125:3560-71.
7. Sadeghipour N , Lotfiyani F, Raoofian R, Noori-Daloii MR , Azimi C, Heidari M. Investigation of mRNA Expression Levels of TGIFLX and OCT1 Homeobox Genes in Colorectal Cancer. Acta Medica Iranica 2018; 56:653-659.
8. Misura KM, Scheller RH, Weis WI. Three-dimensional structure of the neuronal-Sec1–syntaxin 1a complex. Nature 2000;404:355-62.
9. Stamberger H, Weckhuysen S, De Jonghe P. STXBP1 as a therapeutic target for epileptic encephalopathy. Expert OpinTher Targets 2017;21:1027-36.
10. Hamdan FF, Piton A, Gauthier J, Lortie A, Dubeau F, Dobrzeniecka S, Spiegelman D, Noreau A, Pellerin S, Côté M, Henrion E. De novo STXBP1 mutations in mental retardation and nonsyndromic epilepsy. Ann Neurol 2009;65:748-53.
11. Weimer RM, Richmond JE, Davis WS, Hadwiger G, Nonet ML, Jorgensen EM. Defects in synaptic vesicle docking in unc-18 mutants. Nat neurosci 2003;6:1023-30.
12. Ortega-Moreno L, Giraldez BG, Verdú A, Garcia-Campos O, Sánchez-Martín G, Serratosa JM, et al. Novel mutation in STXBP1 gene in a patient with non-lesionalOhtahara syndrome. Neurología 2016;31:523-7.