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

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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 germlinmosaicism. © 2019 Tehran University of Medical Sciences. All rights reserved. Acta MedIran 2019;57(8):518-521.

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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,
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-exome sequencing (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, Mannheim, Germany). 200 μM of each dNTPs, 0.67 μl of 50 mM MgCl₂, 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.
De novo mutation in STXBP1 gene

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](image)

**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 lead 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.

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References