Shokoohi et al., J Genet Disor Genet Rep 2018, 7:3
DOI: 10.4172/2327-5790.1000177

Journal of Genetic Disorders & Genetic Reports

Case Report

A SCITECHNOL JOURNAL

Whole Exome Sequencing found a Novel Truncating Mutation within *CNTNAP2* Gene in an Iranian Patient with Mental Retardation

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Received date: June 30, 2018; **Accepted date:** August 21, 2018; **Published date:** August 28, 2018

Abstract

Intellectual disability (ID) is a major health problem mostly with an unknown etiology, affecting 1–3% of the general population. Discovering the genetic cause of these cases is dramatically challenging. Nowadays, the next-generation sequencing (NGS) technology provides advantages for the genetic diagnosis of ID. Here, by the help of Whole exome sequencing, we report an Iranian family with a child having non-syndromic ID (NS-ID) with autistic behaviors and seizure. The results revealed a novel nonsense mutation (c.3283CGA>TGA) located in an autozygous region on chromosome 7, leading to an amino acid change to terminal codon (p.Arg1095*) in CNTNAAP2. The affected child was homozygous for the mutation and his parents were heterozygous as expected in autosomal recessive inheritance. In conclusion, our study identified a novel mutation in *CNTNAAP2* gene, and showed that WES provides advantages for detecting novel ID associated variants and can greatly facilitate the genetic diagnosis of the disease.

Keywords: Intellectual disability; Whole exome sequencing; *CNTNAP2* gene; Novel nonsense mutation

Introduction

Intellectual disability (ID), a neurodevelopmental disorder, is demonstrated by considerable limitations not only in intellectual functioning but also in adaptive behavior that appears before the age of 18 [1-3]. However, although there is a higher ratio of males to females (Approximately 30%) among milder cases of ID, the ratio decreases followed by intelligence quotient (IQ) reduction [4-6]. Despite the classification of ID by moderate to severe /IQ level, ID can also be divided into two parts; syndromic intellectual disability (SID) and non-syndromic intellectual disability (NS-ID) [7]. On the one hand, In SID, patients suffer from clinical features or co-morbidities in addition to ID like for instance autism, Fragile X syndrome, and so on. On the other hand, there is discussion and challenge about the categorization of NS-ID [8-11].

In all of these cases, non-genetic factors (e.g., infection, trauma and brain damage, prenatal exposure to alcohol and other drugs, environmental exposure to toxins, psychiatric conditions and so on) cannot lead to cognitive impairment as severe as forms of ID with genetic factors and fundamental mutations ranging from large cytogenetic abnormalities to point mutations and even including epigenetic alterations [12,13]. In the past few years, hundreds of genes have been identified which is related to both SID and NS-ID [7,14,15]. However, it has been a challenge to discover genetic cause of individuals with intellectual disability without associated phenotypes [16,17].

Nowadays, exome sequencing which is a variant of next-generation sequencing (NGS) technology focuses on the coding regions of the genome, improves the efficiency of molecular diagnosis and contributes to discover novel mutations present in either sporadic or familial cases with non-specific phenotypes [18-20].

In summary, in this study, we introduced the genetic diagnosis of an Iranian family with one affected child who suffers from Mental retardation by Whole exome sequencing (WES). To our knowledge, this family might be another case of mental retardation caused by mutations located in the Contactin Assosiated Protein-Like 2 (*CNTNAP2*) gene (OMIM*604569). The data can provide the evidence of contribution for this mutation in further diagnosis.

Case Presentation

Clinical information of the patient

The family has two daughters that seem to be normal individuals without any clinical signs, and one son with features of mental retardation. These children were born of a consanguineous marriage (Figure 1). The patient had an abnormal behavior with below-normal IQ and impairment of adaptive skills. He was born after normal pregnancy, and his growth parameters were within the normal ranges. He had seizure several times since he was 2 years old. Afterward, he had language developmental delay and his language skill was limited to single words. Another serious problem included autistic behaviors which were displayed in this case. He started to walk at the age of 18 months and his muscle tonus was within normal.

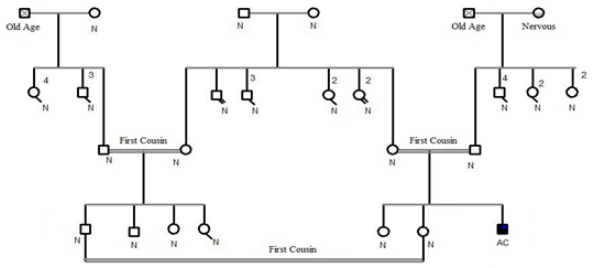

Figure 1: The pedigree of Iranian family with mental retardation patient.

DNA isolation

Genomic DNA (gDNA) was isolated from peripheral blood of the proband and family members by Magcore HF16/HF48/Compact Nucleic Asid Extraction Kit.

Whole exome sequencing

DNA extracted from case 1 was sent to Macrogene company (South Korea) for whole exome sequencing to reveal if pathogenic variants exist.

Data analysis and validation

The Mutation Taster (http://www.mutationtaster.org), CADD, and DANN were applied to predict if the variants are pathogenic. Then, the variants were traced through the family members.

Results

Our patient had motor delay and limitations in motor skills as well as limited language comprehension. There were no distinguishing physical features. Severe intractable seizures began after 2 years old. He showed aberrant social interactions, mental retardation as weel as autistic features.

The in-silico analysis gave information about a couple of candidate variants which were identified by WES data. Eventually, Sanger validation followed by co-segregation analysis were successfully utilized to detect a novel nonsense mutation in *CNTNAP2* gene (p.Arg1095*), which mapped to exon 20 of the *CNTNAP2* gene in the patient (Figure 2). It was found that the parents of the patient were obligate carriers of this mutation (Table 1).

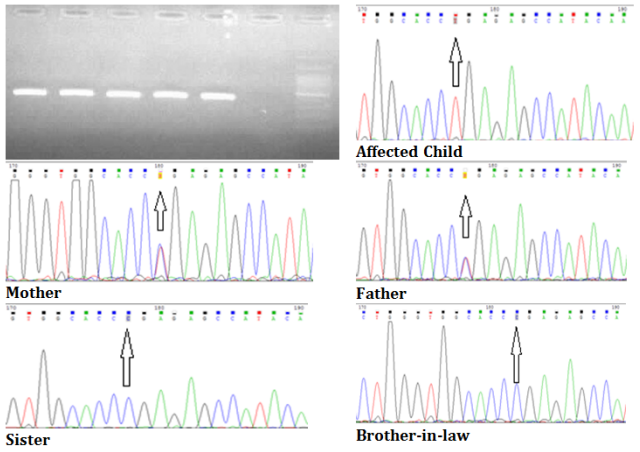

Figure 2: PCR products and its Sanger sequencings.

As can be seen from figures, fragments with 400 base pair in length were extended by PCR method. Afterwards, they were sequenced by sanger sequencing method. Regarding the sequences, a homozygote mutation in *CNTNAP2* gene (p.Arg1095*) was detected in the patient, and the parents of the patient were obligate carriers of this mutation. Additionally, the patient's sister and her husband were normal for this mutation.

Case No.	Sex	Age	Amino acid change	Nucleotide change	Zygosity
1	Male	-	Arg1095*	c.3283CGA>TGA	Het
2	Female	-	Arg1095*	c.3283CGA>TGA	Het
3	Male	17 yr	Arg1095*	c.3283CGA>TGA	Homo

In silico analysis of the variant:

Different *in silico* analysis softwares including SIFT, PROVEAN, CADD, and mutation taster predicted that the variant is pathogenic. The novel variant was neither found in EXAC nor 1000 genome browser (Table 2).

Prediction software	Prediction
Mutation taster	Disease causing
CADD	Deleterious
DANN	Deleterious

Discussion and Conclusion

Intellectual disability (ID) is a widespread neurodevelopmental disorder characterized by low IQ (below 70). Prevalence of ID varies from 1–3% [21-23]. To identify genetic causes of ID in the last decade, it has been suggested that Whole Exome Sequencing (WES) is a single experiment which can check nearly all the coding content of the genome and the region likely to contain most of the disease-causing mutations [24-26]. Apart from this, there is a controversial issue whether functional studies are necessary to validate the causative role of the newly identified mutations [27,28].

In this study, we present the results of exome sequencing in a consanguineous Iranian ID patient. He had intellectual disability, speech problems and seizure. However, he did not have any Brain imaging. Candidate gene sequencing followed by exome and sanger sequencing identified a novel homozygous nonsense mutation (Chr.7: p.Arg1095*), in *CNTNAP2* gene that causes mental retardation, autistic behaviors and seizure, as the two unaffected parents share this rare heterozygous mutation in heterozygous form.

Recent studies have reported: 1) a deletion mutation related to cortical dysplasia, focal epilepsy, relative macrocephaly, and diminished deep-tendon reflexes [29], 2) homozygous or compound heterozygous mutations with clinical features like mental retardation, seizures, and hyperbreathing patterns, reminiscent of Pitt-Hopkins syndrome [30], 3) and homozygous or compound heterozygous truncating mutations and/or intragenic deletions [31] in the *CNTNAP2* gene. Further, it was identified a common variant in the CNTNAP2 gene that was associated with increased risk for autism [32-34]. Pitt-Hopkins-like syndrome-1 (PTHSL1) is an autosomal

recessive neurodevelopmental disorder characterized by delayed psychomotor development, intellectual disability, severe speech impairment or regression, and behavioral abnormalities. Most patients have onset of seizures within the first years of life. Some patients may have cortical dysplasia on brain imaging [31]. Autism, the prototypic pervasive developmental disorder (PDD), is usually apparent by 3 years of age. It is characterized by a triad of limited or absent verbal communication, a lack of reciprocal social interaction or responsiveness, and restricted, stereotypic, and ritualized patterns of interests and behavior [35,36]. As previously mentioned, although the biological function of the gene has not been well studied, knowledge about this novel mutation will provide insights that will increase our understanding of ID development. All in all, there is no doubt that finding the broad range of new mutations can help genetic counseling in families with affected individuals, particularly with ID patients.

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