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Original Article

A Novel Hemizygous Variant in the *AFF2* Gene Causing Fragile XE (FRAXE) Syndrome: First Report from Pakistan

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Abstract

Background: Fragile XE (FRAXE) is an X-linked recessive condition that affects 1 in 50,000 of new born males with intellectual disability (ID). It is characterized by mild Intellectual disability (ID), speech delay cognitive impairment, and in some cases with phenotypes of Autism Spectrum disorder (ASD).
Methodology: In this study, a family was investigated with two male siblings having neuro developmental delay. Whole exome sequencing analysis (WES) was carried out to identify the pathogenic variant. Sanger sequencing was performed in normal and affected family members and co-segregation analysis was done.
Results: Two probands were affected in a family diagnosed with intellectual disability. A novel hemizygous variant (c.3348G>T, p.Asp1150Tyr) in *AFF2* gene was identified as the causal variant cause in affected individuals. This variant was novel from Pakistani population.
Conclusion: In this study, a novel hemizygous variant (c.3348G>T, p.Asp1150Tyr) identified in *AFF2*. These findings paved the way for further studies on genetic and clinical spectrum of rare X-linked recessive disease involved in ID.
Key words: *AFF2*, hemizygous, intellectual disability, neurological disorders.

Introduction

Cognition is the result of cellular, biological and multiple molecular events in the nervous system. Minor defect in any of these events can results in intellectual disability or cognitive impairment.[1,2] It can also be termed as neuro-development disorder as it results from defect in synapse formation.[3,4]

Overall, in general population, the prevalence of intellectual disability (ID) is 2-3%.[5,6] Its clinical and genetic heterogeneity make the diagnosis challenging for scientists and physicians.[7,8] More than 900 genes are reported to cause intellectual disability so far.[9,10].

The X-linked intellectual disability is a heterogeneous group of genetic disorders. There are more than 141 genes linked with disease located on x-chromosome.[11] Its prevalence is high in males as x-chromosome genes contribute to cognition. Among x-linked intellectual disability, Fragile X syndrome (FXS) is common and characterized by moderate to severe disability (OMIM - 309548).[12] There is high repeat expansion of a CGG in *FMR1* gene which cause methylation, that ultimately halts the production of *FMR1* protein leads to (FXS) syndrome.[13]

The *AFF2* gene (also as *FMR2* gene) cause non-specific x-lined intellectual disability with prevalence as 1/25,000 to 100,000 in new born male. Micro deletion in *AFF2* genes leads to Fragile XE (FRAXE) syndrome [14]. It is characterized by mild to moderate intellectual disability.[15]

In FRAXE syndrome, learning, thinking ability and cognitive function, affected badly. Also there is delay in speech; hyperactivity, poor writing skills, and very short attention span are common symptoms of people affected with this syndrome. It

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is reported that autistic behavior (intense interest in a particular subject, hand flapping, repetitive behavior) were displayed by some individuals. In FRAXE syndrome, cognitive function does not decrease with age as compared to other ID types.[16]

Additionally, some phenotypes are found to be variable among patients including mild facial dysmorphic features, long narrow face, nasal abnormalities, irregular teeth's and thick lips. The *AFF2* protein expression resulted in high expression in mouse model in brain area (involved in memory and learning) that provide impact of the gene.[17]. Chromosomal abnormalities were also reported to cause disruption of *AFF2* gene leading to ID as reported in patients.[18] Micro deletion in *AFF2* gene causing ID was also reported in two patients which provides further evidence of involvement of this gene in ID.[19]

Methodology

The blood samples were collected and DNA extracted by using phenol chloroform method. The suspected family was approached as per convenient sampling. Informed consent was taken from adults and in case of children; consent was taken from parents prior to enrolment.

Microarray genotyping was carried out using Illumina cytoSNP-12v2.0 arrays in both probands to identify the known genes in the family. The microarray typing was done by specialized bead chip to detect cytogenetic abnormalities relevant to human diseases. Whole exome sequencing (WES)was performed commercially on Illumina platform from Macrogen, Seoul, South Korea. WES was performed on two samples (one in each family) by Agilent Sure Select human all exon; V4 exome enrichment kit. Sequence reads aligned to the reference genome UCSC: hg19/ GRCH37 NCBI. GATK (Genome analysis tool kit) tool was used to do the sequence realignment and base quality recalibration. Sam tool methods were used to determine the read depth of the sequences. This software was also used to detect insertion and deletions and single nucleotide variations (SNVs).[19,20] The annotation of data was carried out by Annovar.[21] Primers were designed by primer3plus tool.[22]

PCR (polymerase chain reaction) was performed on genomic DNA of the patient (proband) and family members and PCR products were purified. Sequencing was performed by using Big Dye terminator kit (Applied Biosystems). The purified sequencing reactions were performed. Sequences were reviewed manually and comparison was made to reference sequence NM_002025.4 of AFF2 gene using Codon Aligner software.

Psipred [23] and I-Tasser [24,25] models were used to predict secondary and three dimensional protein models. The reliability of predicted models was checked by using RAMPAGE.[26] Visualization of models were done by UCSF Chimera.[27] The protein pockets on three dimensional structure of protein was identified by CASTp server.[28,29] The impact of protein stability was checked by Meta SNP,[30] I-mutant 2.0 [31] and PREDICT SNP [32] Different in-silico tools were used to determine the pathogenicity of the variant. Multiple sequence alignment was performed using Clustal Omega.

The Ethical approval was obtained from the Ethical Review Committee of International Islamic University, Islamabad.

Results

A family was enrolled with size of 06 members containing two probands. The probands are named as IV: 2 and IV: 3 (Figure-1A).

Proband IV:2

The Proband (IV:2) was male and aged 08 years old. He was born with normal gestational age. The birth weight, birth length and OFC (occipitofrontal circumference) were 2.2 kg, 41.6 cm and 31 cm, respectively at birth.

Few early developmental milestones were delayed in first 2 years of life. At 14 months, he was able to walk, but could not speak single word. He started uttering few words and crying at need when he was at 02 years of age. The cranial MRI performed at 3 years was normal. Speech testing and language evaluation indicated lack of expressive speech. At 04 years, he was referred to a local hospital for neurological checkup. His parents were having consanguineous marriage, but no other genetic disorders were reported in the family. The weight was 11.56 kg and height was 88.6 cm; with head circumferences as 46.5 cm. There was learning disability, speech delay and flattening of occiput with clinical characteristics of ID. Also other features like large ears and nasal tip was noted. He joined school for special children at 04 years and required support for social, communication skills and cognition.

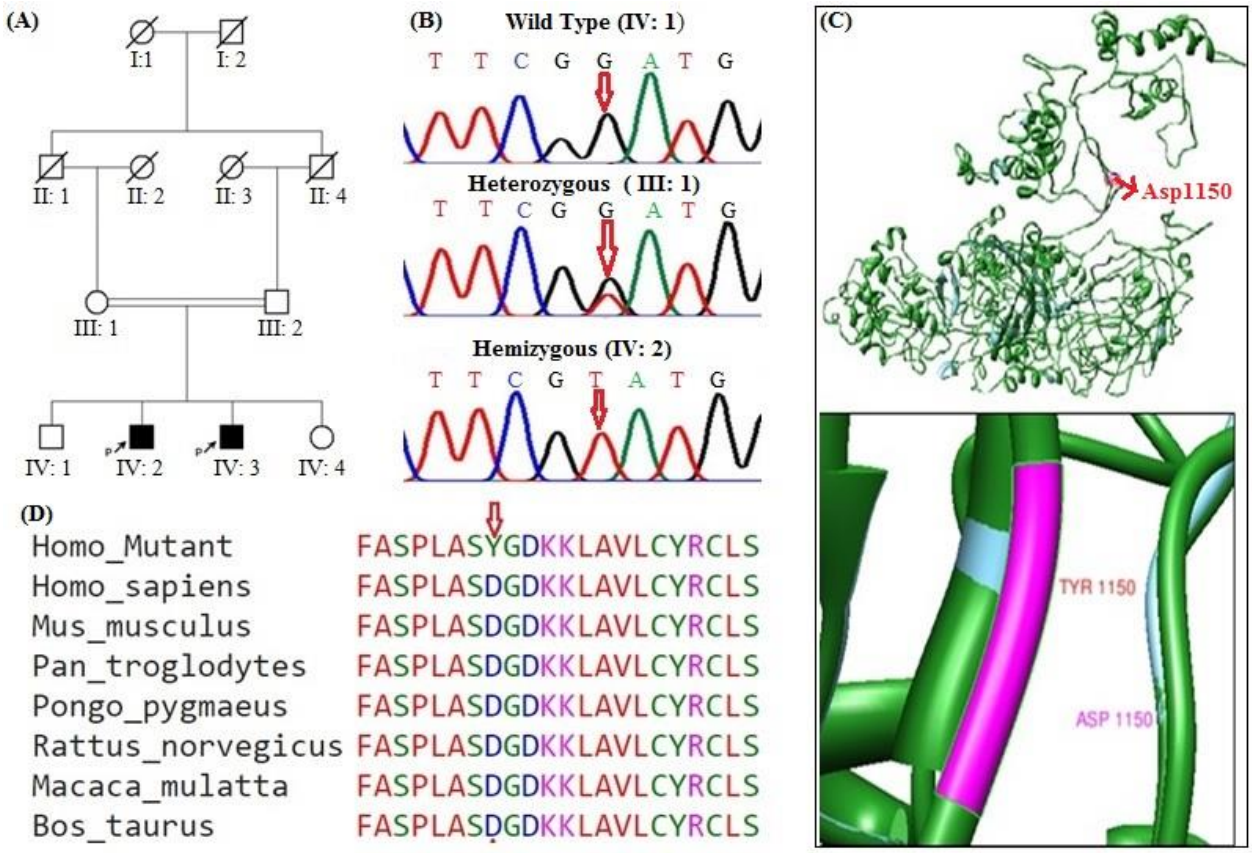

Figure: Figure-1A Pedigree of the family with probands indicated in black filled blocks. (B) Chromatograms showing variant of AFF2 (c.3448G>A,p.Asp1150Tyr) in heterozygous (III: 1), wild type (IV: 1), and affected individual (IV: 2) in AFF2 gene, exon 17. (C) The Superimposed AFF2 structure (normal and mutant) highlighting substitution of variant of Asp (magenta color) with Tyr (red color) at position 1150. Protein structure in green and cornflower blue color showing wild and mutant types respectively. (D) Multiple alignment of mutated protein showing conservation in nearby amino acid.

Patient IV: 3

The second patient (proband) was male, 06 years old and born after uneventful pregnancy. His parents were having consanguineous marriage. His weight and OFC at birth was 3 kg and 30 cm. He started walking with few steps at 18 months, utter few words at 2.5 years with cognitive and motor milestones were not fine.

He was admitted to the district hospital Dera Ismail Khan, KPK at 3 years age for neurological examination. He has speech delay, global developmental delay and behavioral problems. He also exhibited behavioral problems, including aggression, agitation with attention deficits. Hand flapping was also noted during clinical examination but seizures were not reported by the parents. Intellectual disability was indicated in proband with developmental delay.

Genetic analysis

Whole Exome sequencing was performed in the selected patient (IV:2). Genomic library was prepared by using exon kit (Sure Select, Agilent, Santa Clara, CA) and enrichment of exome was performed on illumina HiSeq4000. The variants were annotated and aligned with human reference genome (GRCH37). The filtration was carried out by variant type, allelic frequency and mode of inheritance for selection of causal variants. Firstly, the variants that were more common were removed on the basis of frequency. The duplicate alignments were removed and variants with allelic frequency <0.005 were retained. This resulted in 4 candid variants. The frequency of variants was checked in gnomAD and HGMD. The variants were not found in South Asian population category of gnomAD database (https://gnomad.broadinstitute.org/). The ACMG guidelines were followed to classify the variants. [33]. Benign and likely benign variants were excluded and one variant (NM_002025.4: c.3448G>T; p.Asp1150Tyr) in the *AFF2* gene was selected (OMIM: 309548) for further analysis.

In-silico results indicated that the variant (p.Asp1150Tyr) is deleterious and disease causing.

The pathogenicity of the variant with different software predictions are mentioned in Table. Sanger sequencing analysis was carried out.

Co-segregation analysis was performed in normal and affected members of the family. The analysis revealed complete co-segregating with family members. Two affected males (IV: 2 and IV: 3) were found hemizygous for mutant allele of *AFF2* gene mother (III: 1) was heterozygous and the other family members (IV: 1 and IV: 5) were of wild type (Figure-1B). The Amino acid (aspartic acid) which was converted into tyrosine, was found present in evolutionary conserved position (p.Asp1150Tyr) as per UCSC Human Genome (GRCh37/hg19).

Table: Software predictions for the variant.

Pathogenicity software	*Prediction*	*Score*
FATHMM-MKL	Damaging	0.88
DEOGEN2	Damaging	-
PROVEAN	Damaging	-2.75
DANN and	Damaging	0.99
GERP	Damaging	5.98

Effect of variant on protein

The pathogenicity of p.Asp1150Tyr variant was assessed by using I-TASSER Tool for the structure of *AFF2* protein using template 6R80 [34] (Figure-1C). The result revealed that template triggers the release of polymerase II enzyme from promoter proximal pausing site. Paused polymerase-II played role in the maintenance of structure of promoter region. The loss of protein structure with no change in protein length was observed. The paused polymerase II also showed alteration in protein conformation and loss of c-terminal helices. Therefore the variant p.Asp1150tyr is probably pathogenic for the family.

Multiple sequence alignment showed conservation of mutated amino acid (p.Asp1150Try) and nearby amino acids in 7 different species. Variant site denoted with red arrow (Figure-1D).

Discussion

X linked intellectual disability is a heterogeneous group of genetic disorder with different clinical manifestations. The features include developmental delay, cognitive impairment and low IQ in males mostly. Most common cause of this disorders an expansion of CGG repeats in the UTR region of the FMR1 gene.[35] About 141 genes were reported to be responsible for this disorder.[11] Micro deletion and variants in *FMR2* (*AFF2*) genes leads to FRAXE syndrome in the absence of CGG repeats expansion.[14]

Fragile XE (FRAXE) is an x-linked syndrome characterized by speech delay, cognitive impairment, and autistic behavior. The gene (*AFF2*) is responsible for protein present in the cell's nucleus. This protein serves as a transcription factor, binds to specific regions of DNA and helps in controlling activity of other genes.[36]

In this study, a family with two sibling patients (probands) showing symptoms of FRAXE syndrome. The common symptoms were speech impairment, behavioral problems, hand flapping and developmental delay. Genetic testing showed novel missense variant i.e. c.3448G>T (p.Asp1150Tyr) in coding exon 17 of *AFF2* gene.

The high prevalence of fragile x syndrome was reported from Spain, Chile and Columbia.[37] It has been reported that pharmacological, behavior and cognitive interventions are important for improvement in the quality of life of patients.[38] There is a need to conduct large scale studies to determine the prevalence and identify the public health burden of fragile x syndrome. Diagnosis and timely screening is also important for early diagnosis of the disease to prevent morbidity in future. Our physicians and health care provider need to be trained to provide genetic counseling to the patient.[39]

Genetic analysis identified a novel missense variant (c.3448G>T; p.Asp1150Tyr) in the coding exon 17 of AFF2 gene as the pathogenic variant in a family identified as FRAXE syndrome. Combinational approach of exome sequencing and Sanger sequencing can provide better clinical diagnosis of the ID families.

Conflict of interest: None declared.

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