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Original Research Article

Whole exome sequencing reveals the first c.7456C>T p.Arg2486X mutation in ATM gene in Iranian population

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ABSTRACT

Mutations in Ataxia-Telangiectasia Mutated (*ATM*) gene are prominently responsible for the condition. *ATM* gene encodes a serine/threonine protein kinase, a crucial component in DNA repair systems. Whole exome sequencing was performed on a nine year old male subject with clinical features of A-T. Alpha fetoprotein and immunoglobulins levels in the serum sample were also measured by biochemical testing. Sequencing test revealed c.7456C>T (p.Arg2486X) mutation in exon 50 of *ATM* gene in this patient. This mutation was previously described as a missense pathogenic variant that could lead to truncation or lack of protein.

***Keywords*:** *ATM* gene, whole exome sequencing, missense variant, cerebral ataxia

INTRODUCTION

Ataxia-Telangiectasia (A-T), also known as Louis-Bar syndrome (OMIM 208900), is a progressive and rare multisystemic disorder. [1] A-T is a neurodegenerative autosomal recessive disorder that stems from biallelic *ATM* gene loss of function mutations. Ataxia-Telangiectasia Mutated

(*ATM*) is a housekeeping gene spanning approximately 150 kb of genomic DNA at the long arm of chromosome 11 (11q22.3). [2] *ATM* consists of 66 exons, and its coding region size is about 6 kb. The translational start codon lies within the fourth exon. The last exon also is the largest, with 3.8 kb in length. Both 3' and 5'UTRs are highly prone to undergo the alternative splicing process, which results in formation of structurally and functionally varied *ATM* mRNA transcripts [3-5].

ATM gene encodes a protein kinase from the PI3-kinases (PI3K) family. This 350 KDa protein is an essential factor in DNA repair, genome stabilizing, cell cycle arrest, and apoptosis signaling pathways [6]. Other PI3Ks, like DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and ataxia telangiectasia and Rad3-related (ATR), also participate in the cellular response to DNA damage. *ATM* is a Serine/Threonine protein kinase which contributes to four different DNA repair systems [7]:

- **Double-Strand DNA Break (DSB) repair**: In both homologous recombination and non-homologous end joining pathways, *ATM* gene can participate as a regulator in the upstream side of important genes like *BLM*, *BRCA1*, and *XRCC4*.
- **Single-strand DNA break (SSB) repair**: By activating checkpoint kinase 2 (CHK2), *ATM* can alter the *XRCC1* regulation, which is the major mediator involved in Base Excision Repair (BER) of DNA damage.
- **Removal of topoisomerase cleavage complexes**: Enzymatic activity of topoisomerase regulates DNA topology during replication and transcription, leading to the formation of Topoisomerase Cleavage Complexes (TOP1cc and TOP2cc), which produce DSBs. *ATM* plays a key role in cleavage complexes removal by regulating tyrosyl-DNA phosphodiesterase 1(TDP1) and DNA Topoisomerase II Alpha (TOP2A).
- **Transcription-related DNA lesion**: the most common one is R-loop, which consists of an unpaired DNA-RNA hybrid. They are physiologically labeled as harmful, as they interrupt gene expression. In addition to XP proteins and helicases like RNase H, *ATM* could

participate in R-loops removal or prevent their formation.

Although the prevalence of A-T in consanguineous populations is significantly higher than in others, no difference has been reported in terms of ethnicity, geographical region, and sex. [8] Based on previous studies, the prevalence of this disease is within the range of 1:40000-1:300000. [9]

Clinical manifestations of A-T occur in a wide range and mainly consist of progressive cerebellar degeneration, oculocutaneous telangiectasia, humoral and cellular immunodeficiency, cancer susceptibility, and sensitivity to radiation. [10-12] Despite its complexity and age-related variability, A-T can be subcategorized into two main forms [13, 14]:

- **Early-onset or severe A-T:** It is also known as classic A-T. About 65 percent of these patients develop acute cerebellar ataxia before age two. Recurrent infection and a high probability of malignancy have also been reported.
- **Late-onset or mild A-T:** This form of A-T is associated with less progressive symptoms and a more extensive lifespan. It also occurs later in patients' life. One possible explanation for this mild phenotype is more enduring ATM kinase activity due to a less pathogenic mutation.

Cerebrum-related disorders manifest in A-T children, including hypotonia, body balance dysfunction, and dyssynergia. Moreover, progressive freezing gait disorder and truncal ataxia are two of the most critical clinical features in individuals with A-T. The onset of these conditions is mostly between the ages of 1 to 4 years and leads the patients to use a wheelchair in their twenties. [15-17] Brain atrophy (loss of neurons or synapses) in cerebellar hemisphere and vermis of patients results in dysregulation of basket cells (intraneuronal GABAnergic inhibitors), Purkinje cells (largely branched neurons that control motor movements), and granule neurons (One of the smallest neurons in brains extending into hippocampus). Interruption of these cells leads to a condition called progressive cerebellar degenerations that widely happens in A-T patients [16].

Telangiectasia (or spider veins) is defined as abnormally dilated blood vessels that can be seen in the eyes' sclera and skin. It is one of the prominent clinical manifestations of A-T patients that usually occurs within the

age range of 2 to 5 years. Ocular (or conjunctival) telangiectasia does not lead to visual impairment but appears in most A-T patients. [18] This clinical feature is the most well-known sign of these patients, so that, its absence in a minority of them delays the diagnosis.[17] Although no molecular explanation has yet been identified on how telangiectasia pathogenesis occurs, some proposed that overexpression of *HIF-1*, a transcriptional factor, may have a role [19]. Diagnosis of A-T is confirmed mainly by observation of cerebral ataxia and oculocutaneous telangiectasia. Even though, in some cases, absence or heterogeneity makes the disease less likely to be detected [20,21]. In these cases, serology tests like Alpha-Fetoprotein (AFP) and Immunoglobulin (Ig) levels are more helpful tools. Augmented level of AFP in blood serum in A-T patients is a reliable indication for diagnosis. It is possibly a sign of liver dysfunction (fibrosis and fatty liver disease), which results in metabolic toxicity and, therefore, possible nervous system dysregulation [22-24].

In about two-thirds of individuals with A-T, Primary Immune Deficiency Disorders (PIDDs) have been reported. It's been established that ATM is crucial in the development of T and B lymphocytes, especially in v(D)J somatic recombination processes. Furthermore, patients with A-T are also classified based on serum Ig levels into five groups [25,26] :

1. Normal Ig level
2. selective IgA deficiency (SIgAD)
3. Hypogammaglobulinemia
4. IgG deficiency
5. Hyper IgM or CSR defect

The average life expectancy of A-T patients is about 25 years. There hasn't been any significantly effective treatment for A-T patients that prevents the progression of the disease. However, some management methods, including prophylactic antibiotics and Ig Replacement Therapy (IRT), can prolong their lifespan, which gets terminated primarily due to cancer or chronic pulmonary disease [27,28].

In the present study, we examined the application of Whole Exome Sequencing (WES) to an Iranian child with A-T manifestations. We reported a known pathogenic mutation in the *ATM* gene for the first time in Iran's population.

MATERIALS AND METHODS

In this study we evaluated a 9-year-old male patient with A-T manifestations. He was the first born of a non-consanguineous family of four children in Zahedan, Sistan

and Baluchistan, Iran. (Figure 1) All other member of the family, including parents, were healthy. Moreover, no history of similar condition have been observed in the family. We collected 10 mL of the subject blood sample. We sent it for laboratory tests including genetic testing, biochemical analysis of AFP and Ig. The patient has undergone Magnetic Resonance Imaging (MRI) of his brain and his cognitive function was assessed by Mini-Mental State Exam (MMSE) and Montreal Cognitive Assessment (MoCA), as well. This study was conducted with approval of the ethic committee of Golestan University of Medical Sciences.

DNA Extraction

DNA was extracted from patient's whole blood sample using Favorgen DNA kit based on the manufacturer's instructions. Then genomic DNA (gDNA) concentration was controlled using spectrophotometry.

Whole Exome Sequencing

To capture entire exon sequence of the genomic sample, we used Agilent SureSelect DNA enrichment kit (Agilent Technologies Inc Santa Clara, CA) for exome enrichment purpose of the isolated DNA. Whole exome sequencing technique was performed with Illumina platform. Evaluation is focused on coding exons along with flanking intron regions. The target region is not covered 100 %. Data analysis including base calling demultiplexing, alignment to the hg19 human reference genome (genome reference consortium GRCh37) and variant calling is performed using a validated in-house software. Relevant reported variants in HGMD ClinVar are considered.

Biochemical and Immunological Analysis

Blood serum isolation on subject's sample performed by centrifugation. Then we used the product to evaluate AFP and Ig levels. The level of total IgA, IgG and IgM assessed with ELISA method.

RESULTS

Clinical characteristics

Based on parent's declaration, the 9-year-old boy was born normally until the first signs of difficulties in maintaining neck mobility and body balance appeared 6 months and 1 years later, respectively. Following that, in a gradual process, problems in walking, hand and neck maintaining and rational verbal communication, progressively increased. As seen in Figure 2, the patients has redness in his conjunctiva with normal eye movements. Brain MRI results reported

atrophy in superior cerebellar peduncles and pons, and normal size of cerebral ventricles and sulci. The score of Mini-Mental State Exam (MMSE) was 27 out of 30, which is classed as normal. Moreover, Montreal Cognitive Assessment (MoCA) score was 23/30. Range of 18 to 25 is considered as mild cognitive impairment.

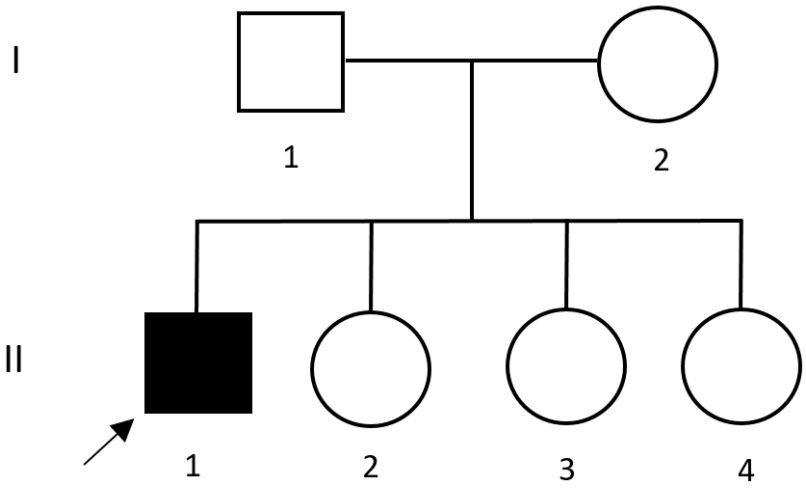

Figure 1. Pedigree analysis of the study subject. None of the other proband family members were affected. As A-T inherited by autosomal recessive pattern, both parents are most likely to have heterozygous genotype.

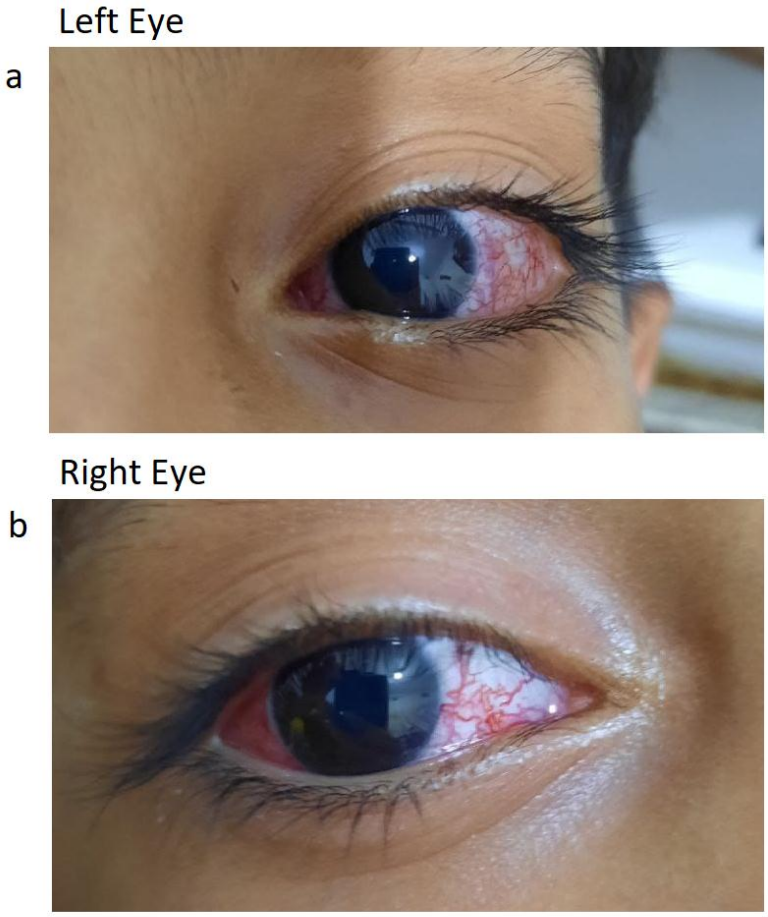

Figure 2. Presentation of ocular telangiectasia in the study subject. Section 2a and 2b illustrate wide network of blood vessels in sclera the left and right eyes, respectively.

Genetic testing

ATM variant c.7456C>T (p. Arg2486) identified in the proband, which creates a premature stop codon with probable pathogenicity. Table 1 shows the results of sequencing test, in detail. The variant was classified as class 1 variant. According to the ACMG guidelines, genetic variants are grouped into five classes:

1. Class 1: Pathogenic
2. Class 2: Likely pathogenic
3. Class 3: Variant of Uncertain Significance (VUS)
4. Class 4: Likely benign
5. Class 5: Benign

Serological testing

The serum AFP levels were significantly increased, whereas serum level of IgA was decreased. Serological level of IgM and IgG were in normal range (Table 2).

DISCUSSION

In this study, we applied Whole Exome Sequencing (WES) to a subject with clinical presentation of A-T. The results report a stop codon variation (c.7456C>T / p.Arg2486*) in *ATM* gene. The mutation is located at exon 50 of the *ATM* gene transcript and leads to a truncated protein or zero concentration due to nonsense-mediated mRNA decay. This premature translational termination signal was previously characterized as an A-T causative variant by Buzin and his colleagues in 2003 and has been observed in breast cancer and other A-T-related malignancies. ClinVar also has listed this variation as a pathogenic mutation (Variation ID: 127445). Here we reported c.7456C>T (p.Arg2486*) mutation in A-T for the first time in the Iranian population. There are 120 reported A-T cases in Iran's population. Types of variants are listed in Table 3. Inducing ATM signaling pathway is canonically a response to DSBs in human cells. [41] In the resting state, ATM protein inhibits itself as a polymerized form. In DNA lesion site, a complex of three proteins (MRE11, RAD50, and NBS1) called MRN complex, triggers conformational changes in ATM molecule, which results in its monomeric active state. Thereafter, activated ATM kinase enzyme targets the downstream pathways as an initiator of DNA repair system [42,43].

Mutations in ATM gene cause interruptions in cellular DNA response. According to HGMD Professional 2021.4 data, and as demonstrated by Figure 3, there are 2050 mutations in ATM gene. More than 500 are characterized as missense or nonsense, like the variant in this study. This variant changes nucleotide 7456 from cytosine (C) to thymine (T), which prematurely terminates translation after 2486 amino acids near the C-terminal. In terms of protein architecture, this mutation leads to the elimination of PIKK and FACT and part of FAT domains. In other words, ATM protein in this patient possibly lost its ability to activate into monomeric form and to perform kinase activity. Hence, downstream DNA repair cascade may not initiate from scratch.

Table 1. Results of whole exome sequencing test. This variant in ATM gene creates stop codon (*Genome Aggregation Database **based on ACMG recommendations)

Gene	Variant coordinates	Zygosity	Allele frequency*	Type and classification**
ATM	Chr11(GRCh37):g.108201089C>T NM_000051.3:c.7456C>T pp.(Arg2486*) Exon50	Hom.	gnomAD: 0.000012	Stop gain Pathogenic (class 1)

Table 2. Results of biochemical and serological tests. Elevated leves of alpha fetoprotein is a biochemical hallmark in A-T patients. In cases that telangiectasia and ataxia are not observed, serological test is the preferred diagnostic tool

Test	Result	Unit	Risk	Normal Ranges
AFP (CL)	146.1	IU/mL	H	0-5.5
IgG (Total)	678	mg/dL	-	656-1351
IgM (Total)	120	mg/dL	-	34.9-255
IgA (Total)	44	mg/dL	L	86-320

Table 3. Mutations in ATM gene in Ataxia-Telangiectasia patients in Iran. Based on clinical diagnosis 120 A-T patients have been identified in Iran

Type of mutation	Number of case	References
c.829G>T (p.E277*)	1	[29]
c.8250C>T(p.2622ala>val)	1	[30]
p.S2761LfsX2805 p.I2628fsX2630	1	
p.S2761LfsX2805 p.I2628fsX2630	1	
p.S2761LfsX2805 p.I2628fsX2630	1	
p.K2756T p.I2628fsX2630	1	[31]

p.E2778DfsX2805 p.H2552PfsX2563	1	
p.R2792TfsX2795	1	
p.I2628fsX2630	1	
p.I2628fsX2630	1	
p.I2628fsX2630	1	
p.I2628fsX2630 p.H2552PfsX2563	1	
p.I2628fsX2630 p.T2556fsX2563	1	
p.I2628fsX2630	1	
p.I2628fsX2630	1	
p.I2628fsX2630	1	
p.L1851fsX1856 (p.Gln1852ProfsTer5) (c.5552_ 5553insC)	1	[32]
p.A2622V	1	Overlap with (2)
p.E1622X new patient	1	[32]
p.E277X new patient	1	
p.Y2969X c.2639-1G>A new patient	1	
p.A1299PfsX1348 new patient	1	
p.R1875X (c.5623C > T)	1	Overlap with [33]
c.8046-8047delTA p.Thr2682ThrfsX5	2	[34]
c.3895delG p.A1299Pfs*50	1	[35]
Del EX 37-48	1	
c. 6658C>T p.Q2220*	1	
c.3244-3245insG P.His1082fs	1	[36]
Deletion EX62-63	1	[37]
c.67C>T p.R23*	1	
c.1537C>T p.Q513* c.8050C>T p.Q2689*	1	
c.3244-3245insG p.H1082Rfs*14	1	
c.3895delG p.A1299Pfs*50	1	
c.6658C>T p.Q2220*	1	
c.3600-3601delTT p.F1201Wfs*3	1	

c.8907T>G p.Q2684* c.8050C>T p.Q2969*	1	
c.6452G>C p.R2151T	1	
Deletion EX37-48	1	
c.5585delA p.S1863Lfs*54	1	
c.6658C>T p.Q2220*	1	
Deletion EX1	1	
c.6259delG p.E2087Kfs*9 c.6658C>T p.Q2220*	1	
c.5552_5553insC p.Q1852Pfs*5	1	
c.6047A>G p.D2016G	1	
c.6198+1G>A c.6047A>G p.D2016G	1	
EX 18-61 duplication c.7788G>A	1	
c.9097_9101dupAATTT p.L3035Ifs*8	1	
c.7308-6T>G	1	
c.8046-8047delTA p.I2683Tfs*4	1	
c.6658C>T p.Q2220*	1	
c.829G>T p.E277*	1	
c.6658C>T p.Q2220*	1	
Deletion EX62-63	1	
Deletion EX62-63	1	
c.3600_3601delTT p.F1201Wfs*3	1	
c.5003A>G p.L1668P	1	
c.3102T>G p.Y1034*	1	
Deletion EX62-63	1	
c.5712dupA p.S1905fs*12	1	
c.1159A>C p.K387Q	1	
Deletion EX62-63	1	
Deletion EX61-62	1	
c.6807+1G>C	1	
c.829G>T p.E277*	1	
Deletion EX62-63	1	
c.6199-1G>T	1	
c.664C>T p.Q222*	1	
c.8741T>A p.I2914N	1	
c.1834C>A p.L612I	1	
c.6067G>C p.G2023R	1	
Deletion EX37-48	1	
c.7308-6T>G	1	[38]

c.4236_4236del p. Pro1412fs	1	[39]
c.8907T>G p. Tyr2969Ter	1	
c.2251-4A>G c.3576G>A	3	[40]
c.7456C>T p.Arg2486*	1	Present Study

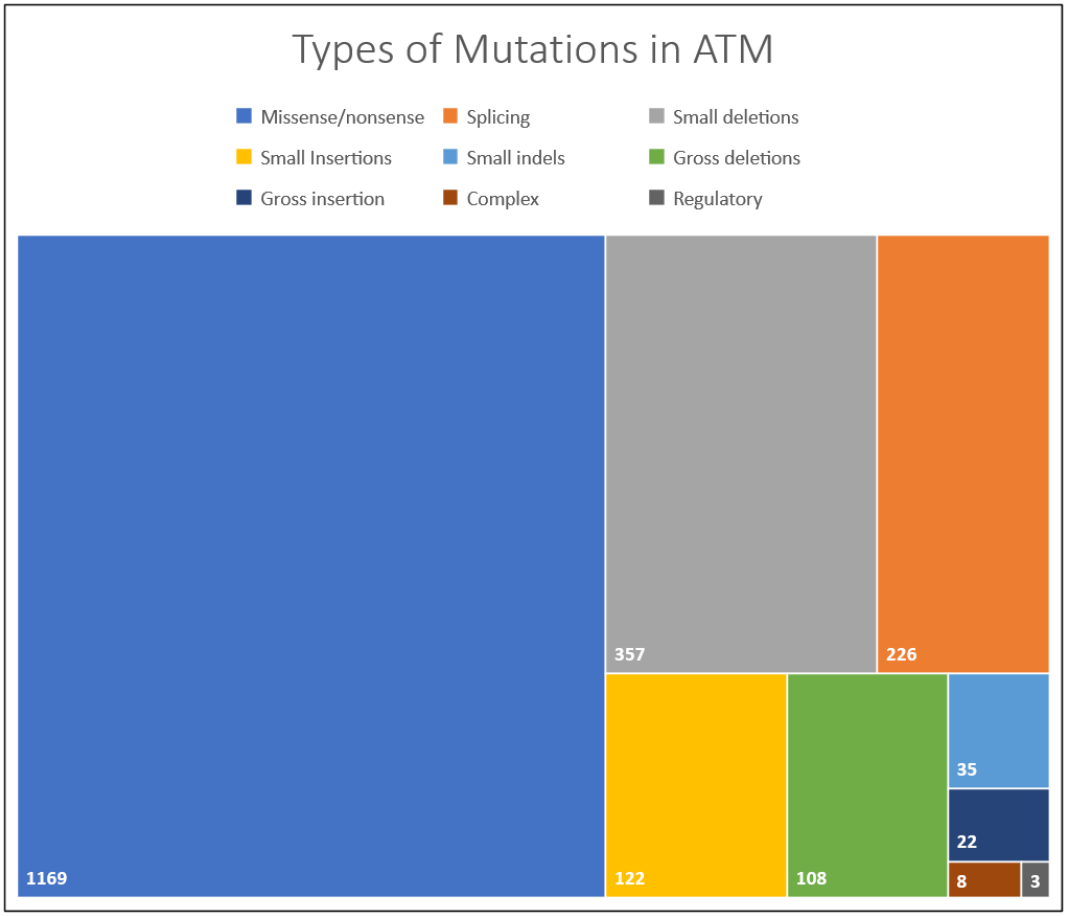

Figure 3. Types of mutations in ATM gene. According to latest data available on HGMD database, 2050 mutations have been reported within the ATM gene. These include 57 % Missense/nonsense, 11 % splicing, 17.4 % small deletions, 6 % small insertions, 1.7 % small indels,

CONCLUSION

A 9-year-old patient with initial characterization of A-T including body balance difficulties and abnormal MRI, was recommended for following genetic and biochemical tests, which revealed a c.7456C>T single base substitutions in ATM gene. According to ACMG this was a pathogenic variant. Significant increase in serum AFP level was also identified. This is the first report of this genetic mutation in Iranian population for a rare disease like A-T. Since consanguineous marriage is frequent in the country, it is necessary for future evaluations of A-T patients to perform genetic test and report the variants.

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