Case Report:

Novel Single Base Pair Deletion in ATM Cause Ataxia Telangiectasia in an Iranian Proband

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ABSTRACT

Ataxia-telangiectasia is a rare disorder with neurological manifestations, caused by mutations in *ATM* gene. This gene produces a serine/threonine protein kinase, an activator of the DNA damage response in the face of DNA DSBs, which phosphorylates downstream substrates, integrating with DNA repair procedure. Most *ATM* mutations are private mutations and, there are no mutational hotspots in the *ATM* gene. This study tries to unveil a new mutation in this gene in an 8 years old A-T patient. This mutation led to fundamental alterations in ATM protein structure and representation of AT.

Keywords: Ataxia Telangiectasia; *ATM*; Novel mutation; Homozygous; Frame shift

INTRODUCTION

Ataxia-telangiectasia (AT, OMIM#208900) also named Louis-Bar Syndrome is an infrequent autosomal recessive disorder characterized by progressive neurological degeneration, ocular and skin related telangiectasia[1], cerebellar ataxia, immune deficiency[1-3], and a predisposition to malignancies[3]. AT is the most common cause of progressive cerebellar ataxia in infants in which chromosome breakage is quite common[4]. Cellules of such patients are sensitive to killing by ionizing radiation (IR)[5], and their lymphoblastoid cells are abnormally persistent to inhibition of DNA synthesis in response to ionizing radiation[6]. The recent feature utilizes for classification of different complementation groups of the disease[2]. Since 1995, when the defective gene in AT was discovered [7], several mutations have been discovered in the *ATM* gene, resulting in ataxia telangiectasia [8-10]. At least 4 of these groups (A, C, D, and E) are mapped to chromosome 11q23[11] and are accompanied with mutations in *ATM* gene. ATM, a 370kDa serine/threonine protein kinase[12], is a pioneer activator of the DNA (DSBs). ATM phosphorylates downstream substrates which are involved in DNA repair and administration of cell cycle [13]. However, recent pieces of evidence show the case of involvement of ATM protein epigenetic regulation [14]. On the other hand, epigenetic silencing of *ATM* is reported in different cancers like breast cancer and this molecule is an epimarker[15]. Epigenetic silencing or dysregulation has been evidenced to be important in many human diseases like infertility or breast cancer [16]. Epidrug therapies for these genes, such as ATM might prove to be promising in future treatment options. Then, knowing different pathogenic mechanisms and

mutations in ATM seems undeniable [17-19].
Most *ATM* mutations are private mutations and there are no mutational hotspots in *ATM* gene. This study reports an Iranian patient enduring AT who was found to be homozygous for a novel single base pair deletion in *ATM* gene.

CASE REPORT

One female patient with chief compliment of ataxia and recurrent infections was referred to Sarem Medical Genetics Laboratory for genetic counselling and mutation detection.

Clinical findings

An 8 year old female patient who is clinically considered for progressive ataxia, vertigo and tremor since age 30th month is the case of the present study. The patient is mentally normal and has been suffering from recurrent diarrheal and respiratory infections for years. Her parents were healthy persons who had a consanguineous ethnic Iranian marriage (pedigree is shown in figure 1A). Dystonic cerebral palsy was diagnosed at first 2-3 years of her life and she was considered for unsteady gait and frequent falls since the age of 3. At first, ataxic walking was diagnosed at the age of 30 months, and then it remained stable, became progressive, leading to loss of walking ability in referring time. She suffered from delayed slurred speech movement patterns for many years, demonstrating ataxia. Investigations revealed low lymphocyte count and IgA while Alpha-fetoprotein (AFP) testing was not available. The child received symptomatic and supportive treatments for recurrent infections. Bilateral ocular telangiectasia and ocular-motor apraxia was present at the age of 7 years. Neurologic symptoms were progressive in recent two years, conveyed by involuntary movements of the lips and tremor in superior limbs. All clinical findings supposed the proband to be affected by ataxia telangiectasia; she then was evaluated for pathogenic variations in her *ATM* gene.

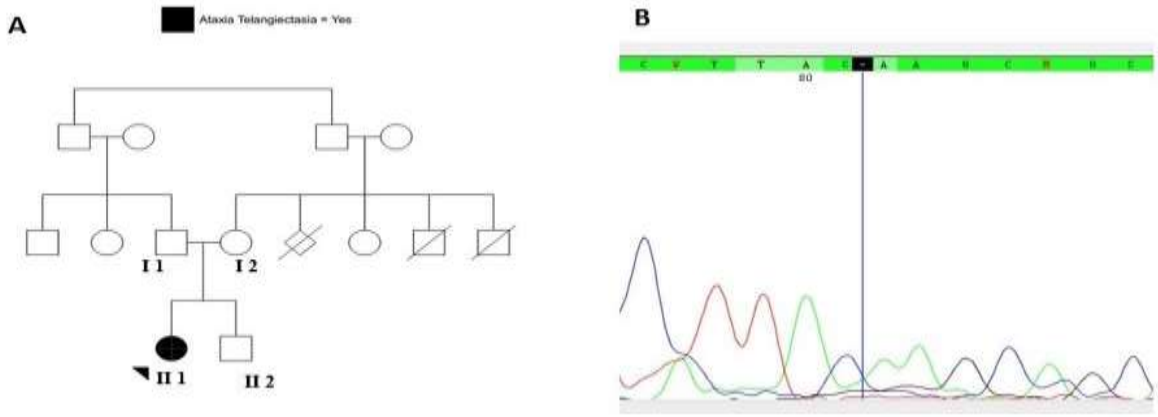

Figure 1. A) Female patient who was born in a consanguineous marriage. B) Sanger sequencing of targeted region to detect the mutation in homozygous state.

Molecular genetic assays

Family members received genetic counseling and they were aware about the study. Informed consent obtained from patient's parents indicating their satisfaction to be a participant in this study. The genomic DNA was isolated from peripheral blood leukocytes of all family members using DNA purification kit (Roche, Switzerland). Extracted DNA was used to perform targeted gene capture using a custom capture kit (QIAGEN, Germany). The libraries were sequenced to mean >80-100X coverage on Illumina sequencing platform (Illumina, San Diego, CA, USA). The obtained sequences were aligned to the GRCh37/hg19 human reference genome using BWA program[20, 21] and analyzed using Picard and GATK-Lite toolkit [22, 23] was used to identify variants in the targeted genes relevant to clinical indication. Annotation of the variant was performed against the

Ensemble release 75 gene model (http://www.ensembl.org/index.html). Clinically relevant mutations were annotated using published variants in literatures and a set of databases including ClinVar (https://www.gwascentral.org/), OMIM(http://www.omim.org/), GWAS (http://www.gwascentral.org/), HGMD (http://www.biobase-international.com/product/hgmd) and SwissVar (http://swissvar.expasy.org/). Using best recommendations, only pathogenic variants were extracted and common variants registered in dbSNP build 137 (Minor allele frequency ≥ 0.01) (http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=316787363&g=snp137Common&hgTracksConfigPage=configure) were excluded. The pathogenic effects of all candidate variants were predicted using SIFT (http://sift.jcvi.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org/) software. Segregation analysis was performed to confirm the mutation inheritance through parents and interpretation of unveiled mutation. Moreover, candidate variants were validated in all family members by Sanger sequencing.

Molecular genetic findings Diagnosis was confirmed by the identification of a novel deletion in the *ATM* gene in studied patient. A homozygous single base pair deletion in exon 43 of the *ATM* gene (chr11:108188201delC) resulting in a frameshift and premature termination of the protein 19 amino acids downstream to codon 2101 (p.Gln2101LysfsTer19:ENST00000278616) was detected. Ataxia telangiectasia (OMIM#208900) is caused by homozygous or compound heterozygous mutations in the *ATM* gene (OMIM*607585). This novel variant was not reported in both the 1000 genomes and ExAC databases (Figure 2). The region is conserved across some vertebrates (Figure 3) representing its importance. This variant was not registered in house database, 1000 Genomes database, and NHLBI Exome Sequencing Project (ESP6500). The followed Sanger sequencing revealed the presence of this mutation in proband (Figure 1B).

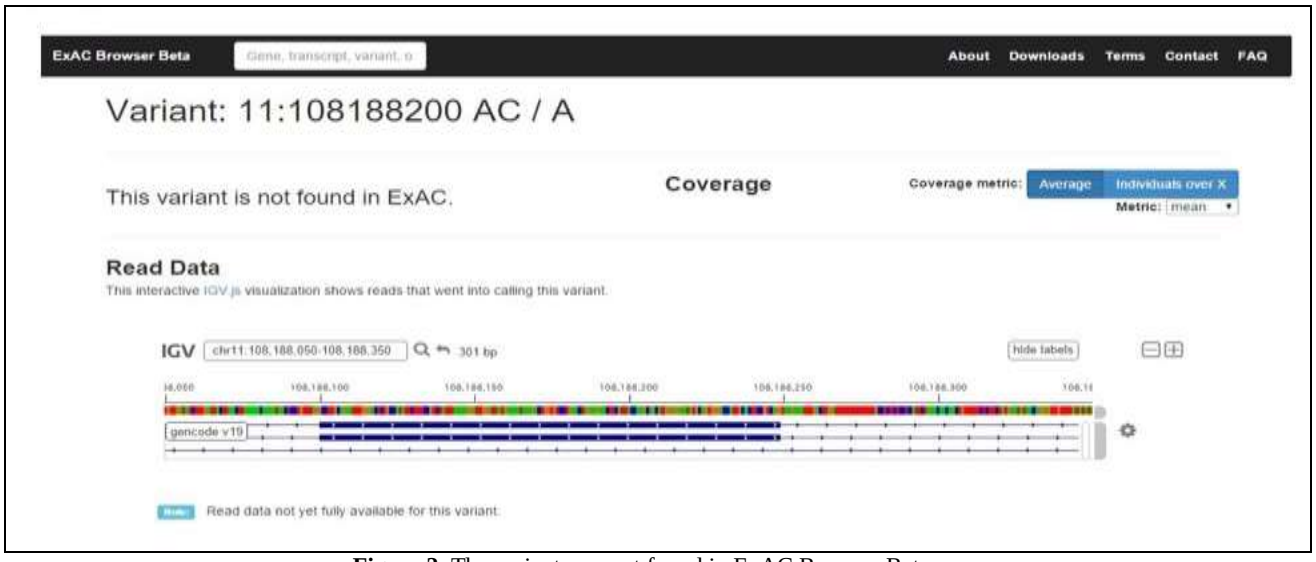

Figure 2. The variant was not found in ExAC Browser Beta.

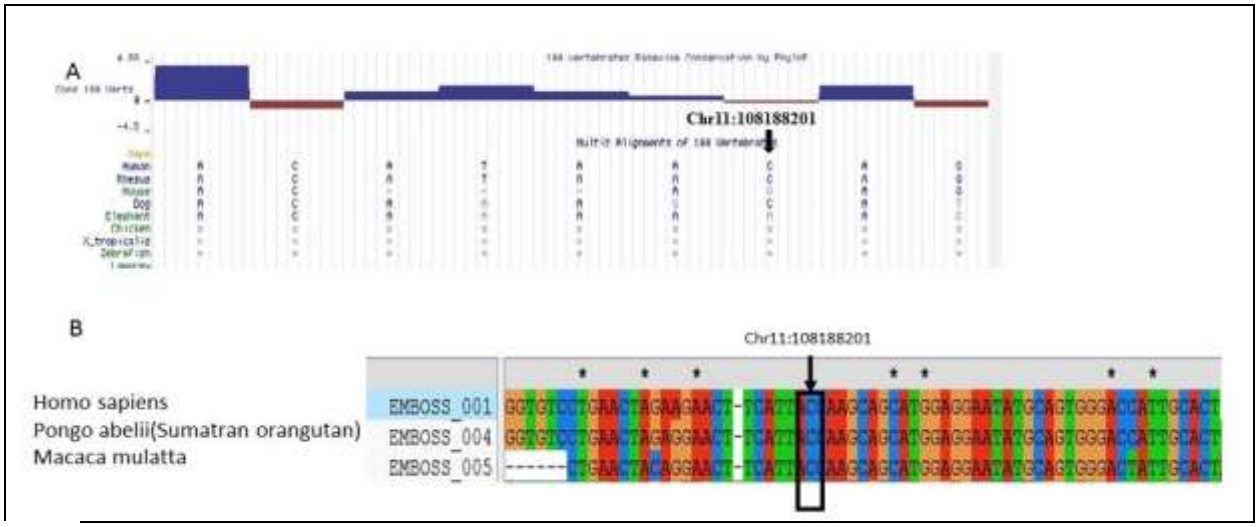

Figure 3. Region chr11:108188195-108188205 in ATM gene. A) The mentioned position in ATM gene is conserved across most vertebrates. B) The arrow pinpoints to the position of deletion.

DISCUSSION

Incidence of AT among patients with primary immune deficiency disorders is high in Iran, probably due to prevalence of consanguineous marriage [24]. Based on declared indications, this *ATM* variation is classified as a possibly noteworthy variant and has to be carefully correlated with the clinical symptoms. The reported mutation led to frameshifting, Nonsense Mediated Decay (NMD) and Ataxia Telangiectasia through affecting structure of ATM protein in the end (supplementary Figure). Referring to Multiz alignment of 100 vertebrates, the base which is deleted in the patient is conserved between human, rhesus and dog (Figure 3), demonstrating the importance of this position and catastrophic consequences of deletion in this region.

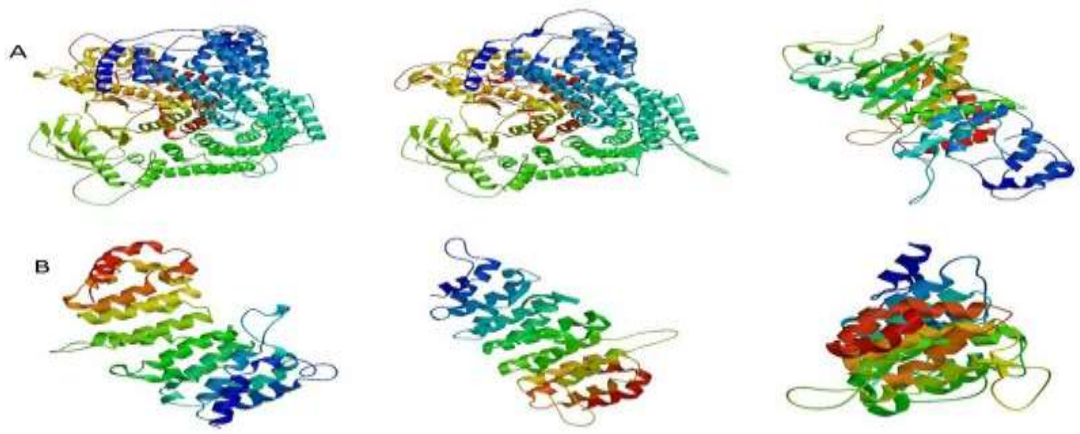

Figure 4. Supplementary Figure.A) Normal ATM protein. B) Mutated ATM protein. (The mutated protein was not probably synthesized due to NMD mechanism.)

In spite of rarity of AT disease, heterozygous mutations in *ATM* gene occur at 1% of general population [7] then discovering new mutations in this gene can improve quality of diagnostic tests and health in general population.

ATM protein has four conserved domains which are critical for its function. 75% of these conserved domains span through amino acid number 2097 to 3056 (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?INPUT_TYPE=live&SEQUENCE=AAB65827.1). Our declared mutation in ATM protein deranges NMD and production of truncated protein. Regarding the position of discovered mutation and termination of its translation after 19 amino acids, this truncated ATM has no conserved PIKKc_ATM (accession:cd05171), FATC (accession: pfam02260), and FAT (accession pfam02259) domains(supplementary Figure 1). Complete lack of ATP binding site, catalytic loop, activation loop [25], and FAT [26]domains conclude malfunctioning of the truncated ATM protein where Ataxia Telangiectasia is a probable consequence. Moreover, the mutated protein was not probably synthesized due to NMD mechanism.

The findings are contextulalixed, where this mutation interpreted as a pathogenic mutation that causes AT in the patient. Every patient with a suspicion of AT needs to be genetically tested and the pathogenic variant needs to be found for future guidance in the family and the patient. This novel mutation should be considered for PND in future pregnancies and upcoming managements of such families. As the newly-found mutation is a truncating mutation that disrupts the protein and eliminates most parts of the protein and its important functional domains, the pathogenicity does not need be confirmed through functional studies. The proband and her parents should have a standard genetic counseling for future pregnancies. PND, based on direct and indirect methods to ensure the absence of aforementioned mutation and affected allele of the family is recommended for next pregnancy of patient's mother.

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"The authors declare no conflict of interest"

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