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RESEARCH ARTICLE

Identification of Two Novel Mutations in the *ATM* Gene from Patients with Ataxia-Telangiectasia by Whole Exome Sequencing

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Abstract: ***Background*:** Ataxia telangiectasia (AT) is one of the most common autosomal recessive hereditary ataxia presenting in childhood. The responsible gene for AT designated ATM (AT, mutated) encodes a protein which is involved in cell cycle checkpoints and other responses to genotoxicity. We describe two novel disease-causing mutations in two unrelated Iranian families with Ataxia-telangiectasia.

***Methods*:** The probands including a 6-year-old female and an 18-year-old boy were diagnosed with Ataxia-telangiectasia among two different Iranian families. In this study, Whole-Exome Sequencing (WES) was employed for the detection of genetic changes in probands. The analysis of the co-segregation of the variants with the disease in families was conducted using PCR direct sequencing.

***Results*:** Two novel frameshift mutations, (c.4236_4236del p. Pro1412fs) and (c.8907T>G p. Tyr2969Ter) in the ataxia telangiectasia mutated ATM gene were detected using Whole-Exome Sequencing (WES) in the probands. These mutations were observed in two separate A-T families.

***Conclusion*:** Next-generation sequencing successfully identified the causative mutation in families with ataxia-telangiectasia. These novel mutations in the ATM gene reported in the present study could assist genetic counseling, Preimplantation Genetic Diagnosis (PGD) and prenatal diagnosis (PND) of AT.

Keywords: Ataxia-telangiectasia, mutation detection, whole exome sequencing, protein, probands, frameshift mutations.

1. INTRODUCTION

Ataxia-telangiectasia (AT), also known as Louis Bar Syndrome is one of the most prevalent autosomal recessive disease, which is characterized by cerebellar ataxia, immune deficiency, a predisposition to cancers, progressive neurological degeneration, ocular and skin-related telangiectasia, insulin-resistant diabetes, poor growth, delayed pubertal development and gonadal atrophy [1-3]. Newborn patients are normal at birth, but after the age of 3 years, they lose muscle coordination and typically need a wheelchair in 10 years' age [4]. It is well documented that AT is a clinically and genetically heterogeneous hereditary disorder. Therefore, all patients do not show similar laboratory findings and/or clinical presentation and symptoms [5]. Although Ataxia-telangiectasia is reported in all regions of the world, its incidence is variable. This disease is often considered as a chromosomal instability syndrome, genome instability syndrome, and neurocutaneous syndrome, which are caused by mutations in the ATM gene [6].

The ATM gene is located on chromosome 11 (11q23), encodes a 370kDa serine/threonine-protein kinase, which plays a crucial role in the regulation of cell cycle [7]. Besides, studies showed that this gene can act as a signal transducer in response to genotoxic stress [8, 9]. Herrup (2013) indicated that ATM protein is involved in the regulation of epigenetic processes [10]. Also, it has been frequently reported that ataxia-telangiectasia disease is caused by homozygous or compound heterozygous mutation. Until now, more than 1000 different AT mutations have been reported on the ATM gene, and compound heterozygous mutations inherited in most patients [11-13]. Therefore, the identification of pathogenic mutations in the

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ATM gene can be useful in early detection such as Pre-implantation Genetic Diagnosis (PGD) and Prenatal Diagnosis (PND).

Here, we report two Iranian AT families with affected children. Two novel homozygous frameshift deletion mutations were identified in the ATM gene by Whole Exome Sequencing (WES) and the molecular confirmation of mutations causing this disorder carried out by Sanger sequencing.

2. MATERIALS AND METHODS

2.1. Patients and Sample Collection

The first case was a six-year-old female, whereas the second one was an 18 years old male diagnosed with AT, referred to Aria Gene Medical Genetics Laboratory (Qom-Iran) in different families. Both patients were born after a full-term pregnancy without serious complications and neurological examination showed dysarthria and retardation in psychomotor development. In both families, the probands were offspring of consanguineous marriages (Fig. **1**). Although they presented clinical heterogeneity, clinical symptoms such as swallowing difficulties, uncoordinated movement and dysarthria were observed; whereas their parents were healthy persons. Initially, both of the studied patients were diagnosed with AT (without molecular analyzes to determine mutation(s) causing AT). Also, the 100 healthy age and ethnically matched subjects, referred to the hospitals affiliated to Tehran University of Medical Sciences for a routine examination, were selected as healthy controls. To exclude the epidemiological bias, the healthy controls were selected from the population of Tehran-Iran, which were unrelated genetically, and matched the age and ethnicity. According to the ethical standards of the Declaration of Helsinki, the patient and their parents, and all healthy controls were informed about the study and an informed consent was signed. The study was performed with the approval of the Institutional Review Board (IRB) Tehran University of Medical Sciences (Ethical Code Number; ERC/S/277) and informed consent was obtained from the patient, or authorized representative/guardian, and controls before genetic testing. The pedigree analysis was carried out using Cyrillic 2.1 software.

2.2. Genomic DNA Extraction and Whole-exome Sequencing (WES)

In the present study, a 5mL peripheral blood sample was drawn from the patient, their parents and all healthy controls. Genomic DNA extraction was performed using a DNA purification kit (Roche, Switzerland) according to the manufacturer's instructions. The quantity of extracted genomic DNA samples was evaluated using a nanodrop instrument (Thermos Fisher Scientific, USA) and OD 260/280 ratio between 1.7-1.9 was selected for molecular study. Also, the quality of extracted genomic DNA samples was evaluated using electrophoresis on 1% agarose gel. The extracted genomic DNA samples with desirable quantity and quality were selected and stored at -20°C until molecular analysis.

Whole Exome Sequencing (WES) was employed to enrich all genomic coding regions and some important other genomic regions. Next-generation sequencing was conducted to sequence close to 100 million reads on Illumina Sequencer (Illumina, San Diego, CA, USA). In general, the test platform examined >95% of the targeted with the sensitivity of above 99%. In this point mutations and micro-insertion/deletions and duplication (<20bp) can be simultaneously detected. Bioinformatics analysis of the sequencing results was performed using international databases. Genetic variants such as point mutations and indels were identified using SAMtools and annotated by ANNOVAR software, as described previously by Hua1 and Wan (2019). A 45 candidate gene was considered a variant that fulfilled the following criteria: (i) missense, nonsense, frameshift, and splice-site variants; (ii) absent or rare (frequency below 1%) in the two databases (dbSNP, 1000G); and (iii) homozygous variants in the patient.

2.3. Confirmation and Validation of WES Results by Sanger Sequencing

The target exons containing mutations of ATM gene were typically amplified using 10pmole of primer, 0.2U Taq DNA polymerase (Roche, Mannheim, Germany), 200 µM of each dNTPs, 0.67µl of 50mM $MgCl_2$, 60ng DNA and 2.5 µl of PCR buffer in 25µl 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).

3. RESULTS

3.1. ATM Mutation Detection Using WES

Molecular genetic testing using whole-exome sequencing was carried out to identify disease-causing mutations in the ATM gene of two affected individuals with AT. The exomes were covered at least 50-fold and the mean read depth was 100. After the exclusion of variants found in healthy individuals, and by further filtering against in-house exomes database, two homozygous mutations remained for segregation analysis.

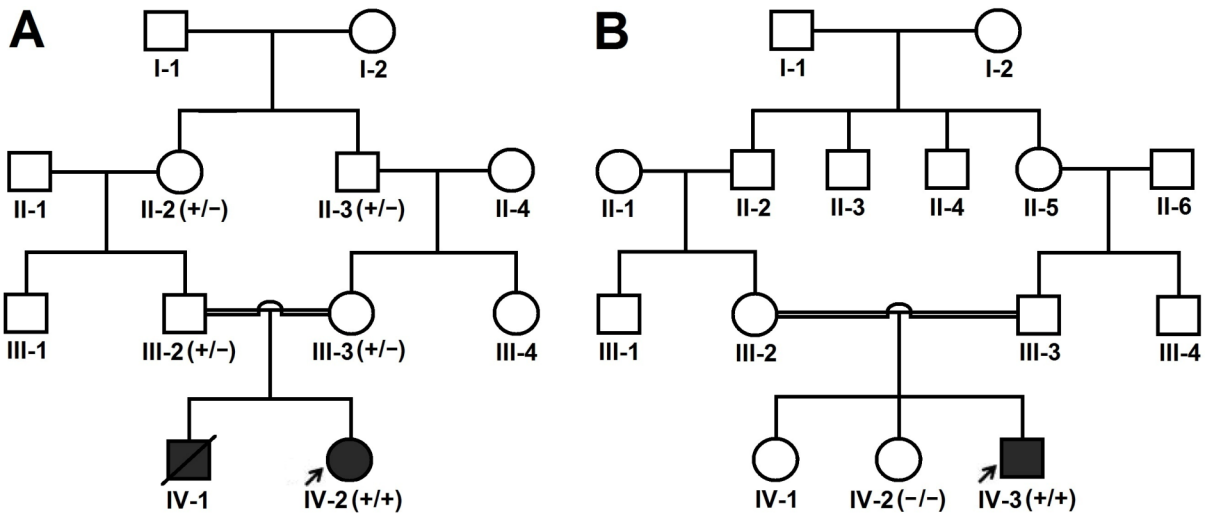

Fig. (1). Pedigrees of Ataxi Telangectasia families with novel homozygous mutations in the ATM gene. A) In pedigree 1, +/+ and +/− represent the homozygous and heterozygous (c.4236_4236del p. Pro1412fs) mutation, respectively. **B**) In pedigree 2, +/+ and +/− represent the homozygous and heterozygous (c.8907T>G p. Tyr2969Ter) mutation, respectively. −/− represents the wild type. 'Male' and 'female' are indicated by squares and circles, respectively, and the filled-in symbols represent individuals affected with AT. The arrow shows the proband.

3.2. Confirmation and Validation of WES Results Using Sanger Sequencing

To confirm and validate the novel mutations (c.4236_4236del p. Pro1412fs) and (c.8907T>G p. Tyr2969Ter) obtained from WES method in ATM gene in probands, their parents and healthy family members, Sanger sequencing was conducted using forward and reverse primers. We found the genetic variants of ATM in the homozygous state in probands, while their parents were heterozygous normal (Fig. **1**).

4. DISCUSSION

Currently, the diagnosis of ataxia telangiectasia remains predominantly based on clinical manifestation and supported by laboratory tests. The genetic dissection of this lethal disease assists to establish the diagnosis of familial AT and to predict neonatal patients and young people, even when the clinical symptoms are not yet evident. This disorder is due to various mutations in the ATM gene. In the current study, we screened the whole ATM gene and its upstream non-coding region by next-generation sequencing (NGS) technology. Two novel ATM alterations, (c.4236_4236del p. Pro1412fs) and (c.8907T>G p. Tyr2969Ter) affecting AT in ATM gene in two Iranian families were detected. These genetic variants were not recorded in the dbSNP (https://www.ncbi.nlm. nih.gov/snp/) version 150 and gnomAD version 2.0.2 (http://gnomad. broadinstitute.org/) databases. These mutations were co-segregated with Ataxia telangiectasia in two Iranian families.

In family one, the proband (IV-2) with AT was diagnosed to harbor a novel ATM homozygous deletion, c.4236_4236del: p. Pro1412fs, which resulted in a frame-shift mutation and truncated the original 3,056 amino-acid full-length proteins to a 1,149-residue protein. This mutation is in exon 28, located within domain 1 (HEAT repeat). The HEAT repeat of ATM mediates protein-protein interaction which is important for the function and regulation of this kinases protein [14, 15].

In another family, the proband (IV-3) with AT was demonstrated to harbor a novel ATM homozygous nonsense mutation, c.8907T>G: p. Tyr2969Ter, which caused translation of the protein to stop at the 2969th amino acid residue. This mutation, located in exon 62, is an AT pathogenic mutation. The affected proband in the present study presented ATM phenotypes which were similar to those of previous cases [16].

Studies on the characterization of cellular defects caused by ATM-deficiency in cells derived from Ataxia telangiectasia patients have led to the discovery of phenotypes associated with functional impairment of ATM [17, 18]. The phenotypic consequences are mainly in the degree of neurological deterioration, levels of immunodeficiency, levels of cellular radiosensitivity, and presence or absence of tumors [19]. One of the most prominent phenotypes of ATM-deficient cells is hypersensitivity to ionizing radiations [20, 21]. Also, many malignancies such as breast cancer and non-small cell lung cancer, lymphoma and leukemia were showed as phenotypic consequences of somatic mutations in the ataxia telangiectasia mutated gene [22, 23].

Various studies have frequently reported that AT is a progressive disorder; however, the timetable for this progression process is not predictable in an individual affected patient. All patients with AT do not indicate the same phenotype. Even among the same family members, where the familial mutation is the same, there is a huge variable expressivity in the problems experienced by each patient [24]. Phenotypic variations among affected persons carrying the same genetic change suggest that at least in some cases this variation could be a result of modification from the genetic background and environment factors. Also, clinical variations could be due to genetic modifiers and gene-gene interaction [25].

CONCLUSION

We, therefore, conclude that our study characterizing two novel ATM mutations extends the mutational spectrum in AT.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Board (IRB) Tehran University of Medical Sciences, Iran (Ethical Code Number; ERC/S/277).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All experimental procedures on human subjects were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from the patients for the study.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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