

ATM Gene Mutations Detection in Iranian Ataxia-Telangiectasia Patients

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ABSTRACT

Ataxia-Telangiectasia (AT) is an autosomal recessive disorder involving cerebellar degeneration, immunodeficiency, radiation sensitivity and cancer predisposition. The ATM gene on human chromosome 11q22.3 has recently been identified as the gene responsible for ataxia-telangiectasia (AT). The gene mutated in AT, which has been designated as the ATM gene, encodes a large protein kinase with a PI-3 kinase-related domain. More than 100 mutations are broadly distributed throughout the ATM gene. The large size of the ATM gene (66 exons spanning ~150kb of genomic DNA) together with the diversity and broad distribution of mutations in AT patients, greatly limits the utility of direct mutation screening as a diagnostic tool.

In this study, 20 families with at least one affected child clinically suspected to have ataxia-telangiectasia were examined and their DNA was extracted and amplified with standard methods. Sequencing methods were used to detect the new point mutation.

Four exons which were hot spots for point mutations in ATM gene were detected by PCR-SSCP or PCR-RFLP.

Keywords: Ataxia-Telangiectasia, Gene, Mutations

INTRODUCTION

Ataxia-Telangiectasia (AT) is an autosomal recessive disorder that affects ~1:40000-1:300000 children in various ethnic groups¹⁻³, characterized by neurological and immunological symptoms, radio sensitivity, dilated blood vessels in the eyes and skin (telangiectasia) and cancer predisposition.¹ The typical phenotype is caused by ATM null alleles that either truncate or severely destabilize the ATM protein. Cultured cells from AT patients are also hypersensitive to ionizing radiation and show defective activation of radiation-induced cell cycle checkpoints, including retarded p53 stabilization.^{2,4} Heterozygous carriers of mutation are clinically

unaffected, but there is evidence reported from epidemiological studies that AT heterozygosity predispose patients to some epithelial cancers, in particular breast cancer.^{5,6} The gene mutated in AT has been localized on chromosome 11q23;⁷ which spans ~150 kb of genomic sequence and contains 66 exons.^{8,9} The ATM gene gives rise to a ubiquitously expressed transcript of ~13kb, which encodes a nuclear 350 kDa protein with homology to PI-3 kinases and related proteins involved in DNA damage responses and cell cycle regulation.^{10,11} While the precise function of the ATM gene is still unknown, the ATM gene product is believed to sense DNA double-strand breaks and to regulate physiological responses via p53- or Chk1-mediated pathway.³ The p53 protein also mediates another radiation-induced pathway leading to a programmed cell death (apoptosis). AT cells seem to have a low threshold for triggering p53-dependent apoptosis, and their radio

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sensitivity might stem from induction of apoptosis usually by non-lethal doses. These observations led to the proposal that the AT gene product might be a component of a radiation-induced signal transduction pathway which was activated by a specific type of DNA damage and involved the induction of p53, leading to the activation of cell cycle checkpoints and the enhancement of DNA repair mechanisms. Furthermore, the non-receptor tyrosine kinase c-Abl has been identified as a downstream target of the ATM protein.³ Numerous different mutations in the ATM gene have been identified in classical AT and in some patients with variant forms of AT.¹² The majority of the published mutations in the ATM gene are truncating, although missense substitutions and in-frame-deletions have also been found.¹³⁻¹⁵ Now that the exon-intron structure of the ATM gene has been fully elucidated⁹ and the complete sequence information is available for the entire ATM gene⁸, mutation scanning methods using genomic DNA have become feasible. We applied PCR-SSCP, PCR-RFLP, PCR-mediated site directed mutagenesis (PSDM),¹⁶ and sequencing to detect point mutation in 4 exons as hot spots⁵ in 20 AT families diagnosed in Iran.

MATERIALS AND METHODS

The diagnosis was established on the basis of typical clinical features, such as ataxia, telangiectasia, and elevated [alpha]-fetoprotein levels. Blood samples of 20 families were collected from the AT patients and, from their parents as well. Genomic DNA of 20 AT families were extracted from white blood cells according to standard procedures.¹⁷ PCR conditions were established to separately amplify four exons of the ATM gene for every patient sample. PCR amplifications were performed in 25µl reaction volume containing 100ng of genomic DNA, 0.2 mM of each dNTP and 2U Taq DNA polymerase in the reaction buffer supplied by manufacturer (Cinagen, Iran). Primer concentrations were each 0.5µM.

Primers for PCR-SSCP:

Exon 53,54

5'-TAGTTAGTGAAGTTTTGTTAAC-3' (forward)

5'-AGGAAAGACTGAATATCACAC-3' (reverse)

Exon 55

5'-GACTGTTTTGTTTGTATCTGAGG-3' (forward)

5'-TCTACAGAGTAACACAGCAAG-3' (reverse)

Exon59

5'-ATGCTTTGCACTGACTCTGATAG-3' (forward)

5'-CTTAGTATCTTTGACAATTACCTG-3' (reverse)

Primers for PSDM

5'-GATTCATGATATTTTACTCTAA-3' (forward)

5'-AAGACAGCTGGTGAAAAATC-3' (reverse)

As standard procedure, 32 PCR cycles were performed within 1 min denaturation at 94°C, 1 min annealing at 54 or 59°C, depending on the primer pair, and 1 min elongation at 72°C. SSCP and RFLP analysis were performed on PCR products. The PCR products were purified by kit (Roche). SSCP was performed as described in the original paper¹¹ in 6% polyacrylamide. RFLP was performed by DdeI enzyme for exon 39. For this exon, a primer was designed to set up a PSDM system (PCR-mediated site directed mutagenesis).¹¹ Variable bands in SSCP gel were sequenced and their results in blast search were analyzed.

RESULTS

We designed a set of 4 PCR primer pairs based on the published genomic sequence information to amplify all 4 exons of the ATM gene. All primers and PCR conditions are listed in the section of Materials and Methods. From genomic DNA samples of all patients, PCR products were obtained for 4 exons. During screening for mutations in the ATM gene with SSCP, variable band patterns were observed in exons 54, 55 and 59, and sequenced (Table 1). In SSCP gel for exon 59 (Figure 1) one nonspecific band was observed and then sequenced and one of the mutations was detected (Table 2).

In the SSCP analysis of exon 54 variable bands (Figure 2) and in the sequencing results two mutations were observed (Table 1). Exons 55 and 59 have variable bands in SSCP gel (Figures 1, 3). The sequencing of these exons showed an 8bp deletion in exon 55 that present in 65% of patients and two deletions, G at 8334 and T at 8280, and one insertion, C at 8375 in exon 59. One sample in our study was homozygote for G>A5557 polymorphism in the exon 39 (Figure 4).

Table 1. Ataxia telangiectasia mutations

Mutation	Location	Predicted effect	Patient ID (status)
Deletion T at 8280	59	Truncation	F07
			F014
			F016
A>C at 8268	59	Truncation	F05(hom)
Deletion of G at 8334	59	Truncation	F10(het)
Insertion of C at 8375	59	Truncation	F09(het)
Deletion of 8T at 7883	55		4,5,6,7,8,9,11,12, 13, 14, 16, 17, 18 (hom)
Deletion of T at 7655	54		F010(het),F011(het)
Deletion of T at 7668	54		F013(unk)
G>A at 5557	39	polymorphism	F011(het)

het=heterozygote, hom=homozygote, unk=unknown

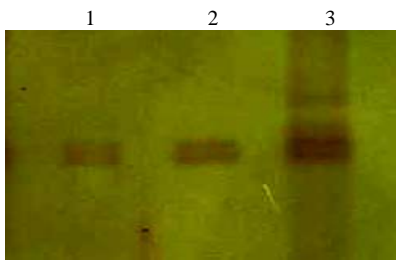


Figure 1. SSCP analysis (Acrylamide 6%) of exon 59 in AT patients. Line1: control sample. Line 2: AT patient without mutation in this exon. Line3: AT patient with 130790A>C mutation.

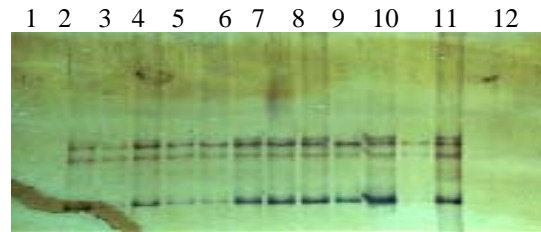


Figure 3. SSCP analysis (Acrylamide 6%) of exon 55 in AT patients. Line12: control sample. 3,6,7,8,10: patients without deletion in this exon. 1,2,4,5,9,11: patients with this deletion.

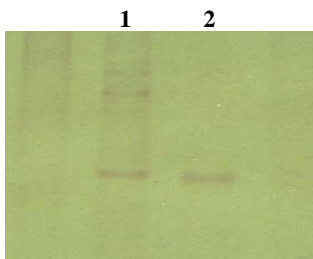


Figure 2. SSCP analysis (Acrylamide 6%) of exon 54 in AT patients. Line2: control sample. Line1: AT patient with 119444delT.

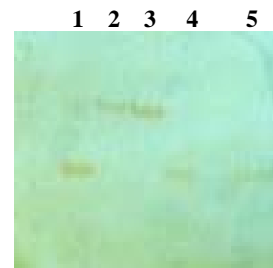


Figure 4. RFLP analysis of exon 39 by DdeI in AT patients, Line 1: control digested; 2: control undigested; 3: patient digested with 5557G>A mutations (hom); 4, 5: patients without this mutation

DISCUSSION

In recent years, researchers have identified several genetic mutations in ATM which may lead to the onset of AT. Approximately 70% of reported ATM mutations would result in a truncated protein.¹⁸

In this study, we have presented seven mutations in three end exons of ATM gene which encode ATM protein segments and have homology with PI3 kinase, and a polymorphism in one of the exons. We sequenced ~950bp of this gene (exons 54, 55, 59) in 20 unrelated ataxia-telangiectasia patients and we utilized a natural polymorphism, G>A5557, in exon 39 of the ATM gene of one patient by PSDM analysis. The sequencing results in exon 54 show two deletions of T at nucleotides 7655 and 7668. In this exon a 4bp deletion at 7668 was reported,¹⁹ which resulted in protein truncation. In the present study of mutation, deletion of 8T at 7883 in exon 55, was observed in 13 families, 5 of which were homozygotes and 8 heterozygotes for this mutation. This mutation has a high frequency in Iranian ataxia-telangiectasia patients. In this exon, a 5bp deletion at 7883,^{9,20} which is similar to an 8bp deletion at the same position in our results, as reported. We do not know which segment of protein is encoded by this exon of ATM gene. Nevertheless, as in our study, patients with this mutation showed severe disease symptoms, we suggest that it encodes an important segment of ATM protein. Sequencing results in the exon 59 revealed three mutations, deletions T8280 and G8334 and one substitution A>C 8268. In this exon a 10bp deletion at 8385, a 150bp deletion at 8269,¹⁹ TC deletion at 8283 and G>A substitution at 8307²⁰ have been reported. Although numerous mutations have been previously identified in AT families, currently there is no cure for AT and most afflicted individuals die in early adolescence. Therefore, achieving a better understanding of the etiology of the disease, and an ability to diagnose the disease earlier, may give rise to the development of better treatments. Moreover, the identification of ATM mutations can be advantageous for other applications, for example, heterozygous AT females may be five times more likely to develop breast cancer than non-carriers. Therefore, mutation analysis of ATM may also play a significant role in the etiology of breast cancer as well as that of AT.

ACKNOWLEDGEMENT

We thank the Immunology Department in Children Hospital. We would also like to thank AT patients' families.

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