

# Investigation of tRNA<sup>Lys/Leu</sup> and ATPase 6/8 gene mutations in Iranian ataxia telangiectasia patients

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## Abstract

**Introduction:** Ataxia telangiectasia (AT) is a rare human neurodegenerative autosomal recessive multisystem disease. AT is the result of mutations in the AT-mutated (ATM) gene. ATM protein is required for radiation-induced apoptosis and acts before mitochondrial collapse. The tRNA genes are considered one of the hot spots for mutations causing mitochondrial disorders. Due to the important role of ATM in apoptosis and its effect on the cell cycle it might be possible that it has a central role in mtDNA mutations. On the other hand, the tRNA<sup>Lys/Leu</sup> gene and also ATPase6 and ATPase8 genes are important for many mitochondrial diseases and many causative mutations have been reported from these genes.

**Material and methods:** In the present research, we performed mutation screening for these genes in 20 patients who were diagnosed with ataxia telangiectasia by a PCR sequencing method.

**Results:** The results showed a significant level of mtDNA variations in AT patients. Among 20 patients in this study, 12 patients (60%) were detected with point mutations, among which 8 mutations (40%) belonged to the MT-ATP6 gene. There was probably a second effect of mtDNA mutations in AT disease and mtDNA plays a main role in establishment of AT.

**Conclusions:** MtDNA mutations might be responsible for the decline of mitochondrial function in AT patients. Mitochondrial investigation can help to understand the mechanism of damage in AT disease.

**Key words:** ataxia telangiectasia, mitochondrial tRNA gene, ATPase 6/8 genes.

## Introduction

Ataxia telangiectasia (AT) is a rare, neurodegenerative, autosomal recessive inherited disease that affects a number of different organs in the body and causes severe disability during childhood. About 20% of patients with AT develop cancer, particularly acute lymphocytic leukaemia or lymphoma. Many of these patients have been reported to have an impaired immune system, making them susceptible to recurrent respiratory infections. Ataxia telangiectasia is caused by mutations to the ATM (ataxia telangiectasia mutated) gene, which has been identified, sequenced and

is located primarily on chromosome 11q22-23. Ataxia telangiectasia patients have shown more than 400 distinct ATM mutations, of which 85% are null mutations [1, 2]. The ATM protein is a member of the phosphatidylinositol 3-kinase-like family of serine/threonine protein kinases (PIKK) [3, 4]. Ataxia telangiectasia mutated protein is required for radiation-induced apoptosis and acts before mitochondrial collapse [5]. This protein is also involved in cell cycle control, intracellular protein transport, and the DNA damage response. Apoptosis is induced either through the death receptor pathway of apoptosis, or the mitochondrial pathway of apoptosis. This protein also plays a role in normal development and activity of body systems such as the nervous and immune system, which obviously indicates that any problem in the functions of the ATM protein could lead to immune system related diseases.

Mitochondria are present in almost all mammalian cells, and are responsible for energy generation. Despite the fact that the mitochondrial genome is very small, mitochondrial DNA mutations have an important role in genetic diseases [6, 7]. Mitochondrial DNA (mtDNA) mutations can result in both maternally inherited and sporadic diseases. The mitochondrial genome is 16.6-kb double-stranded circular DNA encoding 37 genes, including 13 proteins, 22 transfer RNAs, and 2 ribosomal RNAs. A number of mutations in the mitochondrial (mt) tRNA genes have been reported to be associated with human mitochondrial diseases.

It has been reported that the ATM protein is involved in regulation of cell check points and mutations leading to apoptosis, leaving us to hypothesize that mitochondrial DNA could play a role in AT patients who develop cancer, especially in the apoptotic pathway [8]. Many exhaustive screening approaches have been applied to investigate the mutations in mtDNA causing various human diseases. Many scientists have reported that point mutations in tRNA<sup>Lys/Leu</sup> genes and ATPase 6 and 8 genes are the leading causes of mitochondrial disorders [9]. To elucidate the molecular and functional role of mtDNA in patients with AT, it is required to establish that the mutations to mitochondrial (mt) tRNA could lead to the mitochondrial pathway of apoptosis in AT patients.

In this study we performed screening of mutations to the tRNA<sup>Lys/Leu</sup> genes and ATPase 6 and 8 genes in Iranian patients with AT who were referred to our centre. Primers for mitochondrial tRNA<sup>Lys/Leu</sup> and ATPase 6 genes were used on the DNA extracted from the blood samples of these patients and amplified by PCR. Automated DNA sequencing of the amplified DNA led to detection of possible DNA mutations in the mitochondrial (mt) tRNA<sup>Lys/Leu</sup> and ATPase 6 genes. Our additional

analysis suggested that the presence of these mutations could be one of the causes of mitochondrial dysfunction in these AT patients leading to cancer.

## Material and methods

### Diagnosis of ataxia telangiectasia patients

All the patients were clinically defined as having AT by a neurologist according to generally accepted diagnosis criteria of AT [10]; all of the patients had gait ataxia, oculocutaneous telangiectasias, apraxia of eye movement or immunological defects that include immunoglobulin deficiencies (particularly IgA and IgE), high serum  $\alpha$ -fetoprotein concentration and lymphopenia. If the diagnosis was uncertain, molecular genetic tests for ATM mutations were performed to confirm the diagnosis [2].

### Analysis of tRNA<sup>Lys/Leu</sup> genes and ATPase genes

Mutation screening of tRNA<sup>Lys/Leu</sup> genes and also ATPase6 genes was performed in 20 patients who were diagnosed based on the typical clinical features of ataxia telangiectasia. Peripheral blood samples were obtained and the DNA was purified using a Diatom DNA Extraction Kit (Genefanavar, Tehran).

Two pairs of primers were used to amplify the targeted genes. The primers and the PCR conditions were replicated from previous studies [11, 12]. The nucleotide sequences were directly determined by automated sequencing in a 3700 ABI machine using forward primers (Macrogen Seoul, Korea). The obtained mtDNA sequences were aligned with a multiple sequence alignment interface CLUSTAL\_X with comparison to Mitomap references (<http://www.gen.emory.edu/mitomap/mitoseq.html>).

## Results

The sequence analysis of samples showed the following mutations: A8774G, A8982C and C8684T in the mt-ATPase6 gene. Each mutation was found in one patient out of 20. These mutations in the MT-ATPase6 gene result in amino acid replacements. In 6 patients, an mtDNA polymorphism without changing the amino acid was identified. The findings showed six known polymorphisms in the mt-ATP6 gene. All of the variations were homo-plasmic based on the sequencing results. Nucleotide changes in mitochondrial genes found in our patients are shown in Table 1 [18-26]. Nucleotide changes in mitochondrial tRNA<sup>Leu</sup> were not found in the studied patients.

## Discussion

A prominent characteristic of mtDNA diseases is that tissues with a high energy demand, such as

**Table I.** Nucleotide changes in mitochondrial genes found in this study

Number	Nucleotide position	Locus	Nucleotide change	Amino acid change	Report	Reference
1	8774	MT-ATP6	A-G	N-S	No	
2	8684	MT-ATP6	C-T	T-I	Yes	Unpublished MtDNA polymorphism, Arabs from Saudi Arabia
3	8277	MT-NC7	T-C	Non-coding region	Yes	18
4	8697	MT-ATP6	G-A	Syn	Yes	19-21
5	8574	MT-ATP6	C-T	Syn	No	
6	8562	MT-ATP6	C-T	Syn	No	
7	8982	MT-ATP6	A-C	Q-H	No	
8	8730	MT-ATP6	A-G	Syn	No	
9	8562	MT-ATP8	C-T	P-L	No	
10	8292	MT-NC7	G-A	Non-coding region	No	
11	8269	MT-COII	G-A	Non-coding region	Yes	21-23
12	8856	MT-ATP6	G-A	Syn	Yes	19, 24-26

muscle and brain, are mostly affected [7]. Based on other investigations on Iranian AT patients, mtDNA deletions have been observed in about 54% of the patients. The discovered deletions remove or impair some structural genes including ATPase 6 and 8, COIII, ND3, ND4L, ND4, ND5, ND6, Cytb and eight tRNA genes. Such deletions in AT patients may result in multiple respiratory chain deficiencies [13]. The human mtDNA encodes 13 polypeptides that are essential for the mitochondrial energy generating system, oxidative phosphorylation (OXPHOS), as well as the tRNA genes which are necessary for their expression; thus, any mutation in the mtDNA coding region will alter mitochondrial energy production. Series of enzyme complexes are present in the mitochondria inner membrane which are known collectively to constitute the electron transport chain which consists of five complexes. Complex V (ATP synthase) is composed of 14 subunits. The protons expelled from other complexes create an electrochemical gradient for ATP production. Human mtDNA encodes 2 subunits (ATPases 6 and 8) of complex V subunits [14]. So any mutation in these genes may also disorganize the mitochondrial energy production processes. Defective energy metabolism resulting from loss of ATP production is certainly a common attribute of most OXPHOS diseases [6]. For example, point mutations in the ATP synthase subunit 6 have been observed in some disease including neuropathy ataxia retinitis pigmentosa (NARP) and Leigh syndromes [15].

Mitochondria use OXPHOS to generate most of the cellular ATP and as a toxic product they produce

most of the endogenous reactive oxygen species (ROS). The ROS can damage the OXPHOS enzymes and mtDNA in turn, eroding mitochondrial function. In the presence of mtDNA mutations which may be caused by ROS during aerobic metabolism, sensitive cells such as skeletal muscles are deprived of ATP (due to the defective respiratory functions of mitochondria). Apoptosis is induced either through the death receptor pathway of apoptosis, or the mitochondrial pathway of apoptosis. When mitochondrial energy production gets too low and/or mitochondrial ROS damage becomes too high, similar to the condition happening in AT patients, the mitochondrial permeability transition pore (mtPTP) is activated and the cell is removed by apoptosis, thus resulting in more profound oxidative damage [16].

Since point mutations of mitochondrial tRNA genes are the hot spots of mitochondrial disorders, in this research the sequence of mitochondrial tRNA<sup>Lys/Leu</sup> genes and ATPase genes of 20 AT patients were analysed by PCR and automated sequencing methods. The results showed a significant level of mtDNA variations in AT patients. In one case a nucleotide variation (C8684T) resulting in amino acid change from Thr to Ile was observed in the MT-ATPase6 gene. Thr is categorized in polar amino acids, but Ile contains non-polar properties. This alteration may strongly affect the tertiary structure of protein that will impair its interaction with ATP.

Moreover, two samples in this study showed novel point mutations in the MT-ATP6 gene causing amino acid replacement. The first one was A8982C

mutation in an affected patient which changes Gln to His. Gln has neutral properties but His is a basic amino acid. Therefore, it could deeply influence the structure of the subunit of respiratory complex V and the activity of this complex as a result. Regarding the second sample, we found a point mutation at position 8774 in the MT-ATP6 gene that alters Asn to Ser, where both of the amino acids have similar properties. Neither of these variations have been reported previously.

In the present research, approximately 60% of our patients had mtDNA mutations. Among the 12 mitochondrial mutations in this study, four of them (~30%) were missense mutations. Three of the patients (25%) in this study had mutations in the non-coding region of the MT-NC7 gene. All of the mutations in the coding region belonged to the mt-ATP6 gene with the exception of one variation in the mt-ATP8 gene.

Recent studies proposed involvement of mitochondrial pathway mediated apoptosis in the development of cancers in this group of patients and in immunopathogenesis of AT. In fact, apoptosis in AT could be via the mitochondrial pathway, which would offer a basis for establishment of mtDNA-targeted therapeutic interventions in AT [8].

Ataxia telangiectasia mutated is the gene mutated in ataxia telangiectasia and numerous different mutations in the ATM gene have been identified in classical AT and in some patients with variant forms of AT [17-26]. It was explained by Eaton *et al.* (2007) that their results show a novel function for ATM in mtDNA maintenance. This study hypothesizes that since ATM influences the *de novo* pathway for dNTP synthesis which is required for mtDNA replication and repair, and also since AT cells have higher amounts of ROS, the loss of ATM will affect the mtDNA copy number. Finally they offered novel roles for ATM in mitochondrial homeostasis.

Based on our results, there was probably a second effect of mtDNA mutations in AT disease and mtDNA plays a main role in establishment of AT. Perhaps mtDNA mutations are responsible for the decline of mitochondrial function in AT patients. In conclusion, we suggest more analysis to unravel the precise connection between ATM, mtDNA mutations and AT disease.

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