Short Communication

Role of mitochondria in Ataxia-Telangiectasia: Investigation of mitochondrial deletions and Haplogroups

Massoud Houshmand[1]*, Mohammad Hossein Sanati[1], Baharak Hooshiar Kashani[1], Mehdi Shafa Shariat Panahi[1], Mohammad Mehdi Banoei[1], Anna Isaian[2], Mostafa Moin[2], Abolhasan Farhoudi[2]

[1]Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology (NIGEB), P.O. Box 14155-6343, Tehran, I.R. Iran [2]Department of Allergy and Clinical Immunology, Children Medical Center, Tehran University of Medical Sciences, P.O. Box 14185-863, Tehran, I.R. Iran

Abstract

Ataxia-Telangiectasia (AT) is a rare human neurodegenerative autosomal recessive multisystem disease that is characterized by a wide range of features including, progressive cerebellar ataxia with onset during infancy, occulocutaneous telangiectasia, susceptibility to neoplasia, occulomotor disturbances, chromosomal instability and growth and developmental abnormalities. Mitochondrial DNA (mtDNA) has the only non-coding regions at the displacement loop (D-loop) region that contains two hypervariable segments (HVS-I and HVS-II) with high polymorphism. We investigated mt-DNA deletions and haplogroups in AT patients. In this study, 24 Iranian patients suffering from AT and 100 normal controls were examined. mt-DNA was extracted from whole blood and examined by 6 primers for existence of mitochondrial deletions. We also amplified and sequenced the mtDNA HVS-I by standard sequencing techniques. mtDNA deletions were observed in 54.1% (13/24) of patients (8.9 kb deletion in all samples, 5.0 kb in one and 7.5 kb in two patients), representing mtDNA damage which may be due to oxidative stress in mitochondria. Our results showed that there is no association between mtDNA haplogroups and AT. This data may indicate involvement of mitochondrial damage in the pathogenesis of AT.

Keywords: *Ataxia-Telangiectasia; Mitochondrial DNA Deletion; Haplogroup*

Correspondence to:* **Massoud Houshmand, Ph.D.
Tel: +98 21 44580390; Fax: +98 21 44580399
E-mail: massoudh@nrcgeb.ac.ir

Ataxia-Telangiectasia (AT) is a rare progressive neurodegenerative disorder causing a predisposition to cancer, with a hallmark of onset in early childhood (Gatti *et al*., 1991). AT is seen in approximately 1 in every 40 000 live births in the USA, although the frequency varies from country to country (Chun and Gatti, 2004). At birth, infants appear normal and begin walking at a normal age (approximately age 1 year); however, by age 2-3 ataxia (loss of muscle co-ordination) becomes visible and generally by age 10 patients are confined to a wheelchair (Chun and Gatti, 2004). AT is the result of mutations in the ataxia telangiectasia mutated (*ATM*) gene, which was discovered in 1995 (Savitsky *et al*., 1995). AT patients suffer as a result of over 400 distinct *ATM* mutations, of which 85% are null mutations in the *ATM* gene (Becker-Catania *et al*., 2000). The ATM protein is a member of the phosphatidylinositol 3-kinase-like family of serine/threonine protein kinases (PIKK) (Goodarzi *et al*., 2003). ATM protein is required for radiation-induced apoptosis and acts before mitochondrial collapse. (Vit *et al*., 2000) Apoptosis is induced either through the death receptor pathway of apoptosis, or the mitochondrial pathway of apoptosis.

Several reports showed a relationship between diseases and mitochondrial haplogroups (Abe *et al*., 1998; Makino *et al*., 2000). Hofmann *et al*. (1997) concluded that certain European mtDNA haplogroups determine a genetic susceptibility to various disorders. It was proposed that polymorphisms characteristic for haplogroups in fact influence the respiratory chain

activity and cooperate in disease formation. Most studies of mtDNA variation have been conducted by use of one of two methods that assay different portions of mtDNA: Direct sequencing of control region (CR) and digestion of the entire molecule by means of standard set of restriction enzymes. To investigate the association of mtDNA haplotypes with ATM mutations and alterations in HVS-I region, the nucleotide sequence in the D-loop region was determined in an Iranian population of AT patients. The mitochondrial deletions were also examined in our patients to evaluate any possible mtDNA damage.

For this purpose, 24 representative Iranian AT patients and 100 normal controls were evaluated. Peripheral blood samples were obtained and DNA was extracted after lyses of white blood cells using DNA extraction kit (Diatom DNA Extraction Kit, Genefanavaran, Tehran). The reaction mixture for multiplex PCR contained 10 pmol of each primer, 1 unit Taq polymerase (Roche, Mannheim, Germany), each dNTP at a final concentration of 200 μM, and 2.5 μl PCR buffer at a final volume of 25 μl. The PCR reactions were performed in a thermal cycler (MWG-Biotech Primus) for 35 cycles with denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min and primer extension at 72°C for 35 sec. The amplified fragments were separated on gel electrophoresis in 1.5% agarose. The primers and the investigated regions of mtDNA are illustrated in Figure 1. The deletion-prone region between nucleotide 5461 of light strand and nucleotide 15000 of heavy strand was investigated in all patients using the primers ONP 86, ONP 89, ONP 10, ONP 74, ONP 25 and ONP 99. The distances between the primers were long enough to allow amplification only if a part of the DNA between respective primers was deleted. Primer pair ONP 86, 89 was used to amplify a normal internal mtDNA fragment in a region which is seldom afflicted by deletions and served as a control of the preparation and PCR analysis (Wallace *et al*., 1993). Deletion breakpoints were analyzed by direct sequencing of mtDNA fragments amplified by the PCR reactions using ABI 3700

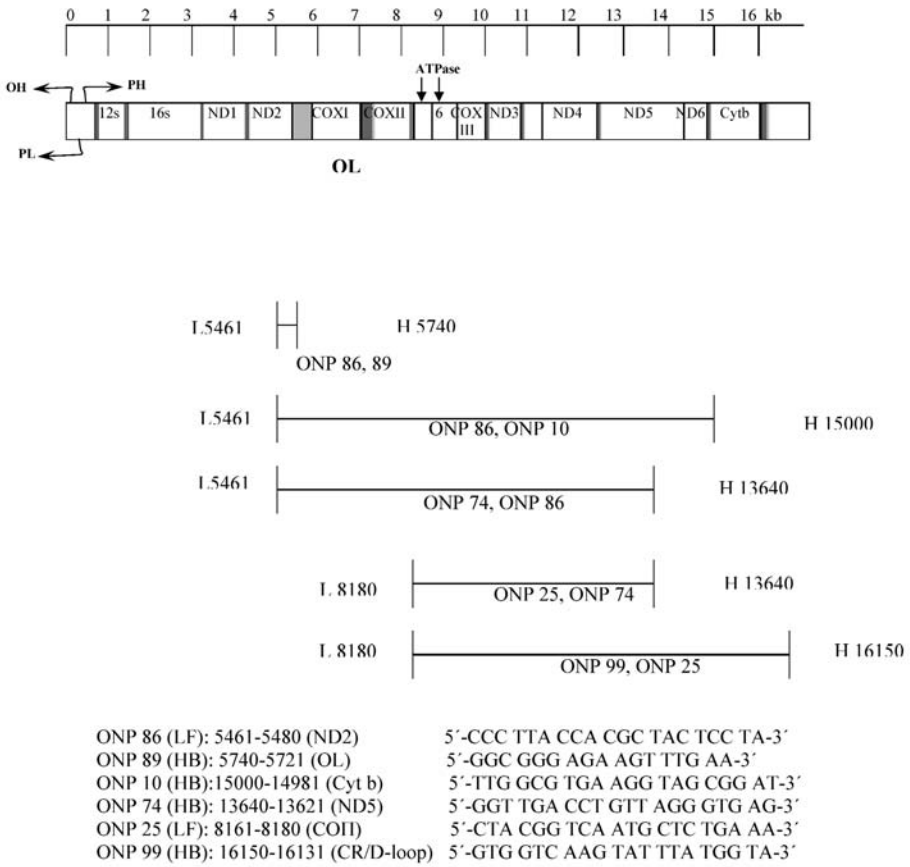

Figure 1. Mitochondrial gene map, location of mtDNA deletions, primers position and their corresponding sequence. LF=Light chain Forward, HB=Heavy chain Backward, ND2=NADH dehydrogenase subunit 2, OL=L-strand origin, Cytb=Cytochrome b, ND5=NADH dehydrogenase subunit 5, COII=Cytochrome c oxidase subunit II, CR/D-Loop=Control Region, including Displacement loop

capillary sequencer (Genfanavaran, Macrogene, Seoul, Korea). Sequences were compared with a comprehensive mitochondrial databank (Mitomap database).

To study the relationship between AT patients and mtDNA haplogroups, we sequenced HVS-I from 17 AT patients under 4 years of age and 100 normal controls. All the patients were clinically defined, as having AT by a neurologist based on generally accepted diagnosis criteria of AT (Sedgwick and Boder, 1991). All of the patients had gait ataxia, oculocutaneous telangiectases, apraxia of eyes movement or immunologic defects that include immunoglobulin deficiencies (particularly IgA and IgE), high serum alphafetoprotein concentration and lymphopenia. If diagnosis was uncertain, molecular genetic tests for ATM mutations performed to confirm the diagnosis. Controls were randomly chosen from people who had no AT symptoms or family history of the disease. All of the patients and controls were informed of the aims of the study and gave their informed consents to the genetic analysis. Peripheral blood samples were obtained and DNA was purified after lyses of white blood cells by use of DNA extraction kit (Diatom DNA Extraction Kit, Genefanavaran, Tehran). PCR amplification was carried out in a final volume of 50 µl containing 200-300 ng total DNA, 10 pmol each primers, 2.5 mM MgCl2, 200 µM of each dNTP and 2 Units *Taq* DNA polymerase. (Roche Applied Science, Mannheim, Germany) Primers were as follows: Primer ONPF206 (15340-15360 nt) 5´- ATC CTT GCA CGA AAC GGG ATC -3´ and primer ONPR 77 (110-91 nt) 5´-GCT CGG GCT CCA GCG CTC CG-3´. These primers amplified a 1366 bp sequence encompassing HVS-I in the D-Loop of the mtDNA to fetch the 359 bp sequence (16024-16383 nt) for HVS I. The nucleotide sequence of the amplicon was directly determined by automated sequencing 3700 ABI machine, using primer ONPR 77 (Macrogene Seoul, Korea). The obtained mtDNA sequences were aligned with a multiple sequence alignment interface CLUSTAL_X with comparison to rCRS (http://www.gen.emory.edu/mitomap/mitoseq.html).

Haplotypes were assigned to haplogroups (hgs) according to the West Eurasian mtDNA genealogy (Macaulay *et al*., 1999). Hg assignment proceeded by using the following algorithm (all numbering is according to Anderson *et al.* (1981) minus 16,000 in the control region for brevity): 069T 126C 223C assigned to hg J ; 126C 223C 294T assigned to T; 129A 223T 391A assigned to I ; 223T 292T assigned to W; 189C 223T 278T assigned to X; 223C 224C 311C assigned to K; 223C 249C and either 189C or 327T assigned to U1; 129C 223C assigned to U2 : 223C 343G assigned to U3; 223C 356C assigned to U4; 223C 270T assigned to U5; 172C 219G 223C assigned to U6; 223C 318T assigned to U7; 223C 298C assigned to V; 067T 223C assigned to HV1; 126C 223C 362C assigned to preHV; 145A 176G 223T assigned to N1b; 223T 278T 390A assigned to L2; and 187T 189C 223T 278T 311C assigned to L1. Fisher's exact probability test was used to examine the association between two groups. Values of $P< 0.05$ were regarded as statistically significant. mtDNA deletions were present in 13 patients out of 24 (54.1%). The sizes of deletions were 8.9 kb, 7.5 kb and 5.0 kb. (Fig. 2) We found also a 10kb deletion in three patients with AT. Our study showed 2 individuals with 7.5 kb deletion and one with 4977 bp (common deletion). 8.9 kb deletion was also found in all 13 patients. Deletions mostly occurred in the region between np 5461 and np 15000 (Primers ONP86 and ONP10). Healthy controls showed no deletions in their mtDNA. None of our patients had multiple deletions. The mtDNA haplogroups of 17 AT patients and 100 normal subjects were characterized by direct sequencing of mtDNA HVS-I. Our results for the distribution of mtDNA haplogroups among 17 patients and 100 normal controls are summarized in Table 1.

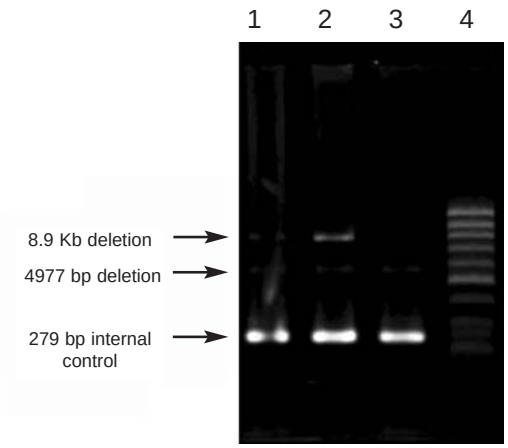

Figure 2. Detection of mtDNA deletion in Ataxia-Telangiectasia by multiplex PCR. Lane 1. Normal control, Lane 2. Patient with 8.9 kb deletion, Lane 3. Patient with 4977 bp deletion, Lane 4. 100 bp molecular weight marker

One of the genetic factors suspected of modulating multifactoral diseases is the haplogroup. Haplogroups H, I, J, K, T, U, W, V, X cover most of the European mtDNAs. To the best of our knowledge, this is the first study investigating correlations between AT with a specific mtDNA haplogroup. In this study, lack of association was found between mitochondrial haplogroups and AT. The rich variability within HVS-I

Table1. Distribution of mtDNA haplogroups among AT patients and normal controls.

mtDNA Haplogroups	AT Patients	Normal Controls	P-Value
H	1/17 (5.8%)	3/100 (3%)	0.471
L	0/17 (0%)	14/100 (14%)	0.217
J	1/17 (5.8%)	20/100 (20%)	0.302
T	4/17 (23.5%)	16/100 (16%)	0.487
U	2/17 (11.7%)	3/100 (3%)	0.152
K	1/17 (5.8%)	0/100 (0%)	0.145
W	1/17 (5.8%)	5/100 (5%)	1

compared with the relatively constant constellation within the gene regions provides useful criteria for pathogenetic studies. A particular mtDNA haplotype may signal, through a founder effect, a population subgroup that has inherited a group of detrimental or protective nuclear genes. Substitutions in the D-loop may be part of a haplotype with mutations elsewhere in the mtDNA. Mutations in mtDNA HVS-I may cause energy deficiency in stressful situations during a vulnerable developmental period (Arnestad *et al*., 2002).

In the presence of mtDNA deletions which may caused by Reactive Oxygen Species (ROS) or free radicals generated during aerobic metabolism, sensitive cells as skeletal muscles are deprived of ATP (due to the defective respiratory functions of mitochondria) and then they run into a state of energy crisis through a "vicious cycle" as proposed by Wei (1998). This "vicious cycle" may have catastrophic consequences and is accelerated by electron leakage from defective mitochondria; as such, it may play an important role in the pathophysiology of Ataxia-Telangiectasia patients. The molecular mechanisms responsible for generating mtDNA deletions are not well known (Hirano *et al*., 2001). Several mechanisms have been proposed, including slipped-mispairing (Shoffner *et al*., 1989), oxidative reactions elicited by free radicals (Poulton *et al*., 1993), and DNA strand break affected by a topoisomerase or DNA recombinase (Lestienne *et al*., 1995). Large-scale deletions of mtDNA are frequently found in the affected tissues of patients with mitochondrial myopathy (Holt *et al*., 1989) or elderly subjects (Yen *et al*., 1991). The 4977 common deletion of mtDNA causes removal or truncation of multiple structural genes (ATPase 6/8, COIII, ND3, ND4L, and ND4) and five tRNA genes. The 7.5 and 9.0 kb deletions cause a loss or truncation of the structural genes of ATPase 6/8, COIII, ND3, ND4L, ND4, ND5, ND6, Cytb and eight tRNA genes. Such deletions in AT patients may result in multiple respiratory chain deficiencies as described before in human aging (Lee and Wei, 1997). Defective respiratory enzymes containing protein subunits encoded by the deleted mtDNA may further enhance free radical production, resulting in more profound oxidative damages in AT patients. The human mtDNA encodes 13 polypeptides that are essential for the mitochondrial energy generating system, OXPHOS, plus the tRNA genes necessity for their expression. Thus, any mutation in the mtDNA coding region will alter mitochondrial energy production. Mitochondria use OXPHOS system to generate most of the cellular ATP and produce most of the endogenous ROS as a toxic product. The ROS can damage the OXPHOS enzymes and mtDNA in turn, eroding mitochondrial function. When mitochondrial energy production gets too low and/or mitochondrial ROS damaging becomes too high, the mitochondrial permeability transition pore (mtPTP) is activated and the cell is removed by apoptosis (Kokoszka *et al*., 2001) and resulting in more profound oxidative damages in AT patients.

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