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Association ofMitochondrial T16519C polymorphism with Coronary Artery Disease (CAD) in Iranian patients underwent coronary angiography

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ABSTRACT

Early diagnosis, before manifestation of symptoms or identifying susceptibility to diseases can reduce the severity and prevalence and may even control or inhibit the disease progression. Now the relationship between the incidence of cardiovascular diseases and certain types of haplotypes of human mtDNA (mitochondrial DNA) have been substantiated and investigation of the frequency of particular types of cases and controls can reveal this connection. Substitution of T to C at nucleotide position 16189 in the hypervariable D-loop of the control region (CR) of mitochondrial DNA (mtDNA) has attracted research interest because of its suspected association with various multifactorial diseases. The objective of the current study was to identify and evaluate different haplotype mtDNA and examine their relationship to the incidence of coronary artery disease in different haplogroups in Iranian patients visiting SINA hospital in Tehran. A total of 70 participants (including 40 males and 30 females) visiting the Cath Lab (angiography) of SINA hospital were selected according to the index of stenosis (CSI). After obtaining informed consent, approximately 5cc of blood sample from each patient was taken and sent to the laboratory. Total DNA (50 µg) of blood samples were extracted using primers of D-Loop- a specific region of mtDNA, the replicative samples (HVS regions) were then seperated by electrophoresis process and quality of amplicon (product) was confirmed. This step was followed by sequencing and compared with the reference sequence. According to the SNP, polymorphisms and mutations, and the percentage of their presence in case and control populations were studied and the data analyzed. Items have been composed of haplotype 263 A>G (maximum) with 62 samples from 34 patients and 23 healthy and 5 mild cases, 310-311 insC with 61 samples from 35 patients, 21 healthy and 5 slight, variant 750 A>G with 59 persons of 33 patients, 20 healthy and 6 average, other SNP 73 A>G with 45 samples from 23 patients, 17 healthy and 5 average, 40 varieties of 16519 T>C with 24 patients, 13 healthy and 3 middle and 32 of the 309-310 ins C with 17 patients, 10 healthy and 5 are mild cases. Some changes, such as C>T and insCC, regardless of position, as well as history and ethnic studies were evaluated. The 263 A>G, 310-311 insC, 750 A>G, 73 A>G, 16519 T>C and 309-310 insC with high frequency are more important in patients with cardiovascular disorders. These changes (except 750 A>G and sex variable) have been previously established with certain diseases, however in the present study no particular allelic association were revealed. In this study susceptibility of men against women is 6.6 times and 750 A>G significantly increases 1.6 times the risk of CAD.

Keywords: Coronary Artery Disease (CAD), Reference Sequence, Mitochondrial DNA (mtDNA), Haplotype, Haplogroup, Single Nucleotide Polymorphism (SNP), Coronary Stenosis Index (CSI)

INTRODUCTION

By virtue of the functional role of the mitochondrion in energy and reactive oxygen species production, mutations in mitochondrial DNA (mtDNA) are potential candidates for cardiovascular-related disorders [1].The maternally inherited human mitochondrial DNA (mtDNA) has around 16,500 base pairs. It contains ribosomal RNA, tRNA, and 37 genes supporting metabolic oxidative phosphorylation Mitochondria produce energy to sustain cell growth and function, but they also generate harmful free radicals and mediate apoptosis and is composed of double-stranded, closed and circular DNA molecule [2]. All of the mitochondrial protein coding genes encode subunits of the OXPHOS enzymes that are responsible for the energy generating pathway. In addition, mtDNA contains a non-coding region called a displacement loop (D-loop), which is involved in the control of replication and transcription of mtDNA [3]. It is independent of the nuclear genome.The D-loop is a region of 1124 base pairs (position 16024-576 on the mtDNA), which acts as a promoter for both heavy and light strands of mtDNA and contains essential transcription and replication elements [4]. This loop contains two hypervariable regions (HVS-I at positions 16024-16383 and HVS-II at positions 57-372) [5] .Specific large mutation, large deletions or duplications of the mitochondrial genome cause mitochondrial diseases. Rare pathogenic mutations of the mtDNA that either impair mitochondrial protein synthesis or impair proteins encoded by the mtDNA have been associated with more than 70 human diseases [6]. Since many diseasesassociated with mitochondrial mutations have been found, these mutations have beenreported in MITOMAP (a human mitochondrial database).

The very high mitochondrial evolutionary rate compared to the nuclear genome has provided for the accumulation of diverse mtDNA variations, marking a number of haplotypes, thus providing an excellent toolfor studying human evolution, migration, and population histories [7]. Over the last two decades, analysis of modern human mtDNA variations has revealed modern human origins in Africa and subsequent migrations to Asia and Europe [8].It has been found that some mtDNA haplotypes not only elucidate population structures, but may also predispose to, or protect against, certain diseases [9].

In addition to the mutations in genes carried on the nuclear chromosomes, mitochondrial mutations are a significant cause of human genetic disease. The mitochondrial genome is small (only 16.5 kb) but it is highly mutable in comparison with the nuclear genome, probably because mitochondrial DNA replication is more error-prone and the number of replications is much higher. Mitochondrially encoded diseases have two unusual features, matrilineal inheritance and frequent heteroplasmy [10].

Coronary artery disease (CAD) is the most common cause of death in industrialized countries and is rapidly increasing in prevalence in developing countries. It results from atherosclerosis, a process that takes place over many years and involves deposition of lipid in the subendothelial space (intima) of arteries with a consequent narrowing of their lumina the first stage involves the deposition of lipid in the arterial wall that is determined by hemodynamic factors. Monocytes adhere to areas of the endothelial surface of arterial walls with lipid deposits and enter the vessel wall, proliferate and differentiate into macrophages. The macrophages scavenge the lipids, producing the classic fatty streaks, and through the action of cytokines, growth factors and adhesion molecules induce smooth muscle proliferation and the formation of extracellular matrix, resulting in the development of the fibrous atherosclerotic plaques. The narrowing of the coronary arteries compromises the metabolic needs of the heart muscle, leading to myocardial ischemia, which, if severe, results in myocardial infarction [10].

High levels of LDLs are associated with an increased risk of coronary artery disease. Conversely, high levels of HDLs are inversely correlated with a risk of coronary artery disease. Consequently, the LDL : HDL ratio has been used as a risk predictor for coronary artery disease and as an indicator for therapeutic intervention. Statins are effective drugs for lowering LDL-cholesterol levels.

For the majority of persons their risk of coronary artery disease is multifactorial or polygenic in origin. A variety of different genetic and environmental risk factors has been identified that predispose to early onset of the atherosclerotic process. Well publicized environmental risk factors include lack of exercise, dietary cholesterol and smoking. The advice with respect to the potential for prevention of developing coronary artery disease for these factors is obvious [10].

There polymorphisms in some genes also increase the risk of disease. For example the insertion-deletion polymorphism in the gene of angiotensin-converting enzyme (ACE) has also been extensively studied for association with diseases. DD homozygotes are at increased risk of myocardial infarction and coronary artery disease [11].

We now know of hundreds of DNA variants that are clearly associated with susceptibility to one or another complex disease. In most cases, these variants probably do not themselves directly confer susceptibility but are associated with susceptlbility because they are in linkage disequilibrium with the actual causative variant. Whether or not this is the case, genotypes for these variants reliably predict variations in disease susceptibility.This has been a major advance in biomedical science [11].

Mitochondrial functional differences are thought to be among the most important risk factors of coronary artery diseases (CAD) [12]. Mitochondria are the primary site of superoxide production in vascular endothelial cells. Mitochondrion-derived reactive oxygen species play an important role in the pathogenesis of atherosclerosis and CAD [13]. Consequently, polymorphisms in the mtDNA are expected to associate with CAD.

The mtDNA variant 16189T>C increased risk of CAD and MI in Saudi Arabs [14] and of CAD in Austrians [15], while the mitochondrial haplogroup N9b was reported to be protective against MI in Japanese males [16]. However, in a large study on the Danish population, no association was found between mitochondrial haplogroups and risk of ischemic cardiovascular disease [17].

A mitochondrial haplogroup is a cluster of phylogenetically related mitochondrial genotypes (haplotypes). These haplogroups are defined by ancient mutations [18]. These changes appeared and survived; therefore, they could not be deleterious mutations. Most of them probably did not have a phenotypic effect and were neutral. Some of them had a beneficial effect and were positively selected. However, this positive effect was related to a particular environment and nowadays, in other environmental conditions, may have different effects on the phenotype [18-20].In other words human haplogroups are defined by special polymorphisms in human mitochondrial DNA (mtDNA). These haplogroups trace the matrilineal inheritance of modern humans back to human origins in Africa and the subsequent spread across the globe.Most of the mutations observed in both mtDNA coding and non-coding regions have occurred in pre-existing haplogroups and have defined the individual mtDNA types or haplotypes [5, 21].

Since CAD risk is both genetically and environmentally determined, and family history [22] along with ethnicity [23] play a major role in disease occurrence, the strong association of mtDNA with population structure may explain the inconsistent implication of CAD among mtDNAhaplogroups, both in terms of correlation with inherited autosomal mutations, as well as correlation with cultural and environmental risks. Studies exploring population structures associated with CAD and incorporated into admixed populations at a resolution finer than ethnicity are lacking [24].

Population origins and ancestry have previously been found to be important determinants of coronary artery disease(CAD) [25].Substitution of T to C at nucleotide position 16189 in the hypervariable D-loop of the control region (CR) of mitochondrial DNA (mtDNA) has attracted research interest because of its suspected association with various multifactorial diseases [26].

In this case-control study, our aim is to investigate theassociation of Mitochondrial T16519C polymorphism with Coronary Artery Disease (CAD) in an iranian population of CAD cases and controls from SINA Hospital using mtDNA lineages that may infer mitochondria functional associations.

MATERIALS AND METHODS

The local ethical committee approved the research proposal before beginning the project and a consent form was taken from each patient to do this research.

Sampling

The study consists of 70 patients randomly chosen from Cath Lab (angiography ward). The subjects were classified as 30 controls and 40 cases) Controls have a normal angiogram defined by the absence of any atherosclerosis and/or any lesions in all coronary arteries. Cases were diagnosed with >50% stenosis in any of the coronary arteries. They answered an extensive questionnaire regarding their current health and medical history. All of the patients and controls were informed of the aims of the study and gave their informed consent to the genetic analysis. The mean age was 63 for patients and 52 for normal controls. The other clinical information and biochemical information are shown in table 5 & 6.

10 ml of blood were taken under sterile conditions and with informed consent. 5 ml for biochemical test (blood clot) sent to the hospital laboratory and another 5 ml tube containing EDTA and maintaining the cold chain for DNA

extraction procedure was sent to the university laboratory. A total of 70 samples (including cases and controls) to extract DNA and coding and preparation, then we have the documentation to start the next stage.

DNA extraction

Total DNA corresponding blood samples (about 50 µg) using an extraction kit (Takapouzist, Tehran, Iran) and was isolated and purified according to the protocol. Using a special spectrophotometer (Nanodrop) the presence and concentration of extracted DNA was studied. The registration process to execute code each time PCR (polymerase chain reaction) is kept at freezing temperatures.

The Polymerase Chain Reaction (PCR)

PCR amplification was carried out in a final volume of 25 µl containing 200-300 ng total DNA, 70 µM of each dNTP, 10 pmol of each primer, 2.5 mM MgCl2, 1 U of *Taq*DNA polymerase (Cinnagen, Tehran, Iran). The PCR profile was as follows: 94°C for 5 min, 30 cycles of 94°C for 50 s, 55.5°C for 50 s and 72°C for 50 s, followed by 72°C for 10 min.

Table 1. Information on PCR process

NO.	Primer name	F/R	Sequence	NT	Gene	Concentration
1	ONP-38	F	5'-GAT CAC AGG TCT ATC ACC CT-3'	1-20	D-loop	10 Pmol
2	ONP-77	R	5'-GCT CCG GCT CCA GCG TCT GC-3'	110-91	D-loop	10 Pmol
3	ONP-79	R	5'-GAG CTG CAT TGC TGC GTG CT-3'	780-761	12S rRNA	10 Pmol
4	ONP-98	F	5'-ATC ATT GGA CAA GTA GCA TC-3'	15791-15810	12S rRNA	10 Pmol

No. of cycles	temperature	time
1	94	5
30	94	50"
	55.5	50"
	72	50"
1	72	10

Materials	V
mix	7.5
Primer F/R	0.4/0.4
DNA	1
H_2O	
Final volume	25

After the process of PCR the sequenced products were analyzed on 1.5% agarose gel. The amplified sequences of of the D loop region are shown in figure 1.

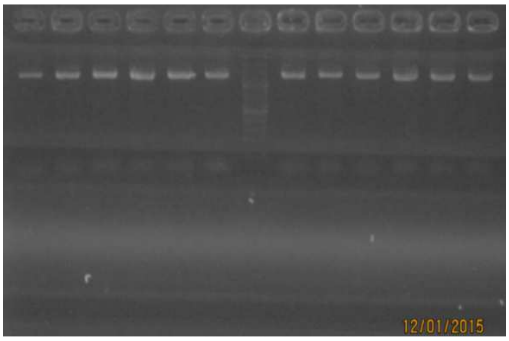


Figure 1. Gel electrophoresis image of isolated region

Sequencing of samples

At the end of practical stages the samples were sent to specialized laboratories and were sequenced. The nucleotide sequence of the amplicon was directly determined by automated sequencing on an ABI 3730 XL machine sequencingusing primer mt91R (Gene Fanavaran, Macrogene Seoul, Korea). The obtained mtDNA sequences were aligned with a multiple sequence alignment interface CLUSTAL X with comparison to revised Cambridge reference sequence or rCRS (http: /www.gen.emory.edu/ mitomap/mitoseq.html) and analysis of the resulting electrophorotic 1-4-0 with software version FINCH TV were examined (Figures 5 & 7). At this stage the important practical work has been completed and the results analysis (*in silico*) began. NCBI nucleotide sequence of the two areas mentioned in the application by the rCRS were compared and blast. Finally, the statistical analysis was performed to determine the significance of the results.

RESULTS

Our sample, a total of 40 men and 30 women with the consult of cardiologist and according to the results of angiography (stenotic index of the arteries in figure 3) and, if necessary biochemical test (Figure 2), in three groups with 39 numbers (29 men and 10 women), 25 healthy (9 men and 16 women) and average number of 6 patients (2 males and 4 females) were classified. After the experimental stages and bioinformatics, mutations and

polymorphisms have been discovered and confirmed their previous association with certain diseases listed and analyzed. The following are examples of tables (Table 1) that relates to a patient (patient sample code 1) is given.

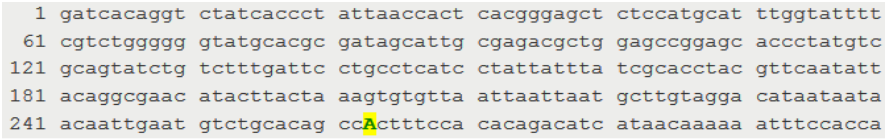


Figure 2. Represents the position of largest part of sequence variants (263 A>G) in this study in the Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (from mitomap.org)

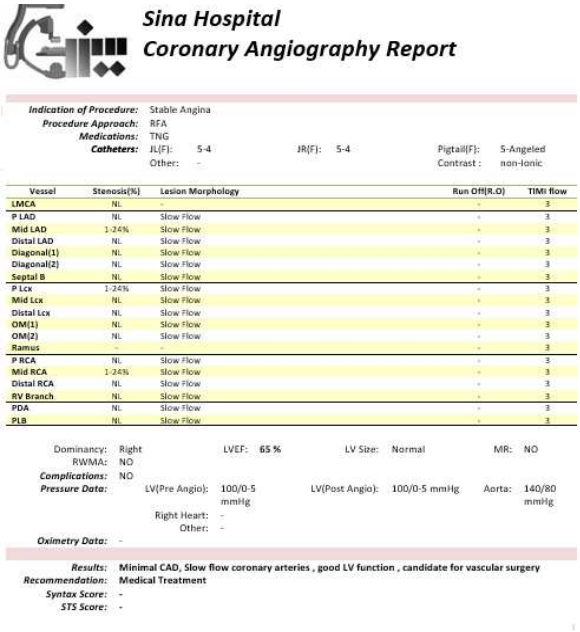
Table 2. An example of a known position along each polymorphism (code 1, case)

Change position	Change type	hom/het	Pathological status
73	A>G	homo	Polymorphisms/Somatic Mutation(aging brains, POLG/PEO & control muscle, buccal cell, thyroid & prostate tumors
263	A>G	homo	Polymorphisms/Somatic Mutation (POLG/MNGIE muscle)
309-310	ins C	homo	Polymorphism/mutation(AD-weakly associated)
750	A>G	homo	Polymorphisms
310-311	ins C	homo	Polymorphism/mutation(Melanoma patients)
15924	A>G	homo	Reported Mitochondrial DNA Base Substitution Diseases: rRNA/tRNA Mutations (LIMM Disease) Polymorphisms
16256	C>T	homo	Polymorphisms
16270	C>T	homo	Polymorphisms
16399	A>G	homo	Polymorphisms/mtDNA Somatic Mutations (gastric carcinoma)
16320	C>T	homo	Polymorphisms

Among the studied samples 70 samples in total (39 patients, 25 patients and 6 healthy mild or moderate) in terms of changes and polymorphisms most sequence variations such as polymorphisms or mutations, respectively, are described below. The proportion of patients in terms of gender seems interesting. Because the patient samples of 39 patients, formed 29 men (74%) and 10 women (26%) whereas conversly the ratio between control group of 25 samples consists of 9 men (36%) and 16 women (64%) and even in mild cases, this ratio was 4 to 2 in favor of women (table 3).

Table 3. Sex ratio in patients and healthy subjects and with mild subjects

	Total	Male	Female	Percent(M)	Percent(F)
Case	39	29	10	%74	%26
Control	25	9	16	%36	%64
Moderate	6	2	4	%33	%67



Sina Hospital
Coronary Angiography Report

Indication of Procedure: Stable Angina
Procedure Approach: RFA
Medications: TNG
Catheters: JL(F): 5-4 JR(F): 5-4 Pigtail(F): 5-Angeled
Other: - Contrast : non-Ionic

Vessel	Stenosis(%)	Lesion Morphology	Run Off(R.O)	TIMI flow
LMCA	NL	-	-	3
P LAD	NL	Slow Flow	-	3
Mid LAD	1-24%	Slow Flow	-	3
Distal LAD	NL	Slow Flow	-	3
Diagonal(1)	NL	Slow Flow	-	3
Diagonal(2)	NL	Slow Flow	-	3
Septal B	NL	Slow Flow	-	3
P Lcx	1-24%	Slow Flow	-	3
Mid Lcx	NL	Slow Flow	-	3
Distal Lcx	NL	Slow Flow	-	3
OM(1)	NL	Slow Flow	-	3
OM(2)	NL	Slow Flow	-	3
Ramus	-	-	-	3
P RCA	NL	Slow Flow	-	3
Mid RCA	1-24%	Slow Flow	-	3
Distal RCA	NL	Slow Flow	-	3
RV Branch	NL	Slow Flow	-	3
PDA	NL	Slow Flow	-	3
PLB	NL	Slow Flow	-	3

Dominancy: Right LVEF: 65 % LV Size: Normal MR: NO
RWMA: NO
Complications: NO
Pressure Data: LV(Pre Angio): 100/0-5 mmHg LV(Post Angio): 100/0-5 mmHg Aorta: 140/80 mmHg
Right Heart: -
Other: -
Oximetry Data: -

Results: Minimal CAD, Slow flow coronary arteries , good LV function , candidate for vascular surgery
Recommendation: Medical Treatment
Syntax Score: -
STS Score: -

Figure 3.An example of angiography sheets and indicators of coronary stenosis (minimal CAD)

By visiting the website MITIOMAP we could find interpretation of the changes obtained in this way, the impact of the changes we identified in previous studies. Some changes were only polymorphism, some associated with various pathologic conditions, and some variants had not been reported previously and in this study were first time observed.

MITOMAP: mtDNA Coding Region Sequence Polymorphisms

Nucleotide Position	Locus	Nucleotide Change	Codon Number	Codon Position	Amino Acid Change	GB Sequences	References
750	MT-RNR1	A-G	-	-	rRNA	30156	references

Figure 4. Search for interpretation of the variant 750 A>G (for example) on MITOMAP

From among total of 166 observed change (often SNPs) Frequent variants of the 263 A>G (nucleotide position 263 by replacing G instead of A), which has 62 of the 34 patients (87%) and 23 controls (92% normal) and the rest is composed of 5 medium addition to polymorphism role was in association with POLG / MNGIE muscle (previous reseaches). Next change 310-311 insC (the insertion of a C between positions 310 and 311) also including 61 samples from cases exists no superset that includes 35 patients (89%), 21 healthy (84%) and 5 moderate and accompanied only the Melanoma patients is shown. Variant 750 A>G with 59 sample consists of 33 patients (84%), 20 control (80%) and 6 medium that is only a single nucleotide polymorphism in past association studies. Single nucleotide change 73 A>G with 45 samples, which include samples of 23 patients (59%), 17 healthy subjects (68%) and the rest of the five sample average has been formed. This polymorphism and somatic mutation previously associated with aging brains, POLG / PEO & control muscle, buccal cell, thyroid & prostate tumors is known. 40 samples with variant 16519 T>C with 24 patients (61%), 13 years old (52%) and 3 medium and in conjunction with glioblastoma, gastric, lung, ovarian, prostate tumors, Cyclic Vomiting Syndrome with Migraine / has been metastasis.32 samples with mutations 309-310 ins C with 17 patients (43%), 10 controls (40%) and 5 average, in keeping with Melanoma patients previously known. Another case related to 152 T>C with 22 samples of 11 patients (28%), 9 healthy (36%) and 2 average in relation to aging brains, elderly fibroblasts, ovarian carcinoma, breast tumor is. 20 samples with changes 16126 T>C of 12 patients (30%), 7 healthy (28%) and 1 medium composition and glioblastoma, normal tissues is shown.The latest varieties frequent among 195 T>C with 16 cases, of which 7 patients (18%), 8 healthy (32%) and 1 medium consists in keeping with Tumors: lung, thyroid, ovarian, prostate BD -associated, melanoma. The rest of the changes in the samples is low and even rare, as most of them only in one or two instances have been sporadic and generally below ten. The summary of statistical information of these changes is shown in table 4.

Except for the foregoing the common features with different ratios up,some changes have been seen only in patient samplesmore unique variants in a sample of patients only accounted for.The important thing is that regardless of the

status in patient samples most frequent type C>T at 32, which is reminiscent of the frequency of these mutations in the human genome.

Healthy patient samples are similar in most cases exclusively polymorphisms, only one sample is included. Here the polymorphism C>T at 22 is the highest. There was no difference compared to the patient samples of 4 insertion C and the second is about the insertion CC.

It should be noted that polymorphisms in the study population T16189C Saudi and Central Europe was evaluated.Its association with cardiovascular disease in which the two had been proved.In this study, 12 patients with a frequency of 8 patients, 3 healthy and one of the relative importance is mild.The SNP previously associated with prostate tumor, normal buccal swab, Reported Substitution Disease NIDDM / Cardiomyopathy / Endometrial cancer risk / mtDNA copy nbr / Metabolic Syndrome melanoma patients had been proved that some of the studies mentioned in the bibliography.

Interestingly, polymorphisms 16126 T>C at the study of Mir Rahim Fakhraz and colleagues tribes Persian, Turkish, Gilaks, and Sistani were frequent,In this study, the prevalence higher than 20 of the 12 patients and 7 healthy and mild consist 1 and in previous studies, but also play the role of polymorphisms associated with glioblastoma has shown. Also variant 16223 C>T at the same exists no superset of the haplotype in Turkmen and Baluchi ethnic group with 9 and 3 of 6 patients healthy.This is in addition to polymorphism has been associated with some tumors. These two cases can be a patient's ethnicity in the project refer to the range.

Table 4. Frequency of most mutations

Change	263 A>G	310-311 insC	750 A>G	73 A>G	16519 T>C
Total	62	61	59	45	40
Case	34	35	33	23	24
Moderate	5	5	6	5	3
Control	22	21	20	17	13
Frequency in case	%87	%89	%84	%59	%61
Frequency in control	%92	%84	%80	%68	%52
Total percent	%88	%87	%84	%64	%57

Not reportedmutations

Among 166 mutations (totally) observed 9 changes for the first time has been observed and reported in this study. These variants include: Case 219T>C, Case 265T>G, Control 389G>A, Control 567-568 insCC, Case 509A>G, Case 683G>A, Control 757A>G, 2 Controls 15545-15546 insC, Control 16538-16539 insC.As you can see these changes in small amounts and have no significant correlation with CAD.

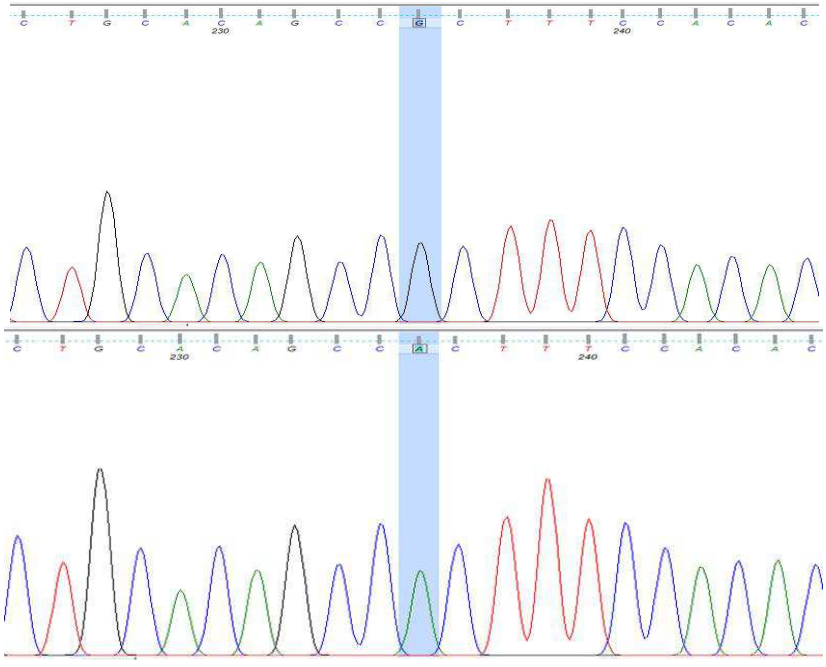


Figure 5. The most frequent variants (263 A> G) were observed (62)

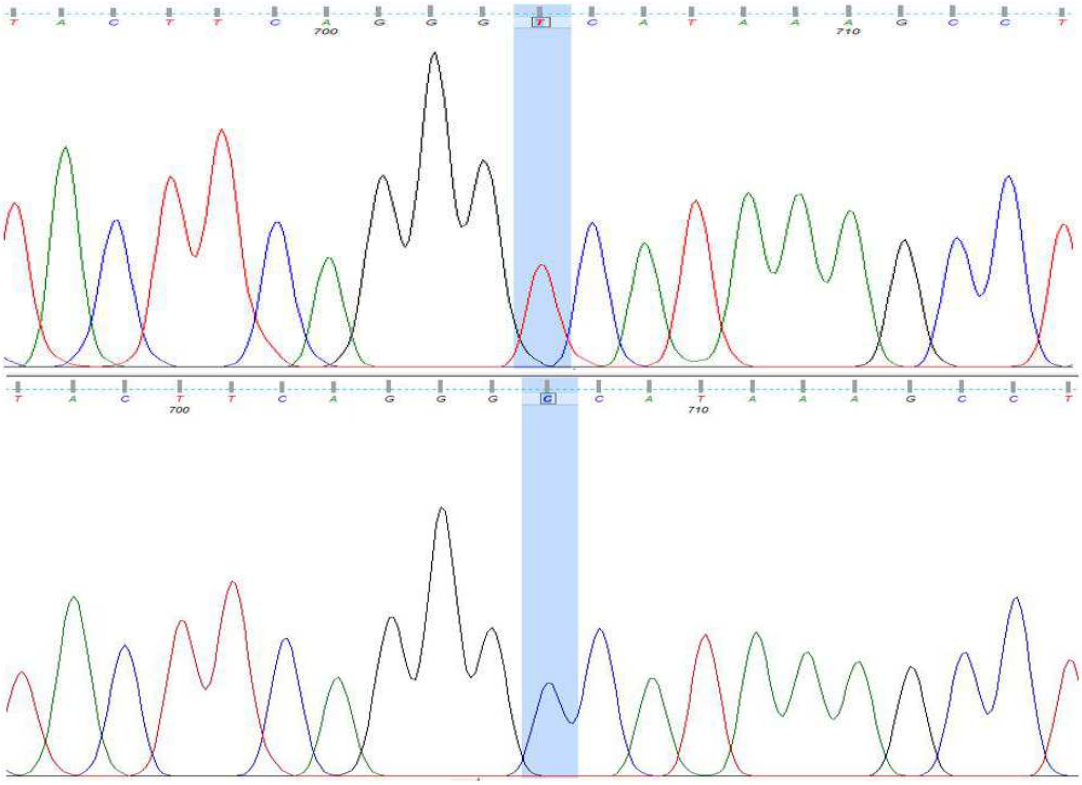


Figure 5. This variant (16519T>C) were observed (62)

Statistical analysis

Tests of disease susceptibility seek to compare an individual's risk of developing a disease with the general population risk. Available measures of the relative risk include the likelihood ratio and the odds ratio. For case-control studies, the usual measure is the odds ratio (OR). In contrast with the likelihood ratio, this can be calculated directly from the results of the study.Odds ratios must be interpreted with great caution, especially for high-frequency risk alleles.Despite being less intuitive, ORs are used in case-control studies because of their statistical properties, particularly in relation to logistic regression, the statistical technique used to tease out single effects from a complex set of factors. If we consider a genetic risk allele, the OR will probably be different for people heterozygous or homozygous for that allele. Unless there is clear evidence of dominance or recessiveness, susceptibility alleles are usually assumed to have a multiplicative effect [25].

Fisher's exact probability test was used to examine the association between the two groups. A P-value of less than 0.05 determines the statistical significance of the relationship between CAD and the proportion of mtDNA with the mitochondrial haplogroups.

Statistical analysis was performed with 95% confidence that the direction of the relationship between mutations with cardiovascular disease as well as other variables that could have the The effect of confounding (such as gender, fasting blood glucose, urea, blood pressure measurement, disease kidney, smoking and alcohol and drug addiction) this analysis was carried out. The association of each variable alone with cardiovascular disease once through the analysis chi-square ($\chi 2$) were analyzed (analysis of raw materials) and once with other variables through logistic regression analysis using Backward method was used (adapted or adjusted) the significance level (P.value) and the risk of both crude and adjusted as shown in the table have been reported.

In this study, 45 patients as cases or patients (with integration mild) and 25 healthy people as a control group were considered. In healthy subjects (control group), mean age 52 years, age range from 28 to 78 years and an average age of about 63 years in patients aged 32 to 82 years. Number and percentage of patients and healthy in all other variables are presented in Table 5.

The results showed that between gender and risk of cardiovascular disease both univariate analysis (crude) and multivariate analysis (adjusted) There is a significant relationship between cardiovascular disease in men and 4.7

times that of women in raw mode (PV = 0.003, oR = 4.7, 95% CI [1.66-14.28]) and if it is matched against women is 6.6. (P.V = 0.006, OR = 6.6, 95% CI [3.22-10.01]).

Among the mutant 750 A>G significantly associated with cardiovascular disease were observed in the match. So that this mutation increases the risk of heart disease 1.6 times. (P.V = 0.02, OR = 1.6, 95% CI [0.24-3.01]). Among other mutations as well as other variables, no significant association with coronary artery disease.

Table 5. Compare the relationship between CAD (Coronary Artery Disease) clinical records

Variable	(CAD)		SE	P value	OR (95% CI)	P value	OR (95% CI)
	No	Yes		(Crude)	(Crude)	(adjusted)	(adjusted)
Sex				0.002	6.16 (1.45-4.82)	0.001	5.52 (1.054-9.49)
Male	8 (21.6%)	29 (78.4%)	1.29				
Female	17 (63%)	10 (37%)					
Age				0.02	0.84 (0.71-0.99)	0.98	1.37 (0.001-1.99)
40≥	4(%100)	0 (0%)	0.13				
>40	21 (35%)	39 (65%)					
BP				0.26	2.24 (0.69-7.28)	0.549	0.025 (0.001-36.45)
≥15	5(%26.3)	14(73.7%)					
<15	20(44.4%)	25 (55.6%)					
Renal				0.17	3.45 (0.67-17.5)	0.608	0.039 (0.001-9.31)
YES	2 (18.2%)	9 (81.8%)					
NO	23 (43.4%)	30 (56.6%)					
Smoking				0.19	2.21 (0.72-6.74)	0.956	0.884 (0.03-13.35)
YES	6 (27.3%)	16 (72.7%)					
NO	19 (45.2%)	23 (54.8%)					
Alcohol							
YES	0	0					
NO	25 (39.1%)	39 (60.9%)					
Addiction				0.83	1.29 (0.11-15.11)	0.922	0.001 (0.0001-6.88)
YES	1 (33.3%)	2 (66.7%)					
NO	24 (39.3%)	37 (60.7%)					

Table 6. Compare the relationship between CAD with biochemical variable

variable	(CAD)		SE	P value	OR (95% CI)	P value	OR (95% CI)
	No	Yes		(Crude)	(Crude)	(adjusted)	(adjusted)
Chol				0.8	1.58 (0.95-26.52)	0.999	0
<200	24 (38.7%)	38 (61.3%)					
≥200	1 (50%)	1(50%)					
HDL				0.51	1.05 (0.98-1.13)	0.999	4.2 (0.001-19.95)
<25	0 (0%)	2 (5.1%)					
≥25	25 (100%)	37(94.9%)					
LDL				0.96	1.04 (0.16-6.73)	0.999	33 (0.001-14.71)
≥130	2 (8%)	3 (7.7%)					
<130	23 (92%)	36 (92.2%)					
TG				0.62	0.93 (0.2-4.28)	0.931	0.80 (0.005-9.81)
≥200	3 (12%)	5 (12.8%)					
<200	22 (88%)	34 (87.2%)					
FBS				0.43	0.62 (0.2-1.82)	0.931	7.8 (0.001-15.05)
70< , ≤116	7 (28%)	15 (38.5%)					
70-115	18 (72%)	24 (61.5)					
Urea				0.24	0.43 (0.12-1.52)	0.55	3.14 (0.369-28.22)
15< , ≤41	4 (25%)	12 (75.8%)					
15-40	21 (43.8%)	27 (56.3%)					

Table 7. Compare the relationship between CAD with changes

Variable	(CAD)		SE	P value	OR (95% CI)	P value	OR (95% CI)
	No	Yes		(Crude)	(Crude)	(adjusted)	(adjusted)
16519 T>C				0.51		0.21	
YES	13 (32.5%)	27 (67.5%)	1.17		1.38 (0.51-3.71)		4.43 (0.43-45.22)
NO	12 (40%)	18 (60%)			Reference		Reference

DISCUSSION AND CONCLUSION

This study evaluated the potential role of mitochondrial haplogrouping and mtDNA variants in CAD in the Iranian population. Our results didn't revealed any association of the 16189T>C with the disease. Since mitochondria play pivotal roles in metabolism and energy consumption cells the aim of this study was to evaluate the different mitochondrial haplotypes associated with the risk of coronary artery diseaseand as we know, along with a statistical

correlation of risk of disease that Can be either due to the direct impact of the changes to the pathology of the disease or linkage disequilibrium or linkage with a gene involved in disease.

Linkage disequilibrium is defined formally as the association of two alleles at linked loci more frequently than would be expected by chance, and is also referred to as allelic association. The concept and the term relate to the study of diseases in populations rather than families [26]. In this study the statistical relationship with one of the mutations were observed, as well as with gender. Other mutations, as well as variables as shown in the tables have been ineffective.

According to Table 5 clinical variables such as gender, age, blood pressure, addiction and also studied and except for the gender variable in both matched and not matched no significant relationship with the disease.As seen in Table 6 biochemical variables such as cholesterol, LDL, glucose, etc. associated with the disease and have not been included in the study (the effect of confounding).

By examining the variations exists no superset (frequency more than 10) single nucleotide changes in the genome of the mitochondria and the D loop it was determined that the mutations associated with certain diseases and pathologic proof exists no superset already sentas well as in other studies indicate that polymorphisms in the corresponding position.Associated mutation in this study (750 A>G) in previous studies lacked communication with patients and their only show in the role of polymorphism.Mitomap site and view this information by referring to the interpretation of any of the changes achieved statistical correlation with patients, Most of these mutations was studied in detail in previous studies and some do not and have been associated relationship. For example, in a large study on the Danish population, no association was found between mitochondrial haplogroups and risk of ischemic cardiovascular disease (Benn et al., 2008).

Most variants in this project has not been mentioned as 263 A>G is that in previous studies with cases of the disease have been identified relationship. Other mutations have been associated so on.As will become clear later all common mutations in addition to the polymorphism has been associated with some diseases. Except for a significant variant in this study, previous research has only been in the role of polymorphism.

The results sent exists no superset mutations in detail in this section of mutations have been evaluated more than 30 repetitionsand significant mutations of the 750 A>G is compared.

The most frequent single nucleotide change in this study, 263 A>G respectively.That by visiting the site Mitomap and interpret mutations we find that in previous studies with POLG / MNGIE muscle is associated with the resources referred to But in this study, as can be seen in Table 7 has been correlated with disease (Table 7). This study listed in the Resources section [27].

MITOMAP: mtDNA Somatic Mutations

Locus	Nucleotide Position	Nucleotide Change	Homoplasmy	Heteroplasmy	Cell or Tissue Type	GB Sequences	References
MT-DLOOP	263	A-G	-	+	POLG/MNGIE muscle	28559	references

Figure 9. Interpretation of the relevant variants (263 A> G) on mitomap

The next variant 310-311 insCtotally with 61 cases that have been associated with pathological conditions such as Melanoma patients.This mutation in present study showed no relationship (Table 7). This study has also been implied in the literature [28].

MITOMAP: Reported Mitochondrial DNA Base Substitution Diseases: Coding and Control Region Point Mutations

Locus	Disease	Allele	Nucleotide Change	Amino Acid Change	Homoplasmy	Heteroplasmy	Status	GB Sequences	Reference
MT-CR	Melanoma patients	T310TC	T-TC	non-coding	.	.	Reported	-	references

Figure 10. Interpretation of the relevant variant (310-311 insC) on mitomap

Another single nucleotid change 73 A>G with a frequency of 45 in the study has no correlation but in previous researchsassociated with aging brains, POLG / PEO & control muscle, buccal cell, thyroid & prostate tumors. Relatedresearches also found on the resource categories [29-35].

MITOMAP: mtDNA Somatic Mutations

Locus	Nucleotide Position	Nucleotide Change	Homoplasmy	Heteroplasmy	Cell or Tissue Type	GB Sequences	References
MT-DLOOP	73	A-G	+	+	aging brains, POLG/PEO & control muscle, buccal cell, thyroid & prostate tumors	22551	references

Figure 11. Interpretation of the relevant variants (73 A> G) on mitomap

The next single nucleotide change 16519 T>C is that somatic mutations in glioblastoma, gastric, lung, ovarian, prostate tumors, Cyclic Vomiting Syndrome with Migraine / has been metastasis associated with it are explained in detail in the results section.This mutation also showed no relationship (Table 7). These studies also come in the resource list [36-41].

MITOMAP: mtDNA Somatic Mutations

Locus	Nucleotide Position	Nucleotide Change	Homoplasmy	Heteroplasmy	Cell or Tissue Type	GB Sequences	References
MT-DLOOP	16519	T-C	+	+	glioblastoma, gastric, lung, ovarian, prostate tumors	18961	references

Figure 12. Interpretation of variance (16519 T>C) on mitomap

309-310 insC as last mutation in this section has been associated with AD-weakly patients but in this study with no association and plays polymorphism role (Table 7). As that study is also in the Resources section [42].

MITOMAP: Reported Mitochondrial DNA Base Substitution Diseases: Coding and Control Region Point Mutations

Locus	Disease	Allele	Nucleotide Change	Amino Acid Change	Homoplasmy	Heteroplasmy	Status	GB Sequences	Reference
MT-CR	AD-weakly associated	C309CC	C-CC	non-coding	.	.	Reported	274	references

Figure 13. Interpretation of the relevant variant (309-310 insC) on mitomap

As demonstrated in table 7, variant 750 A>G is significantly more abundant and have association with CAD in patients (OR=1.62 (0.24-3.01) and P=0.02) and in adjusted analysis. This finding is suggestive of a significant role for mtDNA haplotype in CAD development and risk. Our data showed that patients with coronary artrydesease clustered in above haplogroup have a significantly higher frequency when compared with controls, implicating a possible association of this SNP with CAD. We concluded that mitochondrial polymorphisms might play a genetic role in predisposing to this desease.Substitutions in the D-loop may be part of a haplotype with mutations elsewhere in the mtDNA. Also mtDNA HVS-I mutations may cause energy deficiency in stressful situations during a vulnerable developmental period [43]. This variant play a polymorphism role in all of past studies (Mitomap and figure 4).

The hypothesis is that on their own some polymorphisms are selectively neutral, but in specific combinations they act in a synergistic,deleterious manner with established pathogenic mtDNA mutations to increase the risk of disease expression or to produce a more severe clinical outcome.The rich variability within HVS-I compared with the relatively constant constellation within the gene regions provides useful criteria for pathogenetic studies. This is the first study to trace mtDNA HVSI variants in CAD patients of the Persian population from SINA hospital.We concluded from the tested data that haplogroup 750 A>G is considerably more frequent in CAD patients (Table 7). Thus, mtDNA variant might constitute a risk factor for CAD.

It is important, However, along with these changes (except 750 A>G) has been established with certain diseases, in this study their relevance in the particular allelic polymorphism and unaccompanied show.

As mentioned earlier mutations 750 A>G and the gender variable has been associated with disease. Between mutations 750 A>G significantly associated with cardiovascular disease were observed in data matching. So that this mutation increases the risk of heart disease 1.6 times.The mutation in all previous studies of polymorphisms that this is important to study. And if that is matched against women is 6.6.

It should be noted that according to what scientific source that by increasing the sample size can be documented results in terms of statistical significance achieved.

In conclusion, our data suggest an associationof haplogroup750 A>G with CAD in Iranian patients. However, more studies of both genders with stratification of the data set by sex are necessary and also further investigations on haplotype and other genes must be performed to shed new light on the molecular pathogenesis of CAD.

In this contribution we found a significant association of 750 A>G with CAD in Iranian patients so it can be concluded that mtDNA this haplotype might constitute a risk factor for coronary artery desease.

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