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Association of -77T>C and Arg194trp polymorphisms of *XRCC1* with risk of coronary artery diseases in Iranian population

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ABSTRACT

Objective(s): Coronary artery disease (CAD) is the leading cause of death in both male and female worldwide. The main cause of CAD is the atherosclerosis of coronary arteries, which is, mostly caused by genetic alteration. 50% of such cases occur in mitotic cells where single-strand breaks occur spontaneously or due to ionizing radiation. X-ray repair cross-complementing protein 1 (XRCC1) as a key element, participate in the base excision repair (BER) and Single-strand Break Repair (SSBR) pathways. It has been suggested that XRCC1 functions as a scaffold protein able to coordinate and facilitate the various steps of DNA repair pathways. Two Single Nucleotide Polymorphisms (SNPs) (Arg194Trp and -77T>C) were reported to affect the function and expression of XRCC1, respectively.

Materials and Methods: A case-control study was performed to investigate the relation between these polymorphisms and the CAD development. A population of 406 individuals was screened for SNPs by Restriction Fragment Length Polymorphisms (RFLP) method.

Results: XRCC1 Arg194Trp polymorphism was associated with increased risk of CAD in examined population under a dominant model (Odds-ratio=2.604, *P*-value=0.001). Also the SNP of -77T>C revealed a protective role in the population under a dominant model (Odds-ratio=0.618, *P*-value=0.032).

Conclusion: Our findings demonstrated a contributory role of these two SNPs in CAD. Furthermore, our results support the role of DNA damages and the malfunctions of DNA repair system in cardiovascular disease development in Iranian patients.

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Introduction

Coronary artery disease (CAD) is the most common type of cardiovascular disease, which nowadays has become one of the leading causes of death all around the world. The main cause of CAD is the atherosclerosis of coronary arteries characterized by atherosclerotic plaques (1). As a consequence, plaques can narrow the coronary arteries, which in turn lead to decreased or total cessation of blood flow towards the heart, a phenomenon called hardening. A complete blockage can eventually cause heart attacks (2). The molecular mechanism involved in the development of CAD is not fully understood. However, several studies have documented the multifactorial nature of this disease on the basis of interaction between environmental risk factors and predisposing genes (3).

During past years, several researches on CAD, tried to find predisposing genotypes focusing mainly on single nucleotide polymorphisms (SNPs) which play a vital role in lipid metabolism (4, 5), coagulation (6), endothelial function (7) and hypertension (8). Along these lines, however, it has been pointed out that novel SNPs which decrease DNA repair efficiency may play key role in individual's susceptibility to different type of heart diseases (9).

Recent consensus in atherosclerosis holds that different DNA lesions are frequent in both circulating cells and the vessel wall cells (10-12). Chief among factors are reactive oxygen species (ROS) that seems to play pivotal role for DNA damages in atherosclerosis (13-15). ROS can be generated continuously as byproduct of enzymatic reactions (16, 17) and mitochondrial oxidative metabolism as well as inflammation in the course of pathological processes. DNA strand breaks, base modifications and rough nucleotides are the main lesions, which are induced by ROS (18-21).

Naturally, several DNA repair pathways like base excision repair (BER), nucleotide excision repair

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(NER) and DNA strand break repair are alertly active in repairing of such DNA damages. Different proteins have been discovered to participate in DNA repair pathways so far. One of the most important proteins which plays critical roles in BER and DNA strand breaks repair is X-ray repair cross-complementing protein 1 (XRCC1) (22, 23). This multidomain protein acts as a scaffold and is essential in gathering various components of DNA repair pathways together (24). It has been reported that XRCC1 deficiency results in embryonic lethality in mice and no human cell line lacking XRCC1 has been identified, yet (25). Based on the critical function of XRCC1 in DNA repair, it's been suggested that any decline in its efficiency may cause an individual susceptibility to ROS-induced DNA damages (9). Supporting this notion, several SNPs have been identified to cause functional amino acid substitutions in codons 194 (Arg to Trp), 280 (Arg to His), and 399 (Arg to Gln). These changes are located in regions that may affect XRCC1 activity (26). Moreover, there are some evidences that another SNP (-77T>C) in the 5' untranslated region of XRCC1 can alter promoter activity (27).

On the basis of these findings XRCC1 polymorphisms may be associated with CAD. There are only few and controversial studies about the association of these polymorphisms with CAD in few populations. The aim of this study was to examine the association of Arg194Trp and -77T>C polymorphic variants with the risk of developing CAD in Iranian population.

Materials and Methods

Study and control groups

A total of 203 patients with coronary artery disease (CAD) and a population of 203 healthy people without any evidence of CAD were participated in this study. All participants were referred to Tehran Heart Center outpatients' clinic and underwent coronary angiography from 2013 to 2014. There were 247 males and 159 females who participated in this project. The Medical Ethics Committees of the Tehran Heart Center and Tarbiat Modares University approved the study. A written informed consent was obtained from all participants prior to blood sampling.

Genomic DNA extraction

Three milliliters (ml) of peripheral blood were collected from each volunteer in test tubes containing 0.5 M Ethylenediaminetetra acetic acid **(**EDTA) and DNA was extracted using DNP™ Kit (CinnaGen, Iran). Briefly, lysis solution was used to lyse blood cells and then genomic DNA from white blood cells was selectively precipitated by isopropanol. The precipitated DNA was washed and desalted by ethanol and dissolved in Tris-EDTA buffer (TE) and stored in -20 °C till molecular analysis. The quantity and quality of extracted DNA was examined spectrophotometrically and visually by electrophoresis on 1% agarose gel respectively.

DNA genotyping

100 to 150 ng of purified genomic DNA used for genotyping. Two SNPs were determined by Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) method. The fragment which contains Arg194Trp SNP (rs#1799782) was amplified using following primers: forward: 5'-CTGACTCCCACCCCTCCTTT-CCCAG-3' and reverse: 5'-GGACTCCACTACCCTCCT-CCCTCAG-3' to form a 427 base pairs PCR product. The thermal condition was as follow: an initial denaturation at 95 ˚C for 5 min, followed by 40 cycles of denaturation at 95 ˚C for 30 sec, annealing at 60 ˚C for 30 sec and extension at 72 ˚C for 40 sec. Final extension step was conducted at 72 ˚C for 5 min. PCR products were digested with Pvu II (Thermo scientific) in a total volume of 20 µl at 37 ˚C for an overnight according to manufacturer's instruction and then analyzed by electrophoresis on 2% agarose gel. Digestion of Arg/Arg genotypes results in 427 bp products, while digestion of Arg/Trp and Trp/Trp genotypes results in 427, 285 & 142 bp products and 285 & 142 bp products respectively (Figure 1A).

For second Polymorphism, -77T>C (rs#3213245) which is located in promoter, a fragment of DNA was amplified with primer pairs 5'-AGAGGCGCGACTGGG-CTTG-3' and 5'-ACGCCGGAACGTCCCTAATTC-3'. The PCR product was subjected to digestion using MbiI (Thermo scientific) enzyme for overnight. Digestion of TT genotypes results in 110 and 296 bp products, while digestion of CT and CC genotypes results in 406, 296 and 110 bp products and 406 bp product respectively (Figure 1B). The designed method for genotyping was confirmed by some randomly selected from each genotype by DNA sequencing with ABI automated DNA sequencer (Macrogen, Korea). All primers were designed using PRIMER EXPRESS software (Applied Biosystems, USA).

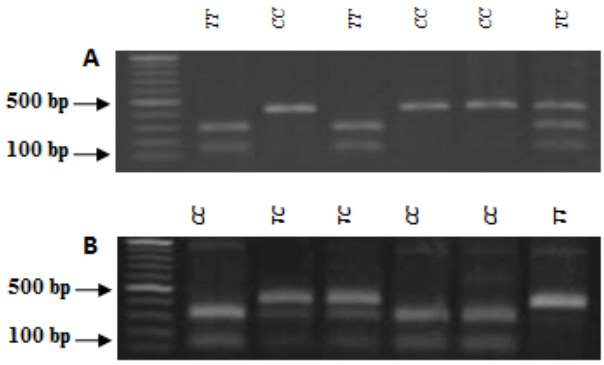

Figure 1 The result of restriction digestion of PCR products by PuvII (A) and MbiI (B) on 2% agarose gel for polymorphisms of genotyping of rs#1799782 and rs#3213245, respectively. The genotype of each sample and SNPs are shown on top of the gels

Table 1. Baseline characteristics of the study population among cases and controls

Characteristics	With CAD n=203	Without CAD n=203	*P*-value
Sex (males)	79.6% (160)	52.7% (108)	.000
Age (years)	54±9.55	59.97 ± 10.36	.000
BMI (Kg/m^2)	27 ± 4.6	28± 4.91	0.877
Family history of CAD	29.1%	16%	.000
Hypertension	47%	44.6%	0.629
Current smoking	49.3%(99)	29.1% (59)	.000
Serum triglycerides (mmol/l)	186 ± 48	185.27 ± 43	0.845
Serum cholesterol (mmol/l)	184±50.1	185±43.05	0.912
HDL (mmol/l)	38.98 ±10.3	44.6± 10.4	.000
LDL (mmol/l)	109.43±41.21	108.65±35.51	0.893
FBS (mmol/l)	118±46.67	105.49±28.54	0.027
Aspirin	93.5%	78.1%	0.000
ACE inhibitors and ARBs	55.3%	33.3%	0.000
Beta-blockers	83.9%	74.1%	0.016

Data are presented as mean±SD or as number and percentage. *P*<0.05 was considered statistically significant and bold numerals represent the *P*-values which were significant when compared between two groups
BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; ARB: Angiotensin II receptor blockers

Statistical analyses

The calculated genotype frequencies of studied SNPs were compared with expected genotype frequencies according to the Hardy–Weinberg law. Differences in the distribution of XRCC1 polymorphisms genotypes or allele frequencies between case and controls were tested using Chi-square test and Fisher's exact test. The study power was also determined as 94.8%. Quantitative variables represented as mean±SD and were also compared using a t-test or Mann-Whitney U test. The groups were compared using the Student t-test for the continuous variables and the χ^2 -test or the Fisher exact test for the categorical variables. Comparing frequencies of each genotype of the examined polymorphisms were tested using multivariate logistic regression model after adjustment for age, sex, body mass index (BMI) and CAD risk factors such as FBS, hyperlipidemia, hypertension as well as drug history. Odds ratio and 95% confidence intervals for these analyses were obtained. Another data analysis was conducted by PHASE 2.1.1 software (28, 29) to reveal haplotype frequencies and association of different haplotypes and risk of disease. A *P*-value of ≤ 0.05 was considered as significant. All statistical analysis was conducted using SPSS software, version 22.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Baseline characteristics

Demographic characteristics of population study were summarized in Table 1. The rate of CAD was more abundant in male and old people but also a strong correlation with familial history of the disease was observed. Groups of people with CAD showed higher level of FBS as well as lower levels of HDL (High Density Lipoprotein). Although people with CAD had a more smoking habit, also there were no striking differences for hypertension and BMI. Contrastingly, regarding to statistical analysis, no meaningful differences were observed between people with and without CAD for total plasma triglyceride and triglycerides. Finally, the results showed CAD positive patients remarkably had more stiffened vessels compared to controls.

-77T>C and Arg194Trp SNPs of XRCC1 influence the risk of CAD

Based on obtained results, both SNPs were conformed to be in Hardy-Weinberg equilibrium expectation in each group (*P*>0.05). Frequency of alleles and genotypes for both polymorphisms in patients and controls are presented in Table 2. The results confirm the association between the

Table 2. Genotype distribution and allele frequencies of Arg194Trp and -77T>C SNPs among cases and controls

Gene polymorphism	Genotypes	CAD group N=203	Allele frequency	Non-CAD group N= 203	Allele frequency
Arg194Trp	CC	155/ 76.4%	C/ 76.4%	185/ 89.4%	C /89.4%
rs1799782	CT	44 /21.7%	T/ 23.6%	19/ 9.2%	T /10.6%
	TT	4/ 2%		3 /1.4%	
-77T>C	CC	32/ 15.4%	T/ 66.7%	28 /13.6%	T /76.4%
rs3213245	CT	104/ 51.2%	C/ 33.3%	127/ 62.8%	C/ 23.6%
	TT	67/ 33.3%		48 /23.6%	

Data are presented as mean±SD or as number and percentage; CAD: Coronary artery disease

Table 3. Odds ratio and 95 % CI of the XRCC1 polymorphisms Arg194Trp and -77T>C among cases and controls

	Before adjustment			After adjustment		
Genotype	OR	95% CI	*P*	OR	95% CI	*P*
Arg194Trp						
Arg/Arg	1.0 (Reference)	-	-	1.0 (Reference)	-	-
Arg/Trp	2.714	1.522-4.841	0.001	1.997	1.054-3.785	0.034
Trp/Trp	1.563	0.345-7.088	0.569	1.724	0.346-8.598	0.506
Arg/Trp +Trp/Trp	2.604	1.506-4.504	0.001	2.557	1.479-4.422	0.001
-77T>C						
TT	1.0 (Reference)	-	-	1.0 (Reference)	-	-
TC	0.588	0.373-0.927	0.022	0.518	0.328-0.818	0.005
CC	0.777	0.413-1.462	0.434	0.852	0.606-1.198	0.357
TC+CC	0.618	0.399-0.960	0.032	0.58	0.329-1.040	0.68

P <0.05 was considered statistically significant; CI: confidence interval; OR: odds ratio

heterozygous genotype of Arg194Trp and -77T>C with the risk of CAD (OR = 2.77, *P*-value = 0.001 and OR = 0.58, *P*-value = 0.022, respectively). However, no risk was observed with the different homozygous genotype for Trp/Trp at codon 194 and CC at -77 positions. Furthermore, it was found that Arg194Trp is associated with CAD in dominant model. Also it is revealed that the C allele carriers at -77 position are less susceptible to CAD (OR=0.618, *P*-value =0.032) (Table 3) There were no association between different genotypes and various CAD risk factors including TG, LDL, FBS and BMI (Data not shown).

No haplotype is in association with the risk of coronary artery disease

To investigate whether there was a relation between XRCC1 polymorphisms and to be at risk for CAD, haplotype analysis was performed. Since two SNPs were in linkage disequilibrium (data not shown) the theory was conducted. Among all, TT haplotype (-77T/Trp) was the most abundant type. By considering T/Trp as a reference, the haplotype did not show the association with risk of CAD (Table 4). No association has been seen with CAD risk factors in mentioned cases.

Discussion

It is clearly approved that atherosclerosis represents a leading universal cause of death through the thickening of arteries. Although it is considered that environmental factors could affect CAD, unraveling the genetic characteristics of disease is supposed to be informative criteria for prognosis and treatment.

It is also an inevitable part of personalized medicine, which is employed in order to identify high-risk individuals and provide early and profitable interventions (30, 31).

Increasing evidences show a relation between ROS and risk of CAD. It has been shown that ROS generated by dyslipidemia and diabetes mellitus have profound effects on the development of CAD through endothelial dysfunction (32). One of the aspects of endothelial dysfunctions could be a DNA lesion through rough nucleotides or DNA strand breaks produced by ROS (19, 33, 34). Hence, it seems that any polymorphism, which decreases proper ROS scavenging or repair of DNA damages capacity, would play important roles in the initiation or development of CAD as well as other risk factors (35, 38). Among DNA repair genes, XRCC1 is investigated enough because of its approved fatal roles in different repair pathways particularly in response to oxidative DNA damages. Single stranded breaks and modified bases are repaired via the BER pathway, in which XRCC1 has a critical role. Some of DNA double-strand breaks (DSBs) will form after an SSB-induced replication fork collapse. It has been

Table 4. Haplotype frequencies of two polymorphisms and risk of association with CAD

	Frequencies		Before adjustment		After adjustment	
Haplotypes	With CAD	Without CAD	OR (95% CI)	*P*	OR (95% CI)	*P*
-77T/Trp (ref)	51%	50%	1 (reference)	-	1(reference)	-
-77T/Arg	36%	43%	3.123 (CI= 1.230-7.929)	0.470	3.693 (CI= 1.259-10.829)	0.470
-77C/Arg	5%	2%	0.819 (CI= 0.609-1.101)	0.186	0.807 (CI= 0.589-1.107)	0.183
-77C/Trp	7%	3%	1.205 (CI= 0.661-2.197)	0.544	1.089 (CI= 0.577-2.057)	0.793

P <0.05 was considered statistically significant; CAD: coronary artery disease; CI: confidence interval; OR: odds ratio

shown that XRCC1 may also contribute to backup pathways of DSB repair (37, 38). In the present study, we aimed to evaluate if two SNPs of XRCC1 (Arg194Trp and -77T>C) could suggest an individual susceptibility to CAD.

One of examined polymorphisms was Arg194Trp because of its strategic location in exon 6 (distance between N-terminal and BRCT-1 domain) which could influence the interaction of XRCC1 with proliferating cell nuclear antigen (PCNA) (26, 39). Based on the data from several literatures, this SNP might be associated with the development of malignant diseases (40, 41), cataract (42) and some other types of age-related disease (43). In contrast, some controversial data were published about the lower value of chromosomal breaks and thus the protective role of this SNP (44). Therefore, the association of Arg194Trp with CAD was examined in a sample from Iranian population. The result showed that, the Trp allele increases the risk of CAD in our population (OR=2.604). In contrast, Bazo *et al* (45) reported that Arg194Trp was not associated with CAD in Brazilian population, however, an association was showed between Trp allele and CAD susceptibility in a Chinese population (46). Hence, our result confirmed their statement, but in opposite view to Bazo *et al*. This discrepancy may be due to some reasons, including large ethnic differences or variable risk factors in these populations (47). We previously showed that, there is no meaningful inter-subpopulation variations in Iranian ethnic groups therefore, obtained allelic frequencies are not result of variable ethnic our population (48) and can be attributed to the differences in power of studies. In order to be confident about the power of study, we computed it using an online sample size calculator (OSSE) as 94.8% (49), so it appears that our research about this SNP has an acceptable power of study. Altogether our results suggest that Trp allele was responsible for individual susceptibility in our population, however, its mechanism of action is remained imperfectly understood.

The second SNP of -77T>C of XRCC1 gene seems to be the first case-control study that had explored the association and the risk of developing CAD. This SNP is located in the promoter region and reported to affect the expression level of XRCC1. It is reported that underlined polymorphic site at -77T is within the core region of the SP1 element in the 5′ UTR of XRCC1 would affecting the binding ability of this zinc finger transcription factor (49, 50). Up to know several studies were subjected SP1 as a transcription inhibitor as well as an activator conflicting and inclusive role. It was reported that when T is mutated to C, SP1 will attach to the GC box with more affinity and the result will be the inhibition of XRCC1 that may increase susceptibility to lung cancer (27). In contrast, Brem *et al* reported that C allele has no effect on transcriptional activity of the promoter (50). Our data analysis revealed that C allele may has a protective role against CAD (OR=0.618), so it seems that SP1 may act as an activator in cells which are affected in atherosclerosis. The reason for the differences about the reported function of -77C allele between these studies is unclear. But it may be due to cell type specific function of SP1 (50). More research is needed to unravel doubtful role of this polymorphism in different types of cells.

In the present study, the C allele was found at a frequency of 0.263 and the T allele at 0.764, whilst in Chinese subjects the allele frequencies were 0.10 for the C allele and 0.90 for the T allele (46). These frequencies were reported to be 0.401 and 0.599 in France respectively (50). It is also determined that the most frequent haplotype in Iranian population contains T allele at position -77 in combination with wild-type allele at codon 194. In contrast, it was reported that the most frequent haplotype in France population contains C allele at position -77.

Conclusion

Whilst this study has some limitations, it revealed the important role of XRCC1 polymorphisms in individual susceptibility to CAD and may be used as a candidate in personalized medicine studies. Our results provide more supportive documents for the role of DNA damages and repair gene in the development of cardiovascular disease.

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Conflict of interest

The authors declare that they are no conflict of interest.

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