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APE1 and *XRCC3* Polymorphisms and Myocardial Infarction

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Abstract. *Background: In most cells, DNA is regularly damaged by mutagens. Different DNA repair mechanisms operate on specific types of damaged DNA. When DNA damage resulting from free radicals is not repaired, it might lead to deteriorated gene expression, the development of a number of diseases such as cancer, diabetes, vascular diseases, and aging. In the present study, APE1 and XRCC3 gene polymorphisms were investigated in patients with myocardial infarction. Materials and Methods: Forty-five first time elective coronary artery bypass grafting (CABG) patients with cardiopulmonary bypass (CPB) and 40 healthy individuals were studied. Gene polymorphisms were determined by a polymerase chain reaction–restriction fragment length polymorphism method. Results: For the APE1 gene, the AG genotype was significantly higher in the patient group than in the control group. The patient group had significantly more G carriers but there was no statistically significant difference between patient and control groups the A allele. The XRCC3 TT genotype was found to be significantly more frequent in the patient group than it was in the control group. Conclusion: The results of our study suggested that the XRCC3 gene TT genotype and the APE1 gene AG genotype might increase the risk of myocardial infarcts.*

Coronary artery disease is a multifactorial disease of inherited and environmental factors (1). The exact cause of atherosclerotic artery formation is still unknown (1). It is thought that disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defence plays a vital role in formation of atherogenic plaques (1). ROS are thought to affect more than one mechanism contributing to this process: for example, oxidation of low density lipoprotein (LDL), endothelial dysfunction, vascular smooth muscle cell growth, monocyte migration and DNA damage (1, 2). Available evidence has shown that if DNA damage resulting from free radicals is not repaired, it might lead to deteriorated gene expression, the development of a number of diseases such as cancer, diabetes, neurodegenerative and vascular diseases and aging (3, 4).

Apurinic/apyrimidinic endonuclease (AP endo) is one of the key enzymes in the repair of abasic sites of DNA (5). Spontaneous base loss can occur in DNA by depurination, by action of glycolyses and by DNA oxidation; abasic products of these can be mutagenic by inhibiting topoisomerase actions, replication and transcription (5). This is usually prevented by the natural repair systems located in the cell (5). Mammalian cells have the AP endo 1 isoenzyme; it is a multifuctional enzyme, hydrolyzing phosphodiester bond 5' to an abasic site to be able to generate 3' hydroxyl terminus suitable for extension by a DNA polymerase and a downstream 5' terminus with a deoxyribose phosphate residue to be removed and so replaced, so two strands can be ligated in order to complete the repair (5, 6). APE1 also plays a major role as 3'-phosphodiesterase in initiating repair of single-strand breaks resulting from DNA damage by free radicals (7-9). Previously, it was shown that reduced functional AP endo activity resulted in increased sensitivity to several oxidizing agents and ionizing radiation (5).

In the cell cycle, the S-phase checkpoint in response to DNA damage supresses the initiation of replication and elongation and the restarting of the action of the replication fork requires several factors such as *XRCC3*-directed repair (10). XRCC3 functions through complex interactions with other relevant proteins to repair double-strand breaks and maintain genome integrity in multiple phases of homologous recombination (11, 12). Polymorphisms of *XRCC3* have been associated with breast cancer susceptibility (10).

In a previous study on the roles of AP endo in embryonic development, it was suggested that *APE1* gene knockouts of zebrafish developed a range of abnormalities in the eyes, notochord, brain and especially in the heart (5). In another study it was shown that overexpression of APE1/ref-1 suppressed TNF-α-induced expression of vascular cell

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Table I. *Distribution of APE1 and XRCC3 genotypes in the study groups.*

	APE 1			*XRCC3*			
	AA	GG	AG	CC	TT	CT	Total
MI patients n (%)	20 (44.4)	4 (8.9)	21 (46.7)**	9 (20)	14 (31.1)*	22 (48.9)	45 (100)
Control group n (%)	28 (70)	5 (12.5)	7 (17.5)	7 (17.5)	5 (12.5)	28 (70)	40 (100)
Total	48 (56.5)	9 (10.6)	28 (32.9)	16 (18.8)	19 (22.4)	50 (58.8)	85 (100)

n: Number of individuals. Significantly different from the control group at *p=0.002, **p=0.040.

adhesion molecule-1(VCAM-1) in endothelial cells and consequent monocyte adhesion (2). Due to these findings, we aimed to evaluate the possible relationships of *APE1* and *XRCC3* gene polymorphisms with myocardial infarct.

Materials and Methods

The study population consisted of 45 first time elective coronary artery bypass grafting (CABG) patients with cardiopulmonary bypass (CPB) admitted to the Cardiovascular Surgery Department of Marmara University, Faculty of Medicine Hospital. The control group consisted of 40 healthy individuals with no known history of any disease. The study had the approval of the Institutional Ethical Committee for Biomedical Research. Clinical examination was followed by a series of laboratory biochemical investigations. For DNA extraction studies, venous blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and centrifuged immediately at 3000 ×g for 10 min (13).

Polymorphism analysis. Previously described methods were used for *APE1* (14) and *XRCC3* (15).

The primers used for polymerase chain reaction (PCR) for *APE1*/exon 5 polymorphism were forward: 5'-CTG TTT CAT TTC TAT AGG CTA-3' and reverse: 5'-AGG AAC TTG CGA AAG GCT TC-3' from IDT (Integrated DNA Technologies Inc, Iowa, USA). These fragments were amplified using a 50 μL reaction mixture containing approximately 50-200 ng of template DNA, 0.2 μL of each primer, all four deoxyribonucleoside 5' triphosphates (each at 0.2 mM), 2.5 mM $MgCl_2$ and 2.25 U of *Taq* polymerase in 1× reaction buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100] (MBI Fermentas, Vilnius, Lithuania). The reaction was carried out with an initial melting step of 2 min at 95˚C, followed by 40 cycles of 15 s at 94˚C, 45 s at 57˚C, 45 s at 75˚C, and a final elongation step of 5 min at 72˚C.

The restriction endonuclease *Bfa1* was used to distinguish the *APE1*/exon5 G/A polymorphism. The products of this reaction were separated by electrophoresis using 10% polyacrylamide gel. Bfa1 digestion generated the following fragments: Asp/Asp, Asp/Glu and Glu/Glu genotypes creating 164, 164/144/20 and 144/20 bp length bands.

To evaluate *XRCC3*/codon 241 polymorphism, the primers used were forward: 5'-GGT CGA GTG ACA GTC CAA AC-3' and reverse: 5'-TGC AAC GGC TGA GGG TCT T-3' (Integrated DNA Technologies Inc). PCR amplification was performed using a total reaction mixture of 50 μL containing 100 ng of template DNA, 300 ng of each primer, all four deoxyribonucleoside 5' triphosphates (each at 0.1 mM), 2.5 U of *Taq* polymerase in 1× reaction buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.45% Triton X-100]. The reactions were started using an initial melting step of 5 min at 95˚C, followed by 35 cycles of 1 min at 94˚C, 1 min at 62˚C, 1 min at 72˚C, and a final elongation step of 10 min at 72˚C. The final PCR product was expected to be 456 bp. After the usage of restriction endonuclease $N1_{aIII}$, the final digested products were 315 and 141 bp.

Statistical analysis. Student's *t*-test was used to determine whether or not significant differences in sex and ages existed between patient and control groups. Pearson's Chi-square analysis was used to examine differences in genotype distribution between cancer patients and controls. Statistical analyses were performed with SPSS for Windows, standard version 7.5 software (SPSS Inc, Chicago, IL, USA).

Results

There were no statistically significant differences between patient (n: 45) and control (n:40) groups in regard to sex and age. The age, as mean±SEM, of the patient group was 60.81±2.02 and of the control group was 57.88±1.79.

For the *APE1* gene, the AG genotype was found at a significantly higher frequency in the patient group than in the control group (p=0.002) (Table I). The patient group had significantly more G carriers (p=0.018) (Table II); but there was no statistically significant difference between the frequency of patients and controls carrying the A allele.

For the *XRCC3* gene, the TT genotype was found to be significantly more frequent in the patient group than it was in the control group (p=0.040) (Table I).

Discussion

Reactive oxygen species were shown to contribute to the cardiovascular damage process in many ways (1, 2); in this study we aimed to focus on two DNA repair genes (*APE1* and *XRCC3*) and evaluate whether their polymorphisms increased the tendency for myocardial infarct or not. For this reason, we took 45 myocardial infarct patients and 40 healthy control individuals. Both groups were matched in sex and age. Previously, similar studies were carried out for various kinds of cancer. But although cardiovascular diseases are known to be more common than cancer cases, this was

Table II. *Distribution of APE1 and XRCC3 alleles in the study groups.*

	APE 1				*XRCC3*				
	A+	A–	G+	G–	C+	C–	T+	T–	Total
MI patients n (%)	41(91.1)	4 (8.9)	25 (55.6)*	20 (44.4)	9 (20)	36 (80)	14 (31.1)	31 (68.9)	45 (100)
Control group n (%)	35(87.5)	5 (12.5)	12 (30)	28 (70)	7 (17.5)	33 (82.5)	5 (12.5)	35 (87.5)	40 (100)
Total	76(89.4)	9 (10.6)	37 (43.5)	48 (56.5)	16 (18.8)	69 (81.2)	19 (22.4)	66 (77.6)	85 (100)

n: Number of individuals. Significantly different from the control group at *p=0.018.

the first of such studies regarding myocardial infarct cases. Reactive oxygen species were studied several times in this type of patient group usually for the damage to vascular structures *via* the oxidation of several kinds of molecules (1, 4). We especially focused on the DNA repair mechanisms that might protect those structures from damage to DNA.

In our study, we found there was a greater *APE1* G allele carrying tendency in the patient group than in the control group. The G allelle may cause a decrement in AP endo enzyme expression and cause a lack of strong repair mechanisms; this would decrease the strength of defense to ROS in these patients. This seemed to be valid especially in heterozygous AG people. Other genotypes did not seem to affect the tendency to creat myocardial infarcts. We also found that for the *XRCC3* gene, the TT genotype was significantly more frequent in patients than in the control group, this indicates the increased tendency of TT homozygous people to creat myocardial infarcts.

In conclusion, these findings could show the effects of homozygosity and heterozygosity on phenotype. We found that the *XRCC3* gene TT genotype and the *APE1* gene AG genotype might increase the risk of creating myocardial infarcts. But despite these findings, myocardial infarct is likely to be a result of multifactorial conditions. To evaluate the effects of genetic tendencies, it would be better to analyze more related genes altogether in a larger population.

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