Heart Vessels (2007) 22:355–360
DOI 10.1007/s00380-007-0986-9

© Springer 2007

ORIGINAL ARTICLE

Mehmet Guven · Gulgun S. Guven · Erdinc Oz
Ahmet Ozaydin · Bahadir Batar · Turgut Ulutin
Seniba Hacihanefioglu · Nergiz Domanic

DNA repair gene XRCC1 and XPD polymorphisms and their association with coronary artery disease risks and micronucleus frequency

Received: October 17, 2006 / Accepted: March 28, 2007

Abstract Coronary artery disease (CAD) is a multifactorial process that appears to be caused by the interaction of environmental risk factors with multiple predisposing genes. In this study, we investigated the effects of the XPD Lys-751Gln and XRCC1 Arg399Gln polymorphisms on the presence and the severity of CAD. We also investigated the presence of DNA damage in the peripheral lymphocytes of patients with CAD by using the micronucleus (MN) test and the effect of XPD Lys751Gln and XRCC1 Arg399Gln polymorphisms on this damage. The study population consisted of 147 patients with angiographically documented CAD and 48 healthy controls. No association between XPD Lys751Gln or XRCC1 Arg399Gln polymorphisms and the presence or the severity of CAD was observed. On the other hand, a significantly higher frequency of MN was observed in CAD patients compared with controls (5.7 ± 1.9 vs 5.0 ± 2.1, respectively, $P = 0.018$). We found an elevated frequency of MN in CAD patients with the XPD 751Gln allele (Gln/Gln genotype) or the XRCC1 399Gln (Arg/Gln or Gln/Gln genotypes) allele compared with the XPD 751Lys (Lys/Lys genotype) allele or XRCC1 399 Arg (Arg /Arg genotype) allele, respectively. These preliminary results suggest that XPD Lys751Gln and XRCC1 Arg-399Gln polymorphisms may not be a significant risk factor for developing CAD. In addition, our results indicate that the MN frequency is associated with presence, but not severity, of CAD and is related to the XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms, suggesting an elevated frequency of MN in CAD patients with the XPD 751Gln or XRCC1 399Gln alleles.

M. Guven (✉) · G.S. Guven · A. Ozaydin · B. Batar · T. Ulutin · S. Hacihanefioglu
Department of Medical Biology, Cerrahpasa Medical School, Istanbul University, 34098 Istanbul, Turkey
Tel. +90-212-414-3035; Fax +90-212-586-1548
e-mail: mgguven@istanbul.edu.tr

E. Oz · N. Domanic
Department of Cardiology, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey

Key words Coronary artery disease · XRCC1 · XPD · Micronucleus · Polymorphism

Introduction

Coronary artery disease (CAD) results from atherosclerosis, a chronic inflammatory disease of the arterial intima characterized by the formation of atherosclerotic plaque.[1] Family and epidemiological studies as well as those based on animal models have documented that CAD is a multifactorial disease with an important genetic component.[2] The genetic model underlying a multifactorial disease is often complex since it may be related to multiple genes and to the presence of environmental factors.[2] Genetic research on CAD has traditionally focused on investigation aimed at identifying disease-susceptibility genes. Several polymorphisms that may play role in the development and progression of atherosclerosis have been identified in lipid metabolism, coagulation, endothelial function.[2,3] and hypertension.[4] On the other hand, polymorphisms in DNA repair genes may be associated with differences in the repair efficiency of DNA damage and may influence an individual's risk of atherosclerosis. Published studies have demonstrated that DNA damage contributes significantly to the development and the progression of atherosclerosis.[5,6] It has been suggested that oxidative stress and the generation of reactive oxygen species may play an important role in the induction of DNA damage in atherosclerosis.[7] Repair of oxidative DNA damage is mediated by both base excision repair (BER) and nucleotide excision repair (NER) mechanisms.[8] Genetic polymorphisms in DNA repair genes may influence individual variation in DNA repair capacity, which may be associated with risk of developing CAD.

XRCC1 (X-ray cross-complementing group 1), a DNA repair protein involved in single-strand breaks (SSBs) and BER pathway, has been reported to be responsible for the efficient repair of DNA damage caused by active oxygen, ionization, and alkylating agents.[9] XRCC1 is a multidomain protein that interacts with the nicked DNA and participates with at least three different enzymes, poly-ADP-ribose

polymerase, DNA ligase III, and DNA polymerase β.[9] Three polymorphisms occurring at conserved sequences in the XRCC1 gene were reported by Shen et al.[10] These coding polymorphisms, resulting in amino acid substitutions, were detected at codons 194 (Arg-Trp), 280 (Arg-His) and 399 (Arg-Gln). In particular, 399Gln polymorphism resulting from a guanine to adenine nucleotide substitution occurs in the PARP binding domain and may affect complex assembly or repair efficiency.

XPD (Xeroderma pigmentosum complementation group D) encodes a helicase, which participates in both NER and basal transcription as part of the transcription factor IIH.[11] Mutations destroying enzymatic function of the XPD protein are manifested clinically in combinations of three severe syndromes: Cockayne syndrome, xeroderma pigmentosum, and trichotiodystrophy.[11] Because XPD is important in multiple cellular tasks and rare XPD mutations result in genetic diseases, XPD polymorphisms may operate as genetic susceptibility factors. Several single nucleotide polymorphisms in XPD gene exons have been identified.[10] The XPD 751Gln variant substantially modifies the amino-acid electronic configuration in a domain important for the interaction with helicase activator p44 and may produce the most relevant change in XPD function.[12]

On the basis of the key role, the XRCC1 and XPD genes play in DNA repair mechanisms, we examined whether the polymorphisms in XPD codon 751 (Lys→Gln) and XRCC1 codon 399 (Arg→Gln) modulate the risk of developing CAD. We also examined the associations between these polymorphisms and severity of CAD and DNA damage, as measured by the micronucleus (MN) test, a sensitive and reliable system for the evaluation of spontaneous and mutagen-induced DNA damage in human peripheral blood lymphocytes.[13]

Materials and methods

Study sample

One hundred and eighty-three patients who required coronary angiography due to symptoms relating to CAD were studied. These patients were randomly selected at Istanbul University Cerrahpasa Medical School Cardiology Department. Coronary angiograms were performed, and stenoses were confirmed in orthogonal views. Significant CAD was defined as a diameter stenosis of ≥50% in any of main coronary arteries. According to this evaluation, 147 patients with significant CAD was included in the patient group. A total of 60 of them had one-vessel disease, 44 had two-vessel disease, and 43 had three-vessel disease. The severity of CAD was also estimated by calculating the Gensini score.[14]

At the time of blood sampling, all patients gave a complete clinical history that included cardiovascular risk factors such as hypertension, diabetes, family history of CAD, dyslipidemia, and smoking habit. Hypertension has been defined by blood pressure values of 140/90 mmHg or higher, or by the use of antihypertensive treatment. Subjects with diabetes history, or receiving any antidiabetic medication or with confirmed fasting glycemia >126 mg/dl (7.0 mmol/l), were considered to be diabetic. A positive family history was considered when a first-degree relative with CAD was present at the age of 55 years for men and 65 years for women. Subjects were deemed dyslipidemic when their total cholesterol concentration level was ≥220 mg/dl (12.2 mmol/l), triglyceride concentration was ≥200 mg/dl (11.1 mmol/l), or if they were receiving lipid-lowering drugs. We considered as smoker individuals who smoked at least three cigarettes per day at the time of the analysis; ex-smokers who stopped smoking for at least 6 months, and non-smokers who never smoked.

Patient exclusion criteria were acute or chronic inflammatory disease, immunological disease, and neoplastic disease. Medications used by patients generally included nitrates, oral aspirin, calcium-channel blockers, and ACE-inhibitors. No patient was receiving vitamin and/or antioxidant therapies.

The control group included 48 healthy control subjects matched with the patients for age, sex, and smoking habits. They had normal electrocardiographic findings. Their histories were negative for diabetes, hypertension, heart disease, and cancer, and their serum cholesterol and triglyceride levels were within the normal levels. Control subjects did not receive antioxidant therapy or vitamin supplements in the previous 2 months.

Written informed consent was obtained from all patients and control subjects. The institutional ethical committee approved the protocol. Venous blood samples were taken before angiographic procedure. After an 8-h fast, blood was drawn from patients for the MN assay and for the determination of the XRCC1 and XPD genotypes.

Genotyping of XRCC1 codon 399

DNA was extracted using a NucleoSpin DNA purification kit (Macherey-Nagel GmbH, Duren, Germany) according to the manufacturer's instructions. XRCC1 genotypes were determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). An Arg→Gln substitution in exon 10 (codon 399) was amplified to form an undigested fragment of 242 bp using the primer pair 5′-CCCCAAGTACAGCCAGGTC-3′ and 5′-TGTCCC GCTCCTCTCAGTAG-3′. After initial denaturation at 94°C for 4 min, there were 35 cycles of 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C, and a final extension step of 10 min at 72°C. PCR products were digested with *Msp*I at 37°C overnight and analyzed on 2% agarose gel. Arg/Arg individuals had 94 and 148 bp fragments, Arg/Gln individuals had 94, 148 and 242 bp fragments, and Gln/Gln individuals had only 242 bp fragments.

Genotyping of XPD codon 751

XPD genotypes were determined PCR-RFLP. A Lys→Gln in exon 23 (codon 751) was amplified to form an undigested

fragment of 436 bp using the primer pair 5′-GCCCGCTCT GGATTATACG-3′ and 5′-CTATCATCTCCTGGCCC CC-3′. After initial denaturation at 94°C for 3 min, there were 38 cycles of 45 s at 94°C, 45 s at 60°C, and 60 s at 72°C, and a final extension step of 7 min at 72°C. PCR products were digested with *Pst*I at 37°C overnight and analyzed on 3% agarose gel. *Pst*I digestion resulted in two fragments of 290 and 146 bp for the wild-type homozygotes (Lys/Lys); three fragments of 227, 146, and 63 bp for the variant homozygotes (Gln/Gln); and four fragments at 290, 227, 146, and 63 bp for the heterozygotes (Lys/Gln).

Cytokinesis block micronucleus (CBMN) in peripheral blood lymphocytes

Peripheral blood was collected by using heparin as an anticoagulant. Lymphocyte cultures were initiated by adding 0.5 ml of whole blood to 4.5 ml of RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 15% fetal calf serum (Gibco), 1% antibiotics (penicillin and streptomycin) and 1% L-glutamine. The lymphocytes were stimulated to divide with 1.5% phytohemagglutin (Gibco). Two replicates of each individual were made. The cultures were incubated at 37°C for 72 h and at 44 h after the initiation, cytochalasin B (Sigma Chemical, St. Louis, MO, USA) at a final concentration of 6 µg/ml was added to arrest cytokinesis. At the end of this period, the cultures were harvested by cytocentrifugation and treated with a hypotonic solution (2–3 min in 0.075 M KCl at 4°C). Cells then were centrifuged and methanol–acetic acid (3 : 1, v/v) solution was gently added. This fixation step was repeated twice and the resulting cells were resuspended in a small volume of fixative solution and dropped onto clean slides. The slides were prepared in triplicate, air-dried, and stained with 10% Giemsa solution for approximately 10 min. About 1000 binucleated cells from each subject were scored on coded slides to evaluate MN frequency. MN frequency was expressed as the number of micronuclei per 1000 binucleated cells. Criteria for selection of binucleated cells and micronuclei were as described by Fenech.[15]

Statistical analyses

Mean and standard deviations (SD) are presented in case of continuous variables. Conformity to Hardy–Weinberg equilibrium was determined by χ^2 analysis. Differences in noncontinuous variables and genotype distribution were tested by χ^2 analysis. Differences between the means of the two continuous variables were evaluated by the Student *t*-test. The data for three or more independent groups were analyzed by analysis of variance, and significant differences among pairs of means were tested by Scheffe's test. Multiple regression analysis was applied to investigate the effect of each variable (age, smoking status, hypertension, diabetes, dyslipidemia, family history, Gensini score) in determining MN frequency. A value of $P < 0.05$ was considered statistically significant. Analysis was performed with SPSS 11.5 statistical software.

Results

Demographic and clinical characteristics of patients and control participants are shown in Table 1. The groups were not statistically different with respect to age, gender, and risk factors ($P > 0.05$).

The distribution of patients according to genotype and extent of CAD is presented in Table 2 and the results demonstrate no association between the XPD codon 751 or XRCC1 codon 399 polymorphism, the presence or the extent of CAD. As shown in Table 2, the variant allele frequency for the XPD codon 751 polymorphism in controls and all CAD patients was 37% and 39%, respectively. On the other hand, the variant allele frequency for the XRCC1 codon 399 polymorphism in controls and all CAD patients was 41% and 40%, respectively. There were no significant differences in allele frequencies of XPD 751A > C and XRCC1 399G > A between controls and CAD patients or CAD subgroups. The distributions of the XPD-Lys751Gln and XRCC1-Arg399Gln genotypes were in accordance with the Hardy–Weinberg equilibrium among the controls ($P =$

Table 1. Characteristics of the population studied

	Control	Extent of CAD			*P* value
		One vessel	Two vessel	Triple vessel	
N	48	60	44	43	
Age (years)	55.3 ± 14.9	57.6 ± 12.7	60.0 ± 11.7	62.1 ± 12.2	0.07[a]
Age range (years)	20–84	31–85	33–88	33–85	
Male/Female	28/20	40/20	29/15	28/15	0.814[b]
Smoking	21	20	14	13	0.509[b]
Family history of CAD	–	7	6	7	0.797[c]
Hypertension	–	26	19	23	0.527[c]
Diabetes	–	16	15	18	0.270[c]
Dyslipidemia	–	18	8	8	0.260[c]

CAD, coronary artery disease
[a] *P* value based on ANOVA test
P value based on Chi-square statistic: [b] (df = 3), [c] (df = 2)

Table 2. Distribution n (%) of the XPD and XRCC1 genotypes according to the presence and extent of coronary artery disease

	Control (*n* = 48)	CAD (*n* = 147)	Extent of CAD			P^a	P^b
			Single vessel (*n* = 60)	Double vessel (*n* = 44)	Triple vessel (*n* = 43)		
XPD							
Lys/Lys	21 (43.8)	52 (35.3)	23 (38.3)	12 (27.3)	17 (39.5)	0.93	0.91
Lys/Gln	18 (37.5)	76 (51.7)	29 (48.3)	26 (59.1)	21 (48.9)		
Gln/Gln	9 (18.7)	19 (13.0)	8 (13.4)	6 (13.6)	5 (11.6)		
A (Lys) allele frequency	0.63	0.61	0.63	0.57	0.64	1.00	0.73
C (Gln) allele frequency	0.37	0.39	0.37	0.43	0.36		
XRCC1							
Arg/Arg	12 (25.0)	50 (34.0)	19 (31.6)	17 (38.5)	14 (32.5)	0.15	0.56
Arg/Gln	33 (68.7)	76 (51.8)	33 (55.0)	20 (45.6)	23 (53.5)		
Gln/Gln	3 (6.3)	21 (14.2)	8 (13.4)	7 (15.9)	6 (14.0)		
G (Arg) allele frequency	0.59	0.60	0.59	0.61	0.59	0.88	0.99
A (Gln) allele frequency	0.41	0.40	0.41	0.39	0.41		

Pearson chi-square test (Control + CAD)[a] and (Control + CAD groups)[b].

Table 3. Micronucleus (MN) frequencies according to XPD and XRCC1 genotypes

	Control (*n* = 48)	CAD (*n* = 141)	Extent of CAD		
			Single vessel (*n* = 57)	Double vessel (*n* = 42)	Triple vessel (*n* = 42)
Mean	5.0 ± 2.1	5.7 ± 1.9*	5.7 ± 1.8	5.7 ± 1.8	5.8 ± 2.1
XPD					
Lys/Lys	5.5 ± 2.4 (*n* = 21)	5.2 ± 2.0 (*n* = 49)	5.0 ± 1.9 (*n* = 21)	4.9 ± 1.8 (*n* = 13)	5.7 ± 2.2 (*n* = 15)
Lys/Gln	4.8 ± 2.0 (*n* = 18)	6.0 ± 1.9 (*n* = 72)	6.2 ± 1.6 (*n* = 28)	6.0 ± 1.9 (*n* = 23)	5.7 ± 2.3 (*n* = 21)
Gln/Gln	4.2 ± 1.6 (*n* = 9)	6.3 ± 1.6[a] (*n* = 20)	6.6 ± 1.5 (*n* = 8)	6.3 ± 1.4 (*n* = 6)	6.6 ± 1.8 (*n* = 6)
XRCC1					
Arg/Arg	4.1 ± 1.6 (*n* = 12)	4.6 ± 1.5 (*n* = 47)	4.7 ± 1.5 (*n* = 20)	4.6 ± 1.4 (*n* = 15)	4.5 ± 1.5 (*n* = 12)
Arg/Gln	5.2 ± 2.2 (*n* = 33)	6.1 ± 2.0[b] (*n* = 74)	5.9 ± 1.8[d] (*n* = 30)	6.1 ± 1.9[c] (*n* = 20)	6.3 ± 2.3[d] (*n* = 24)
Gln/Gln	5.0 ± 2.0 (*n* = 3)	7.0 ± 1.4[b] (*n* = 20)	7.1 ± 1.1[b] (*n* = 7)	7.0 ± 1.4[b] (*n* = 7)	6.8 ± 1.7[d] (*n* = 6)

MN test was successfully performed in 141 CAD patients. MN frequency values are expressed as micronuclei/1000 binucleated cells
*$P < 0.02$ compared with control group (Student's *t*-test)
[a]$P < 0.02$ compared with relevant XPD 751 Lys/Lys genotype (analysis of variance [ANOVA] test)
[b]$P < 0.01$, [c]$P < 0.03$, [d]$P < 0.05$ compared with relevant XRCC1 399 Arg/Arg genotype (ANOVA test)

0.69, $P = 0.09$, respectively) and the cases ($P = 0.76$, $P = 0.77$, respectively).

Micronucleus data of the patients has been summarized in Table 3 according to genotype and extent of CAD. We found that the mean MN frequency in all CAD patients was higher than that found in the controls (5.7 ± 1.9 and 5.0 ± 2.1, respectively, $P = 0.018$). However, when the patients with CAD were grouped according to affected coronary vessel number, there was not a statistically significant difference among the three groups of CAD patients ($P = 0.944$), neither between patients nor control subjects ($P = 0.128$). Patients with the XPD 751 Gln/Gln genotype in CAD group exhibited higher levels of MN with respect to the patients with the XPD 751 Lys/Lys genotype ($P = 0.02$). On the other hand, MN frequencies were significantly higher in all CAD patients and patients with single-double-, or triple-vessel CAD with the XRCC1 399 Gln/Gln or Arg/Gln genotype than in those with the Arg/Arg genotype ($P < 0.05$).

Multiple regression analysis showed that dyslipidemia and diabetes appeared to be the only two independent determinant factors in determining MN frequency in our patient population (Table 4). Micronucleus frequency was significantly increased in CAD patients with dyslipidemia (6.0 ± 1.9, $P = 0.023$) or diabetes (6.1 ± 1.9, $P = 0.011$) compared with the control group (5.0 ± 2.1). There were no significant correlations between MN frequency and Gensini score index, age, smoking, hypertension and family history.

Table 4. Multiple regression between MN frequency and risk factors

Variable	Regression coefficient	Significance (*P*)
Age	0.013	0.857
Smoking	0.114	0.100
Hypertension	0.023	0.743
Diabetes	0.172	0.014
Dyslipidemia	0.165	0.020
Family history of CAD	0.023	0.744
Gensini score	0.042	0.54

Discussion

The potential role of DNA damage in atherosclerosis represents an important issue, which is still not fully understood. It seems likely that reactive oxygen species generated by many routes could cause DNA damage in atheroscléro-

sis[6,16] which, in turn, could be influenced by susceptibility polymorphisms that can alter the activity of relevant DNA repair genes as well as that of carcinogenic-activating and carcinogenic-detoxifying genes.

The present study examined whether polymorphisms in two DNA repair genes involved in BER and NER pathways are related to the development of CAD. This is, to the best of our knowledge, the first case–control study that has explored the association between DNA repair gene polymorphisms and risk of developing CAD. We found no association between CAD and the XRCC1 Arg399Gln, and XPD Lys751Gln polymorphisms in this study population.

Cytogenetic studies of human plaque smooth muscle cells have revealed the presence of a variety of karyotypic abnormalities, primarily involving aneuploidies.[17,18] Chromosome breakage (double strand breaks) and chromosome loss were assessed by the cytokinesis-block MN test by using cytochalasin-B to inhibit actin polymerization during in vitro cell division. Several studies in patients with CAD have demonstrated the presence of somatic DNA damage in circulating human lymphocytes using the MN test and comet assay as sensitive biomarkers of DNA damage.[19,20] We also found elevated levels of MN frequency in CAD patients compared with controls. Although MN levels were increased in patients with CAD, these values did not vary in patients grouped into affected vessel number.

Several well-known atherosclerotic risk factors lead to DNA damage.[6] The effects of dyslipidemia and diabetes mellitus on DNA damage in CAD have been demonstrated by means of increased production of reactive oxidative species.[21,22] In our study, we found that DNA damage in patients with dyslipidemia or diabetes mellitus was significantly higher than without dyslipidemia or diabetes mellitus, and dyslipidemia and diabetes mellitus were an independent risk factor of DNA damage.

Several studies have suggested that genetic polymorphisms can affect the level of chromosome damage associated with genotoxic exposures, but may also influence the background level of cytogenetic alterations.[23,24] Genotype effects seen in both the exposed and the controls or only in the controls probably represent genotype effects on the baseline level of the cytogenetic biomarkers.[24] Micronuclei are used as one of the cytogenetic biomarkers, and intra- and inter-individual variations in this frequency have been reported in human blood lymphocytes.[13] Polymorphisms in a few metabolic enzyme genes seem to account for a proportion of this variability, but the impacts of specific genetic variants on the MN frequency have not yet been clarified. In the future larger studies could be targeted to healthy individuals under controlled lifestyle conditions, known to interfere with MN formation (e.g., nutrient dietary intake and smoking habits). This approach will allow us a better chance to investigate the role of this polymorphic DNA repair genes on MN frequency. Human cells have several different DNA repair mechanisms. The main repair pathway involved in repair of endogenous damage is BER. In addition, some oxidized bases may also be processed via NER.[8] In this part of our study, we assessed the effect of XRCC1 codon 399 and XPD codon 751 polymorphisms on MN frequency in our study population. Our results indicate an association between the frequencies of MN and polymorphisms in XPD codon 751 and XRCC1 codon 399. Carriers of the XRCC1-399 Gln variant allele had an increased frequency of MN; a similar effect was seen for carriers of XPD-751 Gln variant allele.

The functional significance of most DNA repair gene polymorphisms is unknown or poorly characterized. Functional assays suggest that the XPD codon 751 and XRCC1 codon 399 genotypes impact DNA repair, but these studies are not consistent regarding which alleles would be predicted to increase sensitivity to DNA damage.

Polymorphisms in XRCC1 have been thought to alter BER proficiency. XRCC1 protein interacts with PARP by binding to it by way of its central domain.[9] This domain contains codons 301–402. Therefore, codon 399 is within this binding region. Thus, the XRCC1 Arg399Gln polymorphism may affect the interaction of XRCC1 with other proteins. The 399Gln allele of the XRCC1 gene has been suggested to be associated with reduced DNA repair efficiency.[26] The XRCC1 exon 10 variant allele (Gln) has previously been observed to be associated with increased levels of SCEs in lymphocytes.[27] and glycophorin A variant erythrocytes in smokers,[26] polyphenol DNA adducts in mononuclear cells,[23] and bulky DNA adducts in leukocytes in never-smokers;[28] however, it did not influence SCE or CA levels in nonsmokers.[23,27,28] The XRCC1-Arg399Gln gene polymorphism has been studied as a risk factor for various cancers. The variant Gln allele variant has been associated with squamous cell carcinoma of the head and the neck, gastric cancer, lung cancer, bladder cancer, but not breast cancer, prostatic cancer, lymphoma, and laryngeal cancer.[29,30]

The XPD Lys751Gln polymorphism has been suggested to be the most important functional polymorphism in the gene due to major change in the electronic configuration of the respective amino acid in an important interaction domain of the protein.[11] Different XPD alleles have been associated with chromosomal aberrations,[24] frequency of sister chromatid exchanges,[23] DNA adducts,[28] reduced efficiency of DNA repair as measured by the host cell reactivation assay,[31] and increased UV-induced DNA strand breaks detected by the alkaline comet assay.[32] Functional assays for the XPD codon 751 polymorphism do not provide consistent results regarding which alleles would be predicted to increase cancer risk. Previous molecular epidemiologic studies have found that the XPD 751Gln allele is associated with increased risks for head and neck cancer, melanoma skin cancer, and lung cancer.[11,33] However, inconsistent findings were also reported, including absence of any association with lung, and paradoxical inverse associations with esophageal adenocarcinoma and basal cell carcinoma.[11,33]

In conclusion, we did not find an association between XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms and the risk of developing CAD. However, we should note that the sample size of our study has limited power for assessing these associations. Nevertheless, this study is the first report to demonstrate the effects of XRCC1 codon 399 and XPD codon 751 polymorphisms on MN frequency in a

CAD population. Our study suggests that the variant XPD 751Gln and XRCC1 399Gln genotypes could result in an increase in the MN frequency. However, these preliminary findings need to be validated in larger populations because of relatively small sample size.

Acknowledgment This work was supported by the Research Fund of The University of Istanbul. Project number: 204/29042004.

References

1. Ross R (1999) Atherosclerosis – an inflammatory disease. N Engl J Med 340: 115–126
2. Hauser ER, Pericak-Vance MA (2000) Genetic analysis for common complex disease. Am Heart J 140:36–44
3. Winkelmann BR, Hager J (2000) Genetic variation in coronary heart disease and myocardial infarction: methodological overview and clinical evidence. Pharmacogenomics 1:73–94
4. Zhang M, Ma H, Wang BS, Zhao YZ (2006) Angiotensin II type 2 receptor gene polymorphisms and cardioprotective role in essential hypertension. Heart Vessels 21:95–101
5. Andreassi MG, Botto N (2003) DNA damage as a new emerging risk factor in atherosclerosis. Trends Cardiovasc Med 13:270–275
6. Andreassi MG (2003) Coronary atherosclerosis and somatic mutations: an overview of the contributive factors for oxidative DNA damage. Mutat Res 543:67–86
7. Botto N, Masetti S, Petrozzi L, Vassalle C, Manfredi S, Biagini A, Andreassi MG (2002) Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis 13:269–274
8. Frosina G (2000) Overexpression of enzymes that repair endogenous damage to DNA. Eur J Biochem 267:2135–2149
9. Caldecott KW (2003) XRCC1 and DNA strand break repair. DNA Repair (Amst) 2:955–969
10. Shen MR, Jones IM, Mohrenweiser H (1998) Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res 58:604–608
11. Clarkson SG, Wood RD (2005) Polymorphisms in the human XPD (ERCC2) gene, DNA repair capacity and cancer susceptibility: an appraisal. DNA Repair (Amst) 4:1068–1074
12. Benhamou S, Sarasin A (2002) ERCC2/XPD gene polymorphisms and cancer risk. Mutagenesis 17:463–469
13. Tucker JD, Preston RJ (1996) Chromosome aberration, micronuclei, aneuploidy, sister chromatid exchanges, and cancer risk assessment. Mutat Res 365:147–159
14. Gensini GG (1983) A more meaningful scoring system for determining the severity of coronary heart disease. Am J Cardiol 51:606
15. Fenech M (2000) The in vitro micronucleus technique. Mutat Res 455:81–95
16. Olinski R, Gackowski D, Foksinski M, Rozalski R, Roszkowski K, Jaruga P (2002) Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. Free Radic Biol Med 33:192–200
17. Matturri L, Cazzullo A, Turconi P, Lavezzi AM (1997) Cytogenetic aspects of cell proliferation in atherosclerotic plaques. Cardiologia 42:833–836
18. Casalone R, Granata P, Minelli E, Portentoso P, Giudici A, Righi R, Castelli P, Socrate A, Frigerio B (1991) Cytogenetic analysis reveals clonal proliferation of smooth muscle cells in atherosclerotic plaque. Hum Genet 87:139–143
19. Botto N, Rizza A, Colombo MG, Mazzone AM, Manfredi S, Masetti S, Clerico A, Biagini A, Andreassi MG (2001) Evidence for DNA damage in patients with coronary artery disease. Mutat Res 493:23–30
20. Andreassi MG, Botto N, Rizza A, Colombo MG, Palmieri C, Berti S, Manfredi S, Masetti S, Clerico A, Biagini A (2002) Deoxyribonucleic acid damage in human lymphocytes after percutaneous transluminal coronary angioplasty. J Am Coll Cardiol 40:862–868
21. Harangi M, Remenyik E, Seres I, Varga Z, Katona E, Paragh G (2002) Determination of DNA damage induced by oxidative stress in hyperlipidemic patients. Mutat Res 513:17–25
22. Dincer Y, Akcay T, Ilkova H, Alademir Z, Ozbay G (2003) DNA damage and antioxidant defence in peripheral leucocytes of patients with Type I diabetes mellitus. Mutat Res 527:49– 55
23. Duell EJ, Wiencke JK, Cheng TJ, Varkonyi A, Zuo ZF, Ashok TD, Mark EJ, Wain JC, Christiani DC, Kelsey KT (2000) Polymorphisms in the DNA repair genes XRCCl and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis 21:965–971
24. Vodicka P, Kumar R, Stetina R, Sanyal S, Soucek P, Haufroid V, Dusinska M, Kuricova M, Zamecnikova M, Musak L, Buchancova J, Norppa H, Hirvonen A, Vodickova L, Naccarati A, Matousu Z, Hemminki K (2004) Genetic polymorphisms in DNA repair genes and possible links with DNA repair rates, chromosomal aberrations and single-strand breaks in DNA. Carcinogenesis 25: 757–763
25. Norppa H (2004) Cytogenetic biomarkers and genetic polymorphisms. Toxıcol Lett 149:309–334
26. Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA (1999) XRCC1 polymorphisms: effect on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res 59:2557–2561
27. Lei YC, Hwang SJ, Chang CC, Kuo HW, Luo JC, Chang MJ, Cheng TJ (2002) Effects on sister chromatid exchange frequency of polymorphisms in DNA repair gene XRCC1 in smokers. Mutat Res 519:93–101
28. Matullo G, Palli D, Peluso M, Guarrera S, Carturan S, Celentano E, Krogh V, Munnia A, Tumino R, Polidoro S, Piazza A, Vineis P (2001) XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. Carcinogenesis 22:1437–1445
29. Hung RJ, Hall J, Brennan P, Boffetta P (2005) Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. Am J Epidemiol 162:925–942
30. Hu Z, Ma H, Chen F, Wei Q, Shen H (2005) XRCC1 polymorphisms and cancer risk: a meta-analysis of 38 case-control studies. Cancer Epidemiol Biomarkers Prev 14:1810–1818
31. Qiao Y, Spitz MR, Shen H, Guo Z, Shete S, Hedayati M, Grossman L, Mohrenweiser H, Wei Q (2002) Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic XPC and XPD/ERCC2 genotypes. Carcinogenesis 23: 295–299
32. Moller P, Wallin H, Dybdahl M, Frentz G, Nexo BA (2000) Psoriasis patients with basal cell carcinoma have more repair-mediated DNA strand-breaks after UVC damage in lymphocytes than psoriasis patients without basal cell carcinoma. Cancer Lett 151: 87–92
33. Matullo G, Dunning AM, Guarrera S, Baynes C, Polidoro S, Garte S, Autrup H, Malaveille C, Peluso M, Airoldi L, Veglia F, Gormally E, Hoek G, Krzyzanowski M, Overvad K, Raaschou-Nielsen O, Clavel-Chapelon F, Linseisen J, Boeing H, Trichopoulou A, Palli D, Krogh V, Tumino R, Panico S, Bueno-De-Mesquita HB, Peeters PH, Lund E, Pera G, Martinez C, Dorronsoro M, Barricarte A, Tormo MJ, Quiros JR, Day NE, Key TJ, Saracci R, Kaaks R, Riboli E, Vineis P (2006) DNA repair polymorphisms and cancer risk in non-smokers in a cohort study. Carcinogenesis 27:997–1007