ORIGINAL ARTICLE

Arg399Gln Polymorphism of the XRCC1 Gene is Associated with Coronary Artery Disease in a Turkish Population

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ABSTRACT

Objective: *Coronary artery disease, the leading cause of morbidity and mortality worldwide, is an inflammatory disease. The X-ray repair cross complementing 1 (XRCC1) gene plays the role of scaffolding protein for the base excision repair (BER) and single strand break (SSB) repair.*
Methods: *The study population consisted of 402 participants living in the same region and classified into case group (n = 201) and control group (n = 201). Phenol-chloroform method was used to extract DNA from blood samples of the study participants. The X-ray repair cross complementing 1 genotypes were determined using polymerase chain reaction/ restriction fragment length polymorphism (PCR/RFLP) methods.*
Results: *No statistically significant difference was found between the study groups in terms of allele and genotype frequencies in XRCC1 Arg194Trp polymorphism. However, distribution of XRCC1 399Gln allele frequency was found to differ at a statistically significant level between the case and the control groups (*p *= 0.003; OR = 1.56). Regarding the Arg/Arg genotype in Arg399Gln polymorphism, a statistically significant difference was detected in the distribution of Gln/Gln genotype (*p *= 0.017; adj OR = 3.11). Statistically significant differences were also recorded for Arg399Gln polymorphism among the smoking male participants with hypertension (*p *= 0.009;* p *= 0.031;* p *= 0.032, respectively).*
Conclusion: *The study suggests that XRCC1 399Gln/Gln genotype may be a significant risk factor for coronary artery disease.*

Keywords: Coronary artery disease, genetic polymorphism, X-ray repair cross complementing 1

El Polimorfismo Arg399Gln del gen XRCC1 se Halla Asociado con la Enfermedad de las Arterias Coronarias en una Población de Turquía

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RESUMEN

Objetivo: *La enfermedad de las arterias coronarias, la principal causa de morbilidad y mortalidad en todo el mundo, es una enfermedad inflamatoria. El gen de rayos X de reparación de complementación cruzada grupo 1 (XRCC1) desempeña el papel de proteína de andamiaje*

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para la reparación por escisión de la base (REB) y la reparación de roturas simples de cadenas (RSC).

Métodos: *La población de estudio consistió en 402 participantes que vivían en la misma región, y que fueron clasificados en el grupo de caso (n = 201) y el grupo de control (n = 201). El método de fenol-cloroformo fue utilizado para extraer el DNA de las muestras de sangre de los participantes del estudio. Los genotipos de rayos X de reparación de complementación cruzada grupo 1, fueron determinados usando métodos de reacción en cadena de la polimerasa/ polimorfismos de longitud de fragmentos de restricción (RCP/PLFR).*

Resultados: *No se encontraron diferencias estadísticamente significativas entre los grupos de estudio en términos de alelos y frecuencias genotípicas en el polimorfismo XRCC1 Arg194Trp. Sin embargo, se halló que la distribución de la frecuencia del alelo de XRCC1 399Gln difería a un nivel estadísticamente significativo entre los grupos de caso y control (*p *= 0.003; OR = 1.56). Respecto al genotipo Arg/Arg en el polimorfismo Arg399Gln, se detectó una diferencia estadísticamente significativa en la distribución del genotipo de Gln/Gln (*p *= 0.017; adj OR = 3.11). También se registraron diferencias estadísticamente significativas para el polimorfismo Arg399Gln entre los participantes varones fumadores con hipertensión (*p *= 0.009;* p *= 0.031;* p *= 0.032, respectivamente).*

Conclusión: *El estudio sugiere que el genotipo de XRCC1 399Gln/Gln puede ser un factor de riesgo significativo para la enfermedad de la arteria coronaria.*

Palabras clave: Enfermedad de las arterias coronarias, polimorfismo genético, rayos X de reparación de complementación cruzada grupo 1

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INTRODUCTION

Coronary artery disease (CAD), is a major cause of disability and sudden death all over the world (1). Epidemiological studies have shown that CAD incidence frequency in Turkey is 5.1 per thousand among males and 3.4 per thousand among females (2). An inflammatory disease, CAD is characterized by a modification of atherosclerotic plaques, calcified regions, modified lipids, inflamed smooth muscle cells, endothelial cell, leukocytes and foam cells (3). Genetic polymorphisms in DNA repair genes may be associated with differences in DNA repair capacity and may influence an individual's risk of atherosclerosis.

X-ray repair cross complementing 1 gene (XRCC1), a DNA repair gene, plays an important role in base excision. This gene consists of 17 exons and is located on chromosome 19q 13.2–13.3, with a length of 33 kilobase. The XRCC1 gene encodes DNA-repair proteins, which interact with other proteins such as Lig3, Polb and PARP, creating a complex involved in short patch BER [base excision repair] (4). This study concentrated on two polymorphisms of XRCC1: codon 194 and codon 399. One of these two polymorphisms, namely codon 194, is "an arginine (Arg) to tryptophan (Trp) change" (Arg194Trp). The X-ray repair cross complementing 1, Arg399Gln polymorphism is located in the area coding for the binding side of poly (ADP-ribose) polymerase [PARP]. Poly (ADP-ribose) polymerase is a zinc-finger containing enzyme that detects DNA strand breaks (5). In this scope, this study aimed to investigate the association between XRCC1 Arg194Trp and Arg399Gln polymorphisms and CAD. This is the first study investigating the association between this polymorphism and demographic parameters in Turkish population.

METHODS

Study Population: After approval by the Ethics Committee of the Medical School of Cumhuriyet Universty, data of 201 cases and 201 controls were analysed (Number of Ethical Committee 2011–02/04). Control and case groups are composed of Özbilüm's previous study; because this study is a continuation of our previous studies; we selected cases and the control group based on our previous publication (6).

Genotyping: Blood samples (2 mL) were collected into EDTA tubes. Leukocytes DNA (100 ng) were extracted using standard phenol-chloroform methods was used as a template in PCR-based restriction fragment length polymorphism (RFLP) assays.

Polymerase chain reaction-restriction fragment length polymorphism condition is carried out based on a previous publication (7). Polymerase chain reaction-restriction fragment length polymorphism analyses of XRCC1 Arg194Trp and Arg399Gln polymorphism are shown in Figure.

Ten per cent of the study population was further confirmed for each genotype *via* direct sequencing, using an ABI PRISM 377 automatic sequencer (Applied Biosystems).

Statistical analysis: Statistical analysis was performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA) and EH for haplotype (8). Pearson's Chi-square test was used to calculate the statistical significance of the differences in genotypes and demographic and clinical parameters of the case and the control group participants. *T*-test was made to evaluate the age distribution of the case and the control group populations. Multivariate logistic regression analysis (adjusted for all demographic and clinical parameters) was performed to assess the independent contribution of genotype to CAD. X-ray repair cross complementing 1 gene and Arg399Gln polymorphism was also adjusted for demographic parameters as well. For each odds ratio (OR), confidence interval (CI) was calculated to be 95%. In all cases, statistical significance was set at $p \leq 0.05$.

RESULTS

Table 1 shows the demographic and clinical parameters of the study population. A total of 201 patients with CAD and 201 healthy control subjects were included in this study. Demographic parameters were found to be statistically significantly different between the case and the control group's subjects, except for age and diabetic mellitus (Table 1).

Table 2 presents the Arg194Trp and Arg399Gln allele and genotype distributions of study groups. No statistically significant difference was detected between the case and the control groups in terms of allele and genotype frequencies in Arg194Trp (Table 2). Gln frequency in Arg399Gln polymorphism were found to statistically significantly differ ($p = 0.003$; OR = 1.56). Comparison of the Arg/Arg genotype with Gln/Gln genotypes produced a statistically significant difference between case and the control groups ($p = 0.017$; OR=1.98). Comparison between the Arg399Gln geno-type and wild genotype also revealed a statistically significant difference between the study groups ($p = 0.013$; OR = 1.73). The study also revealed four possible haplotypes with no statistically significant difference between the case and the control groups (Table 2).

Table 1. Demographic and clinical parameters of patients with coronary artery disease and healthy control subjects.

	Cases n (%)	Controls n (%)	OR 95%CI	*p*-value
Total	201 (100)	201(100)		
Age (mean ± SD)	63.12 ± 6.56	60.26 ± 6.82		0.274
Gender				
Female	63 (31.34)	126 (62.69)		
Male	138 (68.66)	75 (37.31)	3.68 (2.43–5.56)	< 0.001
Smoking status				
Non-smoker	74 (36.82)	118 (58.71)		
Smoker	127 (63.18)	83 (41.29)	2.44 (1.63–3.64)	< 0.001
Hypertension				
Absent	72 (35.82)	146 (72.64)		
Present	129 (64.18)	55 (27.36)	4.75 (3.11–7.26)	< 0.001
Diabetes				
Absent	124 (61.69)	137 (68.16)		
Present	77 (38.31)	64 (31.84)	1.33 (0.88–2.00)	0.174
Hypercholesterolaemia				
Absent	127 (63.18)	148 (73.63)		
Present	74 (36.81)	53 (26.37)	1.63 (1.06–2.49)	0.024

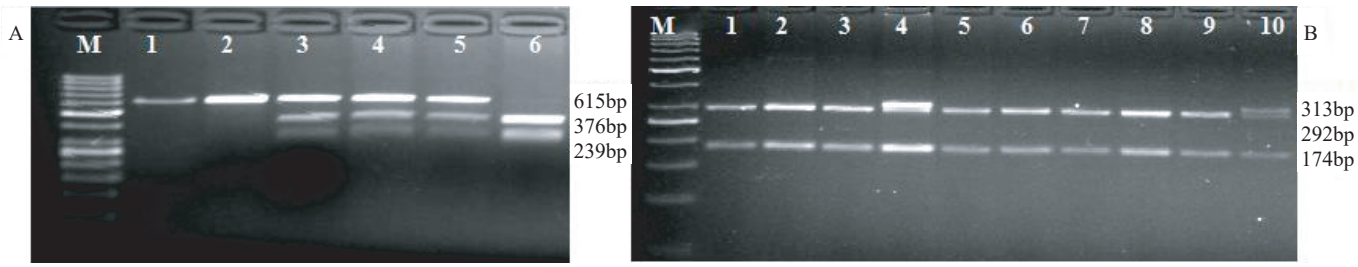

Figure 1: Polymerase chain reaction-restriction/ fragment length polymorphism analysis of *Arg399Gln* and *Arg194Trp* polymorphisms.

A) For *Arg399Gln* polymorphisms, Arg/Arg genotype yielded 239 bp and 376 bp; **line** 6. Arg/Gln genotype yielded 615 bp, 376 bp and 239bp; **line** 3, 4 and 5. Gln/Gln genotype yielded 615 bp.
B) For *Arg194Trp* polymorphism, Arg/Arg genotype yielded 292 bp, 174 bp and 21 bp; **line** 1, 2, 3, 5, 6, 7, 8 and 9. Arg/Trp genotypes yielded 313 bp; 292 bp 174 bp and 21 bp; line 4 and 10. M is a molecular weight marker (50 bp DNA ladder, Fermentas).

Table 3 also shows risk estimates and genotype frequencies for all demographic parameters in Arg399Gln polymorphism. Comparison of the Arg/Arg genotype with both Arg/Gln and Gln/Gln genotypes produced a statistically significant difference between the male CAD patients and the control group participants (p = 0.005; OR = 2.49; p = 0.009; OR = 3.00, respectively).

DISCUSSION

Coronary artery disease, a multifactorial disease, originates from an interaction between environmental influences and genetic tendencies (9). DNA damages are observed in both circulating cells and atherosclerotic plaques of patients with CAD (10). Some studies suggest that DNA alterations are present in atherosclerotic tissues and may play a critical role in initialization of this disease (11). The X-ray repair cross complementing 1 gene, is a major gene in the base excision repair (BER) system, interacting with several BER enzymes to modify and stabilize their activity (12).

The present study tested the hypothesis whether SNPs in the genes encoding different DNA repair enzymes interact with CAD risk. While the allele frequency of XRCC1 194Trp in the present study was calculated to be 8.96% in the control group (Table 2), it was reported as 6.48% in the Czech Republic (13) and 8.47% in the United States of America (14). In a study investigating the relationship between CAD polymorphism and Arg194Trp polymorphism, Bazo (15) *et al* determined Trp allele frequency as 2.6% in a Brazilian population. Results of the present study agree with the results of the latter study as both studies did not reveal any relationship between this polymorphism and CAD.

The present study revealed a relation between XRCC1399 Gln/Gln genotype and CAD patients compared with healthy individuals (p = 0.017): the individuals with Gln/Gln genotype have approximately three-times greater CAD risk compared to those carrying Arg/Arg genotype (adj OR = 3.11) and there is a statistically significant difference in Arg399Gln allele distributions between CAD patients and the control group (p = 0.003).

There was a significant association between Arg399Gln polymorphism and CAD in different ethnic groups (15, 16). On the contrary, some studies previously carried out in Turkey did not show a statistically significant relationship between CAD and Arg399Gln polymorphism (17). The X-ray repair cross complementing 1 gene, polymorphism was also found to have a statistically significant association with many types of diseases in a Turkish population (18). The X-ray repair cross complementing 1 gene protein interacts with PARP's central domain. This domain has codon 301–402; therefore, codon 399 is within this binding region. The X-ray repair cross complementing 1 gene 399Gln allele is linked to decreased BER mechanism.

Table 2. Risk estimates and frequencies of allele, genotypes and haplotypes of Arg194Trp and Arg39Gln

		Cases n (%)	Controls n (%)	p-value	Unadjusted OR (95%Cl)	[a]Adjusted OR (95%Cl)
Arg194Trp						
	Arg	375 (93.28)	366 (91.04)			
	Trp	27 (6.72)	36 (8.96)	0.238	0.73 (0.43–1.23)	
	Arg/Arg	174 (86.57)	165 (82.09)			
	Arg/Trp	27 (13.43)	36 (17.91)	0.217	0.71 (0.41–1.22)	
	Trp/Trp	0 (0.00)	0 (0.00)	–	-	
Arg399Gln						
	Arg	235 (58.46)	276 (68.66)			
	Gln	167 (41.54)	126 (31.34)	0.003	1.56 (1.16–2.08)	
	Arg/Arg	74 (36.82)	103 (51.24)			
	Arg/Gln	87 (43.28)	70 (34.83)	0.013	1.73 (1.12–2.67)	
	Gln/Gln	40 (19.90)	28 (13.93)	0.017	1.98 (1.13–3.51)	3.11 (1.65–5.85)
Haplotypes						
Arg	Arg	224 (55.72)	242 (60.20)	Ref	Ref	Ref
Arg	Gln	154 (38.31)	128 (31.84)	0.083	1.30 (0.97–1.75)	1.48 (0.85–2.59)
Trp	Arg	16 (3.98)	28 (6.96)	0.137	0.62 (0.33–1.17)	0.69 (0.26–1.84)
Trp	Gln	8 (1.99)	4 (1.00)	0.203	2.16 (0.64–7.27)	2.41 (0.41–13.98)

[a]Adjusted for age, gender, hypertension, smoking habit, hypercholesterolaemia and diabetes

Table 3: Subgroup analysis of genotype frequencies in X-ray repair cross complementing 1 gene Arg-399Gln polymorphism

Arg/Gln	Cases n (%)	Controls n (%)	*p*-value	OR (95%Cl)	Adjusted OR (95%Cl)
Female					
Arg/Arg	24 (38.10)	58 (46.03)	Ref		
Arg/Gln	29 (46.03)	49 (38.89)	0.288	1.43 (0.74–2.77)	
Gln/Gln	10 (15.87)	19 (15.08)	0.601	1.27 (0.52–3.13)	0.94 (0.41–2.16)
Male					
Arg/Arg	50 (36.23)	45 (60.00)	Ref		
Arg/Gln	58 (42.03)	21 (28.00)	0.005	2.49 (1.31–4.72)	
Gln/Gln	30 (21.74)	9 (12.00)	0.009	3.00 (1.29–7.00)	2.03 (0.91–4.57)
Smoking					
Arg/Arg	49 (38.58)	43 (51.81)	Ref		
Arg/Gln	52 (40.94)	31 (37.35)	0.209	1.47 (0.80–2.69)	
Gln/Gln	26 (20.48)	9 (10.84)	0.031	2.53 (1.07–6.00)	2.11 (0.94–4.78)
Hypertension					
Arg/Arg	49 (37.98)	28 (50.91)			
Arg/Gln	53 (41.09)	22 (40.00)	0.356	1.37 (0.69–2.72)	
Gln/Gln	27 (20.93)	5 (9.09)	0.032	3.09 (1.06–8.92)	2.64 (0.96–7.29)
Diabetes					
Arg/Arg	26 (33.77)	31 (48.44)			
Arg/Gln	36 (46.75)	26 (40.62)	0.174	1.65 (0.80–3.41)	
Gln/Gln	15 (19.48)	7 (10.94)	0.072	2.56 (0.90–7.21)	1.97 (0.75–5.18)
Hypercholesterolaemia					
Arg/Arg	31 (41.89)	20 (37.74)			
Arg/Gln	31 (41.89)	24 (45.28)	0.644	0.83 (0.38–1.81)	
Gln/Gln	12 (16.22)	9 (16.98)	0.770	0.86 (0.31–2.41)	0.95 (0.37–2.44)

Thus, Arg399Gln polymorphism is thought to be associated with reduced DNA repair efficiency (19). On the other hand, increased DNA damage plays an important role in the early phases of atherogenesis. DNA damage causes smooth muscle cell proliferation in the intima of arteries. This is the first study examining haplotype frequencies by linkage analysis. Although no relationship was found between the case and control haplotype frequencies, TG haplotype was recorded to be associated with increased CAD risk (Adj OR = 2.41).

Since Arg399Gln polymorphism is significant for CAD; this study, for the first time, analysed the relationship between genotype frequencies and demographic parameters for Arg399 in a Turkish population (Table 3). This analyses produced a statistically significant difference in distribution of genotype frequency among male participants ($p = 0.009$). Due to some risk and hormonal factors effective during development periods of males and females (20), male individuals had two-times higher-risk than female individuals of CAD [for male OR = 3.00; for female OR = 1.27] (Table 3).

Findings of the present study confirmed that smoking is an important risk factor for CAD since smoker frequency is higher than non-smoker frequency in the case group (Table1). Table 3 presented genotype distribution frequency between Arg399Gln and CAD in smokers. Smokers with Gln/Gln genotype have 2.11 times higher-risk than those with Arg/Arg genotype for XRCC1 Arg399Gln. Recent studies have supported the mutagenic and mitogenic role played in atherosclerosis by the chemicals in cigarette smoke. Moreover, there is growing evidence that DNA damage is not only confined to cancer patients but also present in cardiovascular disease patients (21). Cigarette contains some chemicals that produce free radicals which induce oxidative damage to DNA. Weng *et al* (22) indicated smoking status and age as important variables affecting individuals' basal DNA damage in XRCC1 194 and 399.

The present study also analysed the relationship between Arg399Gln and hypertensive CAD individuals. A statistically significant difference was found between the case and the control group in Arg399Gln (Table 3).

Yu et al (23) found no significant relationship between Arg399Gln and hypertensive CAD patients. These conflicting results may be related to differing ethnic groups and sample sizes. Hypertension is one of the most prevalent population risk factors, especially in patients with established CAD (24).

A limitation of the study is that the sample size could have been larger. There was a statistically significant difference between the case and control groups in terms of gender, smoking status, hypertension and hypercholesterolaemia. In our study, frequency of male patients was found to be higher than female patients as seen in other studies. Similar to gender, smoking status, hypercholesterolaemia and hypertension are risk factor for CAD, so in general population frequencies of these parameters are high in our population.

In conclusion, XRCC1 Arg399Gln polymorphism has been found to be statistically significant for CAD. This study also revealed a statistically significant association between XRCC1 399Gln/Gln genotype and CAD in males, smokers and hypertensive individuals. Thus, XRCC1 399Gln/Gln genotype may be a significant risk factor for CAD.

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REFERENCES

1. World Health Organization. Prevention of Cardiovascular disease; guidelines for assessment and management of cardiovascular risk. WHO Library 2007; ISBN 978 92 4 154717 8.
2. Onat A. Erişkinlerimizde Kalp Hastalıkları Prevalansı, Yeni Koroner Olaylar ve Kalpten Ölüm Sıklığı. Erişim: http://tekharf.org/ Erişim tarihi 28.07.2011 (2009).
3. Ross R: Atherosclerosis an inflammatory disease. N Eng J Med 1999; **340:**115–126.
4. Sterpone S, Cozzi R. Influence of XRCC1 genetic polymorphisms on ionizing radiation-induced DNA damage and repair. J Nucl Acids 2010; 25.
5. Chacko P, Rajan B, Joseph T, Mathew BS, Pillai MR. Polymorphisms in DNA repair gene XRCC1 and increased genetic susceptibility to breast cancer. Breast Cancer Res Treat 2005; **89:**15–21.
6. Özbilüm N, Arslan S, Öcal B, Yanartaş M, Aydemir EI. The role of NF-κB1A promoter polymorphisms on coronary artery disease risk. Basic&Clinical Pharmacology & Toxicology 2013; **113:** 187–92.
7. Improta G, Sgambato A, Bianchino G, Zupa A, Grieco V, La Torre G et al. Polymorphisms of the DNA repair genes XRCC1 and XRCC3 and risk of lung and colorectal cancer: a case–control study in a Southern Italian population. Anticancer Res 2008; **28:** 2941–946.
8. Terwilliger JD, Ott J. A novel polylocus method for linkage analysis using the lodscore or affected sib-pair method. Genet Epidemiol 1993; **10:** 477–82.
9. Zaman AG, Helft G, Worthley SG, Badimon JJ. The role of plaque rupture and thrombosis in coronary artery disease. Atherosclerosis 2000; **149:** 251–66.
10. Botto N, Masetti S, Petrozzi L, Vassalle C, Manfredi S, Biagini A et al. Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis 2002; **13:** 269–74.
11. Lee SH, Blair IA. Oxidative DNA damage and cardiovascular disease. Trends Cardiovasc Med 2001; **11:**148–155.
12. Fortini P, Dogliotti E. Base damage and single-strand break repair: mechanisms and functional significance of short- and long-patch repair subpathways. DNA Repair (Amst) 2007; **6:** 398–409.
13. Pardini B, Naccarati A, Novotny J, Smerhovsky Z, Vodickova L, Polakova V et al. DNA repair genetic polymorphisms and risk of colorectal cancer in the Czech Republic. Mutat Res 2008; **638:** 146–53.
14. Brevik A, Joshi AD, Corral R, Onland-Moret NC, Siegmund KD, Le Marchand L et al. Polymorphisms in base excision repair genes as colorectal cancer risk factors and modifiers of the effect of diets high in red meat. Cancer Epidemiol Biomarkers Prev 2010; **19:** 3167–73.
15. Bazo AP, Salvadori D Jr, Salvadori RA, Sodré LP, da Silva GN, de Camargo EA et al. DNA repair gene polymorphism is associated with the genetic basis of atherosclerotic coronary artery disease. Cardiovasc Pathol 2011; **20:** e9–15.
16. Narne P, Ponnaluri KC, Singh S, Siraj M, Ishaq M. Arg399Gln polymorphism of X-ray repair cross-complementing group 1 gene is associated with angiographically documented coronary artery disease in South Indian type 2 diabetic patients. Genet Test Mol Biomarkers 2013; **17:** 236–41.
17. Gokkusu C, Cakmakoglu B, Dasdemir S, Tulubas F, Elitok A, Tamer S et al. Association between genetic variants of DNA repair genes and coronary artery disease. Genet Test Mol Biomarkers 2013; **17:** 307–13.
18. Engin AB, Karahalil B, Karakaya AE, Engin A. Association between XRCC1 ARG399GLN and P53 ARG72PRO polymorphisms and the risk of gastric and colorectal cancer in Turkish population. Arh Hig Rada Toksikol 2011; **62:** 207–14.
19. Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCC1 polymorphisms: effect on afl atoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res 1999; **59:** 2557–561.
20. Chen Z, Ma G, Qian Q, Yao Y, Feng Y, Tang C. Toll-like receptor 8 polymorphism and coronary artery disease. Mol Biol Rep 2009; **36:** 1897–901.
21. Murgia E, Maggini V, Barale R, Rossi AM. Micronuclei, genetic polymorphism and cardiovascular disease mortality in a nested case control study in Italy. Mutat Res 2007; **621:** 113–8.
22. Weng H, Weng Z, Lu Y, Nakayama K, Morimoto K. Effects of cigarette smoking, XRCC1 genetic polymorphisms, and age on basal DNA damage in human blood mononuclear cells. Mutat Res 2009; **679:** 59–64.
23. Yu X, Liu J, Zhu H, Xia Y, Gao L, Dong Y et al. Synergistic association of DNA repair relevant gene polymorphisms with the risk of coronary artery disease in northeastern Han Chinese. Thromb Res 2014; **133:** 229–34.
24. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM et al. Heart Disease and Stroke Statistics–2011 Update: A Report From the American Heart Association. Circulation 2011; **123:** e18–e209.