International Journal of Toxicology, 25:419–422, 2006
Copyright © American College of Toxicology
ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810600870567

XRCC1 Arg399Gln Genetic Polymorphism in a Turkish Population

Neslihan Aygün Kocabaş and Bensu Karahalil
Gazi University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey

Humans are routinely exposed to mutagenic and carcinogenic chemicals. These chemicals can form DNA adducts in vivo and thus lead to DNA damage. The integrity of most of the so-damaged DNAs is typically restored as a consequence of the action of certain DNA-repairing enzymes. In several DNA repair genes, polymorphisms may result in reduced repair capacity, which has been implicated as a risk factor for various types of cancer. *XRCC1* is a base-excision repair protein that plays a central role in the repair of DNA base damage and strand breaks. Amongst the known genetic polymorphisms of the DNA-repair genes, X-ray repair cross-complementing groups 1 and 3 (*XRCC1* and *XRCC3*) have been studied most commonly. Inconsistent results have been reported regarding the associations between the *Arg399Gln* (exon 10) polymorphism of *XRCC1* and either functional significance or the risk of tobacco-associated cancers. The *Gln* allele of this polymorphism was associated with higher levels of DNA adducts. Therefore we genotyped one of the polymorphism of *XRCC1, Gln* allele. The frequency of the polymorphic alleles varies among populations, suggesting an ethnic distribution of genotypes. There has been no information on interindividual variability of *Arg399Gln* genotype in the Turkish population. Due to the association between the *Arg399Gln* polymorphism of *XRCC1* and the risk of tobacco-associated cancers, we preferred to evaluate the allelic frequencies of *Arg399Gln* genotype than the other polymorphisms in *XRCC1* gene in healthy Turkish population by polymerase chain reaction–restriction fragment polymorphism (PCR-RFLP) analysis to enable to show interindividual differences and compare to other populations.

Keywords *Arg399Gln* Genotype, Genetic Polymorphism, PCR-RFLP Analysis, Turkish Population, *XRCC1* Gene

Humans are routinely exposed to mutagenic and carcinogenic chemicals. These chemicals can form DNA adducts in vivo and thus lead to DNA damage. DNA repair enzymes continuously monitor chromosomes to correct damaged nucleotide residues generated by exposure to carcinogen and cytotoxic compounds (Wood et al. 2001). A variety of mutagenic and cytotoxic DNA lesions are formed by oxidative DNA damage, especially reactive oxygen species (ROS) (Krokan 1997; Lindahl 1997). The accumulation of ROS leads to oxidative stress, which is a risk factor for cancer development (Shirai et al. 1995). ROS can initiate lipid peroxidation, oxidize proteins, and cause damage to DNA directly or indirectly (Joenje 1989). Base damage and single-strand breaks (SSBs) are repaired through the base excision repair (BER) pathway. This pathway is a multistep process that requires the activity of several proteins (Dianov et al. 2001). Substantial evidence indicates an important role for X-ray repair cross complementing 1 (*XRCC1*) in single-strand break repair (SSBR) and BER. This protein is thought to act as a scaffolding protein for other repair factors. A large number of molecular epidemiological studies have analyzed the impact of polymorphisms in the human *XRCC1* gene on cancer risk (Brem and Hall 2005). Amongst the known genetic polymorphisms of the DNA-repair genes, the xeroderma pigmentosum group D (*XPD*, also known as *ERCC2*) and X-ray repair cross-complementing groups 1 and 3 (*XRCC1* and *XRCC3*) have been studied most commonly (Yeh et al. 2005). The gene *XRCC1* encodes a protein involved in DNA base-excision repair. A common polymorphism (*Arg* → *Gln*) at codon 399 of the *XRCC1* gene has been previously linked to functional changes of the gene product and risk of cancers.

A large number of studies have been conducted on the associations between cancer risk and *XRCC1 Arg399Gln* genetic polymorphisms (Kelsey et al. 2004). There has been no information on interindividual variability of *Arg399Gln* genotype in the Turkish population. Therefore, we aimed to determine the allelic frequencies of *Arg399Gln* genotype in healthy Turkish population by polymerase chain reaction–restriction fragment polymorphism (PCR-RFLP) analysis to enable to show interindividual differences and compare to other populations.

MATERIALS AND METHODS

Subjects

Whole blood samples (EDTA) were collected from 166 healthy volunteers after obtaining written informed consent, and detailed questionnaires were applied (age, smoking status,

Received 10 March 2006; accepted 12 June 2006.
The authors wish to thank all subjects who volunteered to participate. This research was financially supported by Gazi University, Pharmacy Faculty, Department of Toxicology.
Address correspondence to Prof. Dr. Bensu Karahalil, Gazi University, Faculty of Pharmacy (Eczacilik), Department of Toxicology, 06330, Ankara, Turkey. E-mail: bensu@gazi.edu.tr

TABLE 1
The *XRCC1* genotype at codon 399 in a Turkish population

	XRCC1 genotype frequency*# (%)			Allele frequency	
Healthy subjects	*Arg399Arg*	*Arg399Gln*	*Gln399Gln*	*Arg399*	*Gln399*
Total ($n = 166$)	37.3	45.2	17.5	0.60	0.40
Smoking status					
Smokers ($n = 60$)#	40.0	40.0	20.0	0.60	0.40
Nonsmokers ($n = 106$)#	36.8	48.1	15.1	0.60	0.40

Note. Calculation for wild/wild genotype versus wild/mutant and mutant/mutant genotypes.
#*Comparison by Fisher's exact test, two sided, $p > .05$.

medication, etc). All subjects were apparently healthy and did not have any chronic disease. The age was ranged from 26 to 78 years. 60 subjects were smokers.

DNA Extraction

Whole blood samples (5 to 10 ml) were removed via venipuncture into tubes containing EDTA and samples were stored at −20°C until DNA extraction. Genomic DNA was extracted from whole blood using a sodium perchlorate/chloroform extraction as described by Karahalil and Kocabaş (2005). Lysis buffer (10 mM Tris-HCl, 320 mM sucrose, 5 mM $MgCl_2$, 1% Triton), Reagent B buffer (400 mM Tris-HCl, 60 mM EDTA, 150 mM NaCl, 1% sodium dodecyl sulfate [SDS]), and 5 mM Sodium perchlorate were used in extraction procedure. All reagents were obtained from Sigma and Merck.

XRCC1 Arg399Gln Genotype

The *XRCC1* alleles were detected using PCR-RFLP technique. This technique is a modification of the method described by Sturgis et al. (1999). The sequences of the PCR primers were 5′-CAGTGGTGCTAACCTAATC-3′ for the forward and 5′-AGTAGTCTGCTGGCTCTGG-3′, which generate an 871-bp product. A 30-μl PCR reaction was performed using approximately 0.2 to 0.5 μg genomic DNA, 0.25 μM each primer, 200 μM of each dNTP, 1.5 U *Taq* polymerase (Promega, Madison, WI) in the 1 × PCR buffer supplied by the manufacturer (50 mM KCl, 10 mM Tris-HCl, pH 9.0, 1% TritonX-100). After an initial melting step at 95°C for 5 min, amplification was carried out for 35 cycles by denaturing at 95°C for 30 s, annealing at 58°C for 45 s, extending at 72°C for 45 s, and a final extention at 72°C for 10 min for 1 cycle. The PCR products were digested with *Nci* I restriction enzyme (New England Biolabs) to distinguish the 28152 polymorphism of exon 10. The digestion of 6 μl of PCR products was carried out using 8U *Nci* I and the 1 × NEB4 buffer at 37°C overnight. The wild allele (i.e., 28152 G) has two *Nci* I restriction enzyme sites of 461 and 182 bp. The mutant allele (i.e., 28152 A) without the *Nci* I restriction enzyme sites has 593 bp. To analyze the restriction fragments, 3% 1:1 ratio of agarose: gamma micropore gel (Prone) was used. The digested products photographed using Genecam system (Spectronics GL-2000) (Sturgis et al. 1999).

RESULTS

We investigated the allelic frequencies of *Arg399Gln* genotype in 166 healthy Turkish population to enable to show interindividual differences and compare to other populations. We found that the *XRCC1* gene allele frequencies were 0.60 for the *399Arg* polymorphism and 0.40 for the 399*Gln* polymorphisms. Sixty subjects were smokers and rest of them ($n = 106$) was nonsmokers. We also compared smoker subjects to nonsmoker subjects. We could not find any statistically significant difference (Table 1).

Table 2 shows the frequency distributions of the *XRCC1* genotype in healthy individuals of our current study and compares these frequencies with those previously published for different ethnic groups. In our study, the allele frequency of the *399Arg* polymorphism in Turkey (0.60) was similar to those previously published by Rossit et al. (2002) for 96 healthy Brazilians from the same region. The observed allele frequency in Turkish population was within the range described for Caucasians (American Caucasian, Italian, and Belgian populations). Wu et al. (2004) found that the frequency of *Arg/Arg, Arg/Gln*, and *Gln/Gln* in *XRCC1* codon 399 in controls was 58% (114/196), 37% (73/196), and 5%, respectively. Ito et al. (2004) also observed similar results regarding the distribution of *XRCC1* genotype in a Korean population (Table 2).

DISCUSSION

Different DNA repair systems maintain the integrity of the human genome, so deficiency in the repair capacity due to mutations or polymorphisms in genes involved in DNA repair can lead to genomic instability that, in turn, is related to chromosomal instability syndromes and increased risk of developing various types of cancer (Duarte et al. 2005). The frequency of the polymorphic alleles varies among populations, suggesting an ethnic distribution of genotypes.

XRCC1, a protein directly involved in the repair of DNA base damage, contains at least three common polymorphisms. One of these, the codon *399 Arg→ Gln* variant, has been associated with several cancer-related biomarkers, suggesting it may have functional significance in exposure-induced cancers (Nelson et al. 2002; Patel et al. 2005). The genotype distribution

TABLE 2
The percentage of genotype frequencies of *XRCC1 Arg399Gln* polymorphism in different populations

References	Individuals	*N*	Genotype frequency (%)			Allele frequency	
			Arg399Arg	*Arg399Gln*	*Gln399Gln*	*Arg399*	*Gln399*
The present study	Turkish population	166	37.3	45.2	17.5	60.0	40.0
Rossit et al. 2002	Brazilian population	96	49.0	34.0	13.0	66.0	34 .0
Park et al. 2002	Korean population	135	60.0	35.6	4.4	77.8	22.2
Ito et al. 2004		448	56.5	37.7	5.8	75.4	24.6
Wood et al. 2001	Belgian population	31	32	55	13	59.5	40.5
Olshan et al. 2002	Americans	161	38.5	50.9	10.6	64.0	36.0
Duell et al. 2002	San Francisco Bay						
	Caucasians	860	48	40	12	68.0	32.0
	African Americans	36	69	31	0	84.5	15.5
	Asians	53	57	33	10	7305	26.5
Kiffmeyer et al. 2004	USA						
	Hmong Population	199	47.0	43.0	10.0	68.5	31.5
	White Population	208	42.5	48.5	9.0	66.8	33.2
Zhou et al. 2003	USA Caucasian (hospital healthy)	1240	44.5	44.0	11.5	66.5	33.5
Shen et al. 2003	Italian population	214	43	46	11	66.0	34.0
Sanyal et al. 2004	German population	246	46	45	9	68.5	31.5
Wu et al. 2004	Taiwanese population	196	58	37	5.0	76.5	23.5
Yeh et al. 2005		736	56.7	36.2	7.1	74.8	25.2
Misra et al. 2003	Finland Caucasian	313	49.2	41.5	9.3	70.0	30.0
Varzim et al. 2003	Portugal Caucasian	178	45.0	44.9	10.1	67.0	33.0

of the *XRCC1* gene does not vary between sexes, but differences between ethnic groups have been suggested (Park et al. 2002; Olshan et al. 2002).

The *XRCC1* gene contains 17 exons and is located on chromosome 19q13.2. Although many polymorphisms have been documented, two nonsynonymous polymorphisms in *XRCC1* (*Arg194Trp* [C → T allelic change] and *Arg399Gln* [G → A allelic change]) have been shown to alter DNA repair capacity in some phenotypic studies and have received considerable attention (Patel et al. 2005).

Three gene polymorphisms resulting in nonconservative amino acid substitutions (*Arg194Trp, Arg280His*, and *Arg399Gln*) have been identified in the *XRCC1* gene. Two of these (*Arg280His* and *Arg399Gln*) were recently predicted to be likely to affect the function of the protein based on the conservation of the amino acids among protein family members. The *XRCC1 Arg280His* polymorphism lies in between the DNA polymerase β and poly (ADP-ribose) polymerase (PARP) binding areas (Metsola et al. 2005).

The proportions of individuals homozygous for *399Arg* allele, heterozygous and homozygous for the *399Gln* allele were 85.8%, 13.7%, and 0.5% among the control group (Jeon et al. 2005). *XRCC1 Arg399Gln* polymorphism is a genetic polymorphism at codon 399 was frequently found in the Korean population.

We have been preparing to publish polymorphisms of the DNA repair genes *XRCC1* and *hOGG1*, relation to environmental exposures, and bladder cancer risk in a case-control study in Turkey. In conclusion, it seems that still there is a growing need for expanding genotype studies with respect to *XRCC1* gene because of its importance in various cancer types.

REFERENCES

Aka, P., R. Mateuca, J. P. Buchet, H. Thierens, and M. Kirsch-Volders. 2004. Are genetic polymorphisms in *OGG1*, *XRCC1* and *XRCC3* genes predictive for the DNA strand break repair phenotype and genotoxicity in workers exposed to low dose ionising radiations? *Mutat. Res*. 556:169–181.

Brem, R., and J. Hall. 2005. *XRCC1* is required for DNA single-strand break repair in human cells. *Nucleic Acid Res*. 33:2512–2520.

Dianov, G. L., N. Souza-Pinto, S. G. Nyaga, T. Thybo, T. Stevnsner, and V. A. Bohr. 2001. Base excision repair in nuclear and mitochondrial DNA. *Prog. Nucleic Acid Res. Mol. Biol.* 68:285–97.

Duarte, M. C., J. Colombo, A. R. B. Rossit, and A. E. Silva. 2005. Polymorphisms of DNA repair genes *XRCC1* and *XRCC3* in a Brazilian population. *Gen. Mol. Biol.* 28:397–401.

Duell, E. J., E. A. Holly, P. M. Bracci, J. K. Wiencke, and K. T. Kelsey. 2002. A population-based study of the Arg399Gln polymorphism in X-ray repair cross-complementing group I (*XRCC1*) and risk of pancreatic adenocarcinoma. *Cancer Res*. 62:4630–4636.

Ito, H., K. Matsuo, N. Hamajima, T. Mitsudomi, T. Sugiura, T. Saito, T. Yasue, K. M. Lee, D. Kang, K. Y. Yoo, S. Sato, R. Ueda, and K. Tajima. 2004. Gene-environment interactions between the smoking habit and polymorphisms in

the DNA repair genes, APE1 *Asp148Glu* and *XRCC1 Arg399Gln*, in Japanese lung cancer risk. *Carcinogenesis* 25:1395–1401.

Jeon, Y. T., J. W. Kim, N. H. Park, Y. S. Song, S. B. Kang, and H. P. Lee. 2005. DNA repair gene *XRCC1 Arg399Gln* polymorphism is associated with increased risk of uterine leiomyoma. *Hum. Reprod*. 20:1586–1589.

Joenje, H. 1989. Genetic toxicology of oxygen. *Mutat. Res*. 219:193–208.

Karahalil, B., and N. A. Kocabaş. 2005. *hOGG1 Ser326Cys* genetic polymorphism in a Turkish population. *Arch. Toxicol*. 79:377–380.

Kelsey, K. T., S. Park, H. H. Nelson, and M. R. Karagas. 2004. A population-based case-control study of the *XRCC1 Arg399Gln* polymorphism and susceptibility to bladder cancer. *CEBP Prev.* 13:1337–1341.

Kiffmeyer, W. R., E. Langer, S. M. Davies, J. Envall, L. L. Robison, and J. A. Ros. 2004. Genetic polymorphisms in the Hmong population: Implications for cancer etiology and survival. *Cancer* 100:411–414.

Krokan, H. E. 1997. DNA glycosylases in the base excision repair of DNA. *Biochem. J*. 325:1–16.

Lindahl, T. 1997. DNA excision repair pathways. *Curr. Opin. Genet. Dev.* 7:158–169.

Metsola, K., V. Kataja, P. Sillanpaal, P. Siivolal, L. Heikinheimo, M. Eskelinen, V.M. Kosma, M. Uusitupa, and A. Hirvonen. 2005. *XRCC1* and *XPD* genetic polymorphisms, smoking and breast cancer risk in a Finnish case-control study. *Breast Cancer Res.* 7:R987–R997.

Misra, R. R., D. Ratnasinghe, J. A. Tangrea, J. Virtamo, M. R. Andersen, M. Barrett, P. R. Taylor, and D. Albanes. 2003. Polymorphisms in the DNA repair genes *XPD, XRCC1, XRCC3*, and *APE/ref-1*, and the risk of lung cancer among male smokers in Finland. *Cancer Lett.* 19:171–178.

Nelson, H. H., K. T. Kelsey, L. A. Mott, and M. R. Karagas. 2002. The *XRCC1 Arg399Gln* polymorphism, sunburn, and non-melanoma skin cancer: evidence of gene-environment interaction. *Cancer Res.* 62:152–155.

Olshan, A. F., M. A. Watson, M. C. Weissler, and D. A. Bell. 2002. *XRCC1* polymorphisms and head and neck cancer. *Cancer Lett.* 178:181–186.

Park, J. Y., S. Y. Lee, H. S. Jeon, N. C. Bae, S. C. Chae, S. Joo, C. H. Kim, J. H. Park, S. Kam, I. N. Kim, and T. H. Jung. 2002. Polymorphism of the DNA repair gene *XRCC1* and risk of primary lung cancer. *CEBP Prev*. 11:23–27.

Patel, A. V., E. E. Calle, A. L. Pavluck, H. S. Feigelson, M. J. Thun, and C. Rodriguez. 2005. A prospective study of *XRCC1* (X-ray cross-complementing group 1) polymorphisms and breast cancer risk. *Breast Cancer Res*. 7:1168–1173.

Rossit, A. R., I. R. Cabral, C. Hackel, R. da Silva, N. D. Froes, and S. Z. Abdel-Rahman. 2002. Polymorphisms in the DNA repair gene *XRCC1* and susceptibility to alcoholic liver cirrhosis in older Southeastern Brazilians. *Cancer Lett.* 180:173–182.

Sanyal, S., F. Festa, S. Sakano, Z. Zhang, G. Steineck, U. Norming, H. Wijkström, P. Larsson, R. Kumar, and K. Hemminki. 2004. Polymorphisms in DNA repair and metabolic genes in bladder cancer. *Carcinogenesis* 25:729–734.

Shen, M., R. J. Hung, P. Brennan, C. Malaveille, F. Donato, D. Placidi, A. Carta, A. Hautefeuille, P. Boffetta, and S. Porru. 2003. Polymorphisms of the DNA repair genes *XRCC1, XRCC3, XPD*, interaction with environmental exposures, and bladder cancer risk in a case-control study in Northern Italy. *CEBP Prev.* 12:1234–1240.

Shirai, T., Y. Fradet, H. Huland, C. Bollack, M. Droller, R. Janknegt, P. Jones, and S. Kagawa. 1995. The etiology of bladder cancer-are there any new clues or predictors of behaviour? *Int. J. Urol*. 2:64–75.

Sturgis, E. M., E. J. Castillo, L. Li, R. Zheng, S. A. Eicher, G. L. Claynab, S. S. Strom, M. R. Spitz, and Q. Wei. 1999. Polymorphisms of DNA repair gene *XRCC1* in squamous cell carcinoma of the head and neck. *Carcinogenesis* 20:2125–2129.

Varzim, G., E. Monteiro, R. A. Silva, J. Fernandes, and C. Lopes. 2003. *CYP1A1* and *XRCC1* gene polymorphisms in SCC of the larynx. *Eur. J. Cancer Prev.* 12:495–499.

Wood, R. D., M. Mitchell, J. Sgouros, and T. Lindahl. 2001. Human DNA repair genes. *Science* 291:1284–1289.

Wu , M. T., C. L. Liu, C. K. Ho, and T. N. Wu. 2004. Genetic polymorphism of *p*53 and *XRCC1* in cervical intraepithelial neoplasm in Taiwanese women. *J. Formos Med. Assoc.* 103:337–343.

Yeh, C. C., F. C. Sung, R. Tang, C. R. Chang-Chieh, and L. L. Hsieh. 2005. Polymorphisms of the *XRCC1, XRCC3*, and *XPD* genes, and colorectal cancer risk: A case-control study in Taiwan. *BMC Cancer* 5:12–19.

Zhou, W., G. Liu, D. P. Miller, S. W. Thurston, L. L. Xu, J. C. Wain, T. J. Lynch, L. Su, and D. C. Christiana. 2003. Polymorphisms in the DNA repair genes *XRCC1* and *ERCC2*, smoking, and lung cancer risk. *CEBP Prev*. 12:359- 365.