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Genetic polymorphisms of *XRCC1* (at codons 194 and 399) in Shiraz population (Fars province, southern Iran)

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Abstract *Objective* Several potential functional polymorphisms in the DNA base excision repair gene X-ray repair cross-complementing group 1 (*XRCC1*) have been reported. There has been no information on interindividual variability of *Arg194Trp* and *Arg399Gln* polymorphisms of *XRCC1* in the Iranian population. Due to the association between the polymorphisms of *XRCC1* and the risk of some types of cancers, the present study was done. *Methods* The genetic polymorphisms of *XRCC1* were detected by PCR-based method in 707 healthy individuals from Shiraz population, (Fars province, southern Iran). *Results* Considering that there was no statistically significant difference between males and females, the sex groups were pooled. The frequencies of *194Trp* and *399Gln* alleles were 9.05% and 33.95%, respectively. When both polymorphisms were considered, the linkage-disequilibrium was observed ($D' = 0.8986$, $r^2 = 0.0413$, $P < 0.00001$). *Conclusion* The present results indicated that the allelic frequencies in Iranian populations showed intermediate frequencies in comparison with European and other Asian countries.

Keywords *XRCC1* · Polymorphism · Iran · Population genetics

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Introduction

The X-ray repair cross-complementing group 1 (XRCC1) protein is essential for mammalian viability. *XRCC1* deficiency in mice results in embryonic lethality, and it is required for the efficient repair of single-strand breaks and damaged bases in DNA. The XRCC1 has no known enzymatic activity and it is thought to act as a scaffold protein for both single-strand break repair and base excision repair activities. The XRCC1 protein complexes with three DNA repair enzymes: DNA ligase III, DNA polymerase β and poly(adenosine diphosphate (ADP)-ribose) polymerase (PARP) involved in excision and recombinational repair pathways [1].

The human *XRCC1* gene has been mapped to the long arm of chromosome 19 and consists of 17 exons and encodes a protein of 633 amino acids. More than 60 validated single nucleotide polymorphisms in *XRCC1* are listed in the Ensembl database, among which approximately 30 variants are located in exons or promoter regions. The most extensively studied single nucleotide polymorphisms are *Arg194Trp* on exon 6 (db SNP no. rs.1799782) and *Arg399Gln* on exon 10 (db SNP no. rs.25487) [2–4].

Because the *Arg399Gln* polymorphism is located in the region of the BRCT-I interaction domain of XRCC1 within a poly(ADP-ribose) polymerase binding region, this polymorphism has been extensively investigated both in its function and its association with cancer risk. The *XRCC1 Arg194Trp* variant occurs in the newly identified proliferating cell nuclear antigen binding region. The presences of the variant (*399Gln* and *194Trp*) alleles have been shown to be associated with measurable reduced DNA repair capacity and increased risk of several types of cancers [2–4].

The distribution of serum proteins, blood groups, and red cell enzymes in Iranian populations has been studied by different investigators [5, 6]. Very recently we had reported the frequencies of some genetic polymorphisms in Shiraz population using DNA analysis [7–13]. In order to get more insight into the genetic structure of Iranian populations the present study was done.

Materials and methods

Subjects

A total of 707 healthy blood donors (304 female, 403 males) were included in the study. The study was done in Shiraz, Fars province (southern Iran). Because *XRCC1* polymorphisms showed significant association with several multifactorial diseases [1–4, 14], we excluded the participants with history for diagnosed cancers, psychiatric disorders, and cardiovascular diseases. The mean age ±SD of participants was 29.1 ± 10.5 years, with age ranging between 15 and 70 years. The study group was unrelated Iranian Muslims. Informed consent was obtained from each subject before the study.

Extraction of DNA and genotyping analysis

Blood samples were obtained from the participants. Immediately after collection, whole blood was stored at –20°C until use. Genomic DNA for PCR was isolated from whole blood using the thawed blood samples. The *XRCC1* genotypes were examined using PCR-RFLP assays as described previously [15]. Genotypic analysis for the *XRCC1* gene was determined in DNA by PCR assay, using the PCR primers described by Lunn et al. for the codons 194 and 399 [16]. These primers generate 491 and 615 bp products containing the polymorphic sites at codons 194 and 399, respectively. The *XRCC1 Arg399Gln* polymorphism was genotyped by restriction enzyme digestion of the PCR product with the *Msp*I enzyme. The *Msp*I restricted products, *Arg/Arg*, *Arg/Gln* and *Gln/Gln* genotypes had band sizes of 374/221 bp, 615/374/221 bp, and 615 bp, respectively. The *XRCC1 Arg/Trp* polymorphism at codon 194 was genotyped by digestion of the PCR product with the *Pvu*II restriction enzyme. The *Pvu*II restricted products, *Arg/Arg*, *Arg/Trp*, and *Trp/Trp* genotypes had band sizes of 490, 490/294/196 and 294/196 bp [17].

To test for contamination, negative controls (tubes containing the PCR mixture, without the DNA template) were incubated in every run. Any sample with ambiguous result due to low yield was retested and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

Statistical analysis

A χ^2 test was performed for each polymorphism to determine if the control sample demonstrated Hardy-Weinberg equilibrium. The software SNPAlyze(TM) ver. 6 Standard (Dynacom Co, Ltd. Kanagawa, Japan) was used to evaluate the status of pair wise linkage disequilibrium for the studied polymorphisms. A probability of $P < 0.05$ was considered statistically significant. All *P*-values were two-tailed.

Results and discussion

Table 1 shows the *XRCC1* genotypes (at codons 194 and 399) of 707 subjects among two sex groups. There was no statistically significant differences between males and females for either polymorphism at codon 194 ($\chi^2 = 1.73$, df = 2, $P = 0.421$) or at codon 399 ($\chi^2 = 1.86$, df = 2, $P = 0.395$). Therefore, the sex groups were pooled.

The allelic frequency of *194Arg* and *194Trp* was 90.95% and 9.05%, respectively. The study population was at Hardy-Weinberg equilibrium ($\chi^2 = 0.018$, df = 1, $P = 0.893$). Also the frequency of *399Arg* and *399Gln* was 66.05% and 33.95%, respectively. It should be noted that there was no obvious deviation from the Hardy-Weinberg equilibrium ($\chi^2 = 1.003$, df = 1, $P = 0.316$).

Table 2 shows the combination genotypes of the two polymorphisms. The observed frequencies of the combined genotypes were remarkably differing from the expected ones. The close physical proximity of these polymorphisms should place them in linkage disequilibrium. Based on the complete dataset, *XRCC1 Arg194Trp* and *XRCC1 Arg399Gln* show a linkage disequilibrium with $D' = 0.8986$, $r^2 = 0.0413$ ($P < 0.00001$). The frequency for *194Arg-399Arg*, *194Arg-399Gln*, and *194Trp-399Gln*

Table 1 Frequencies (percentages) of *Arg399Gln* and *Arg194Trp XRCC1* in Shiraz population (south of Iran)

Genotypes	Females	Males	Total
Arg194Trp polymorphism			
Arg/Arg	84.21	81.39	82.60
Arg/Trp	15.46	17.62	16.69
Trp/Trp	0.33	0.99	0.71
Arg399Gln polymorphism			
Arg/Arg	44.41	41.44	42.71
Arg/Gln	46.71	46.65	46.68
Gln/Gln	8.88	11.91	10.61

Table 2 Combination genotypes of *Arg194Trp* and *Arg399Gln* of *XRCC1*

Codon 194	Codon 399	Observed frequency	Expected frequency
Arg/Arg	*Arg/Arg*	223	255.1
Arg/Arg	*Arg/Gln*	286	262.3
Arg/Arg	*Gln/Gln*	75	67.4
Arg/Trp	*Arg/Arg*	76	50.8
Arg/Trp	*Arg/Gln*	42	52.2
Arg/Trp	*Gln/Gln*	0	13.4
Trp/Trp	*Arg/Arg*	3	2.5
Trp/Trp	*Arg/Gln*	2	2.6
Trp/Trp	*Gln/Gln*	0	0.7

haplotypes was equal to 0.573, 0.339, and 0.088, respectively. The haplotype *194Trp-399Gln* was not observed.

Table 3 shows the allelic frequencies of *194Trp* and *399Gln* in several countries. The frequency of the polymorphic alleles varies among populations, suggesting an ethnic distribution. There were significant differences in terms of the *399Gln* allele frequency between the three major ethnicities. Overall, the frequency of *399Gln* was about 35% among European, 26% among Asian, and 13% among African populations [2]. The allelic frequency of *399Gln* in our samples seems to be more similar to the Caucasians than the Asian populations.

The *194Trp* allele showed high frequency in Asian populations and low frequency among Caucasians and Africans [2]. The *194Trp* allele showed frequency about 9% in our samples, which is very lower than that reported from Asian. The allelic frequency of *194Trp* in our samples is more similar to the Caucasians.

Table 3 Distribution of *Arg194Trp* and *Arg399Gln* polymorphisms of *XRCC1* in African, Asian, and European countries

Country/ethnic	*399Gln* (%)	*194Trp* (%)	Reference
Africa			
Egypt	13.5	5.2	[1]
African-American	13.5	7.5	[18]
Asia			
China	27.0	34.8	[19, 20]
India	21.5	12.6	[21]
Iran	33.9	9.1	Present study
Japan	24.3	–	[22]
Korea	31.5	34.1	[23]
Turkey	37.6	5.2	[24]
Europe and America			
Finland	32.1	2.7	[25]
France	35.9	6.9	[26]
Portugal	32.6	5.1	[27]
USA	36.2	6.9	[18]

Overall, it should be mentioned that for alleles of other genetic polymorphisms, such as *GSTM1*, *GSTT1*, *CC16*, mutations and VNTR-polymorphism of *PHA* [7–13], Iranian populations showed intermediate frequency in comparison with European and Asian countries.

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