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Short Communication

Polymorphisms of the DNA repair genes *XRCC1* and *XRCC3* in a Brazilian population

Márcia Cristina Duarte[1], Jucimara Colombo[1], Andréa Regina Baptista Rossit[2] and Ana Elizabete Silva[1]

[1]*Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas, Departamento de Biologia, São José do Rio Preto, SP, Brazil.*
[2]*Faculdade de Medicina de São José do Rio Preto, Centro de Investigação de Microorganismos, São José do Rio Preto, SP, Brazil.*

Abstract

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. The frequency of the polymorphic alleles varies among populations, suggesting an ethnic distribution of genotypes. We genotyped 300 healthy Southeastern Brazilian individuals (262 of European ancestry and 38 of African ancestry) for polymorphisms of codons 194 and 399 of the *XRCC1* base excision repair pathway gene and of codon 241 of the *XRCC3* homologous recombination repair pathway gene. The allele frequencies were 0.07 for the Arg194Trp and 0.33 for the Arg399Gln codons of the *XRCC1* gene and 0.35 for the Thr241Met codon of the *XRCC3* gene. The genotypic frequencies were within Hardy-Weinberg equilibrium. These frequencies showed ethnic variability when compared with those obtained for different populations from several countries.

Key words: DNA repair, *XRCC1*, *XRCC3*, polymorphism, ethnic variability.

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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 (Mohrenweiser and Jones, 1998; Hansen and Kelley, 2000).

Several polymorphisms in DNA repair genes (*XPD, XPF, ERCC1, XRCC1, XRCC3, XPA, XPB, XPC* and *hOGG1*) representing different repair pathways have been reported (Shen *et al.*, 1998; Ishida *et al.*, 1999; Butkiewicz *et al.*, 2000; Chavanne *et al.*, 2000).

The human genes *XRCC1* and *XRCC3* belong to the X-Ray Repair Cross Complementing family and have been identified by their ability to restore DNA repair activity in Chinese hamster ovary (CHO) mutant cell lines EM9 (Thompson *et al.,* 1990) and irs1SF (AA8) (Fuller and Painter, 1988), respectively. The XRCC1 protein plays a role in base excision repair (BER), interacts with DNA ligase III and complexes with DNA polymerase and PARP (poly ADP-ribose polymerase), facilitating the repair of DNA strand breaks and several types of DNA damage (Dianov *et al*., 2003). Shen *et al*. (1998) identified three polymorphisms in the *XRCC1* gene at conserved sequences, resulting in amino acid substitutions at codons 194 (Arg194Trp; reported allele frequency, RAF = 0.25), 280 (Arg280His; RAF = 0.08) and 399 (Arg399Gln; RAF = 0.25) in only twelve healthy individuals from an unspecified population.

Many authors have analyzed these polymorphisms in human populations and found a significant association between the Arg194Trp and Arg399Gln variants and increased risk of early-onset colorectal carcinoma (Abdel-Rahman *et al.*, 2000; Krupa and Blaviak, 2004) and gastric cardia cancer (Shen *et al*., 2000), besides head and neck cancer (Olshan *et al*., 2002) and skin cancer (Han *et al*., 2004) associated with the Arg194Trp variant and breast cancer (Duell *et al*., 2001), lung cancer (Zhou *et al*., 2003) and esophageal cancer (Yu *et al*., 2004), among others, associated with Arg399Gln polymorphism.

The product of the *XRCC3* gene functions in the homologous recombination repair (HRR) for double-strand breaks (DSBs) and cross-link repair in mammalian cells (Dianov *et al.*, 2003). During HRR the XRCC3 protein in-

Send correspondence to Ana Elizabete Silva. Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas, Departamento de Biologia, Rua Cristóvão Colombo 2265, Jardim Nazareth, 15054-000 São José do Rio Preto, SP, Brazil. E-mail: anabete@ibilce.unesp.br.

teracts with the Rad51C protein and possibly with the Rad51 protein itself, enabling Rad51 protein multimers to assemble at the site of damage (Brenneman *et al*., 2002). A common polymorphism in exon 7 of the *XRCC3* gene results in an amino acid substitution at codon 241 (Thr241Met) that may affect the enzyme's function. The *XRCC3* variant allele has been identified in healthy individuals at a frequency ranging from 0.23 to 0.38 (Shen *et al.*, 1998; David-Beabs *et al*., 2001), and has been associated with increased risk of melanoma (Winsey *et al.*, 2000), bladder cancer (Matullo *et al.*, 2001), breast cancer (Smith *et al.*, 2003) and lung cancer (Jacobsen *et al.*, 2004).

The frequencies of polymorphisms of both metabolic and repair enzymes are distinct in different ethnic groups (Kato *et al.*, 1992; Stephens *et al.*, 1994; Lunn *et al.*, 1999; Abdel-Rahman *et al.*, 2000), based an which we conducted a study to estimate the frequency of the variants Arg194Trp and Arg399Gln in the *XRCC1* BER gene and Thr241Met in the *XRCC3* HRR gene in healthy individuals from a southeastern Brazilian population. As far as we know, this is the first study carried out to evaluate *XRCC3* polymorphism in a Brazilian population.

Blood samples were collected from 300 healthy individuals (164 males and 136 females; mean age of 52 years, range 19 to 93 years) in the São José do Rio Preto region in the southeastern Brazilian state of São Paulo at a general hospital (Hospital de Base) and at the São José do Rio Preto campus of the Universidade Estadual Paulista - UNESP, São Paulo, Brazil. Based on their visual appearance and an interview the participants were ethnically classified as 262 individuals of European descent and 38 of African descent.

This study was approved by the National Research Ethics Committee, and written informed consent was obtained from all individuals.

The DNA from the blood samples was extracted as described by Abdel-Rahman *et al.* (1994). The *XRCC1* genotypes for codons 194 (492 bp) and 399 (615 bp) were detected using multiplex PCR-restriction fragment length polymorphisms (RFLP) (Abdel-Rahman *et al*. 2000), to amplify the fragments and codon 241 of the *XRCC3* gene was genotyped by the PCR-RFLP technique described by David-Beabs *et al.* (2001). The amplified fragments of codons 194 and 399 of the *XRCC1* gene were digested with the *Msp*I restriction enzyme and codon 241 of the *XRCC3* gene with the *Nla*III restriction enzyme and resolved on 2% 1000 agarose gel (Invitrogen, Brazil).

Statistical analyses were performed using the Statdisk v.9.5.5 computer software program and the chi-square test (χ^2) used to compare the genotype frequencies and ethnicity. χ^2 values with a probability (p) value greater than 0.05 being considered as coming from the same statistical population and hence not significant.

We found that the *XRCC1* gene allele frequencies were 0.07 for the 194Trp polymorphism and 0.33 for the 399Gln polymorphisms, whereas the *XRCC3* gene 241Met polymorphism allele frequency was 0.35. Genotypes distributions were within Hardy-Weinberg equilibrium ($\chi^2 = 0.24$ for 194Trp, $\chi^2 = 1.69$ for 399Gln and $\chi^2 = 0.72$ for 241Met; $P = 0.05$). Figure 1 shows the banding patterns of the *XRCC1* (A) and *XRCC3* (B) polymorphisms.

No statistically significant differences in ethnicity were observed with respect to the 194Trp (p = 0.7337), 399Gln (p = 0.4048) and 241Met (p = 0.5306) allele frequencies. According to the literature, it seems that the genotype distribution of the *XRCC1* and *XRCC3* genes does not vary between sexes, but differences between ethnic groups have been suggested (Lunn *et al.*, 1999; Abdel-Rahman *et al.*, 2000; Shen *et al.*, 2000). Table 1 shows the frequency distributions of the *XRCC1* and *XRCC3* genotypes in healthy individuals of our current study and compares these frequencies with those previously published for different ethnic groups.

In our study the allele frequencies of the 194Trp polymorphism in European descent (0.07) and African descent (0.09) were similar to those previously published by Rossit *et al*. (2002) for 96 healthy Brazilians from the same region.

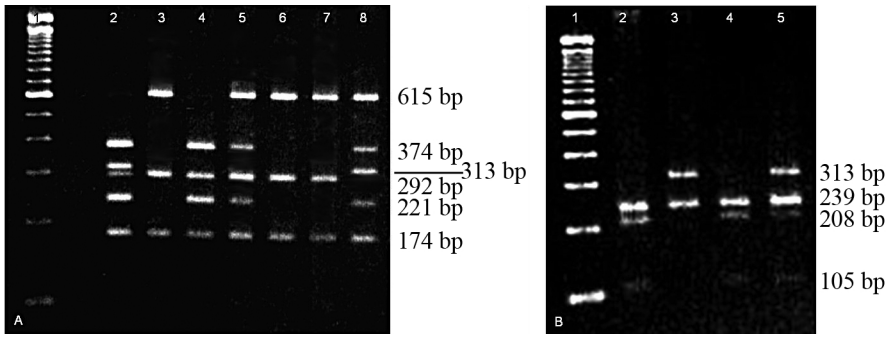

Figure 1 - Representative RFLP analysis of the *Msp*I and *Nla*III digest of the PCR products containing codons 194 and 399 of the *XRCC1* gene (A) and codon 241 of the *XRCC3* gene (B), respectively, separated on 2% 1000 agarose gel. **A** - Lane 1: molecular weight marker; Lane 2: heterozygous Arg/Trp 194 and wild-type homozygous Arg/Arg 399; Lanes 3, 6 and 7: homozygous Arg/Arg 194 and mutant homozygous Gln/Gln 399; Lane 4: wild-type homozygous Arg/Arg 194 and Arg/Arg 399; Lane 5: homozygous Arg/Arg 194 and heterozygous Arg/Gln 399; Lane 8: mutant homozygous Trp/Trp 194 and heterozygous Arg/Gln 399. **B** - Lane 1: molecular weight marker; Lanes 2 and 4: mutant homozygous Met/Met 241; Lane 3: wild-type homozygous Thr/Thr 241; Lane 5: heterozygous Thr/Met 241.

Table 1 - *XRCC1* and *XRCC3* genotypes and allele frequencies for healthy Brazilians of different ethnic ancestry as compared with other populations.

Ethnic groups	Population	Polymorphism											
		194 *XRCC1*				399 *XRCC1*				241 *XRCC3*			
		Arg/Arg	Arg/Trp	Trp/Trp	Allele frequency	Arg/Arg	Arg/Gln	Gln/Gln	Allele frequency	Thr/Thr	Thr/Met	Met/Met	Allele frequency
European-descent	Current study	226 (86%)	36 (14%)	0 (0%)	**0.07**	119 (45%)	107 (41%)	36 (14%)	**0.34**	110 (42%)	115 (44%)	37 (14%)	**0.36**
	Brazilian	50 (86%)	8 (14%)	0 (0%)	**0.07**[a]	25 (43%)	23 (40%)	10 (17%)	**0.37**[a]	ND	ND	ND	**ND**
	North American	150 (89%)	18 (11%)	1 (0.5%)	**0.06**[b]	65 (38%)	83 (49%)	21 (13%)	**0.37**[b]	ND	ND	ND	**ND**
		407 (88%)	54 (12%)	0 (0%)	**0.06**[c]	186 (40%)	217 (47%)	58 (13%)	**0.36**[c]	175 (39%)	210 (46%)	68 (15%)	**0.38**[d]
		264 (88%)	36 (12%)	1 (0.3%)	**0.08**[e]	119 (40%)	150 (50%)	31 (10%)	**0.37**[e]	112 (37%)	141 (47%)	49 (16%)	**0.43**[e]
	Italian	ND	ND	ND	**ND**	53 (43%)	58 (47%)	13 (10%)	**0.39**[f]	33 (26%)	64 (52%)	27 (22%)	**0.35**[f]
African-descent	Current study	32 (84%)	5 (13%)	1 (3%)	**0.09**	20 (53%)	16 (42%)	2 (5%)	**0.26**	18 (47%)	16 (42%)	4 (11%)	**0.31**
	Brazilian	9 (82%)	2 (18%)	0 (0%)	**0.09**[a]	7 (64%)	3 (27%)	1 (9%)	**0.22**[a]	ND	ND	ND	**ND**
	North American	89 (91%)	9 (9%)	0 (0%)	**0.05**[b]	67 (69%)	27 (28%)	3 (3%)	**0.17**[b]	ND	ND	ND	**ND**
		205 (84%)	36 (15%)	2 (1%)	**0.08**[c]	164 (67%)	70 (29%)	9 (4%)	**0.18**[c]	136 (58%)	88 (38%)	10 (4%)	**0.23**[d]
Egyptian	Egyptian	43 (90%)	5 (10%)	0 (0%)	**0.05**[g]	37 (77%)	9 (19%)	2 (4%)	**0.14**[g]	ND	ND	ND	**ND**
Asiatic	Taiwan-ese	67 (56%)	42 (35%)	11 (8%)	**0.27**[b]	63 (53%)	51 (43%)	6 (4%)	**0.26**[b]	ND	ND	ND	**ND**
		ND	ND	ND	**ND**	384 (53%)	291 (40%)	54 (7%)	**0.27**[h]	658 (90%)	74 (10%)	2 (0.3%)	**0.05**[h]
	Chinese	70 (42%)	77 (47%)	19 (11%)	**0.35**[i]	94 (57%)	59 (35%)	13 (8%)	**0.26**[i]	150 (90%)	16 (10%)	0 (0%)	**0.05**[j]
	Korean	ND	ND	ND	**ND**	81 (60%)	48 (36%)	6 (4%)	**0.22**[k]	ND	ND	ND	**ND**

ND = not determined.
[a]Rossit *et al*., 2002
[b]Lunn *et al*., 1999
[c]David-Beabes and London, 2001
[d]David-Beabs *et al*., 2001
[e]Smith *et al*., 2003
[f]Matullo *et al*., 2001
[g]Abdel-Rahman *et al*., 2000
[h]Yeh *et al*., 2005
[i]Shen *et al.*, 2000
[j]Shen *et al*., 2004
[k]Park *et al*., 2002

Our results also agree with those observed for American Caucasians (Lunn *et al.*, 1999; David-Beabs and London, 2001; Smith *et al.*, 2003), African Americans (Lunn *et al.*, 1999; David-Beabs and London, 2001) and Egyptians (Abdel-Rahman *et al.*, 2000) but not with the frequencies reported for Asians (Lunn *et al.*, 1999; Shen *et al*., 2000).

The frequency of 0.34 for the *XRCC1* 399Gln allele in our European descent group was comparable with that described by Rossit *et al.* (2002) in a group of Brazilian Caucasians and in North American (Lunn *et al.*, 1999; David-Beabs and London, 2001; Smith *et al*., 2003) and Italian Caucasians (Matullo *et al.*, 2001), although it was statistically higher (p) than that reported for Asiatic populations (Lunn *et al*., 1999; Shen *et al.,* 2000; Park *et al.*, 2002; Yeh *et al.*, 2005) and Egyptian (Abdel-Rahman *et al.*, 2000). Although the difference observed in our African descent group was not significant ($p > 0.05$), the *XRCC1* 399Gln allele frequency (0.26) was lower than that of the European descent group but higher than that previously reported for African Americans (Lunn *et al.*, 1999; David-Beabs and London, 2001).

We found that allele frequency of the *XRCC3* 241Met polymorphism was similar in Brazilians of European (0.36) and African (0.31) descent, these frequencies being comparable to those observed in North American (David-Beabs *et al.*, 2001; Smith *et al.*, 2003) and Italian (Matullo *et al.*, 2001) studies but statistically higher ($p < 0.05$) than those observed in African American (David-Beabs *et al.*, 2001) and Asiatic (Shen *et al.*, 2004; Yeh *et al*., 2005) populations.

The Brazilian population is very heterogeneous, as a result of sexual congress between different ethnic groups, including native Indians and immigrants from Europe, Africa and Asia. Our results are in accordance with this fact, since no significant difference was found between the two Brazilians ethnic groups studied, although there were differences in the allele frequencies when these two groups were compared to Asiatic and Egyptian populations. Our data suggests that admixture plays an important role in the distribution of the genotypes studied and, because Brazil is large country, there may be differences in genotype distribution in the different States because of the diverse origins of the immigrants which settled in each state. The results reinforce the importance of further studies on polymorphisms of DNA repair genes that may play an important role in cancer susceptibility in different populations.

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