



Research

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Increased Risk of Non-Melanoma Skin Cancer in DNA Repair Gene XRCC1 Polymorphism

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Abstract

Background: DNA repair genes are the genes that protect cellular genome from carcinogenic exposures such as uv radiation, ionizing radiation, nicotine and etc. Any dysfunction in this repair system does significantly increase the risk of cancer induction. XRCC1 (X-ray repair Cross Complementing 1) gene is one of the important DNA repair genes. In this study we aimed to investigate the polymorphism in XRCC1 gene and relationship between the polymorphic genotypes and development of non melanoma skin cancer.

Material and Methods: XRCC1 gene polymorphism was evaluated in a total of 138 patients. Seventyfive healthy individuals were used as the control group and 63 patients with a histopathologic diagnosis of skin cancer as the risk group. Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method was used to define Arg194Arg (Cytosine to Thymidine conversion on exon 6) and Arg399Gln (Guanine to Adenine conversion on exon 10) polymorphisms in XRCC1 gene.

Results: There was no significant difference between the groups with respect to the distribution of XRC194 polymorphism ($p=0.901$). However, there was a significant increase in the number of XRC399 polymorphism in the skin cancer group ($p=0.048$). The genotypes of this polymorphism were Arg/Arg, Gln/Gln and Arg/Gln. Among these Arg/Arg genotype (homozygous Arg) was associated with a higher incidence of skin cancer (2.32 x higher) when compared to heterozygotes (Arg/Gln) ($p=0.022$). There was also no significant relationship between the distribution of polymorphisms and clinical risk factors of age, sex, positive family history, age of tumor detection, tumor type, primary vs recurrent tumor and skin type.

Conclusion: In conclusion, the results of this study suggested that homozygous Arg XRC399 polymorphism was associated with an increased susceptibility to non melanoma skin cancers.

Introduction

Skin cancer is the most common type of cancer in humans. The etiopathogenesis of skin cancers is still unknown. However, a predisposing genetic background is always suspected. In general the polymorphisms in

protooncogenes, oncogenes, and tumor suppressors genes have been the prime suspects in the development of skin cancers as in others [1].

More recently polymorphisms in several DNA repair genes in the normal population have been reported [2, 3].

The variations created between individuals in DNA repair capacity by these mutations may be a risk factor for cancer development [4]. Several studies have documented that the gene involved in DNA repair and maintenance of genome integrity are critically involved in protecting against mutations that lead to cancer and/or inherited genetic disease. DNA repair maintains the integrity of cancer related genes, as well [5].

Recently, nine amino acid substitution variants in several DNA repair genes (e.g in XPD and XPF genes belonging to the NER pathway, and in XRCC1 and XRCC3 genes associated with double-strand /recombination repair) have been identified at the polymorphic frequency in the population studied [3].

XRCC1 gene was mapped to 19cen-19q 13.3. Lamerdin et al. characterized the genomic structure of XRCC1 in humans and mice. The human gene has 17 exons and spans approximately 31.9 kb. XRCC1 plays a role in the base excision repair (BER) pathway, interacts with DNA polymerase, Poly (ADP-ribose) polymerase (PARP) and DNA ligase III [6, 7] and a BRCA1 carboxyl-terminal (BRCT) domain, characteristic of proteins involved in cycle checkpoint function and responsive to DNA damage [8]. The XRCC1 protein interacts with DNA ligase III in rejoicing of DNA strand breaks [9] and DNA polymerase β in base excision repair [10]. XRCC1 is involved in the repair of single strand breaks following BER resulting from exposure to endogenously produced active oxygen, or ionizing radiation of alkylating agents [11]. XRCC1 mutants do display sensitivity to alkylating agents and ionizing radiation and exhibit elevated levels of sister chromatid exchange [12, 13, 14].

Table 1. XRCC1 194 Genotype Frequencies

	XRCC1 194		TOTAL
	Arg/Arg	Arg/Trp	
Control Group (%)	66	9	75
	88.0 %	12.0 %	100 %
Skin Tumor Group (%) within	55	8	63
	87.3 %	12.7 %	100 %
TOTAL (%)	121	17	138
	87.7 %	12.3 %	100 %

It is possible that these inherited polymorphisms of this pathway may effect risk of skin cancer. Therefore we focused on the two reported polymorphisms with greatest allele frequencies. In the first one reported by Broughton et al includes a C-T substitution at position 26304 of the XRCC1 Gene (codon 194, exon 6) and a G-A substitution at position 28152 of the XRCC1 gene (codon 399, exon 10). Shen et al. also found three polymorphisms of the XRCC1 gene, which resulted in amino acid changes at evolutionarily conserved regions of codon 194 (Arg-Trp), 280 (Arg-His) and 399 (Arg-Gln) [2].

In this study, we performed restriction fragment length polymorphism analysis of two polymorphic sites of the XRCC1 gene in a case control study of skin cancer to test the hypothesis that genetic polymorphisms of this gene contribute to susceptibility to skin cancer.

Materials and Methods

Subjects

A 138 patients were included in the study. Sixty-three of them had a documented non melanoma skin cancer and the remaining 75 were otherwise

Table 2. XRCC1 399 Genotype Frequencies

	XRCC1 399			TOTAL
	Gln/Gln	Arg/Gln	Arg/Arg	
Control Group (%)	5	42	28	75
	6.7 %	56.0 %	37.3 %	100 %
Skin Tumor Group (%) within	7	22	34	63
	11.1 %	34.9 %	54.0 %	100 %
TOTAL (%)	12	64	62	138
	8.7 %	46.4 %	44.9 %	100 %

Table 3. Odds Ratios and Results of Hypothesis Testing on XRCC1 399 Gene Polymorphism

Genotypes	Odds Ratio	95 % CI	P
Gln/Gln versus Arg/Arg (reference)	1.15	0.33-4.0	0.824
Arg/Arg versus Arg/Gln (reference)	2.32	1.13-4.76	0.022
Gln/Gln versus Arg/Gln (reference)	2.67	0.76-9.41	0.126

healthy individuals who were used as the control group. Subjects received a detailed description of the study protocol and signed an informed consent. The diagnosis of skin cancer was based on clinic and histopathologic examination. Control subjects were selected among healthy people with no history of cardiovascular disease, cancer, chronic degenerative neurologic disease, chronic obstructive pulmonary disease, hepatitis, allergies in general or alcohol abuse. Documentation of clinical findings included: (a) Sex (b) Age (c) Pathologic types of the skin cancer including SCC, BCC and premalignant lesions (d) Tumour location (e) Familial history of cancer.

Gene Analysis

Genomic DNA was isolated from peripheral lymphocytes using standard techniques. PCR-RFLP assays were used to determine XRCC1 gene genotypes. The primer pairs used were XRCC1 Arg 194 Trp, forward 5'-GCCCGTCCCAGGTA-3' and reverse, 5'-AGCCCCAAGACCCTTCACT-3', XRCC1 Arg 399 Gln, forward 5'TCTCCCTGGTCTCCA-ACCT-3' and reverse 5'-AGTAGTCTGCTGGCTCTGG-3'. PCR was performed in a 25 μ l volume with 20-100 ng DNA, 100 μ M dNTPs, 20 pmol of each primer, 1.5 mM MgCl₂, 1x PCR buffer with (NH4)SO₄ (MBI Fermentas, Vilnius, Lithuania,) and 1U Taq polymerase (MBI Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Genius, Cambridge, England). PCR conditions were 2 min for initial denaturation at 95°C; 35 cycles at 95°C for 30 s for denaturation, 30 s at 56°C for annealing and 1 min at 72°C for extension, followed by 7 min at 72°C for final extension.

PCR products were digested with specific restriction enzymes. Digestion of the PCR product was carried out using 10 U Pvu II (MBI Fermentas, Vilnius, Lithuania) and 10 U MspI (MBI Fermentas, Vilnius, Lithuania) and the 1x buffer (MBI Fermentas, Vilnius, Lithuania) supplied with each restriction enzyme at 37 °C overnight. The Pvu II restricted products of XRCC1 codon 194 Arg/Arg, Arg/Trp and Trp/Trp genotypes had band sizes of 490, 490/294/196 and 294/196 bp, respectively. The Msp I restricted products of XRCC1 codon

399 Arg\Arg, Arg\Gln and Gln\Gln genotypes had band sizes of 269\133, 402\269\133 and 402 bp, respectively 14. The digest products were resolved at 100 V for 20-30 min on a 2.5 % Agarose gel containing 0.5 μ g/ml ethidium bromide. A 100 bp marker (100 bp DNA Ladder, MBI Fermentas, Vilnius, Lithuania) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat, France).

Genotyping was based upon independent scoring of the results by two reviewers who were unaware of case/control status. Following genotype identification, relationships to cancer development and risk proportions were analysed. In addition any possible relationship between patient genotypes and familial cancer histories, gender, age, skin types, pathology types were searched. The patients were examined in three stratified age groups: before 30-year of age, between 30 and 50 years, after 50. Histopathologically the skin cancers were also divided into three groups as BCC, SCC and premalign types. All premalign lesions were actinic keratosis.

Statistical Analysis

The relationships between groups and gene polymorphisms were determined by using binary logistic regression analysis. Pearson Chi-Square test was used to determine the relationships between patient genotypes and their clinical characteristics (familial cancer stories, sex, age groups, skin types, pathology types).

Results

XRCC1 194 and 399 genotype frequencies were depicted in Tables 1 and 2, respectively. In statistical analysis, there was no significant relationship between XRCC1 194 genotype polymorphism and skin cancers ($p=0.901$), the distribution of XRCC1 194 genotypes in both patients and control subjects were similar.

However, there was a significant relationship between XRCC399 polymorphism and skin cancers for certain genotypes ($p=0.048$) (Table 2). Genotypes which carried that risk were shown in Table 3. Statistically, Arg/Arg genotypes had 2.32 times higher risk of skin cancer than Arg/Gln ($p<0.05$). In addition the risk analysis revealed that Gln/Gln genotypes (homozygote Gln) have 1.15 times higher risk in skin cancer than Arg/Arg genotypes however, that difference was not statistically sig-

nificant. In similar, Gln/Gln genotypes were calculated to have more risk than heterozygotes (Arg/Gln), but again this difference was not statistically.

When relationship between the clinical variables and genotypes was analysed, the following results were obtained: Gender had no significant relation with XRCC 194 and 399 genotypes ($p=0.610$, $p=0.282$ respectively). There was no significant relationship between the age groups and XRCC 194 and 399 genotypes ($p=0.413$, $p=0.410$ respectively). There was also no significant relationship between these two genotypes and familial history of cancer. ($p=0.555$, $p=0.673$ for XRCC 194 and 399, respectively). There was no significant relationship between genotypes and skin types ($p=0.306$, $p=0.152$ for XRCC 194 and 399, respectively).

The histopathologic type was had no significant relationship with genotypes, as ($p=0.258$, $p=0.422$ for XRCC 194 and 399, respectively).

Discussion

This study investigated the relationship between the two amino acid substitution variants in DNA repair genes and skin cancer. For this purpose we first examined the frequency of codon 194 (Arg-Trp) and codon 399 (Arg-Gln) polymorphism in XRCC1 in both healthy individuals and non melanoma skin cancer patients. Then we statistically analysed the relationship between these polymorphic genotypes and skin cancers. Our results showed that only homozygote Arg genotypes in codon 399 had more risk than heterozygotes. Neither other variants in that codon, nor polymorphism in codon 194 had an increased risk for skin cancer.

There are several other studies that investigated polymorphic patterns in XRCC1 gene. *Shen et al.* estimated a variant allele frequency of 0.25(194 Trp), 0.08 (280 His) and 0.25 (399 Gln) by sequencing the XRCC1 gene from 12 unidentified individuals [3]. These frequencies are most similar to that observed in a study made by *Lunn et al.* in the Taiwanese population, which revealed that 194 Trp and 280 His alleles were found at a low frequency in blacks [0.05 (194 Trp) and 0.02 (280 His)] and whites [0.06(194 Trp) and

0.03 (280 His)] but were significantly more prevalent in Taiwanese people [0.27(194Trp) and 0.11 (280 His) $P<0.001$] *Matullo et al* [15] found that XRCC1 frequency (0.34) is similar to that reported by *Lunn et al* [15] for the Caucasoid population (0.37), but different from the one estimated by *Shen et al.* in 12 individuals (0.25) [3]. *Sturgis et al.* reported an allele frequency of 72 % for XRCC1 26304 T (codon 194) and of 34.1 % for XRCC1 28152 C in 380 Caucasians [16, 17].

The genetic variant of XRCC1 Arg 399 Gln has been evaluated in cancer of the head and neck, lung and breast [15] was shown that the XRCC1 399 Gln allele was associated with higher levels of DNA adducts and sister chromatid exchange [15]. The differences in allele frequency are consistent with the hypothesis that polymorphisms in DNA repair genes exhibit ethnic variations similar to polymorphisms of carcinogen-metabolizing enzymes [16]. *Dybdahl et al.* reported that in XPD polymorphism, homozygote AA alleles had 4 times higher BCC risk than homozygote CC alleles. They concluded that allel A increased the risk for development of BCC in while allel C had a protective role in cancer genesis. In addition they mentioned that with allel AA cancer develops an earlier age than with other alleles [18].

In our study on XRCC399, individuals who carried Arg/Arg genotype had a 2.32 times higher risk of developing non melanoma skin cancer and this was statistically significant. This result also indicates that homozygote Arg genotypes had a greater risk than heterozygotes. The other genotypical comparisons revealed no significant differences. According to the present study in skin cancer patients, there was no significant relation between XRCC1 194 and 399 polymorphic genotypes to and familial cancer history, sex, age, skin type, or histopathologic tumor type. This result might be contradicting with that of others pointing out to a phenotypic sensitivity risk in patients with a positive familial cancer history and in patients who had cancer at earlier age [17, 19, 20, 21].

In conclusion, the results of this study suggested that homozygous Arg XRC399 polymorphism was associated with an increased susceptibility to non melanoma skin cancers.

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