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ORIGINAL ARTICLE

Polymorphic Variation in Double Strand Break Repair Gene in Indian Population: A Comparative Approach with Worldwide Ethnic Group Variations

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Abstract DNA repair capacity is essential in maintaining cellular functions and homeostasis. Identification of genetic polymorphisms responsible for reduced DNA repair capacity may allow better cancer prevention. Double strand break repair pathway plays critical roles in maintaining genome stability. Present study was conducted to determine distribution of *XRCC3* Exon 7 (C18067T, rs861539) and *XRCC7* Intron 8 (G6721T, rs7003908) gene polymorphisms in North Indian population and compare with different populations globally. The genotype assays were performed in 224 normal healthy individuals of similar ethnicity using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Allelic frequencies of wild type were 79% (C) in *XRCC3* Exon 7 C > T and 57% (G) in *XRCC7* Intron 8 (G > T) 57% (G) observed. On the other hand, the variant allele frequency were 21% (T) in *XRCC3* Exon 7 C > T and 43% (T) in *XRCC7* Intron 8 G > T respectively. Major differences from other ethnic populations were observed. Our results suggest that frequency in these DNA repair genes exhibit distinctive pattern in India that could be attributed to ethnicity variation. This could assist in high-risk screening of humans exposed to environmental carcinogens and cancer predisposition in different ethnic groups.

Keywords DNA repair genes · Polymorphism · Ethnic group · North India

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Introduction

Inherited genetic variation plays a critical but largely uncharacterized role in human differentiation. Variations in the DNA sequences of humans can affect diseases and respond to pathogens, chemicals, drugs, vaccines, and other agents. Genetic variation in human genome is an emerging resource for studying cancer. Molecular epidemiological studies have provided the evidence that an individual's susceptibility to precancerous lesions and cancer is modulated by both genetic and environmental factors. Although many people are exposed to these risk factors, only a fraction of exposed individuals develop cancer, suggesting an individual susceptibility to exposure-related carcinogenesis.

Human genome are continually exposed to a wide range of genotoxic agents from both endogenous and exogenous sources, results in damage to DNA, potentially loss of integrity of the genetic information and elevated cancer risk. To prevent consequences of such detrimental mutations, cells have evolved mechanisms to preserve genomic integrity. The normal function of DNA repair enzymes plays a critical role in maintaining the genome integrity and stability through the reversal of DNA damage. Recently, there has been considerable interest in understanding genetic variability in DNA repair genes and their influence on modifying an individual's susceptibility to cancer.

Several studies while assessing the association of polymorphisms in DNA-repair genes with cancer risk have documented that individuals with "adverse" genotypes resulted reduced DNA repair capacity (DRC) are at a higher risk of developing cancer than the general population [1, 2], highlighting the importance of host genetic factors as determinants of the interindividual and interpopulation differences in susceptibility to cancer disease.

Growing evidence suggest that human cancer can be induced by DNA double strand breaks (DSBs) [3]. This kind of DNA damage is caused by UV light, ionizing radiation and chemical agents. DSBs are repaired by the homologous recombination repair (HRR) and non-homologous end joining (NHEJ) pathways. *XRCC3* participates in HRR and is a member of the Rad-51-related protein family. *XRCC3* deficient cells exhibit defects in RAD51 focus formation after radiation damage and demonstrate genetic instability and increased sensitivity to DNA damaging agents [4]. *XRCC7* also known as PRKDC (protein kinase, DNA-activated, catalytic polypeptide), encodes the catalytic subunit of a nuclear DNA-dependent serine/threonine protein kinase (DNA-PK). The protein mainly participates in the recognition and repair of DSB via the NHEJ mechanism. Genetic variants of the *XRCC3* and *XRCC7*, which is located in Exon 7 and intron 8 lead to amino acid substitution can affect protein function, messenger RNA stability, and splice variants, hence; may influence an individual's capacity to repair DNA damage and thus might contribute to increase cancer risk (Fig. 1).

Therefore, present study is an attempt to investigate frequency distribution of *XRCC3* Exon 7 (C18067T, rs861539) and *XRCC7* Intron 8 (G6721T, rs7003908) genes polymorphism (Fig. 1) by using a PCR-based restriction analysis in unrelated normal healthy individuals from North India and identified a sufficient number of epidemiologic studies to conduct a comparative analysis for genetic polymorphisms in DSB repair pathway genes, focusing on *XRCC3* and *XRCC7*.

Materials and Methods

Subjects

Healthy and genetically unrelated individuals either visiting the hospital for a routine checkup or health awareness camps or healthy hospital employees were recruited as the controls (n = 224). All the controls were similar ethnicity and had no evidence of malignancy or chronic disease. The mean age of the healthy controls were 59.1 ± 10.4 years, respectively. Prior to experiments, written and informed consent for participation in this study was obtained from each subjects enrolled. This study was approved by the hospital ethics committee for Scientific Research.

DNA Extraction

Standard venipuncture was used to collect 3 mL of peripheral blood samples in tubes containing ethylenediaminetetraacetic acid as an anticoagulant from all study participants. The genomic DNA was extracted from the stored peripheral blood samples using salting out method [5]. Obtained DNA was further quantified and analyzed with regard to protein content and purity using Nano drop instrument. Samples were stored at −80 °C for further processing.

Genotyping

All study samples were genotyped for *XRCC3* (Exon 7 C18067T, rs861539) and *XRCC7* (Intron 8 G6721T, rs7003908) genes by polymerase chain reaction (PCR) combined with restriction fragment-length polymorphism using primers implying conditions, as previously described [6, 7]. PCR cocktail comprised of 10 pmol of each primer; 100 ng genomic DNA; 0.25 mM each of deoxyribonucleotide triphosphate; and 1 X PCR buffer containing 10 mM Tris–HCl, pH 8.6, 50 mM KCl, 1.5 mM MgCl2, and 0.5 units of Taq polymerase (Bangalore Genei). Amplified products were digested using restriction enzyme NcoI for *XRCC3* Exon 7 (C > T) and PvuII (MBI Fermentas Inc.) for *XRCC7* Intron 8 (G > T), respectively. Subsequently, fragments of *XRCC3* and *XRCC7* were

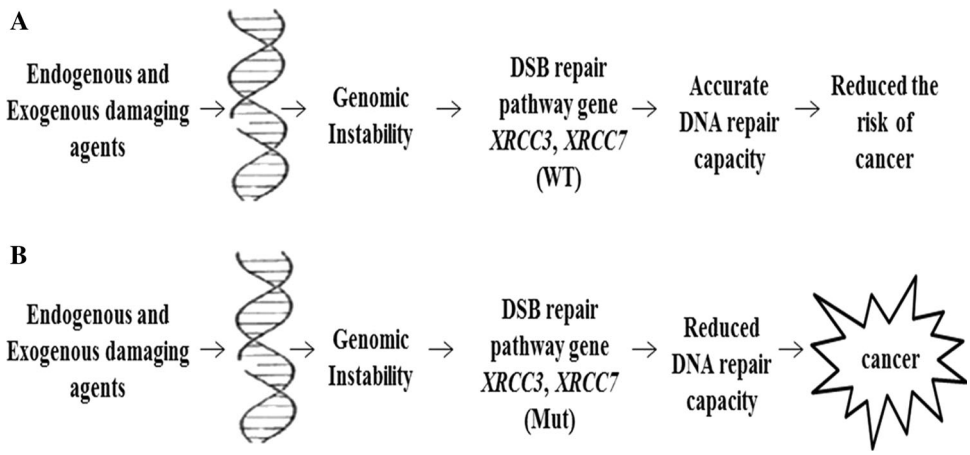

Fig. 1 Hypothesized model of relationship between DNA damage and Double strand DNA repair pathway gene polymorphism. **a** After damaging the DNA Wild type (WT) *XRCC3* and *XRCC7* gene done optimum DNA repair and inhibit cancer risk. **b** Mutant (Mut) *XRCC3* and *XRCC7* would results in reduced DNA repair capacity and increased cancer risk

resolved on 10% PAGE and 2% agarose gels, to identify wild type and polymorphic variant.

Quality Control Procedures

Precise quality control procedures were applied during the genotyping process. As a negative control, a PCR mix without a DNA sample was used to ensure the accuracy of the method used. Samples that failed to genotype were scored as missing and subjected to repetition. Five percent of samples were randomly selected and run in duplicates to evaluate the quality of genotyping, which showed 100% concordance. The results were reproducible with no discrepancy in genotyping.

Prevalence of Gene Variants

We conducted a PUBMED, MEDLINE search using "*XRCC3*", "*XRCC7*", "polymorphism" and cancer. The search was limited to human subjects, without language restriction. For case-control studies, only genotype frequencies for the control population were considered. Studies that reported only allele frequencies and no genotype frequencies were not included. Studies based on fewer than 90 persons were excluded. The most recent publication was included in the study when more than one article was identified for the same study population. We identified 12 publications reported on the prevalence of *XRCC3* Exon 7 polymorphism and 4 publications on *XRCC7* Intron 8 with different ethnicity and subsequently used for comparison with our North Indian study.

Statistical Analysis

Pearson's χ^2 test was done to compare the genotype and allelic frequencies of different populations using the software SPSS (version 16). Court-Lab (web based software) was used to examine Hardy–Weinberg equilibrium (www.tufts.edu). p value 0.05 was considered to be statistically significant.

Results

The genotype distributions of *XRCC3* Exon 7 and *XRCC7* Intron 8 in healthy individuals were in agreement with Hardy–Weinberg equilibrium at all the selected polymorphic sites in two genes (Table 1). The frequency distribution of different genotypes and alleles of these two genes polymorphism with different populations with reference to ours was compared using χ^2 test (Tables 2, 3). The minor allele frequency in our population was 21% and 43% for *XRCC3* Exon 7 and *XRCC7* Intron 8 respectively. In case of *XRCC3* Exon 7 (C > T) significant frequency distribution was observed in United Kingdom, Thailand, Spain, Canada, Japan, Finland, Germany, Italy, Poland, China and USA as compared to our population. Study of *XRCC7* Intron 8 (G > T) polymorphism is not common worldwide, only few studies have been reported. Based on reports, significantly different pattern of genotype and allele frequencies was reported only in China and Japan.

Discussion

Genetic susceptibility is believed to play an important role in determining individual differences in the development of cancer. Currently, major research activities have focused on polymorphisms in DNA repair genes as an important component of susceptibility because DNA-repair activities are critical for the protection of the genome and the prevention of cancer. This information is considered to be extremely useful to design personal-oriented chemotherapy or detect cancer high-risk populations. SNPs were found in nearly all human DNA repair genes that have been investigated so far, and were shown to modulate levels of DNA damage, individual DNA repair capacity and cancer risk [8]. Due to marked differences in the distribution of DNA repair gene polymorphisms between various ethnicities, the data from 'normal healthy' populations are of special interest for the adequate evaluation of the relevance of the investigated genetic markers in susceptibility, manifestation, prognosis or treatment of deadly diseases. However, it is noteworthy to conduct extensive investigations about the distribution of these genes in different ethnic groups. Double-strand breaks in DNA are the most dangerous class of DNA damage because they may lead to either cell death or loss of genetic material and unrepaired can result in genetic instability and ultimately may enhance the rate of cancer development. Hence, targeting DSBr gene in cancer cells could be a tool to tackle these emerging problems and is an attractive target for individual anticancer treatment.

XRCC3 and *XRCC7* play an important role in DSB repair pathway and variants of *XRCC3* Exon 7 and *XRCC7* intron 8 has been reported to be associated with an increased risk of many cancers [9, 10]. In our investigation interesting finding is that, Mutant allele (T) frequency of *XRCC3* in Indian population was 24%, which was significantly higher in United Kingdom, Thailand, Spain, Canada, Japan, Finland, Germany, Italy, Poland and USA and no significant difference was observed from Australia. The (T) allele frequency in *XRCC7* Intron 8 polymorphism was 43% in our population. This was significantly differing in China and Japan as compared to observed in our population. Included study from USA and Iran by Wang et al. [7] and Nasiri et al. [11] the minor variant allele frequencies

Table 1 Genotypes and allele frequency distribution of *XRCC3* Exon 7 and *XRCC7* Intron 8 gene polymorphism in North India

Gene	Genotype	Observed (n) %	Expected (n) %	Minor allele frequency	*p* value (HWE)
XRCC3 Exon 7	CC	137 (61.2)	138 (61.8)	21	0.730
(C18067T)	CT	78 (34.8)	76 (33.6)		
(rs861539)	TT	9 (4)	10 (4.6)		
XRCC7 Intron 8	TT	75 (33.5)	73 (32.4)	43	0.663
(G6721T)	TG	105 (46.9)	109 (49)		
(rs7003908)	GG	44 (19.6)	42 (18.6)		

Table 2 Genotypes and allele frequency distribution of *XRCC3* Exon 7 gene polymorphism in various populations and *p* values in comparison to North Indian population

Gene	Country/ ethnicity	n	Age (years), mean age ± SD	Genotype			*p* value	T€	References
				CC	CT	TT			
XRCC3 Exon 7	North India	224	59.1 ± 10.4	137 (61.2)	78 (34.8)	9 (4)	Ref.	21	Present study
	United Kingdom	317	57.1 ± 9.7	117 (36.9)	148 (46.7)	52 (16.4)	<0.001	63.1	[14]
	Thailand	164	35–88	140 (85.4)	23 (14.0)	1 (0.6)	0.036	7.6	[15]
	Australia	132	69.07 ± 7.99	54 (40.9)	72 (54.5)	6 (4.5)	0.340	31.8	[16]
	Spain	434	63.54 ± 11.33	178 (41.0)	196 (45.2)	60 (13.8)	<0.001	36.4	[6]
	Canada	95	36–59	38 (40)	43 (45)	14 (15)	<0.001	37.4	[17]
	Japan	379	54.6 ± 57.2	295 (77.8)	77 (20.3)	7 (1.85)	0.048	12	[18]
	Finland	306	55–63	149 (49)	134 (44)	23 (7)	0.037	29.4	[19]
	Germany	459	50–71	168 (37)	222 (48)	69 (15)	<0.001	39.2	[20]
	Italy	159		48 (30.2)	92 (57.9)	19 (11.9)	<0.001	40.9	[21]
	Poland	353	–	131 (37.1)	169 (47.9)	53 (15)	<0.001	39	[22]
	China	603	51–67	533 (88.4)	67 (11.1)	3 (0.5)	<0.001	6.1	[23]
	USA	216	49.3 ± 15.2	104 (48)	90 (42)	22 (10)	<0.001	31	[23]

€ Variant allele frequency

Table 3 Genotypes and allele frequency distribution of *XRCC 7* Intron 8 gene polymorphism in various populations and *p* values in comparison to North Indian population

Gene	Country/ ethnicity	n	Age (years), mean age ± SD	Genotype			*p* value	G€	References
				TT	TG	GG			
XRCC7 Intron 8	North India	224	59.1 ± 10.4	75 (33.5)	105 (46.9)	44 (19.6)	Ref.	43	Present study
	China	235	57.1 ± 9.7	118 (50.2)	103 (43.8)	14 (6)	<0.001	27.9	[7]
	Japan	180	58 ± 19	90 (50)	76 (42.2)	14 (7.8)	<0.001	28.9	[24]
	USA	342	43.8 ± 10.6	133 (38.9)	153 (44.7)	56 (16.4)	0.181	38.7	[25]
	Iran	362	44.2 ± 8.6	107 (29.6)	167 (46.1)	88 (24.3)	0.156	47.4	[11]

€ Variant allele frequency

were found to be almost similar with our population (43 vs. 38.7% and 47.4) respectively.

It is well recognized that ethnic background may influence the susceptibility to certain diseases [12]. Therefore, variation in our Indian population in contrast to other populations worldwide signifies the impact of ethnicity. Indian population is believed to be most diverse because of different sociocultural traditions. The study of genetic variation can elucidate critical determinants in environmental exposure and cancer, which could have future

implications for preventive and early intervention strategies. The differences in the allelic frequencies detected among these studies might be due to several reasons such as ethnic variation, heterogeneity of study populations and different sample sizes.

Although the increased/decreased risk associated with individual DNA repair SNPs may be small compared to that conferred by high-penetrance cancer genes, their public health implication may be large because of their high frequency in the general population. Epidemiological investigations of DNA repair polymorphisms are therefore important [13]. Large and combined analyses may be preferred to minimize the likelihood of both false-positive and false-negative results. As there are differences in the prevalence of DNA repair polymorphisms across different populations, hence, it is important to keep in mind that a susceptibility factor in one population may not hold true for another. Such kind of study may form the basis for future establishment of epidemiological and clinical databases.

We concluded that *XRCC3* and *XRCC7* gene polymorphisms may be biomarkers of disease susceptibility and may be contributing factors in the risk of cancer development. Additional single well designed larger study and tissue-specific biochemical and biological characterization is warranted to further evaluate potential gene-to-gene and gene-to-environment interactions on these polymorphisms and cancer risk. It would be an important goal of biological and clinical researches to detect genetic components like *XRCC3* and *XRCC7* DNA repair gene polymorphism as possible indicators of different type of disease including carcinogenesis. In addition, differences in allele distribution of these genes between North Indian healthy population and other ethnic groups may help in building a profile that would contribute towards the study of levels of defective DNA repair pathway and the ability of cancer drug to inhibit or activate DNA repair can dictate an individual treatment strategies.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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