RESEARCH ARTICLE

Genetic Variation in a DNA Double Strand Break Repair Gene in Saudi Population: A Comparative Study with Worldwide Ethnic Groups

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Abstract

DNA repair capacity is crucial in maintaining cellular functions and homeostasis. However, it can be altered based on DNA sequence variations in DNA repair genes and this may lead to the development of many diseases including malignancies. Identification of genetic polymorphisms responsible for reduced DNA repair capacity is necessary for better prevention. Homologous recombination (HR), a major double strand break repair pathway, plays a critical role in maintaining the genome stability. The present study was performed to determine the frequency of the HR gene *XRCC3* Exon 7 (C18067T, rs861539) polymorphisms in Saudi Arabian population in comparison with epidemiological studies by "MEDLINE" search to equate with global populations. The variant allelic (T) frequency of *XRCC3* (C>T) was found to be 39%. Our results suggest that frequency of *XRCC3* (C>T) DNA repair gene exhibits distinctive patterns compared with the Saudi Arabian population and this might be attributed to ethnic variation. The present findings may help in high-risk screening of humans exposed to environmental carcinogens and cancer predisposition in different ethnic groups.

Keywords: DNA repair - homologous recombination - XRCC3- polymorphisms - Saudi Arabia - ethnic variation

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Introduction

Inherited genetic variations play a critical but largely uncharacterized roles in human differentiation. Single-nucleotide polymorphisms (SNPs) are the most common form of genetic variations found in the human population. Nearly 12 million unique reference SNPs are known today and this number is still increasing day by day. Many human diseases including cancer are believed to be induced by both environmental and host factors, in addition with individual genetic susceptibility. Genetic susceptibility is related to chromosomal aberrations or genetic polymorphisms of various genes, including those involved in DNA repair (Schantz et al., 1989; Wang et al., 1998). Maintenance of genome integrity is crucial to preclude development of diseases associated with genomic instabilities such as cancer and other genetic disorders (Hu et al., 2002).

Human genome is continually exposed to a wide range of genotoxic agents from both endogenous and exogenous sources. Normal cellular metabolism can give rise to DNA damage through free radicals production and replication errors, whereas environmental agents, such as ultraviolet (UV) and ionizing radiation (IR), may induce specific types of lesions, which result in potential loss of integrity of the genetic information and possibly elevate many types of malignancies and associated disease risks (Cooke et al., 2003). In order to counteract such type of threat, human cells have developed multiple DNA repair pathways to protect themselves from different types of DNA damage, and it is obvious that defects in DNA repair pathways are mainly involved in various types of human diseases including cancer (Jackson and Bartek, 2009), which highlight the critical importance of systematic and efficient DNA repair for cell and human viability. Earlier studies demonstrated that many healthy individuals (10-15%) exhibit reduced (65-80% of normal) DNA repair capacity phenotypes (Joksić et al., 2009). These damage-specific phenotypes are heritable traits and they are mostly associated with increased disease/ cancer risks.

DNA double-strand breaks (DSBs) are among the most severe and lethal type of DNA damage, even a single DSB is sufficient to kill a cell or disturb its genomic integrity (Jackson and Bartek, 2009). DSBs are predominantly repaired by either homologous recombination (HR) repair or by non-homologous end joining (NHEJ) pathway in mammalian cells (Jackson, 2002). HR is the main DSB repair pathway used during the S and G2 phase when sister chromatids are intact and readily available (Takata et al., 1998). Genetic variations in HR DNA repair genes have been reported earlier and it was found that it has been associated with high incidence of

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chromosomal aberrations, elevated sensitivity to radiation and carcinogenesis, eventually lead to genetic instability (Tebbs et al., 1995; Thompson and Schild, 2001).

XRCC3 (X-ray repair cross-complementing group 3, located in the 14q32.3) is the member of the RAD51 protein family and plays a key role in HR repair pathway to maintain chromosome stability and repair DNA damage (Karagiannis et al., 2004). *XRCC3*-deficient cells exhibit defects in RAD51 focus formation after radiation damage and demonstrate genetic instability and increased sensitivity towards DNA damaging agents (Griffin, 2002). The major polymorphism in *XRCC3* gene involves the change of Threonine (Thr) to Methionine (Met) at codon 241 in exon 7 (Mittal et al., 2012). Recent studies demonstrate that SNPs in DNA repair genes may modulate the DNA repair phenotype, particularly when these SNPs are located within coding or regulating regions. Carriers of *XRCC3* variant allele T (exon 7, C18067T) display relatively high DNA adduct (covalent adducts between cancer causing chemicals and DNA) levels in lymphocyte DNA compared with homozygous C allele carriers, signifying that this polymorphism may be associated with a relatively low DNA repair capacity (DRC) (Synowiec et al., 2008).

Genetic variations of *XRCC3* may contribute to differences of repair capacity in the general population and healthy individuals. Typically, they differ in their intrinsic capacity in repairing DNA damage and this variation could be an effect of variants in DNA repair genes that consequently can modify the individual susceptibility to different kinds of diseases including cancer. The present study is a prospective attempt to investigate the frequency distribution of *XRCC3* exon 7 C18067T polymorphisms in normal healthy individuals from Saudi Arabia and identify a sufficient number of epidemiological studies from global population to perform a comparative analysis for the said polymorphism. To the best of our knowledge this is the first study dealing with evaluation of mutant allele frequency of the *XRCC3* gene in the Saudi population and its comparison with global population to predict the genetically determined DNA repair capacity for increased disease risks.

Materials and Methods

Prevalence of gene variants

We performed a MEDLINE PUBMED search using ''*XRCC3*'' and ''polymorphism'' for research articles published before December 2012. The web search was limited to human subjects, without any language restriction. For case-control research studies, only genotype frequencies for the control population were considered in this study. Reports that stated only allele frequencies and no genotype frequencies were not included. Reports based on less than 95 subjects were excluded. When more than one articles were appeared for the same study population, we included the most recent publication. We identified 14 research articles reporting the prevalence of *XRCC3* Exon 7 (C>T) polymorphism as it is the most extensively studied genetic polymorphism, and subsequently we also used the same for comparison with Saudi Arabian population.

Statistical analysis

Pearson's χ^2 test was performed to compare the genotypic and allelic frequencies of different populations using the software SPSS (version 16) (SPSS, Chicago, IL). Court-Lab (web based software) was used to determine the Hardy-Weinberg equilibrium (HWE). The statistical significance level was considered as 0.05 (p-value < 0.05).

Results

The frequency of *XRCC3* (Exon 7, C>T) genotypes in Saudi Arabian population have been shown in Table 1. Genotypic distributions were in concurrence with HWE for SNPs selected (Table 1). The worldwide frequency distribution of different genotypes and alleles of these SNPs

Table 1. Genotypes and Allele Frequency Distribution of XRCC3 Exon 7 Gene Polymorphism in Saudi Arabia

Gene	Genotype	Observed (n) %	Expected (n) %	Minor allele frequency	p-value (HWE)
XRCC3 Exon 7	CC	101 (40)	95 (37.2)	39	0.240
(C18067T)	CT	106 (42)	119 (47.6)		
(rs861539)	TT	44 (18)	37 (15.2)		

Table 2. Genotypes and Allele Frequency Distribution of XRCC3 Exon 7 Gene Polymorphism in Various Populations and p-Values in Comparison to Saudi Arabian Population

Gene	Country/ ethnicity	(n)	Age (years), Meanage±SD	Genotype CC	CT	TT	P	T€	References
XRCC3	SaudiArabia	251	-	101 (40)	106 (42)	44 (18)	Ref	39.0	Al-Hadyan et al., 2012
Exon7	UnitedKingdom	317	57.1±9.7	117 (36.9)	148 (46.7)	52 (16.4)	0.935	63.1	Matullo et al., 2005
	Thailand	164	35-88	140 (85.4)	23 (14.0)	1 (0.6)	<0.001	7.6	Kietthubthew et al., 2006
	Australia	132	69.07±7.99	54 (40.9)	72 (54.5)	6 (4.5)	0.003	31.8	Dhillon et al., 2011
	Spain	434	63.54±11.33	178 (41)	196 (45.2)	60 (13.8)	0.274	36.4	López et al., 2007
	Canada	95	36-59	38 (40)	43 (45)	14 (15)	0.643	37.4	Casson et al., 2005
	Japan	379	54.6±57.2	295 (77.8)	77 (20.3)	7 (1.8)	<0.001	12.0	Kiyohara et al., 2012
	Finland	306	55-63	149 (49)	134 (44)	23 (7)	<0.001	29.4	Misra et al., 2003
	Germany	459	50-71	168 (37)	222 (48)	69 (15)	0.798	39.2	Popanda et al., 2004
	Italy	159	-	48 (30.2)	92 (57.9)	19 (11.9)	0.769	40.9	Fabiani et al., 2009
	Poland	353	-	131 (37.1)	169 (47.9)	53 (15)	0.761	39.0	Sliwinski et al., 2010
	China	603	51-67	533 (88.4)	67 (11.1)	3 (0.5)	<0.001	6.1	Qian et al., 2011
	USA	216	49.3±15.2	104 (48)	90 (42)	22 (10)	0.015	31.0	Qian et al., 2011
	NorthIndia	224	59.1±10.4	137 (61.2)	78 (34.8)	9 (4)	<0.001	21.0	Mandal et al. 2011

Note: € Variant allele frequency

with reference to the selected Saudi Arabian population were compared using χ^2 test (Tables 2). Minor variant allele frequency (39%) was found in studied population. Significant frequency distributions were observed in case of ethnic populations of Thailand (p=<0.001), Australia (p=0.003), Japan (p=<0.001), Finland (p=<0.001), China (p=<0.001), USA (p=<0.015) and North India (p=<0.001) as compared to our selected Saudi population. However, we failed to observe significant differences between ethnic populations of United Kingdom (p=<0.0935), Spain (p=<0.274), Canada (p=<0.643), Germany (p=<0.798), Italy (p=<0.769) and Poland (p=0.761) in comparison with Saudi Arabian population.

Discussion

Earlier communications reported that genome integrity is continuously challenged by an impressive amount of DNA lesions throughout their life cycle (Lindahl and Barnes, 2000). In healthy individuals, the DNA repair machinery of the cell repairs the consequences of the daily injuries, but, there is a significant variation between healthy individuals in regard with their ability to detect and repair DNA damages. SNPs present in DNA repair genes are of specific importance due to their implications in the pathogenesis of complex genetic diseases including carcinogenesis (Mandal et al., 2010). Additionally, the molecular epidemiological studies have also demonstrated that inheritance of genetic variants at one or more loci and wide population variability, result in a reduced DNA repair capacity and increase in the individual risk of cancer and several other genetic diseases (Shen et al., 1998; Kaur et al., 2000).

Due to remarkable differences in the distribution of DNA repair polymorphisms, between various worldwide ethnic groups, the data from 'normal healthy' populations are of special attention for deciphering the relevance as well as the evaluation of the investigated genetic markers in susceptibility, manifestation, prognosis or treatment of diseases. It has been well established that ethnic background may influence the susceptibility to suffer from certain diseases (Kittles et al., 2003). Therefore, variation in Saudi Arabian population in contrast to other worldwide populations signifies the impact of ethnicity. The study of genetic variations can elucidate critical determinants in environmental exposure and cancer, which could have future implications for preventive and early intervention strategies. Despite good biological reasons for role of DNA repair genetic polymorphisms in many disease and cancer risk modulation, the research reports on functional importance of majority of DNA repair genes remains relatively scanty (Au et al., 2004).

Noteworthy, the most important results considering the *XRCC3* (C>T) polymorphism, the (T) allele frequency in Saudi Arabian population was found to be 39%, which was quite high in United Kingdom, whereas similar frequency has been observed in ethnic populations of Germany, Italy and Poland. In comparison to our selected population the frequency of variant (T) allele was significantly lower in ethnic populations of Thailand, Australia, Japan, Finland, China and North India. The TT genotype was reported to be 0.6% in Thailand population. The differences detected in allelic frequencies among the above mentioned studies possibly found due to ethnic variations, heterogeneity of study populations and different sample sizes. Additionally, there is a well-recognized need to demonstrate the functional significance of polymorphic DNA-repair gene alleles for better interpretation of their effects in human populations (Mohrenweiser et al., 2003).

Although, the increased/ decreased risk associated with individual DNA repair SNPs might be small as compared to that conferred by high-penetrance cancer genes, and their public health implication might be large because of their high frequency in the general population. Epidemiological investigations of DNA repair polymorphisms are therefore important (Wacholder et al. 2004). As we know that there are differences in the prevalence of DNA repair polymorphisms across different populations, hence, it is important to keep in mind that susceptibility factor in one population may not hold true for another. Such type of research study may form the basis for future establishment of epidemiological and clinical databases for human healthcare. However, large and combined comparative analyses may be preferred to minimize the likelihood of both false-positive and false-negative outcomes.

Here, in this study we conclude that *XRCC3* polymorphisms might be a biomarker of disease susceptibility and it may work as contributing factor in the risk of several diseases including cancer. It is an important goal of biological and clinical researches to detect genetic components like, DNA repair gene polymorphism as possible indicators of different type of genetic diseases including carcinogenesis and this study will help in assessment of epidemiological magnitude of the *XRCC3* polymorphism among Saudi population. The differences in DNA double stranded break repair gene polymorphisms distribution between Saudi Arabian healthy population and other ethnic population groups may help in building a profile that would help in assessing the genetic disease predisposition and prevalence.

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