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Polymorphisms in DNA repair and multidrug resistance genes among Sindhis of Central India

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ABSTRACT

Polymorphisms in DNA repair and multidrug resistance genes might contribute to interindividual and interethnic differences in DNA repair capacity and drug disposition respectively. In the present study, we determined the allele and genotype frequencies of four single nucleotide polymorphisms (SNPs) located in the DNA repair genes, *XRCC1*, *XRCC3*, *XPD*, *OGG1*, namely *XRCC1* Arg399Gln, *XRCC3* Thr241Met, *XPD* Lys751Gln, and *OGG1* Ser326Cys, respectively and two SNPs located in the multidrug resistance gene, *ABCB1*, namely *ABCB1* C3435T and *ABCB1* C1236T, in 33–35 healthy and unrelated Sindhi individuals, residing in the Vidarbha region of Central India and compared them with the Maharashtrian population from the same geographical region and some other HapMap populations from the HapMap database. The study findings reveal that the Indian Sindhis are closely related to the Maharashtrians as well as Utah residents with Northern and Western European ancestry and Gujarati Indians in Houston, Texas in the HapMap database.

1. Introduction

Many common, complex diseases in humans are not caused by genetic variation(s) in a single gene and may be the outcome of complex interactions between multiple genetic as well as environmental and lifestyle factors. Even though any two human genomes are roughly 99.9% identical, still there could be millions of differences in the remaining 0.1% of the 3.2 billion base pairs in the genome. These differences account for heritable variation among individuals, including differential susceptibility to disease and differential response to drugs and environmental exposures. Discovering the DNA sequence variants that contribute to common, complex disease risk may lay the groundwork for personalized medicine. Single nucleotide polymorphisms (SNPs) are the preferred marker of choice in genetic mapping studies to identify genes associated with complex diseases because of their high density in the genome, ease of genotyping and their suitability for multiplexing and large-scale analysis.

DNA repair systems play an important role in maintaining genome integrity. Genetic variations in DNA repair genes can modify an individual's capacity to repair damaged DNA and thus, influence susceptibility to various types of cancer. The *XRCC1* (X-ray repair cross-complementing group 1) gene product, an abundant nuclear zinc-finger protein that is part of a DNA-binding protein complex, participates in the base excision repair (BER) pathway and is involved in the repair of DNA single-strand breaks generated by ionizing radiation and alkylating agents. The *XRCC1* Arg399Gln polymorphism at codon 399 of exon 10 (C>T, rs25487) causing non-synonymous substitution of Arginine to Glutamine, is one of the most extensively studied SNPs for cancer risk and has been reported to be associated with increased risk of breast cancer (Ramadan et al., 2014), lung cancer (Guo et al., 2013) and colorectal cancer (Li et al., 2013), although the results are not consistent.

XRCC3 (X-ray repair cross-complementing group 3) gene product encodes a member of the RecA/Rad51-related protein family that plays a critical role in homologous recombination of DNA double-strand breaks for maintaining genome integrity.

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Association between the *XRCC3* Thr241Met polymorphism at codon 241 of exon 8 (G > A, rs861539) causing non-synonymous substitution of Threonine to Methionine and various forms of cancer have been reported including bladder cancer (Andrew et al., 2008), breast cancer (Smith et al., 2003) and lung and colorectal cancer (Improta et al., 2008; Krupa et al., 2011), however, the results are inconclusive.

The protein encoded by the *XPD* (xeroderma pigmentosum complementatation group D, also known as *ERCC2*) gene is involved in transcription coupled nucleotide excision repair pathway, believed to be crucial for removal of bulky DNA adducts. The *XPD* Lys751Gln (T > G, rs13181) polymorphism at codon 751 of exon 23 gives rise to a non-synonymous substitution of Lysine to Glutamine. Association of the 751Gln variant with head and neck cancer (Kumar et al., 2012), bladder cancer (Shao et al., 2007), esophageal squamous cell carcinoma (Huang et al., 2012), aflatoxin B1-related hepatocellular carcinoma (Long et al., 2009) and melanoma in the male population (Kertat et al., 2008) have been reported by different investigators.

Human 8-oxoguanine DNA glycosylase (*OGG1*) is the key enzyme that repairs 8-oxoguanine (8-oxoG) as part of the BER pathway. The *OGG1* Ser326Cys polymorphism causing non-synonymous substitution of Serine to Cysteine at codon 326 of exon 7 (C > G, rs1052133), has been studied most extensively for its association with cancer risk. The association between Ser326Cys polymorphism and cancer is conflicting. The *OGG1* 326Cys allele has been found to be associated with an increased risk of developing several types of cancer including oropharangeal, nasopharangeal, oesophageal and lung, however, its lack of association with breast, biliary tract or colon cancers has also been reported (Kershaw and Hodges, 2012).

The human *ABCB1* gene (also, known as Multidrug resistance gene, *MDR1*) product encodes a 170-kDa plasma membrane glycoprotein [P-glycoprotein (P-gp)]. P-gp is a highly-conserved member of the ATP-binding cassette (ABC) super family of transporters and acts as a transmembrane efflux pump in the energy-dependent export of xenobiotics from intracellular to extracellular region. Genetic polymorphisms in the *ABCB1* gene might affect the expression and function of P-gp, and hence, the absorption, metabolism and clearance of various drugs that are P-gp substrates and consequently, influence the outcome and prognosis of certain diseases like inflammatory bowel disease, colorectal cancer, leukemia and Parkinson's disease. The synonymous C3435T polymorphism in the exon 26 of *ABCB1*, which gives rise to a silent mutation (Ile > Ile), has been found to strongly correlate with P-gp functional expression and drug uptake (Hoffmeyer et al., 2000). Two other SNPs, a synonymous C1236T polymorphism in the exon 12 giving rise to a silent mutation (Gly > Gly) and a non-synonymous G2677T/A polymorphism in the exon 21 causing amino acid substitution (Ala > Ser/Thr), are found to be in linkage disequilibrium with C3435T and these three SNPs have been repeatedly shown to predict the P-gp function in a haplotype-dependent manner (Salama et al., 2006). The C1236T and G2677T/A polymorphisms have also been linked to several diseases like pharmacoresistant epilepsy and Parkinson's disease (Kimchi-Sarfaty et al., 2007).

Genetic studies on Indian Sindhis have been scanty. In this study, we sought to determine the allele and genotype frequencies of four non-synonymous SNPs in the DNA repair genes, *XRCC1*, *XRCC3*, *XPD*, *OGG1*, namely *XRCC1* Arg399Gln, *XRCC3* Thr241Met, *XPD* Lys751Gln, and *OGG1* Ser326Cys, respectively and two synonymous SNPs in the multidrug resistance gene, *ABCB1*, namely *ABCB1* C3435T and *ABCB1* C1236T, in Sindhis residing in the Vidarbha region of Central India and compare them with the Maharashtrian population from the same geographical region and some other HapMap populations from the HapMap database.

2. Material and methods

2.1. Subjects

Protocol of the research study was approved by the Institutional Ethics Committee. The study population consisted of 33–35 healthy and unrelated Sindhi individuals, in the age group of 18–58, residing in the Vidarbha region of Central India. After obtaining prior informed consents from the subjects, 5 ml of blood sample was collected from each.

2.2. DNA extraction and genotyping of samples

Genomic DNA were extracted from whole blood samples of subjects using a standard lysis procedure and DNA were amplified by PCR using respective primers for the *XRCC1*, *XRCC3*, *XPD*, *OGG1* (Pramanik et al., 2011) and *ABCB1* loci (Pramanik et al., 2014). The genotypes of the polymorphisms were determined by digesting the amplified products with appropriate restriction enzymes (Pramanik et al., 2011, 2014) and running the digested products in 2.5% agarose or in 8% polyacrylamide gel.

2.3. Statistical analysis

Hardy–Weinberg equilibrium of determined allele frequencies was assessed using the Chi-square (χ^2) test. The allele and genotype frequencies of the polymorphisms in the Sindhis were compared to the Maharashtrian population (MAH) residing in the same Vidarbha region of Central India and some of the populations in the HapMap database [HapMap Genome Browser release#28 (hapmap.ncbi.nlm.nih.gov) database was used] e.g., Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), Yoruban in Ibadan, Nigeria (YRI), Han Chinese in Beijing, China (CHB), Gujarati Indians in Houston, Texas (GIH), and Japanese in Tokyo, Japan (JPT). Pair-wise Chi-square (χ^2) tests were performed between Sindhis and these populations using the allele frequencies in a 2X2 contingency table, to determine whether the Sindhis were significantly different from other populations.

3. Results

The distribution of the genotypes and the allele and genotype frequencies of *XRCC1* Arg399Gln, *XRCC3* Thr241Met, *XPD* Lys751Gln, *OGG1* Ser326Cys, *ABCB1* C1236T and *ABCB1* C3435T polymorphisms in the Sindhis, residing in the Vidarbha region of Central India, are shown in Tables 1–6.

3.1. Allele and genotype frequencies of XRCC1 Arginine 399 Glutamine (C > T)

The observed C/C (Arg/Arg), C/T (Arg/Gln) and T/T (Gln/Gln) genotype frequencies were 0.343, 0.4, and 0.257, respectively for the *XRCC1* polymorphism (Table 1). The C (major allele) and T (minor allele) allele frequencies were found to be 0.54 and 0.46, respectively. The minor allele frequency ranged from 0.105 among YRI to 0.46 among Sindhis.

3.2. Allele and genotype frequencies of XRCC3 Threonine 241 Methionine (G > A)

The observed G/G (Thr/Thr), G/A (Thr/Met) and A/A (Met/Met) genotype frequencies were 0.235, 0.588, and 0.177, respectively for the *XRCC3* polymorphism (Table 2). The G (major allele) and A (minor allele) allele frequencies were 0.53 and 0.47, respectively.