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Frequency distribution of DNA repair genes *ERCC1* and *ERCC2* polymorphisms in South Indian healthy population

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ABSTRACT

DNA repair genes are crucial in maintaining the integrity of the whole genome. Single nucleotide polymorphisms (SNPs) in DNA repair genes have been attributed to the development of various cancers. SNPs of DNA repair genes (*ERCC1* and *ERCC2*) have been implicated in the causation of various cancers as well as inter-individual variability in the therapeutic outcomes of platinum based therapy. Thus establishing the frequency of these functional SNPs in the healthy population is of significance. The present study was aimed to establish the allele and genotype frequencies of *ERCC1* (19007C > T, rs11615; 8092C > A, rs3212986) and *ERCC2* (Asp312Asn, rs1799793) genes in South Indian healthy population and to compare the data from HapMap populations. The study population consisted of 128 healthy South Indian unrelated individuals of either sex aged between 18 and 60 years. Standard phenol–chloroform method was used to extract DNA from peripheral leukocytes. The genotype of DNA repair gene polymorphisms was determined by quantitative real-time polymerase chain reaction using TaqMan genotyping assay. The observed frequency of the studied polymorphisms followed Hardy–Weinberg equilibrium ($p > 0.05$). The frequencies of the minor alleles of the SNPs rs11615 (T), rs3212986 (A) and rs1799793 (A) were 43.8%, 29.3% and 35.6%, respectively. Gender-based analysis showed no significant difference in the frequency pattern. The observed allele and genotype frequencies showed significant ethnic difference between South Indians and other HapMap populations. This is the first study to provide the normative frequency data of allele and

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genotype distribution of three SNPs of *ERCC1* and *ERCC2* in South Indian healthy population. It might be useful in future genotype–phenotype association studies, especially for predicting the efficacy and adverse events of platinum based drugs.

1. Introduction

DNA repair genes are crucial in maintaining the integrity of the whole genome (Bohr, 1995; Charames and Bapat, 2003; Ma et al., 1995; Radman et al., 1995). A number of DNA repair gene pathways such as nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), direct repair (DR) and double-strand break repair (DSBR) are involved in repairing the DNA damage (De Laat et al., 1999; Smith et al., 2003). Among them, the NER system plays a major role in repairing DNA adducts and UV-induced photolesions (De Laat et al., 1999).

NER pathway is crucial for the repair of various DNA lesions such as thymidine dimers, cross-links, bulky adducts, oxidative damage, and alkylating damage (Braithwaite et al., 1999; Chen et al., 1997; De Silva et al., 2000). Single nucleotide polymorphisms (SNPs) in DNA repair genes may affect the host's capacity to repair DNA damage, resulting in an increased susceptibility to various cancers (Goode et al., 2002). Excision repair cross-complementing 1 (*ERCC1*) and excision repair cross-complementing 2 (*ERCC2*) also called Xeroderma Pigmentosum group D (*XPD*) play a prime role in the NER pathway.

NER is a complex and critical pathway, the mechanism of which is not yet completely elucidated. Studies on DNA repair genes have revealed intermediate steps involved in this pathway. It encompasses DNA damage recognition, recruitment of the several factors and DNA repair proteins at the damaged site, incision and excision of the damaged area, resynthesis of damaged site and ligation of the strands. Xeroderma Pigmentosum group A (*XPA*) and Xeroderma Pigmentosum group C (*XPC*) are involved in damage recognition complex, but various studies have shown that *XPC*-hHR23B (human homologs of RAD23) complex recognizes the damaged area at an early stage, which is succeeded by the recruitment of several repair factors including multicomponent transcription repair factor (TFIIH) that unwinds the damaged DNA and makes space available for the nucleases to act (Masutani et al., 1994; Yokoi et al., 2000; You et al., 2003). The TFIIH involves an essential component helicase, which is encoded by *ERCC2* gene (Sung et al., 1993). Thus, *ERCC2* plays a pivotal role in the regulation of TFIIH function. *XPA* also interacts with replication factor A (RPA) to enhance the repairing capacity of NER system. *XPA* and RPA proteins facilitate the recruitment of *ERCC1–XPF* (Xeroderma Pigmentosum group F) complex that cleaves the 5′end (Biggerstaff et al., 1993). This process is followed by cleavage of the 3′ end by Xeroderma Pigmentosum group G (XPG) (O'Donovan et al., 1994). Eventually most of the complexes unbind, while the gap is bridged by the RPA complex and DNA polymerase δ.

Two common SNPs in *ERCC1* gene, namely *ERCC1* C19007T (rs11615) and *ERCC1* C8092A (rs321986), have been studied in association with various cancers such as glioma, non-small cell lung cancer, head and neck cancers (Zhang et al., 2012). The important SNP of *ERCC2* gene, *ERCC2 Asp312Asn* (rs1799793) has been studied in association with various cancers such as lung, bladder and pancreatic cancers (Jiao et al., 2007; Li et al., 2010a; Wang et al., 2008; Hou et al., 2002). The characteristics of these SNPs are shown in Table 1.

SNPs in DNA repair genes are also important in the context of sensitivity and resistance to platinum based chemotherapy as they also affect the prognosis of such patients treated with these drugs. The commonly used platinum drugs in cancer chemotherapy are cisplatin, carboplatin and oxaliplatin. They cause tumor cell death by the formation of platinum DNA adducts. These platinum DNA adducts are removed by repair genes. High activity of these genes counteracts the action of platinum drugs and results in the development of resistance to chemotherapy. On the other hand single nucleotide polymorphisms of these genes decreases the DNA repair capacity and enhance sensitivity to platinum therapy (Giaccone, 2000; Martin et al., 2008; Rabik and Dolan, 2007).

Thus establishing the normal genotype frequency would provide the reference values of the distribution of these SNPs in a population, so that it can be compared with those of people with cancer risk.

To the best of our knowledge these three SNP's have not been analyzed in the Indian population. Therefore, the present study was aimed to evaluate the frequency of *ERCC1* and *ERCC2* genotypes, alleles, haplotypes and linkage disequilibrium pattern in the population of South India.

2. Materials and methods

2.1. Study population

The study population consisted of 128 unrelated healthy individuals aged between 18 and 60 years. Of these, 73 were males and 55 were females. The mean age (±SD) of the study subjects was 30.2 (±10) years. All the participants reported three consecutive generations residing in South India and spoke a Dravidian language, such as Telugu, Kannada, Malayalam and Tamil as their native language. The study was approved by the Institute Ethics Committee, JIPMER (Jawaharlal Institute of Postgraduate Medical Education and Research), Pondicherry, India. Written informed consent was obtained from all the study participants.

2.2. DNA extraction

Five milliliters of whole blood were collected from each participant using 100 μl of 10% ethylene diaminetetraacetic acid (EDTA) as an anticoagulant. The plasma was separated by