RESEARCH COMMUNICATION

XRCC1 and XPD Gene Polymorphisms in a South Indian Population

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Abstract

DNA repair systems play an important role in maintaining the integrity of the human genome. Deficiency in the repair capacity due to either mutations or inherited polymorphisms in DNA repair genes may contribute to variations in the DNA repair capacity and subsequently susceptibility to cancer. The interindividual variability as well as ethnic differences in DNA repair polymorphisms, stress the importance to establish genotype profiles unique to a population. Hence the present study aimed to determine the frequencies of XRCC1 and XPD gene polymorphisms in 255 healthy random unrelated individuals from South India. DNA was isolated from the peripheral blood sample of these individuals and the XRCC1 and XPD genotypes were determined by PCR-RFLP with Msp1 and Pst1 enzymes respectively. The XRCC1 genotype frequencies revealed 36% Arg/Arg, 47% Arg/Gln and 17% Gln/Gln with Gln allele frequency of 0.41. Analysis of XPD genotypes revealed 51% Lys/Lys, 41% Lys/Gln and 8% Gln/Gln with Gln allele frequency of 0.29. No significant difference in the distribution of genotypes was seen based on gender. Comparison of the frequencies of XRCC1 and XPD polymorphisms observed in the present study with other populations revealed a distinctive nature of the South Indian population. An understanding of DNA repair gene polymorphisms might not only enable risk assessment of humans exposed to environmental carcinogens but also response to therapy, which target the DNA repair pathway.

Keywords: XRCC1 - XPD - PCR-RFLP - polymorphisms - DNA repair

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Introduction

Humans are continuously exposed to mutagenic and carcinogenic aromatic amines via smoking, well-cooked food and other sources. These chemicals can form DNA adducts in vivo and thus lead to DNA damage. The integrity of most of the so-damaged DNA is typically restored as a consequence of the action of certain DNA-repair enzymes. The normal function of DNA repair enzymes is important for maintaining genome integrity and preventing cellular neoplastic transformation (Charames et al., 2003).

In all individuals genetic polymorphisms in DNA-repair enzymes might be able to influence DNA adduct levels (Hou et al., 2002) and the degree of DNA repair capacity has often been associated with the risk of human cancers (Wei et al., 2000; Rajaee-Behbahani et al., 2000; Spitz et al., 2001). However, only a fraction of carcinogen-exposed individuals develop cancer, suggesting an important role of individual susceptibility and possible gene–environment interactions. This indicates the existence of considerable inter-individual variation in DNA repair capacity in the general population.

Amongst the known genetic polymorphisms of the DNA repair genes, the xeroderma pigmentosum group D (XPD, also known as ERCC2) and x-ray repair cross-complementing groups 1 and 3 (XRCC1 and XRCC3) have been studied most commonly (Goode et al., 2002). The XPD gene encodes a helicase that is a component of the transcription factor TFIIH (Sung et al., 1993). This factor is an essential member of the nucleotide-excision repair (NER) pathway that is responsible for effecting repairs to bulky adducts and UV-induced DNA damage (Weeda et al., 1993). Individuals with XPD 751Gln/Gln have been demonstrated to have suboptimal DNA-repair capacity to remove UV photoproducts when compared to the XPD 751Lys/Lys and Lys/Gln genotypes (Qiao et al., 2002).

The XRCC1 gene encodes the XRCC1 protein which is a scaffolding protein directly associated with polymerase beta, DNA ligase III and poly (ADP-ribose) polymerase (PARP) and functions as a complex to facilitate the base-excision repair (BER) and single strand break-repair processes (Caldecott et al., 1996; Kubota et al., 1996; Cappelli et al., 1997). Lunn et al 1999 reported that individuals with XRCC1 399Gln/Gln genotype were significantly associated with higher levels of aflatoxin B1-DNA adducts when compared to individuals with Arg/Arg genotype (Lunn et al., 1999)

DNA repair systems maintain the integrity of the human genome, any deficiency in the repair capacity due to either mutations or inherited polymorphisms in DNA

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repair genes may contribute to variations in DNA repair capacity and susceptibility to cancer within the exposed population. Allelic variants of XRCC1 and XPD have been associated with increased risk of various cancers like head and neck (Sturgis et al., 1999), breast (Duell et al., 2001) and lung (Chen et al., 2002). The allelic and genotypic variations of XRCC1 and XPD have been observed in different populations and ethnic groups in various parts of the world. As India is known for its unique population structure; having about 5000 endogamous populations we here define the allelic profiles and genotype frequencies for XRCC1 and XPD genes in healthy random unrelated individuals from South India.

Patients and Methods

Subjects

The study population comprised of 255 random healthy unrelated individuals of south Indian ethnicity. Of the 255 individuals, 91 were females and 164 were males. The age of the individuals ranged from 20-65 years with mean age of 46 years. Blood samples were collected from these individuals with informed written consent.

DNA Extraction and Genotyping

Genomic DNA was isolated from whole blood by the salting out method (Miller et al., 1988). Genotypes of XRCC1and XPD were determined by PCR followed by restriction fragment length polymorphism (PCR-RFLP). PCR was performed to amplify exon 10 of XRCC1 gene and exon 23 of XPD gene using specific primers (Yeh et al., 2005). The PCR products were visualized by 2% agarose electrophoresis and the genotypes were determined based on the band pattern.

Since the G to A transition in exon 10 of XRCC1 abolishes the recognition site for Msp1 enzyme, the Gln/Gln genotype yields an undigested band of 615 bp, Arg/Arg results in two fragments of 376bp and 239bp and Arg/Gln genotype with three fragments of 615bp, 376 bp and 239 bp. The A35931C transition in exon 23 of XPD gene creates a recognition site for the Pst1 enzyme. The Lys/Lys genotype was characterized by an undigested band of 734bp, Gln/Gln genotype in 646bp and 88bp and the Lys/Gln genotype had three bands of 734bp, 646 and 88bp.

Statistical Analysis

The allele and genotype frequencies of XRCC1 and XPD were calculated. The expected genotype frequencies for each of the genes were calculated to test if the population followed Hardy Weinberg equilibrium. The combined genotype frequencies of XRCC1 and XPD were also determined. Stratified analysis based on gender was performed. Chi square test was applied to compare the allelic frequencies obtained in the present study with that reported in other populations. All the statistical analysis was carried out using the SPSS (version 13) software programme for windows.

Results

The allele and genotype frequency distribution of XRCC1 and XPD are shown in Tables 1 and 2 respectively. For the XRCC1 genotypes, 36% were homozygous Arg/Arg, 47% were heterozygous Arg/Gln and 17% were homozygous Gln/Gln. The Arg allele frequency was 0.59 and that of Gln was 0.41. Analysis of XPD genotypes revealed Lys/Lys in 51% of the individuals, Lys/Gln in 41% and Gln/Gln in 8%. The allele frequency of Lys was 0.71 and that of Gln was 0.29.

The XRCC1 as well as XPD genotypes were found to follow the Hardy Weinberg equilibrium (Tables 1 and 2). Analysis of the combined distribution of the XRCC1 and XPD revealed 21% of the individuals with a combined homozygous wild type Arg/Arg and Lys/Lys, the combined homozygous variants Gln/Gln was found in only 1%, while the combined heterozygous Arg/Gln and Lys/Gln was found in 22% of the individuals. The percentage of the various genotype combinations are as shown in Table 3.

Stratified analysis of the XRCC1 and XPD genotypes based on gender revealed no significant difference in the frequencies between the males and females (Table 4).

Table 1. Distribution of XRCC1 Genotypes and Allele Frequencies (N=255)

Genotypes		Genotype Frequency (%)		Allele frequency Arg	Gln
Arg/Arg	Observed	91.0	(36)	0.59	0.41
	Expected	89.4			
Arg/Gln	Observed	120.0	(47)		
	Expected	123.2			
Gln/Gln	Observed	44.0	(17)		
	Expected	42.4			

Table 2. Distribution of XPD Genotypes and Allele Frequencies (N=255)

Genotypes		Genotype Frequency (%)		Allele frequency Lys	Gln
Lys/Lys	Observed	130	(51)	0.71	0.29
	Expected	129.9			
Lys/Gln	Observed	104	(41)		
	Expected	104.2			
Gln/Gln	Observed	21	(8)		
	Expected	20.9			

Table 3. Distribution of Combined Genotypes of XRCC1 and XPD

XPD	XRCC1 Arg/Arg	Arg/Gln	Gln/Gln
Gln/Gln	11 (4%)	7 (3%)	3 (1%)
Lys/Gln	27 (11%)	57 (22%)	20 (8%)
Lys/Lys	53 (21%)	56 (22%)	21 (8%)

Table 4: Distribution of XRCC1 and XPD Genotypes Based on Gender

Gene	Genotype	Male(N=166)	Female(N=89)
XRCC1	Arg/Arg	57 (34%)	34 (38%)
	Arg/Gln	78 (47%)	42 (47%)
	Gln/Gln	31 (19%)	13 (15%)
XPD	Lys/Lys	87 (52%)	43 (48%)
	Lys/Gln	68 (41%)	36 (41%)
	Gln/Gln	11 (7%)	10 (11%)

Table 5. Comparative Frequency Distribution of XRCC1 Alleles in Various Populations

Study Population	N	XRCC1 Genotypes			Gln Allele Frequency	P	Reference
		Arg/Arg	Arg/Gln	Gln/Gln			
Present Study	255	91	120	44	0.41		
Brazilians	262	119	107	36	0.34	0.07	Duarte et al., 2005
Brazilian	58	25	23	10	0.37	0.5	Rossit et al.,2002
Egyptians	50	37	9	2	0.14	<0.001	Abdel-Rahman et al., 2000
North Americans	169	65	83	21	0.37	0.3	Lunn et al., 1999
Italians	124	53	58	13	0.39	0.16	Matullo et al., 2001
Taiwanese	120	63	51	6	0.26	0.0005	Lunn et al.,1999
Taiwanese	729	384	291	54	0.27	<0.001	Yeh at al.,2005
Koreans	135	81	48	6	0.22	0.000002	Park et al 2002
Chinese	166	94	59	13	0.27	0.0005	Shen et al 2000

Table 6. Comparative Frequency Distribution of XPD Alleles in Various Populations

Study Population	N	XPD Genotypes			Gln Allele Frequency	P	Reference
		Lys/Lys	Lys/Gln	Gln/Gln			
Present Study	255	130	104	21	0.29		
South Koreans	163	145	18	0	0.06	<0.05	Park et al., 2002
Italians	628	216	318	94	0.40	<0.001	Matullo et al., 2001
Japanese	240	217	21	2	0.05	<0.05	Hamajima et al.,2002
African Americans	234	130	91	13	0.25	0.39	David Baebes et al., 2001
Caucasians, US	453	197	198	58	0.35	0.07	David Baebes et al., 2001
United Kingdom	211	72	108	31	0.40	<0.001	Winsey et al., 2000
Chinese	1020	848	166	6	0.09	<0.001	Liang et al., 2003
Swedish	162	69	65	28	0.37	0.01	Hou et al., 2002
Finnish	302	103	153	46	0.41	<0.001	Misra et al., 2003

The allelic and genotype frequency distribution of XRCC1 and XPD in different populations and the present study results are represented in Table 5 and 6 respectively.

Discussion

Polymorphism in genes involved in carcinogen metabolism and DNA repair are reported as a source of inter-individual variability in human response to carcinogens. Many studies have been focused on heritable polymorphisms in genes involved in carcinogen metabolism; however only scanty data is available in relation to the DNA repair capacity and development of cancer (Friedberg et al., 1995). Individuals differ widely in their capacity to repair DNA damage from both exogenous agents, such as tobacco smoke and sunlight exposure, as well as endogenous reactions, such as oxidations. Hence the present study was performed to determine the XRCC1 and XPD genotype distribution among the ethnic South Indians. The study is the first of its kind to report the frequency distribution of XRCC1 and XPD genes in a random population in the Indian subcontinent.

The XRCC1 Arg399Gln genotype frequencies of the present study revealed a significant deviation when compared to that of Taiwanese, Koreans, Chinese and Egyptians (Table 5). However there was similarity between the present study and that of Italians and Brazilians.

With regard to the XPD Lys751Gln polymorphism the frequencies obtained in the present study correlated with that of African Americans and Caucasians in United States and deviated from all the other populations such as Koreans, Italians, Japanese, Chinese, Swedish and Finnish (Table 6)

Establishing the baseline frequency of the different DNA repair alleles in a random population might enable ethnic based risk assessment towards environmental insults and thereby susceptibility towards carcinogenesis. In addition to their role in cancer risk, DNA repair polymorphisms might influence response to treatment and/or survival. Hence DNA repair polymorphisms might prove relevant in pharmacogenetics by modifying the repair capacity in response to cytotoxic or radiation therapy. Future studies on the phenotypic effects of these polymorphisms in random individuals of distinct ethnic origin based on life style and environmental exposures will establish a lucid picture of not only the functional consequences of the various genotypes but also the gene-environment interactions.

In conclusion, our study provides an estimate of the frequencies of XRCC1 and XPD alleles in the South Indian population. The results indicate a distinct molecular profile of polymorphisms at the XRCC1 and XPD loci among South Indians compared to other ethnic groups.

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