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XPD Lys751Gln Polymorphism Analysis in Women with Sporadic Breast Cancer

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Common polymorphism in DNA repair genes may alter protein function and an individual's capacity to repair damaged DNA; deficits in repair capacity may lead to genetic instability and carcinogenesis. In present work we investigated the association between XPD Lys751Gln polymorphism and breast cancer progression.

The polymorphism was analysed in breast cancer patients (n = 92) in blood. Blood samples from age matched healthy women served as control (n = 110). XPD genotypes were measured by PCR-RFLP.

The distribution of the genotypes of the Lys751Gln polymorphism in patients differed significantly ($p < 0.05$) from those predicted by the Hardy-Weinberg equilibrium. There were significant differences in the frequencies of alleles between the breast cancer subjects and controls ($p < 0.05$).

The results support the hypothesis that the Lys-751Gln polymorphism of XPD gene may be associated with the incidence of breast cancer.

Introduction

Breast cancer is one of the major killers worldwide. The genetics of mammary carcinogenesis has not been fully elucidated. Unrepaired or misrepaired DNA damage can lead to gene mutations, chromosomal alterations, and genomic instability, known to have a role in cancer initiation. Accordingly, individuals with reduced DNA repair capacities might have an altered cancer risk [6, 2]. During recent years, several polymorphisms in the genes encoding the DNA repair enzymes have been found and studied in relation to cancer proneness [7].

XPD protein possesses both single-strand DNA-dependent ATPase and 5'-3' DNA helicase activites and is thought to participate in DNA unwinding during the nucleotide excision repair (NER) and transcription [11, 24]. NER repairs DNA damage induced by UV radiation and bulky adducts. However, because XPD is involved in both transcription and NER, it may contribute to repair of others types of damage, such as ionizing radiation. Studies using lymphocytes containing mutant XP genes have an elevated chromatid aberration frequency after exposure to ionizing radiation, suggesting a role for NER proteins in the repair of ionizing radiation-induced damage [18]. Ionizing radiation induces oxidative damage and several studies suggest XP proteins may participate in the repair of this type of damage [20, 13]. Mutations in the *XPD* gene can diminish the activity of TFIIH complexes giving rise to repair defects, transcription defects, and abnormal responses to apoptosis. Because XPD is important in multiple cellular tasks and rare *XPD* mutations results in genetic diseases, *XPD* polymorphism may operate as genetic susceptibility factors.

Single nucleotide polymorphisms (SNPs) in NER genes have been discovered in human populations, and several epidemiologic studies have examined these SNPs as risk for cancer. Several single nucleotide polymorphisms (SNPs) have been described in the *XPD* gene. Two of these SNPs lead to amino acid change, Asp312Asn in exon 10 and Lys751Gln in exon 23, and are in strong linkage disequilibrium with each other [21, 19]. The *XPD-751 Gln* variant allele has been associated with increased DNA adduct levels [22, 25] and suboptimal DNA repair [22, 19], but contrasting results also exist [15]. It has recently been associated with increased risk of smoking-related cancers,

such as lung cancer [10] and squamous cell carcinoma of head and neck [23], and recently a significant effect was also seen for breast cancer [23]. Contrasting results also exist [22, 25, 8, 16, 12].

We studied the association of *XPD* polymorphism located at codons 751 (Lys/Gln) with breast cancer progression. The *XPD* Lys751Gln substitution is attributed to a A→C transversion at exon 23 [7].

Material and Methods

Patients

92 blood samples were obtained from premenopausal (n=30) and postmenopausal (n=62) women with breast carcinoma treated at the Department of Oncology, Institute of Polish Mother's Memorial Hospital between 2003-2005. The patients ranged in age from 32 to 79 years (mean age ± SD, 56 ± 10.97 years). The pathological evaluation report was obtained for each patient. According to the size of the tumor, the samples were divided into four categories: $T_1 \leq$ 2 cm (62 tumors); T_2, 2-5 cm (19 tumors); T_3, > 5 cm (8 tumors); T_4 any size with direct extension to chest wall or skin (3 tumors). According to the appearance of metastasis in the axillary lymph nodes, the samples were allocated to four categories: N_0 (49 tumors), N_1 (26 tumors), N_2 (10 tumors) and N_3 (7 tumors). All tumors were classified as M_0. The average tumor size was 25 mm (range 50-100 mm). 92 samples of ductal breast carcinoma were graded by a method based on the criteria of Scarf-Bloom-Richardson. There were 25 tumours of I° grade, 40 of II° grade and 27 of III° grade in total. Blood samples from age matched healthy women (n=110) served as control.

Genotyping methods

DNA was extracted using commercially available QIAmp Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to manufacturer's instruction. The *XPD* genotypes were determined by PCR-RFLP analysis of DNA samples. The PCR primers for the Lys751Gln were: forward 5' GCC CGC TCT GGA TTA TACG 3' and reverse 5' CTA TCA TCT CCT GGC CCCC 3'. PCR was performed in 25µl containing 2mM $MgCl_2$, 0.04 mM deoxynucleotide triphosphates, 1.0 U of Taq polymerase, and the manufacturer's buffer [20 mM Tris-HCl (pH 8.4) and 50 mM KCl]. After an initial denaturation at 94°C for 3 min, there were 35 cycles of 45 s at 94°C, 45 s at 60°C and 60 s at 72°C, and then a final extension step of 7 min at 72°C. After overnight digestion of the PCR product with *Pst*I, 5 µl of the digested products were resolved on a 3% agarose gel (5V/cm) containing ethidium bromide. The homozygous wild-type allele (Lys751) produced two DNA bands (290 and 146 bp), whereas the variant allele (Gln751) produced three DNA bands (227, 146 and 63 bp). Heterozygotes displayed all four bands (290, 227, 146 and 63 bp) (Fig. 1).

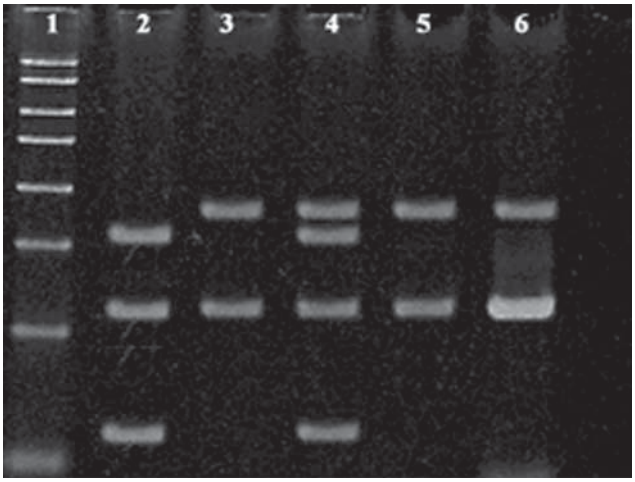

Fig. 1. A typical result of PCR-RFLP performed with a fragment of the *XPD* gene and analysed by 7% polyacrylamide gel electrophoresis, staining with ethidium bromide and viewed under ultraviolet light. Lanes 1 - M — molecular weight markers, 50-2000 bp (Sigma, St. Louis, USA), 2 – Gln/Gln genotype, 3, 5 and 6 – Lys/Lys genotype and 4 display the Lys/Gln genotype

Statistical analysis

The allelic frequencies were estimated by gene counting and the genotypes were scored. The observed numbers of each *XPD* genotype were compared with those expected for a population in Hardy-Weinberg equilibrium by using the χ^2 test. The significance of the differences of the observed allele and genotype frequencies between groups was tested using the χ^2 analysis. *P*-values <0.05 were considered to be significant.

Results

From the PCR analysis, all patients and controls were classified into three genotypes of the Lys751Gln polymorphism: Lys/Lys, Lys/Gln and Gln/Gln.

The distributions of the Lys/Gln genotypes as well as the frequencies of the Lys and Gln alleles for breast cancer patients and control are shown in Table 1. It can be seen

TABLE 1
Distribution of Lys/Lys, Lys/Gln and Gln/Gln genotypes and frequencies of the Lys and Gln alleles in patients with breast cancer and controls

	Patients (n = 92)		Controls (n = 110)	
	Number	Frequency	Number	Frequency
Lys/Lys genotype	16	0.17	26	0.24
Lys/Gln genotype	19	0.21	52	0.47
Gln/Gln genotype	57	0.62	32	0.29
χ^2	23.644[a]		0.309[a]	
Lys allele	51	0.28[b]	104	0.47
Gln allele	133	0.72[b]	116	0.53

[a]$p < 0.05$ as compared with Hardy-Weinberg distribution; [b]$p < 0.05$ as compared with the controls

TABLE 2
Dependency of Lys/Lys, Lys/Gln and Gln/Gln genotypes and frequencies of the Lys and Gln alleles on tumour stage in patients with breast cancer[a]

Stage[b]	I (n = 25)		II (n = 40)		III (n = 27)	
	Number	Frequency	Number	Frequency	Number	Frequency
Lys/Lys genotype	7	0.28	7	0.18	7	0.26
Lys/Gln genotype	11	0.44	20	0.50	11	0.41
Gln/Gln genotype	7	0.28	13	0.32	9	0.33
χ^2	0.360[c]		0.028[c]		0.886[c]	
A allele	25	0.50	34	0.43	25	0.46
G allele	25	0.50	46	0.57	29	0.54

[a]n = 92; [b]according to Scarf-Bloom-Richardson criteria; [c]$p > 0.05$ as compared with Hardy-Weinberg distribution

from the Table that there were significant differences ($p < 0.05$) between the two investigated groups. The frequencies of the Lys and Gln alleles were 0.28/0.72 in patients and 0.47/0.53 in controls. In patients the observed frequencies of the Lys/Lys, Lys/Gln and Gln/Gln genotypes differed significantly ($p < 0.05$) from the distribution expected from the Hardy-Weinberg equilibrium

The dependencies of the distribution of genotypes and frequencies of alleles on the tumour stage evaluated according to Scarf-Bloom-Richardson criteria of patients with breast cancer are displayed in Table 2. There were no significant differences between the distributions of genotypes in the subgroups assigned to the histological stage and the distribution predicted by Hardy-Weinberg equilibrium ($p > 0.05$). There were no differences in the frequencies of the Lys and Gln alleles between the subgroups either ($p > 0.05$).

Discussion

Breast cancer is the commonest malignancy in women and comprises 18% of all cancers in women. Studies conducted over the past few years have identified variant alleles for a number of DNA repair genes, some of which may modify DNA repair capacity. Characterization of these genotypic variations in DNA repair functions d their association with cancer may help to elucidate cancer etiology [9].

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of which may modify DNA repair capacity. Characterization of these genotypic variations in DNA repair functions and their association with cancer may help to elucidate cancer etiology.

The A→C variation of the *XPD*751 gene leads to a change of configuration of the coded protein and may alter the XPD protein's interaction with helicase activator p44 protein inside the TFIIH complex [5]. *XPD* is one of the seven genetic complementation groups encoding for proteins involved in the NER pathway. Although some rare germ line mutations in XPD result in defective NER phenotypes [14, 5], the functional relevance of some common polymorphism in *XPD* has not been determined. Understanding the correlation between DNA genotypes and phenotypes is an important step towards determining how polymorphic genotypes are associated with cancer in the general population.

The *XPD* Lys751Gln polymorphism has been suggested to be the most important functional polymorphism in the gene due to major change in the electronic configuration of the respective amino acid in an important interaction domain of the protein [1]. In a Chinese population polymorphism increased the risk of squamous cell carcinoma of the lung [3]. However, no significant overall association with breast cancer was seen in Metsola et al. study for the *XPD* Lys751Gln genotypes [17]. This was in agreement with the other five studies on the *XPD* Lys751Gln polymorphism and breast cancer risk including one in Finnish [8], one in Danish [16], one in German [12], and two in US Caucasian women [22, 25]. In contrast, a significant association between the *XPD*-751 Gln allele and breast cancer risk was seen in a recent study among American women [26]. Moreover, the *XPD* Asp312Asn polymorphism was recently shown to be associated with breast cancer risk in a German population [12]. We decided not to analyse the *XPD* Asp312Asn polymorphism as it has been shown to be strictly linked with the Lys751Gln polymorphism [21, 19].

In the present work a PCR method was used to screen 92 breast cancer patients for the Lys751Gln polymorphism. We detected a significant difference in distribution frequency of alleles between patients and control ($p < 0.05$). The distribution of the genotypes in the patients differed from one expected from the Hardy-Weinberg equilibrium, with an overrepresentation of Gln/Gln homozygotes. It is possible that the presence of the Gln allele is in linkage disequilibrium with another, so far unknown, mutation located outside the coding region in the *XPD* gene, which may be of importance for the XPD concentration in plasma.

On the other hand we did not detect any significant difference between the genotypes in subgroups assigned to histological stages, which suggests a lack of association between the polymorphism and breast cancer invasiveness.

Our study implies that the Lys751Gln polymorphism of *XPD* gene may be associated with the occurrence of breast cancer. Further studies, conducted on a larger group, are required to clarify this point.

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