The CYP17 and CYP19 gene single nucleotide polymorphism in women with sporadic breast cancer

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The cytochrome P450 family (CYPs) enzymes play an important role in the metabolism of environmental carcinogens and of oestrogen and can affect breast cancer risk. We hypothesise that polymorphisms of CYP17 and CYP19 gene can predict higher incidence of breast cancer. In the present work the distribution of genotypes and frequency of alleles of the T/C polymorphism in promoter region of CYP17 and Trp/Arg polymorphism in codon 39 of CYP19 gene in breast cancer women were investigated. The genetic polymorphisms analysis was performed by amplifying DNA by PCR-RFLP methods in 100 sporadic breast cancer cases. The distribution of the genotypes of the T/C polymorphism of CYP17 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 ($p < 0.05$). However, the distribution of the genotypes of the Trp/Arg polymorphism of CYP19 in both control and patients did not differ significantly ($p > 0.05$) from those predicted by the Hardy-Weinberg distribution. The results support the hypothesis that the T/C polymorphism of CYP17 gene may be associated with the incidence of breast cancer in women from Lodz region of Poland.

Key words: CYP17, CYP19, polymorphism, breast cancer, PCR-RFLP.

Introduction

Breast cancer is the most prevalent cancer type in women. Breast cancer occurs in both hereditary and sporadic forms, and is a great problem in public health all over the world.

Enzymes produced from the cytochrome P450 genes are involved in the synthesis and metabolism of various molecules and chemicals within cells. Cytochrome P450 enzymes play a role in the synthesis of many molecules including steroid hormones, certain fats (cholesterol and other fatty acids), and acids used to digest fats (bile acids). Additional cytochrome P450 enzymes metabolize external substances, such as medications, that are ingested, and internal substances, such as toxins, that are formed within cells. There are 57 *CYP* genes in humans. The enzymes involved in the biosynthesis and metabolism of oestrogens (CYP17, CYP19, CYP2D6, COMT, or CYP1A1) have been the main targets in attempts to identify genetic polymorphisms contributing to a breast cancer risk [2-4].

Cytochrome P450c17α (*CYP17*) encodes for one of the key enzymes, i.e. cytochrome P450c17α (CYP17) that catalyzes the conversion of 17-hydroxy-pregnenolone or dehydroepiandrosterone (DHEA) to androstenedione in the synthesis of oestrogens [5]. Thus, a genetic polymorphism of CYP17 could change the expression levels or activities of the cytochrome P450C17α and, in turn, the risk of breast cancer in relation with the changes in the oestrogen biosynthesis. A single-bp polymorphism in the 5' untranslated region of CYP17 (27 bp downstream from the transcription start site) has been used to identify two alleles, T (formerly designated as A1) and C (formerly designated as A2). CYP17 variant C allele may increase the breast cancer risk in conjunction with the long-term hormone replacement therapy use and high BMI in postmenopausal women [6]. Moreover, a CYP17 A2 allele gene polymorphism

might play a significant role in breast cancer development in young Indian women [7].

Aromatase, a protein product of the *CYP19* gene, is involved in the production of endogenous oestrogens via androgen conversion. Aromatase is expressed in various tissues, including adipose, breast, and bone, where its activity influences local tissue concentrations of oestrogens in a paracrine or intracrine fashion [8]. Aromatase (CYP19) converts adrenal and ovarian androgens into oestrogens, which supports the growth of oestrogen-dependent breast cancers. Miyoshi *et al*. have identified two novel polymorphisms in the *CYP19* gene and showed that one of them (codon 39 Trp/Arg) was significantly associated with the breast cancer risk among the Japanese [9].

In the present work, the association between the T→C polymorphism in the 5'UTR of the *CYP17* gene and *CYP19* gene polymorphism (codon 39 Trp/Arg) and the breast cancer risk in Polish women was investigated.

Materials and methods

Breast cancer samples

Blood samples were obtained from 100 post-menopausal women with node-negative (n = 39) and node-positive (n = 61) ductal breast carcinoma treated at the Department of Menopausal Diseases of the Polish Mother's Memorial Hospital Research Institute, Lodz/Łódź, Poland, between 2006 and 2008. No distant metastases were found in patients at the time of treatment. The patients' age ranged from 46 to 80 years (median age 59 years). The average tumour size was 20 mm (range 17-32 mm). All tumours were graded by a method based on the criteria of Scarff-Bloom-Richardson. Blood samples from age-matched healthy women (n = 106) served as controls. The DNA was extracted using a commercially available OIAmp Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to the manufacturer's instruction.

Determination of the CYP17 and CYP19 genotype

A 459-bp fragment of genomic DNA containing the T to C substitution at -34 bp in the *CYP 17* gene was amplified by PCR [10]. Primer sequences were as follows: forward, 5'-CATTCGCACTCTGGAGTC-3'; and reverse, 5'-AGGCTCTTGGGGTACTTG-3'. The T to C polymorphism creates a recognition site for the restriction enzyme *Msp*AI. After amplification, all samples were digested overnight with 5 U *Msp*AI. In subjects with the C allele, two smaller fragments of 335 and 124 bp were obtained.

Genotypic analyses of the *CYP19* gene were carried out by PCR-RFLP, using primers F1: 5'-ATCTGTACTGTACAGCACC-3' and R1: 5'-ATGTGCCCTCATAATTCCG-3' for the C (Arg) allele and F2: 5'-GGCCTTTTTCTCTTGGTGT-3' and R2: 5'-CTCCAAGTCCTCATTTGCT-3' for the T (Trp) allele. Genomic DNA (30 to 100 ng) was added to 25 μl of reaction medium with 0.15 mmol/l deoxynucleotide triphosphates, 25 pmol of each primer, 5 units AmpliTaq Gold, and 2.5 μl GeneAmp 10 × PCR buffer including 15 mmol/l MgCl2 (Perkin-Elmer, Foster City, CA). Amplification conditions were 10-minute initial denaturation at 95°C followed by 30 cycles of 1 minute at 95°C, 1 minute at 54°C, and 1 minute at 72°C and 5-minute final extension at 72°C. The amplified DNA was visualized on 2% agarose gel with ethidium bromide staining. Genotypes were distinguished as follows: a 200-bp band for the T allele, a 264-bp band for C allele, and a 427-bp common band.

Statistical analysis

The allelic frequencies were estimated by gene counting and genotypes were scored. The observed numbers of each *CYP17* and *CYP19* genotype were compared with those expected for a population in the Hardy-Weinberg equilibrium by using a χ^2 test. The significance of the differences of observed alleles and genotypes between groups was tested using the χ^2 analysis. P-values < 0.05 were considered to be significant.

Results

Based on the PCR analysis, all the patients and controls were divided into three genotypes of the *CYP17* gene promoter region: A1/A1, A1/A2 and A2/A2. Table I shows genotype distribution between breast cancer patients and controls. The Table shows that there were significant differences ($p < 0.05$) between two investigated groups. The frequencies of the A1 and A2 alleles were 0.32/0.68 in patients and 0.42/0.58 in controls. In patients, the observed frequencies of the A1/A1, A1/A2 and A2/A2 genotypes differed significantly ($p < 0.05$) from the distribution expected from the Hardy-Weinberg equilibrium.

Distributions of the T/T, T/C and C/C genotypes of *CYP19* gene as well as the frequencies of the T and C alleles for breast cancer subjects and controls are displayed in Table II. The Table shows that there were no significant differences between these two groups in both genotype distribution and allele frequencies ($p > 0.05$).

The dependence of the distribution of genotypes and frequencies of alleles of both investigated polymorphism on the tumour grade evaluated according to Scarf-Bloom-Richardson criteria in patients with breast cancer are displayed in Tables III and IV, respectively. There were no significant

Table I. Distribution of A1/A1, A1/A2 and A2/A2 genotypes and frequencies of the A1 and A2 alleles of *CYP17* polymorphism in patients with breast cancer (n = 100) and controls (n = 106)

	BREAST CANCER PATIENTS		CONTROLS	
	NUMBER	FREQUENCY	NUMBER	FREQUENCY
A1/A1	10	0.10	17	0.16
A1/A2	44	0.44	55	0.52
A2/A2	46	0.46	34	0.32
χ^2	7.406[a]		0.432	
A1 allele	64	0.32[b]	89	0.42
A2 allele	136	0.68	123	0.58

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

Table II. Distribution of T/T, T/C and C/C genotypes and frequencies of the T and C alleles of *CYP19* in patients with breast cancer (n = 100) and controls (n = 106)

	BREAST CANCER PATIENTS		CONTROLS	
	NUMBER	FREQUENCY	NUMBER	FREQUENCY
T/T	20	0.20	18	0.17
T/C	45	0.45	58	0.55
C/C	35	0.35	30	0.28
χ^2	0.653[a]		1.261	
T allele	85	0.42[b]	94	0.44
C allele	115	0.58	118	0.56

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

Table III. Dependency of genotypes and frequencies of the alleles of *CYP17* gene polymorphism on the tumour grade in patients with breast cancer[a]

GRADE[b]	I (N = 14)		II (N = 64)		III (N = 22)	
	NUMBER	FREQUENCY	NUMBER	FREQUENCY	NUMBER	FREQUENCY
A1/A1	2	0.14	21	0.32	7	0.32
A1/A2	9	0.64	23	0.35	7	0.32
A2/A2	3	0.21	20	0.31	8	0.36
χ^2	1.198[c]		5.088		2.899	
A1 allele	13	0.46	65	0.51	21	0.48
A2 allele	15	0.54	63	0.49	23	0.52

[a]n = 100; [b]according to Scarf-Bloom-Richardson criteria; [c]p > 0.05 as compared with Hardy-Weinberg distribution

Table IV. Dependency of genotypes and frequencies of the alleles of *CYP19* gene polymorphism on the tumour grade in patients with breast cancer[a]

GRADE[b]	I (N = 14)		II (N = 64)		III (N = 22)	
	NUMBER	FREQUENCY	NUMBER	FREQUENCY	NUMBER	FREQUENCY
T/T	1	0.07	18	0.28	3	0.14
T/C	10	0.71	16	0.25	14	0.64
C/C	3	0.21	30	0.46	5	0.23
χ^2	2.962[c]		15.032		1.772	
T allele	12	0.42	52	0.40	20	0.45
C allele	16	0.58	76	0.60	24	0.55

[a]n = 100; [b]according to Scarf-Bloom-Richardson criteria; [c]p > 0.05 as compared with Hardy-Weinberg distribution

differences between the distribution of genotypes in subgroups assigned to histological grades and the distribution predicted by the Hardy-Weinberg equilibrium ($p > 0.05$). There were no differences in frequencies of all alleles between subgroups, either ($p > 0.05$).

Discussion

A higher level of endogenous oestrogens is strongly associated with the risk of breast cancer, and the level of endogenous oestrogens is known to be regulated by the pathway of steroidogenesis in which many enzymes are involved [11]. Oestrogen biosynthesis is catalyzed by a microsomal member of the cytochrome P450 superfamily (CYP), namely aromatase; these are important for the production, bioavailability, and degradation of oestrogens [12].

Several genetic polymorphisms that may influence oestrogen concentrations have been identified in genes involved in oestrogen biosynthesis and oestrogen metabolism [13]. Polymorphisms in these genes have been associated with an increased hormone dependent cancer risk in some populations, but not in others [14, 15].

The location of the T→C polymorphism at the promoter of the *CYP17* gene indicated its possible role in the regulation of its expression at a transcriptional level. A polymorphism of T→C substitution in 5'-untranslated region (UTR) of the *CYP17* gene creates MspAI restriction site (denoted as A1/A2) and has been suggested to create a promoter motif to a transcription factor, Sp-1 (CCACC box) [16]. This polymorphism at +27 relative to the start of transcription, has a potential to enhance the promoter activity and production rate of CYP17 and eventually the levels of endogenous steroid hormones [17-19]. A recent study also found the polymorphism associated with higher levels of dehydroepiandrosterone (DHEA) in premenopausal women and higher levels of oestradiol in postmenopausal women [20, 21].

A few studies have found evidence for an association between this polymorphism and the risk of breast cancer [22-29]; these positive associations were observed for specific subgroups of cases defined by tumour aggressiveness, age at onset, or family history of breast cancer. Two recent meta-analyses [25] showed no overall association of breast cancer with the C (A2) variant, when comparing allele frequencies, or genotypes defined by these alleles under a dominant or recessive model. Results were consistently null in different ethnic groups [25].

Studies of *CYP 19* have focused on the variable number tandem repeats $(TTTA)_n$ in intron 4 of *CYP 19* [30, 31]. The CYP19 TTTA repeat polymorphism is associated with survival in premenopausal women, but not in postmenopausal women, with HR-positive breast cancers. Premenopausal women with the long allele have a greater survival rate and may not benefit from adjuvant chemotherapy [31]. A polymorphism (Trp/Arg) in codon 39 has been previously described and has been associated with the breast cancer risk among the Japanese [9, 32].

Because much knowledge has been gained in recent years on the prognostic values of the the cytochrome P450 superfamily in cancer progression, it is important to know whether polymorphic variants of the gene encoding this protein can be considered as markers of appearance and/or progression of breast cancer.

In the present work, a PCR-RFLP method was used to screen 100 breast cancer patients for the TT→CC of *CYP17* and Trp/Arg in codon 39 of the *CYP19* polymorphism.

We did not find any correlation between occurrence of cancers and the *CYP19* gene polymorphism in Polish women. However, we detected a significant difference in the distribution frequency of A1 and A2 alleles of the *CYP17* gene between patients and controls ($p < 0.05$). The distribution of the genotypes A1/A1, A1/A2 and A2/A2 in the patients differed from the one expected from the Hardy-Weinberg equilibrium, with an overrepresentation of A2/A2 homozygotes. It is possible that the presence of the A2 allele is in linkage disequilibrium with another, so far unknown, mutation located outside the coding region in the *CYP17* gene, which may be of importance for the CYP17 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 the lack of association between the TT→CC of *CYP17* polymorphism and breast cancer invasiveness.

Our study implies that the TT→CC polymorphism of *CYP17* gene may be associated with the occurrence of breast cancer in women from the Lodz/Łódź region of Poland. Further studies, conducted on a larger group, are required to clarify this point.

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