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The Polymorphisms of the *CYP17* and *CYP19* Genes in Endometrial Cancer Patients*

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Endogenous estrogen exposure is an important determinant of endometrial cancer risk. The *CYP 17* and *CYP 19* genes encode 17 hydroxylase/17,20-lyase and aromatase, respectively, both involved in sex hormone synthesis. The genes *CYP17* and *CYP19* are polymorphic and gene variability could contribute to the level of protein biosynthesis. In the present work the distribution of genotypes and frequency of alleles of the C/T polymorphism in promoter region of *CYP17* and G/A polymorphism at position Val^{80} in *CYP19* in subjects with endometrial cancer were investigated. Paraffin embedded tumor tissues were obtained from 100 women with endometrial cancer. DNA from normal endometrial tissue (n=106) served as control. The polymorphisms were determined by PCR-RFLP. The distribution of the genotypes of the C/T polymorphism of *CYP17* and G/A polymorphism of *CYP19* in both control and patients did not differ significantly ($p>0.05$) from those predicted by the Hardy-Weinberg distribution. There were no significant differences ($p>0.05$) in genotype distributions and allele frequencies between subgroups assigned to histological stage. The results suggest that the C/T polymorphism of *CYP17* gene as well as G/A polymorphism of *CYP19* may not be linked with appearance and development of endometrial cancer.

Introduction

The etiology of the endometrial carcinoma is not fully understood. The risk for endometrial cancer increases with late menopause, estrogen replacement therapy and obesity, and decreases with parity and oral contraceptive use; thus risk increases in proportion to the duration of exposure to estrogens unopposed by progestins, probably because unopposed estrogens stimulate endometrial cell division [8]. Estrogen replacement therapy (ERT), in which estrogen is given to postmenopausal women without progestins, was shown to increase endometrial cancer risk; progestins were increasingly added to ERT to reduce this risk.

The enzymes involved in the biosynthesis and metabolism of estrogens (CYP17, CYP19, CYP2D6, COMT, or CYP1A1) have been main targets in attempts to identify genetic polymorphisms contributing to endometrial cancer risk [6, 7].

Cytochrome P450c17 (*CYP17*) gene encodes an enzyme with both 17 hydroxylase and 17,20-lyase activities, the rate-limiting step in androgen biosynthesis [2]. 17 hydroxylase is responsible for hydroxylating pregnelone and progesterone, which are then converted to C19 steroid precursors of testosterone and estrogen by 17,20-lyase activity. 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). Of relevance to endometrial cancer risk, at least two studies have found that the C allele is associated with elevated levels of circulating estrogens in pre- and postmenopausal women [10, 11].

The *CYP19* gene, located on chromosome 15 encodes the enzyme P450 aromatase protein, which catalyzes three consecutive hydroxylation reaction converting C19 androgens to aromatic C18 estrogenic steroids. Aromatase is expressed in various tissues, including adipose, breast, and bone, where its activity influences local tissue concentrations of estrogens in a paracrine or intracrine fashion [7, 26].

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Increased expression of CYP19 in endometrial cancer, but not in normal endometria, suggests a role in promotion of neoplastic proliferation [6]. Changes in aromatase biosynthesis are usually preceded by changes in its gene transcription and mRNA level. Gene variability could contribute to the level of the aromatase biosynthesis. It was shown that a silent polymorphism (G/A at Val80) in exon 3 could be associated with progression of breast cancer [25] but little is known on possible role of the G/A polymorphism in endometrial cancer.

In the present work the distribution of genotypes and frequency of alleles of the *CYP17* and *CYP19* polymorphisms described above in subjects with endometrial cancer was investigated.

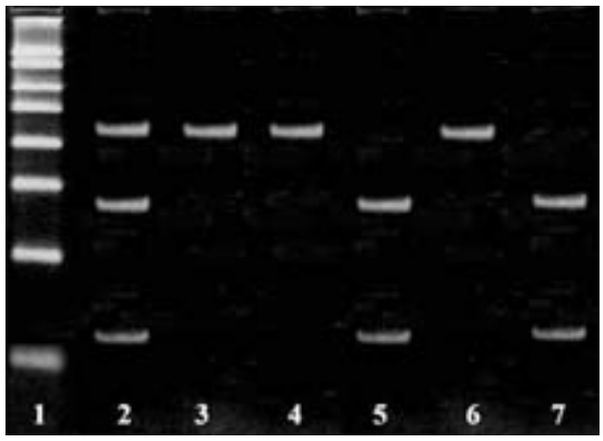

Fig. 1. *CYP 17* genotypes after MspA1 digestion. Lane 1 – molecular weight marker (100-bp ladder); lanes 2 – heterozygote TC; lanes 3, 4 and 6 – homozygote TT; lanes 5 and 7 – homozygote CC.

Material and Methods

Endometrial cancer samples

One hundred patients with histologically-proven diagnosis of endometrial cancer were included in the study (mean age ± SD 63.75 ± 4.72 years). Tumor tissues were obtained from postmenopausal women with endometrial adenocarcinoma treated at Department of Surgical Gynecology at the Institute of Polish Mother's Memorial Hospital in Łódź between 1998 and 2004. All tumors were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). There were 20 tumors of I stage, 45 of II stage and 35 of III stage in total. DNA from normal endometrial tissue (n=106) served as control.

The cancer tissue samples were fixed routinely in formalin and embedded in paraffin. Archival paraffin-embedded tumor sections on slides were deparaffinized in xylene and rehydrated in ethanol and distilled water. DNA was extracted using commercially available OIAmp Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to manufacturer's instruction.

Determination of CYP17 and CYP19 genotype

A 459-bp fragment of genomic DNA containing the T to C substitution at –34 bp in the *CYP17* gene was amplified by PCR [29]. 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*A1. After amplification, all samples were digested overnight with 5 U *Msp*A1. In subjects with the C allele, two smaller fragments of 335 and 124 bp were obtained (Fig. 1).

For determination of the G/A polymorphism at position Val^{80} in *CYP19*, a 188-bp PCR product was generated. The PCR was carried out in a 25- l reaction mixture containing 200 ng genomic DNA, 2.0 mM magnesium chloride, 250 M dNTPs, 0.5 M of each primer, and 0.5 U *Taq* DNA polymerase. Primer sequences were as follows: forward, 5'-AGTAACACAGAACAGTTGCA-3'; reverse, 5'-TCCAGACTCGCATGAATTCTCCGTA-3'. A mismatch (G instead of A) was introduced in the reverse primer to create a restriction site for the enzyme Rsa1. The presence of the G variant in *CYP19* resulted in digestion of the 188-bp amplicon to two smaller fragments of 164 and 24 bp. PCR products were electrophoresed in a 5% polyacrylamide gel (PAGE) and visualized by ethidium bromide staining. Only one 188-bp fragment was seen in subjects with the AA genotype. In subjects with the GA genotype, two bands of 188 and 164 bp were seen, whereas in those subjects homozy-

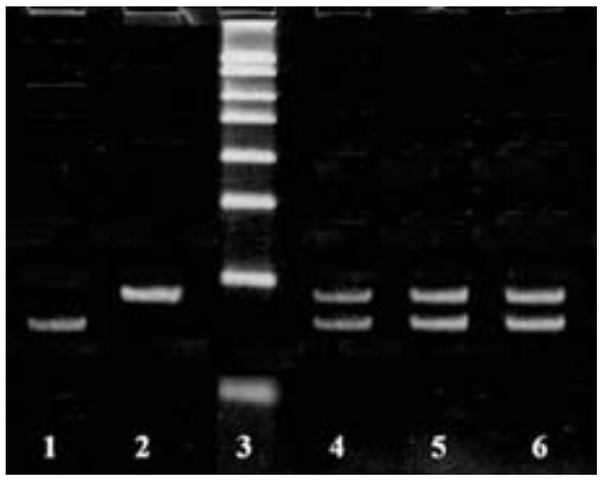

Fig. 2. *CYP 19* genotypes after Rsa1 digestion. Lane 1 – homozygote GG; lane 2 – homozygote AA; lane 3 – molecular weight marker; lanes 4–6 – heterozygote GA.

gous for the G variant (GG), only one 164-bp PCR fragment was seen (Fig. 2). All PCR was carried out in a DNA Thermal Cycler (GeneAmp PCR System 2400; Perkin-Elmer, Norwalk, CT, U.S.A.). After an initial denaturation at 95°C for 5 min, 35 cycles of amplification with denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 30 sec were performed, followed by a final extension step of 7 min at 72°C. The PCR product was digested overnight with 5 U Rsa1. In samples where the genotype was ambiguous, the PCR reaction was repeated, and the genotype verified again. Control samples with known genotypes were also run to check on the reproducibility of the digestion step.

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 that expected for a population in Hardy-Weinberg equilibrium by using a ^{2} test. The significance of the differences of observed alleles and genotypes between groups was tested using the ^{2} analysis. p values <0.05 were considered to be significant.

Results

From the PCR analysis, all the patients and controls were divided into three genotypes of the *CYP17* gene promoter region: T/T, T/C and C/C. Table 1 shows genotype distribution between endometrial adenocarcinoma patients and controls. Both distributions did not differ significantly (p>0.05) from those predicted by the Hardy-Weinberg equilibrium. Additionally, there were no differences in the frequencies of the T and C alleles between patients and controls.

Distributions of the A/A, G/A and G/G genotypes of *CYP19* gene as well as the frequencies of the G and A alleles

TABLE 1
Distribution of T/T, T/C and C/C genotypes and frequencies of the T and C alleles of *CYP17* polymorphism in patients with endometrial cancer (n=100) and controls (n=106)

	Endometrial cancer patients		Controls	
	Number	Frequency	Number	Frequency
T/T	31	0.31	21	0.20
T/C	40	0.40	48	0.45
C/C	29	0.29	37	0.35
^{2}	3.987[a]		0.591[a]	
T allele	102	0.51[b]	90	0.42
C allele	98	0.49[b]	122	0.58

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

TABLE 2
Distribution of G/G, G/A and A/A genotypes and frequencies of the G and A alleles of *CYP19* in patients with endometrial cancer (n=100) and controls (n=106)

	Endometrial cancer patients		Controls	
	Number	Frequency	Number	Frequency
G/G	28	0.28	24	0.23
G/A	42	0.42	44	0.42
A/A	30	0.30	38	0.36
^{2}	3.877[a]		0.572[a]	
G allele	98	0.49[b]	92	0.43
A allele	102	0.51[b]	120	0.57

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

TABLE 3
Dependency of genotypes and frequencies of the alleles of *CYP17* and *CYP19* gene polymorphism on the tumor stage in patients with endometrial cancer[a]

Stage[b]	I (n = 20)		II (n = 45)		III (n = 35)	
	Number	Frequency	Number	Frequency	Number	Frequency
T/T	5	0.25	10	0.22	9	0.26
T/C	10	0.50	20	0.44	16	0.46
C/C	5	0.25	15	0.34	10	0.28
[2]	0.360[c]		0.222[c]		0.886[c]	
T allele	20	0.50	40	0.44	34	0.48
C allele	20	0.50	50	0.56	36	0.52
	Number	Frequency	Number	Frequency	Number	Frequency
G/G	4	0.20	8	0.18	9	0.26
G/A	11	0.55	23	0.51	19	0.54
A/A	5	0.25	14	0.31	7	0.20
[2]	0.357[c]		0.872[c]		0.222[c]	
G allele	19	0.47	39	0.43	37	0.53
A allele	21	0.53	51	0.57	33	0.47

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

for endometrial cancer subjects and controls are displayed in Table 2. It can be seen from the Table that there were no significant differences between these two groups in both genotype distribution and allele frequencies ($p>0.05$).

Dependencies of the distribution of genotypes and frequencies of alleles of both investigated polymorphism on the tumor stage evaluated according to FIGO criteria of patients with endometrial cancer are displayed in Table 3. There were no significant differences between distributions of genotypes in subgroups assigned to histological stage and the distribution predicted by Hardy-Weinberg equilibrium ($p>0.05$). There were no differences in frequencies of the all alleles between subgroups either ($p>0.05$).

Discussion

Sex hormones play a major role in the etiology of several of the commonest cancers, including cancers of the endometrium, breast and ovary in women and cancer of the prostate in men [17]. Estrogens 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 estrogens [15].

Several genetic polymorphism that may influence estrogen concentrations have been identified in genes involved in estrogen biosynthesis and estrogen metabolism [24]. Polymorphism in these genes has been associated with increased hormone dependent cancer risk in some populations, but not others [18, 20]. The goal of this study was to investigate the association between *CYP17* and *CYP19* polymorphism and endometrial cancer progression.

The location of C/T polymorphism at the promoter of the *CYP17* gene indicated their possible role in the regulation of its expression at transcriptional level. The C allele creates an additional Sp1-type (CCACC box) promoter site, and although it was initially suggested to increase expression of the gene [11], a subsequent study did not observe binding to the human transcription factor Sp-1 [23]. There is conflicting evidence indicating that the *CYP17* –34T C polymorphism might influence endogenous steroid hormone levels [9, 12, 22], and the CC genotype has also been reported to be associated with the relative abundance of the 2OHE and 16 OHE forms of estrogen [16]. A recent study also found the polymorphism associated with higher levels of DHEAS in premenopausal women and higher levels of estradiol in postmenopausal women [14].

A few studies have found evidence for an association between this polymorphism and risk of breast cancer [4, 21, 27]. These positive associations were observed for specific subgroups of cases defined by tumor aggressiveness, age at onset, or family history of breast cancer. Two recent meta-analyses [28] showed no overall association of breast cancer with the C (A2) variant, when comparing allele fre-

quencies, or genotypes defined by these alleles under a dominant or recessive model. Results were consistently the null in different ethnic groups [28].

Studies of *CYP19* have focused on the variable number tandem repeats $(TTTA)_n$ in intron 4 of *CYP19* [3]. The $(TTTA)_n$ repeat polymorphism in intron 4 of *CYP19* may be to favor appearance of endometrium cancer throughout a woman's life [6]. The C to T substitution in exon 7 of *CYP19* results in a single amino acid substitution from Arg by Cys at codon 264. Bio-available estrogen was lower in carriers than in non-carriers of the Arg264Cys polymorphism [13]. A silent polymorphism (G/A at Val^{80}) in exon 3 has been previously described and has been associated with breast cancer risk [25].

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

In present work conducted on 100 endometrial adenocarcinoma patients we did not find any correlation between occurrence of cancers and *CYP17* and *CYP19* gene polymorphism. There were no significant differences in alleles frequencies between women with endometrial cancer and controls. The distribution of the genotypes in the patient and in controls did not differ from the distribution that would be expected from the Hardy-Weinberg equilibrium. Moreover, we did not detect any significant difference between genotypes in subgroups assigned to histological stages, what suggests a lack of association between polymorphism and cancers invasiveness.

Our study implies that it is possible that the polymorphism of the *CYP17* and *CYP19* gene may not be directly associated with appearance and development of endometrial cancer but further research, conducted on larger population, are needed to clarify this point.

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