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RAPID COMMUNICATION

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CYP19 gene polymorphism in endometrial cancer patients

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Abstract ***Purpose:*** Initiation/promotion of endometrial cancer is known to be associated with estrogenic influence. Therefore, it is possible that some allelic polymorphisms of the genes involved in steroidogenesis or steroid metabolism contribute to endometrial cancer susceptibility. ***Methods:*** Here, we compared CYP19 (aromatase) gene polymorphism in 85 endometrial cancer patients and in 110 non-affected women. ***Results:*** The genotypes containing the longest alleles (A6 and A7) of CYP19 were found to be over-represented in patients as compared to controls. In addition, these genotypes demonstrated a tendency to be associated with increased concentrations of estradiol and testosterone in postmenopausal patients. ***Conclusions:*** Thus, CYP19 polymorphism might be one of the genetic risk factors for endometrial cancer development.

Key words CYP19 · Polymorphism · Endometrial cancer

Introduction

Existing evidence suggests that excessive estrogen stimulation is involved in the initiation/promotion stages of endometrial cancer (Henderson et al. 1997). Notwithstanding, its precise (gonadal or extragonadal) source, endogenous production of estrogens occurs due to a series of biochemical reactions. Their final and rate-limiting step is catalyzed by aromatase belonging to the XIX class of cytochrome P450 (CYP19) (Miller 1996; Berstein 1998). It was demonstrated recently that the CYP19 gene may function as a low-penetrance gene in breast cancer genetic susceptibility. In particular, alleles containing a high number of intronic TTTA repeats were found to be over-represented in breast cancer patients as compared to controls (Nedelcheva Kristensen et al. 1998; Haiman et al. 1999). However, contradictory data have been reported as well (Probst-Hensch et al. 1999; Healey et al. 2000).

The picture of CYP19 expression is somewhat distinct in breast and endometrial tissues. In particular, CYP19 is transcribed both in normal and transformed mammary cells, whereas its expression is clearly tumor-specific for endometrium. Certain differences exist between estrogen dependence in endometrial and breast cancer as well. Breast tumors are considered more sensitive to the effect of aromatase inhibitors than endometrial adenocarcinomas, although estrogen-replacement therapy in menopausal women is a much more important risk factor for endometrial than for breast cancer (Bulun et al. 1994; Miller 1996; Berstein 1998; Santen 1998). These facts and the above-mentioned evidence prompted us to evaluate the peculiarities of CYP19 allele distribution in endometrial cancer patients.

Materials and methods

Patients and control individuals

The endometrial cancer group included 85 patients treated at the N.N. Petrov Institute of Oncology (St. Petersburg). The age of the patients varied from 39 to 74 years and their average age was 57.3 ± 7.4 years (M ± σ). Twenty-three patients were characterized by a preserved menstrual cycle and 62 were in menopause with a duration of not less than 12 months. The group of control individuals consisted of 110 persons with no oncological or any other serious chronic or acute disease. The age of these women varied from 24 to 88 years and their average age equaled 62.2 ± 12.6 years (M ± σ). Fifty-one of the control individuals were in the reproductive period (aged from 24 to 43 years) and others ($n = 59$) were

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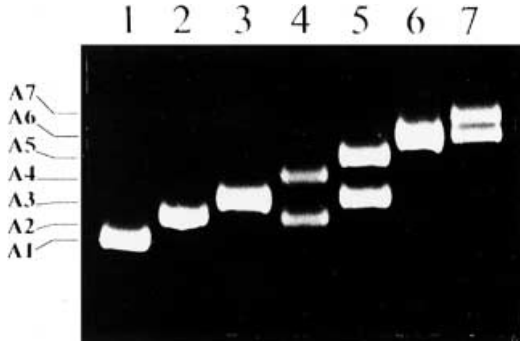

Fig. 1 Examples of CYP19 genotypes. Alleles are designated at the left. *Line 1* – genotype A1/A1; *2* – A2/A2; *3* – A3/A3; *4* – A2/A4; *5* – A3/A5; *6* – A6/A6; *7* – A6/A7

in late postmenopause (age 61–88 years). All the procedures related to group recruitment was carried out in accordance with the requirements of the Ethical Committee of the Institute.

DNA isolation

Blood leukocytes were used as a source of normal DNA. DNA was extracted from the blood samples by a modified salt-chloroform procedure (Mullenbach et al. 1989).

CYP19 PCR genotyping

The following primers were used: 5′-GGT ACT TAG TTA GCT ACA ATC-3′ (upstream, nucleotides 610–630) and 5′-GGG TGA TAG AGT CAG AGC CT-3′ (downstream, nucleotides 721–740). The choice of these sequences, based on the knowledge of aromatase gene structure (Means et al. 1989; GeneBank accession number M30798), was predetermined with the intention of minimizing the size of the amplified polymorphic fragment. Ten-microliter PCR reactions contained 1 μl purified DNA (50–100 ng/μl) or DNA-containing tissue lysate, 0.5 units heat-activated MDP-1 thermostabile polymerase (Your, Moscow), 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 1 μM of each primer, and 2% Triton X-100. The addition of the Triton X-100 turned out to be absolutely critical for the success of PCR amplification. The latter was carried out for 30 cycles, using the following regimens: 95 °C, 35 s – denaturation; 55 °C, 1 min – annealing; 72 °C, 1 min – synthesis. The products were separated in the 15% polyacrylamide (30:1) non-denaturing gel by applying the double running distance in the 20 cm vertical gel apparatus (Helikon, Moscow), and visualized by ethidium bromide staining. This approach allowed the separation of seven CYP19 alleles of different sizes starting from the shortest (A1, 123 bp) to the longest (A7, 146 bp) ones (Fig. 1).

Estradiol and testosterone determination

Measurements of the estrogenic hormone, estradiol, and its androgenic precursor, testosterone, in the blood serum of endometrial cancer patients were performed with the specific commercial radioimmunoassay kits (Beloris, Minsk, Belarus). Blood was taken from the cubital vein in the early morning hours after night fasting. Intraassay coefficients of variation for estradiol and testosterone concentrations were 4.8% and 6.2%, respectively.

Statistical evaluation of the data

Differences between the studied groups were evaluated by Student's *t*-test with the help of Statistica for Windows (version 6.0) software.

Results

Several differences were revealed in the distribution of polymorphic genotypes of the CYP19 (aromatase) gene between endometrial cancer patients and healthy women. In particular, the longest (A6 or A7) alleles, as well as the genotypes containing these variants, were somewhat overrepresented in affected females. On the other hand, an excess of the A2 allele ($P = 0.129$) and A2A2 genotype ($P = 0.028$) was characteristic for control individuals, primarily because of the prevalence of these stigmata in healthy women of reproductive age (Tables 1 and 2). Moreover, the total incidence of the genotypes containing only the shortest alleles (A1A1, A1A2, and A2A2) was significantly higher in healthy women (27.3%) than in endometrial cancer patients (13%, $P = 0.022$). No difference was revealed between groups of endometrial cancer patients and control individuals regarding the rather rare alleles/genotypes A3 or A4.

The prevalence of A6-containing genotypes was higher in premenopausal patients (82.6%) than in menopausal ones (58.1%, $P = 0.034$). On the other hand, in the case of A7-containing genotypes, their incidence was higher in postmenopausal patients (11.3 ± 3.9%), than in premenopausal ones (Table 2), but this difference was not statistically significant ($P = 0.089$). The difference in estradiol and testosterone blood concentrations between postmenopausal endometrial cancer patients with A6 and/or A7 genotypes and with other CYP19 genotypes did not reach statistical significance either ($P > 0.2$), although the tendency to higher hormonal concentrations in the first group was demonstrated (94.4 ± 29.2 pM/l vs 52.9 ± 8.8 pM/l of estradiol and 0.95 ± 0.11 nM/l vs 0.73 ± 0.05 nM/l of testosterone). In the premenopausal group of cancer patients, such data could not be compared because of the small number of hormonal measurements in women with genotypes other than A6 and/or A7.

Discussion

According to our observations, one can conclude that individuals carrying the A6 and/or A7 alleles of CYP19 or not carrying the A2 genotype may have an increased risk of developing endometrial cancer (Tables 1 and 2). These results correspond to some data obtained on breast cancer patients (Nedelcheva Kristensen et al. 1998). It should be mentioned, however, that our postmenopausal group of healthy women was somewhat older than the group of postmenopausal endometrial cancer patients.

Although not statistically significant, the tendency to increased estradiol and testosterone concentrations in A6 and A7 carriers deserves attention. This association could hint at the possible mechanisms of the contribution of CYP19 polymorphisms to endometrial cancer

Table 1 Number and frequency (in %) of CYP19 alleles in endometrial cancer patients (EC) and healthy women. (*preMP* in reproductive age, *postMP* postmenopausal (in the case of healthy women – late postmenopausal)

Group	Number, %	Alleles						
		A1	A2	A3	A4	A5	A6	A7
Healthy (total, $n = 220$)	N	63	44	35	1	8	67	2
	%	28.6	20.0	15.9	0.5	3.6	30.5	0.9
EC (total, $n = 170$)	N	43	24	25	1	4	64	9
	%	25.3	14.1	14.7	0.6	2.4	37.6	5.3
P			$P = 0.129$				$P = 0.136$	$P = 0.01$
Healthy (preMP, $n = 102$)	N	20	32	11	1	3	35	0
	%	19.6	31.3	10.8	1.0	2.9	34.4	0
EC (preMP, $n = 46$)	N	11	3	8	1	0	22	1
	%	23.9	6.5	17.5	2.1	0	47.8	2.1
P			$P = 0.001$				$P = 0.119$	
Healthy (postMP, $n = 118$)	N	43	12	24	0	5	32	2
	%	36.4	10.2	20.3	0	4.3	27.1	1.7
EC (postMP, $n = 124$)	N	32	21	17	0	4	42	8
	%	25.8	16.9	13.7	0	3.2	33.9	6.5
P		$P = 0.074$						$P = 0.064$

Table 2 Distribution of CYP19 polymorphic genotypes among the women studied. Data are presented in absolute numbers and in % (in parentheses). (*HW* healthy women, *EC* endometrial cancer patients)

Genotypes	Total group		Premenopausals		Postmenopausals	
	HW(110)	EC (85)	HW (51)	EC (23)	HW (59)	EC (62)
A1	13 (11.8)	5 (5.9)	3 (5.9)	0	10 (16.9)	5 (8.1)
A2	9 (8.2)	1 (1.2)*	8 (15.7)	0*	1 (1.7)	1 (1.6)
A3	2 (1.8)	0	1 (2.0)	0	1 (1.7)	0
A6	13 (11.8)	9 (10.5)	6 (11.8)	3 (13.1)	7 (11.9)	6 (9.7)
A7	0	1 (1.2)	0	0	0	1 (1.6)
A1A2	8 (7.3)	5 (5.9)	3 (5.9)	0	5 (8.5)	5 (8.1)
A1A3	9 (8.2)	9 (10.5)	2 (3.9)	3 (13.1)	7 (11.9)	6 (9.7)
A1A4	0	1 (1.2)	0	1 (4.3)	0	0
A1A5	4 (3.6)	1 (1.2)	2 (3.9)	0	2 (3.4)	1 (1.6)
A1A6	15 (13.6)	17 (20.0)	7 (13.7)	7 (30.4)	8 (13.5)	10 (16.1)
A1A7	1 (0.9)	0	0	0	1 (1.7)	0
A2A3	6 (5.6)	3 (3.5)	2 (3.9)	0	4 (6.8)	3 (4.8)
A2A5	1 (0.9)	0	0	0	1 (1.7)	0
A2A6	11 (10.0)	13 (15.3)	10 (19.6)	3 (13.1)	1 (1.7)	10 (16.1)*
A2A7	0	1 (1.2)	0	0	0	1 (1.6)
A3A5	2 (1.8)	0	0	0	2 (3.4)	0
A3A6	14 (12.7)	12 (14.1)	6 (11.8)	5 (21.7)	8 (13.5)	7 (11.2)
A3A7	0	1 (1.2)	0	0	0	1 (1.6)
A4A5	1 (0.9)	0	1 (2.0)	0	0	0
A5A6	0	1 (1.2)	0	0	0	1 (1.6)
A5A7	0	2 (2.4)	0	0	0	2 (3.3)
A6A7	1 (0.9)	3 (3.5)	0	1 (4.3)	1 (1.7)	2 (3.3)
All genotypes with A6	54 (49.1)	55 (64.7)*	29 (56.7)	19 (82.6)*	25 (42.4)	36 (58.1)
All genotypes with A7	2 (1.8)	8 (9.4)*	0	1 (4.3)	2 (3.4)	7 (11.3)
All genotypes with A6 or A7	55 (50.0)	60 (70.6)*	29 (56.7)	19 (82.6)*	26 (44.1)	41 (66.1)*

* Difference with data in healthy women in corresponding group is significant (P at least < 0.05)

risk. Naturally, the serum concentration of estradiol and its precursor, testosterone, may not directly reflect the rate of estrogen production in the patients, and the relevant picture appears to be much more complex. Thus, in affected women, the presence of the tumor itself may influence the peripheral concentration of steroid hormones; and appropriate measurements in postmenopausal women are not always informative, since the differences in hormonal level are often more pronounced in the earlier life periods (Henderson et al. 1997). This can partly explain the lack of a statistically significant difference between estradiol and testosterone

concentrations in endometrial cancer patients with different CYP19 genotypes. On the other hand, estrone, but not estradiol, production is more characteristic for the postmenopausal state (Miller 1996; Santen 1998), and thus evaluation of estrone blood levels, as well as the blood levels of its precursor androstenedione, may be more important for the results of gene polymorphism studies, especially because androstenedione concentration is considered to be one of the endometrial cancer risk factors (Potischman et al. 1996). Finally, the process of estrogen formation in endometrial carcinoma tissue – which differs regarding this characteristic from normal endometrium (Bulun et al. 1994) – should be taken into consideration too.

Further studies are required to clarify the impact of CYP19 polymorphism in endometrial cancer susceptibility. It would be of interest to examine whether CYP19 allele distribution is related to some relevant clinicomorphological and biochemical variables, with special emphasis on the aromatase activity, estrogen content, and the presence of steroid receptors in endometrial tumor tissue, the body weight of patients, their smoking habit, etc. Other polymorphisms of the genes involved in steroid metabolism must be taken into consideration. The most promising candidates include CYP17 and catechol-O-methyltransferase (COMT), which, according to some published data (Feigelson et al. 1997; Helzlsouer et al. 1998; Thompson et al. 1998; Huang et al. 1999), could modify susceptibility to breast cancer development.

So far, prospective oncoepidemiological studies directly related to endogenous estrogen production have provided limited information concerning endometrial cancer risk. This report reveals an additional parameter, CYP19 polymorphism, which seems to be promising for research in this field.

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