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CYP1A1 alleles in female genital cancers in the Polish population

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Abstract

Objective: Cytochrome P4501A1 (CYP1A1) plays an important role in the bioactivation processes that transform aromatic hydrocarbons into ultimate carcinogens. Genetically determined differences in activity of this enzyme could modulate individual susceptibility to develop cancers. The role of CYP1A1 in metabolic pathway of estrogens suggests an influence on carcinogenic events in genital tissues. The aim of our study was to elucidate the possible role of CYP1A1 alleles in the pathogenesis of endometrial and ovarian cancers.
Study design: We have compared *CYP1A1* genotype frequency between genital (endometrial and ovarian) cancer groups and 212 healthy women. Cancer patients were stratified using FIGO classification and diagnoses were confirmed histopathologically. The analysis of *CYP1A1* genotypes was performed using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) assay.
Results: We have observed higher frequency of heterozygotic genotypes containing mutation *m4* (*CYP1A1*1/*4*) in cancer groups (5.1% in ovarian and 5.6% in endometrial cancer versus 1.9% in controls).
Conclusion: The higher frequency of mutated *CYP1A1*4* allele connected with lower frequency of *CYP1A1*2A* and *CYP1A1*2B* in endometrial and ovarian cancer groups indicates that differences in the metabolic activity of CYP1A1 could play a significant role in the pathogenesis of genital cancers.

Keywords: Ovarian cancer; Endometrial cancer; Polymorphism; Cytochrome P4501A1; *CYP1A1*

1. Introduction

In humans, the complex of cytochrome P450 enzymes (metalloproteins) contains more than 15 different enzymes, which play a role in drug, steroid hormone and procarcinogen metabolism. Cytochrome P4501A1 (*CYP1A1*) belongs to this complex and is involved in the first phase of biotransformation [1]. *CYP1A1* oxygenates polycyclic aromatic hydrocarbons (PAH), including many procarcinogens (e.g. benzo(a)pyrene), to their final water-soluble derivatives with carcinogenic activity. The strong *CYP1A1* inducers are dioxins and the most common of them is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Dioxins bind to an intracellular aryl hydrocarbon receptor (AhR), inducing mRNA transcription followed by activation of xenobiotic-metabolising enzymes [2]. Additionally, the role of *CYP1A1* in metabolic pathway of estrogens could be important for the outcome of carcinogenic events in genital tissues [3,4].

In the human *CYP1A1* gene, four base substitutions are known (see Table 1). Mutation *m2* exchanges isoleucine 462 for valine; the neighboring *m4* exchanges threonine 461 for asparagine [5]. Mutations *m1* and *m3* are located in the 3′-flanking region. *m3* has been detected only in Africans [6]. Functional consequences of *m1* and *m2* have been presented as an increase in catalytic activity and a higher extent of inducibility, as compared to wild type [7,8]. In molecular–epidemiological studies, mutations *m1* and *m2* were shown

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Table 1
The alleles of *CYP1A1* gene

Allele	Mutation position			
	4887 (*m4*)	4889 (*m2*)	5639 (*m3*)	6235 (*m1*)
*CYP1A1**1 (wild type)	C	A	T	T
*CYP1A1**2A	C	A	T	C
*CYP1A1**2B	C	G	T	C
*CYP1A1**3	C	A	C	T
*CYP1A1**4	A	A	T	T

to increase the risk for lung cancer in their carriers [9,10]. High benzo(a)pyrene diol-epoxide DNA-adducts levels in blood cells occurred in coke oven workers who were carriers of the *m1* mutation [7,9,10].

Little is known about the activity of carcinogen-metabolising enzymes in the etiology of urogenital cancers. Recently, high levels of transcripts of dioxin-induced gene, *CYP1A1*, were found in endometriotic tissues [11,12], and also activity of *CYP1A1* was detected [13]; we could assume that *CYP1A1* is also detectable in ovarian tissue. On the other hand, there are clear evidences that *CYP1A1* genotypes are responsible for different phenotypic induction level in different tissues [2]; we suppose that a similar reaction on different genotypes could occur in endometrial and ovarian tissues. Moreover, recently Schwartz et al. [8] have shown that *CYP1A1* variants could result in different catalytic activity towards estrogens, which are also one of possible risk factors for urogenital cancers.

Since the activity of *CYP1A1* in genital tissues could induce chemical carcinogenesis and influence estrogen metabolism, we can assume that the presence of mutated *CYP1A1* variants could be responsible for different inter-individual susceptibility to genital cancer. Thus, the purpose of our investigation was to elucidate the possible role of *CYP1A1* alleles in the pathogenesis of endometrial and ovarian cancers.

2. Materials and methods

2.1. Patients

We have analysed 71 women with endometrial cancer (age range 44–80 years, mean 64.0 ± 8.7 years) and 39 women with ovarian cancer (age range 30–78 years, mean 52.0 ± 10.7 years). The patients were categorized using FIGO classification and both cancers were confirmed histopathologically as epithelial ovarian cancers and endometrioid adenocarcinomas. A detailed medical history about each patient was collected. Control group consisted of 212 healthy women (age range 35–71 years, mean 52.5 ± 9.1 years), collected from the same area. The patients from both cancer groups and controls were collected between March 2000 and May 2001. All subjects were Caucasians of Polish origin. Before the investigation, the agreement of Ethical Committee of the University of Medical Sciences in Poznań was obtained. All women were informed about the aim of the study and have given their written consents.

2.2. DNA analysis

From each patient, venous blood sample was collected (3–4 ml). Genomic DNA was extracted from peripheral leucocytes by phenol–chloroform method and stored at 4 °C before further analysis. *CYP1A1* mutations were determined by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) assay as previously described [5,9,10].

2.3. Analysis of m1 and m3 point mutations

The presence of *m1* (T6325C) and *m3* (T5639C) mutations was investigated in PCR-amplified 898-bp fragment (primers M3F 5′ GGCTGAGCAATCTGACCCTA and P80 5′ TAGGAGTCTTGTCTCATGCCT: all primers synthesized by Tib Molbiol, Poznan, Poland) by digestion with restriction enzyme *Msp*I (New England Biolabs, Schalbach, Germany). PCR was performed in total volume of 50 μl containing 0.2 μmol/L of each primer, 0.2 mmol/L of each of the four dNTP at 5 μl, 4.8 μl of 25 mM $MgCl_2$, 5 μl of 10× PCR Buffer (supported with *Taq*-DNA-polymerase), one unit of *Taq*-DNA-polymerase (Gene Craft, Rapidozym, Berlin, Germany). Before amplification 1 μl of genomic DNA was added. PCR amplifications were carried out in ABI 9600 or 9700 (ABI, Weiterstadt, Germany) thermocyclers in the following conditions: initial denaturation at 94 °C for 2 min, followed by 35 cycles of annealing (63 °C) for 0.5 min, elongation (72 °C) for 1 min, and denaturation (94 °C) for 0.5 min. PCR process was finished by final elongation for 7 min at 72 °C. PCR products (898-bp) were digested with *Msp*I [8].

2.4. Analysis of m2 and m4 point mutations

The presence of *m2* (A4889G) and *m4* (C4887A) was investigated by amplification of genomic DNA with primers M2F 5′ CTGTCTCCCTCTGGTTACAGGAAGC and M2R 5′ TTCCACCCGTTGCAGCAGGATAGCC in above described conditions [9]. The PCR product (204-bp) was digested separately with restriction enzymes *Bsr*DI or *Bsa*I (New England Biolabs, Schalbach, Germany) for *m2* and *m4*, respectively.

2.5. Allocation of m1 and m2 to alleles

In case of occurrence of two different mutations in one patient, peptide nucleic acid (PNA) mediated specific PCR clamping was applied to locate the mutation to alleles [10].

2.6. Statistical analysis

Statistical analysis was performed with SPSS version 10.0. Results were considered as statistically significant at