Studies on *CYP1A1*, *CYP1B1* and *CYP3A4* gene polymorphisms in breast cancer patients

Badania polimorfizmów genów *CYP1A1, CYP1B1* i *CYP3A4* u chorych z rakiem piersi

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Abstract

Background: *The role of CYP1A1, CYP1B1 and CYP3A4 polymorphism in pathogenesis of breast cancer has not been fully elucidated. From three CYP1A1 polymorphisms *2A (3801T>C), *2C (2455A>G), and *2B variant, which harbors both polymorphisms, the *2A variant is potentially carcinogenic in African Americans and the Taiwanese, but not in Caucasians, and the CYP1B1*2 (355G>T) and CYP1B1*3 (4326C>G) variants might increase breast cancer risk. Although no association of any CYP3A4 polymorphisms and breast cancer has been documented, the CYP3A4*1B (392A>G) variant, correlates with earlier menarche and endometrial cancer secondary to tamoxifen therapy.*

Objective: *The present study was designed to investigate the frequency of CYP1A1, CYP1B1 and CYP3A4 polymorphisms in a sample of breast cancer patients from the Polish population and to correlate the results with the clinical and laboratory findings.*

Material and methods: *The frequencies of CYP1A1*2A; CYP1A1*2C; CYP1B1*3; CYP3A4*1B CYP3A4*2 polymorphisms were determined in 71 patients aged 36-87, with primary breast cancer and 100 healthy individuals. Genomic DNA was extracted from the tumor, and individual gene fragments were PCR-amplified. The polymorphisms were determined by RFLP and were correlated with the patients' TNM stage, grade, estrogen and progesterone receptor status as well as the level of c-erbB-2 protein.*

Results: *CYP1A1 polymorphisms were more frequent in younger patients and in the patients with high level of c erbB 2 protein. No correlation between these polymorphisms and the cancer stage or grade, as well as the receptor status was demonstrated.*

Conclusions: *CYP1A1 polymorphisms probably predispose to an earlier onset of breast cancer and might be associated with higher c-erbB-2 protein level, but further studies on a much larger group are required to substantiate our findings.*

Key words: **breast cancer** / ***CYP1A1*** / ***CYP1B1*** / ***CYP3A4*** / **genetic** / **polymorphisms** /

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Streszczenie

Wstęp: *Rola polimorfizmów genów CYP1A1, CYP1B1 oraz CYP3A4 w patogenezie raka piersi nie została w pełni wyjaśniona. Spośród trzech polimorfizmów CYP1A1*2A (3801T>C), *2C (2455A>G), oraz *2B, który zawiera oba warianty, tylko wariant *2A jest potencjalnie rakotwórczy u afro-amerykanów i mieszkańców Tajwanu, ale nie u rasy kaukaskiej, natomiast polimorfizmy CYP1B1*2 (355G>T) i CYP1B1*3 (4326C>G) mogą zwiększać ryzyko rozwoju raka piersi. Chociaż nie stwierdzono związku żadnego z polimorfizmów CYP3A z rakiem piersi, dowiedziono, że wariant CYP3A4*1B (392A>G) współistnieje z wcześniejszym występowaniem miesiączki oraz rakiem endometrium w następstwie leczenia tamoksyfenem.*

Cel: *Celem badań było oznaczenie częstości występowania polimorfizmów CYP1A1, CYP1B1 i CYP3A4 w grupie chorych z rakiem piersi z populacji polskiej oraz poszukiwanie korelacji z wynikami badań klinicznych i laboratoryjnych.*

Materiał i metody: *Częstości polimorfizmów CYP1A1*2A; CYP1A1*2C; CYP1B1*3; CYP3A4*1B i CYP3A4*2 oznaczono u 71 chorych (w wieku 36-87 lat) oraz 100 zdrowych kobiet. Genomowy DNA wyekstrahowano z tkanki guza i fragmenty poszczególnych genów amplifikowano za pomocą PCR. Polimorfizmy wykrywano techniką RFLP i korelowano ich występowanie ze stopniem zaawansowania klinicznego i histologicznego nowotworu, obecnością receptorów estrogenów i progesteronu jak również poziomem białka c-erbB-2.*

Wyniki: *Polimorfizmy CYP1A1 częściej występowały u młodszych chorych oraz u chorych z wysokim poziomem białka c-erbB-2. Nie wykazano korelacji pomiędzy obecnością polimorfizmów a stopniem zaawansowania nowotworu czy obecnością receptorów.*

Wnioski: *Polimorfizmy CYP1A1 przypuszczalnie predysponują do wcześniejszego występowania raka piersi i mogą wiązać się z podwyższeniem poziomu białka c-erbB-2, lecz potwierdzenie tych spostrzeżeń wymaga dalszych badań w większych grupach pacjentów.*

Słowa kluczowe: **rak piersi** / ***CYP1A1*** / ***CYP1B1*** / ***CYP3A4*** / **polimorfizmy genetyczne** /

Introduction

The *CYP1A1, CYP1B1* and *CYP3A4* gene products, cytochromes P4501A1, P4501B1 and P4503A4, play an important role in the metabolism of both natural and environmental estrogens. Numerous polymorphisms of these genes have been described, but it is not clear whether they affect the enzymatic activity of cytochromes or correlate with breast cancer. Several *CYP1A1* polymorphisms were described. The *CYP1A1*2A* (3801T>C) is localized in the region encoding the 3'UTR and the *CYP1A1*2C* variant (2455A>G), situated in exon 7, causes Ile462Val substitution in the heme-binding domain of cytochrome P450 1A1, while *CYP1A1*2B* variant harbors both polymorphisms [1, 2]. It has been shown, that *CYP1A1*2A* is associated with increased risk of breast cancer in African Americans and the Taiwanese [3-5], but not in Caucasians [6].

The *CYP1B1*2* (355G>T) variant, causing Ala119Ser change and *CYP1B1*3* (4326C>G) causing Leu432Val substitution [7], result in increased 4-hydroxylation of E1 [8, 9] and hence might increase the risk of breast cancer [10-12].

It is not known whether the *CYP3A4*2* (15713T>C) polymorphism localized in exon 7, causing Ser222Pro substitution, and *CYP3A4**3 variant (1437T>C) situated in exon 12, resulting in Met455Thr substitution, affect the 16-hydroxylase activity [13]. To date, no association of any *CYP3A4* polymorphisms with breast cancer has been documented [14]. However, *CYP3A4*1B* (392A>G) variant, localized within the nifedipine response element, coincided with early menarche [15] and endometrial cancer secondary to tamoxifen therapy [16].

Present study was designed to investigate the frequency of *CYP1A1*, *CYP1B1* and *CYP3A4* polymorphisms in breast cancer patients in the sample of Polish population and to associate these polymorphisms with the onset of the disease as well as the cancer stage, grade and the receptor status.

Material and methods

Patients

In this study, 71 patients (aged 36-87, with a medium age of 58.1) with primary breast cancer diagnosed in the Department of Clinical Oncology at Poznan University of Medical Sciences, were enrolled. Clinical characteristics, including TNM stage, grade, expression of estrogen and progesterone receptors in tumor tissue and family history, were obtained from medical records. Samples of breast cancer tissue ($1cm^3$) were collected from every patient and frozen for further analysis. A 100 pregnant patients (aged 23-42, with a medium age of 28.2) from the Department of Gynecology and Obstetrics at Poznan University of Medical Sciences, devoid of any symptoms of breast cancer, served as the control group. The local Ethics Committee approved the project, and a written consent from each patient was obtained.

Methods

The DNA was isolated from breast cancer tissue, while peripheral blood DNA was isolated from the control subjects. Amplification of individual gene fragments was conducted by PCR with the use of specific primers as previously described [6, 13, 17-19]. The polymorphisms were detected by RFLP analysis with the use of the appropriate restriction endonucleases (MspI for *CYP1A1*2A*; NcoI for *CYP1A1*2C*; Eco57I for *CYP1B1*3*; MboII for *CYP3A4*1B* and XcmI for *CYP3A4*2*). The levels of ER and PR proteins in breast cancer tissue were evaluated by immunohistochemistry with the use of monoclonal antibodies (DakoCytomation, Denmark), while C-erbB2 protein level was estimated with the use of HercepTest (DakoCytomation, Denmark) according to the producer's instructions.

The frequencies of genotypes were established with the use of Arlequin 2.001 software, while Hardy-Weinberg's Equilibrium (HWE) was determined with the use of hwe.exe software. Both

were verified with Fisher exact test. The correlation between genotypes and age of disease onset, cancer stage, grade and expression of ER and PR were measured using gamma coefficient and Fisher exact test. The significance of the differences was tested at the level of $p<0.05$.

Results and discussion

Frequencies of all *CYP* polymorphisms, except *CYP1B1*3* and *CYP3A4*1B*, were consistent with Hardy-Weinberg Equilibrium (HWE). The inconsistency of *CYP1B1*3* with HWE resulted from higher frequency of the polymorphic than the wild-type variant. Since *CYP1B1*3* variant, in contrast to the rest of the world, was rare in Caucasians, it was called the "wild type". This was the reason why it was less frequent than the "polymorphic" variant, which resulted in inconsistency with HWE. The distribution of *CYP3A4*2* was inconsistent with HWE because no patients with the variant allele were found. Since frequency of this variant in the general population is about 2.7% [13], the study group was too small to identify carriers of this polymorphism.

Although an increased risk of breast cancer in the patients harboring the *CYP1A1*2A* polymorphism was observed in African Americans and the Taiwanese [3-5, 20], the results of metaanalysis [4] showed that *CYP1A1*2A* and *CYP1A1*2C* polymorphisms did not increase the risk of breast cancer. Our results (Table 1) were similar to those reported in Caucasians [18, 21-23],and were consistent with the data from metaanalyses [21, 22].

Significantly higher ($p>0.003$) frequency of *CYP1A1* polymorphisms was found in pre-menopausal (below 50 years of age) than in postmenopausal (over 50 years of age) women, strongly suggesting an association between the polymorphic alleles and breast cancer onset. (Figure 2).

Similar association concerning *CYP1A1*2A* and *CYP1B1*3* variants, was described by Han *et al.* [11], who suggested that the polymorphisms of both genes resulted in an early onset of the disease. Based on our results, we postulate that the original observation of Han *et al.* might apply at least to all *CYP1A1* polymorphisms, but further investigation on a much larger cohort is necessary to confirm this hypothesis.

We found that the frequency of *CYP1A1*2A* allele in the investigated group was slightly lower (6%) than that obtained in other studies made in Caucasian population, including metaanalyses [21, 24, 25]. All patients participating in our study originated from the western part of the country and this might be the reason why the allele distribution resembled that of the German population and differed from other European ones.

It was reported that *CYP1B1* polymorphism might be a sensitive marker of breast cancer [26]. Our results were consistent with metaanalysis [27] and did not reveal any difference in the frequency of *CYP1B1*3* allele between the patients and the healthy controls.

Although there were no reports of the correlation between *CYP3A4* polymorphisms and breast cancer, it was shown that the breast cancer patients harboring *CYP3A4*1B* polymorphism and treated with tamoxifen, more often developed endometrial cancer [16].

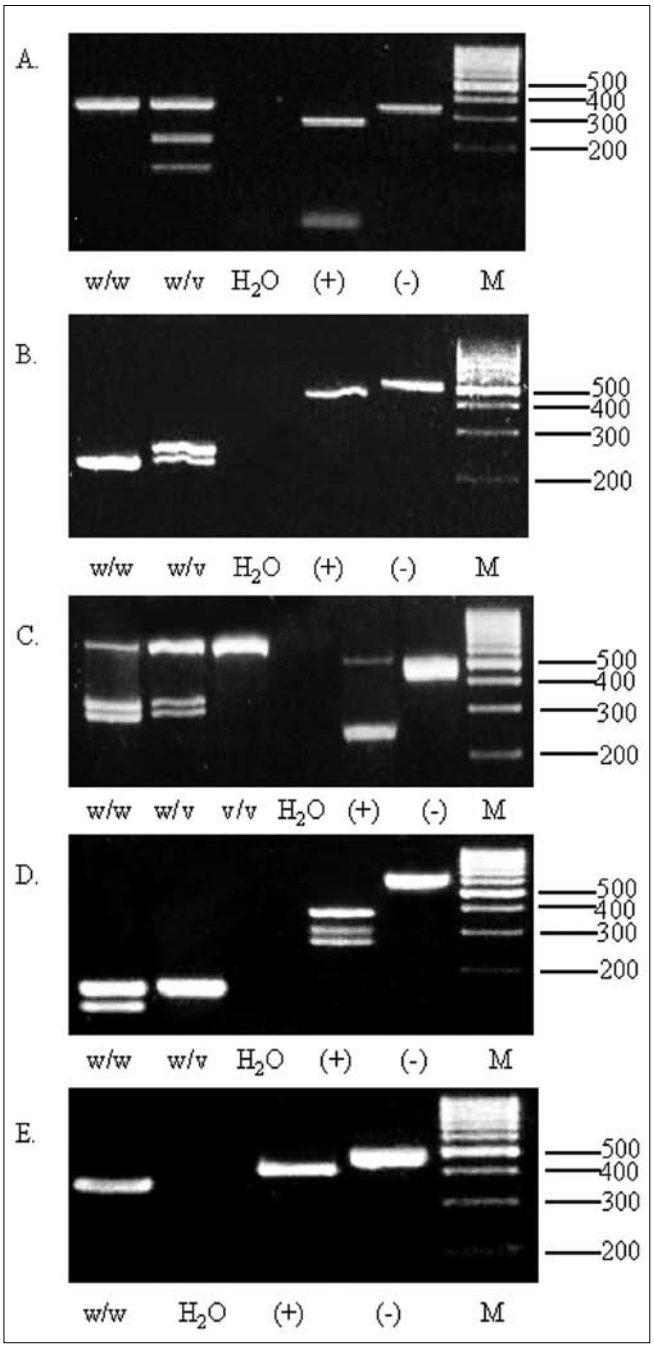

Figure 1. Restriction fragment length polymorphism (RFLP) analysis of *CYP* polymorphisms.
(A) *CYP1A1*2A*. (+), positive control (fragment of *pS2* digested with MspI);
(-), negative control (undigested fragment of the same amplicon), M, size marker. (B) *CYP1A1*2C*. (+), positive control (fragment of *ERβ* digested with NcoI);
(-), negative control (undigested fragment of the same amplicon); M, size marker.
(C) *CYP1B1*3.* (+), positive control (fragment of *C-erbB-2* digested with Eco57I); (-), negative control (undigested fragment of the same amplicon); M, size marker. (D) *CYP3A4*1B.* (+), positive control (fragment of *ERβ* digested with Mbo II);
(-), negative control (undigested fragment of the same amplicon); M, size marker.
(E) *CYP3A4*2*. (+), positive control (fragment of *ERβ* digested with XcmI);
(-), negative control (undigested fragment of the same amplicon); M, size marker;
H_2O, negative control of PCR.

Table I. Distribution of polymorphic alleles in the investigated groups.

Polymorphism	Group	Frequency of alleles n (%)		
		WW	WV	VV
*CYP1A1*2A*	C	82 (82)	18 (18)	0 (0)
	BC	65 (92)	6 (8)	0 (0)
*CYP1A1*2C*	C	87 (87)	13 (13)	0 (0)
	BC	63 (90)	7 (10)	0 (0)
*CYP1B1*3*	C	35 (35)	44 (44)	21 (21)
	BC	12 (18)	13 (20)	41 (62)**
*CYP3A4*1B*	C	97 (98)	2 (2)	0 (0)
	BC	62 (95)	3 (5)	0 (0)
*CYP3A4*2*	C	100 (100)	0 (0)	0 (0)
	BC	69 (100)	0 (0)	0 (0)

C – control group;
BC – breast cancer patients;
WW – homozygote with both wild alleles;
WV – heterozygote with wild allele and polymorphic one;
VV – homozygote with both polymorphic alleles;
** $p<0.05$.

The presence of *CYP3A4*1B* and *CYP3A5*3* polymorphisms in some patients might also influence the metabolism of drugs used in chemotherapy and thus exacerbate the prognosis [28, 29]. However, in our study we did not find any effects of the investigated polymorphisms on survival rate during nearly two years of observation.

In accordance with earlier studies [4], we found no correlation between the presence of any polymorphic *CYP* allele and breast cancer stage or grade as well as the ER or PR status and other breast cancer risk factors (smoking, obesity and family history). Only in the Chinese population, the presence of *CYP1A1* and *CYP1B1* variants were found to exacerbate the prognosis [30].
Our preliminary findings on the content of oncogenic C-erbB-2 protein in the breast cancer tissue, in the patients harboring *CYP1A1* polymorphisms, revealed higher levels in carriers of *CYP1A1* polymorphisms (not shown). The *C-erbB-2* gene encodes a membrane receptor homologous to the EGF receptor [31], which forms dimers with the C-erbB-3 receptor, resulting in stimulation of cell division [32]. Overexpression of the *C-erbB-2* can thus constitute an independent negative prognostic factor in breast cancer. Since it was shown, that the most aggressive form of the disease was found in patients with the higher level of C-erbB-2 protein [33], it is possible that the incidence of *CYP1A1* and *CYP1B1* polymorphisms associated with high levels of C-erbB-2 protein might thus exacerbate the prognosis.

Although our results suggested an association between the *CYP1A1* polymorphisms and earlier breast cancer onset, recent studies [34], which showed that the variant alleles might interact, indicate that the role of polymorphisms of the genes encoding estrogen metabolizing enzymes in breast cancer needs further investigation requiring large cohorts of patients.

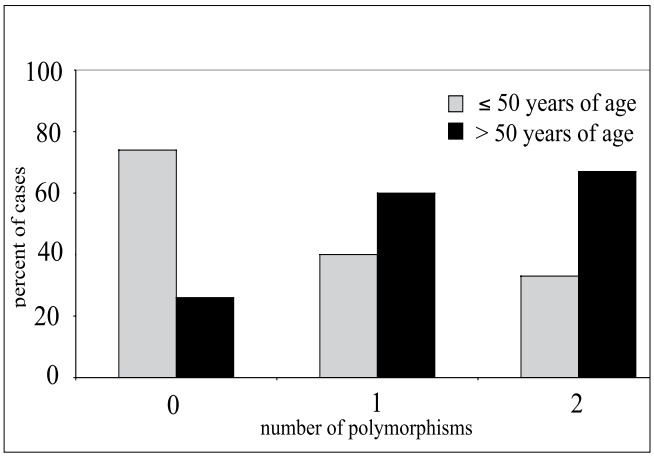

Figure 2. The association between CYP1A1 polymorphisms and the age of onset of breast cancer.
Patients were divided into two groups: below and over 50 years of age and the statistical analysis of the incidence of polymorphisms was conducted as described in Materials and Methods section.
0 – no *CYP1A1* polymorphisms; 1 – one polymorphism; 2 – two polymorphisms.

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