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Is the polymorphism in the promoter region of *CYP17* gene a risk factor for prostate cancer in Polish population?

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Introduction. Prostate cancer is one of the most commonly diagnosed tumors in Poland especially in men over 70 years of age. In the year 2000, the incidence rates were about 9/100 000 and 252/100 000 for men under and over 70 years, respectively. Generally, age is considered the most important risk factor for prostate cancer etiology. It is also known that androgen metabolism can influence its development. Cytochrome P450c17α plays a special role in the biosynthesis of steroid hormones and alterations in the gene encoding this protein (CYP17) can, probably, alter the production of sex-gormones.
Materials and methods. A single-nucleotide polymorphism (T→C) in the promoter of CYP17 gene was examined in patients with prostate cancer and age-matched controls. The DNA was extracted from blood spots or blood samples prepared for PSA analysis. The examined region of CYP17 gene was amplified and the product was digested overnight with MspAI enzyme. The resulted fragments were analyzed by electrophoresis in agarose gel with ethidium bromide.
Results. We were able to find three possible variants in the promoter region of CYP17 gene – TT, TC and CC, however their prevalence did not differ between the examined groups. The groups were divided according to age (<70 and ≥70), and it was found that the CC genotype was more common among younger patients, as compared to the adequate control group.
Conclusion. We conclude that the CC variant can be a risk factor for tumor development, especially in men before their seventies, although further studies are necessary to confirm this hypothesis.

Czy polimorfizm w regionie promotorowym genu *CYP17* może być czynnikiem ryzyka w powstawaniu raka stercza w polskiej populacji?

Wstęp. Rak stercza jest jednym z najczęściej diagnozowanych nowotworów w Polsce. Szczególnie często występuje on u mężczyzn po siedemdziesiątym roku życia. Wiek jest wymieniany jako jeden z głównych czynników sprzyjających powstawaniu raka prostaty. W roku 2000 współczynniki zapadalności dla mężczyzn przed siedemdziesiątym rokiem życia i starszych wynosiły odpowiednio: 9/100 000 i 252/100 000. Dla rozwoju tego nowotworu duże znaczenie ma również metabolizm androgenów. Szczególną rolę w przemianach cholesterolu do hormonów sterydowych odgrywa cytochrom P450c17α, w związku z czym zmiany w genie kodującym to białko (CYP17) mogą wpływać na syntezę hormonów płciowych.
Materiały i metody. U pacjentów z rakiem stercza oraz odpowiednio dopasowanych pod względem wieku kontrolach przeprowadzono analizę występowania jednonukleotydowego polimorfizmu (T→C) w regionie promotorowym genu CYP17. DNA izolowano z plam krwi lub próbek krwi przygotowywanych do badania poziomu PSA. Badany region był powielany, a następnie produkt reakcji poddawano całonocnej inkubacji z enzymem restrykcyjnym MspAI w temperaturze 37°C. Otrzymane fragmenty rozdzielano elektroforetycznie w żelu agarozowym z bromkiem etydyny.
Wyniki. Znaleziono trzy możliwe genotypy: TT, TC i CC, które mogą występować w regionie promotorowym genu CYP17, jednakże częstość ich występowania nie różniła się w badanych grupach. Badana populacja została więc podzielona na dwie grupy wiekowe: <70 i ≥70 roku życia, co pozwoliło na stwierdzenie, że genotyp CC występuje częściej wśród pacjentów przed 70 rokiem życia, w porównaniu do odpowiedniej grupy kontrolnej.
Wnioski. Sugerujemy więc, że wariant CC może stanowić czynnik sprzyjający pojawieniu się nowotworu u mężczyzn przed siedemdziesiątym rokiem życia. Jednocześnie konieczne są dalsze badania w celu potwierdzenia tej hipotezy.

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Introduction

Prostate cancer, besides lung and colon cancer, is the most common tumor in Polish male population, especially over 70 years of age. Every year about 4000 new cases are diagnosed, however this disease is rather rare among men below fifty years of age [1]. The mortality rate from prostate cancer tended to grow during the previous decades and in the early nineties amounted to 10.1/100 000. This phenomenon could have been brought on by better diagnostic methods (USG, test for PSA concentration in serum) as well as by increased patient awareness [2].

Prostate cancer is a multifactorial disease, which means that it can be provoked by environmental and genetic factors. It seems that the most important of these is age; 80% of all cases are diagnosed in men over 65 years [3]. Also, ethnicity is suggested to affect prostate cancer occurrence – the disease is more common in Afro-Americans, as compared to Caucasians and Asians, who present the lowest incidence of this malignancy [4]. Another factor involved in prostate cancer etiology is diet, especially excessive intake of fatty acids can provoke tumor development [5].

Epidemiology indicates that the risk of prostate cancer is about 5-11 times higher in families with two or more cases of the disease among first-degree relatives, however it seems that only about 10% of all prostate cancers are hereditary [3]. Several loci were identified which co segregate with prostate cancer in families and among them were: HPC1, PCAP, CAPB on chromosome 1 (1q24-q25, 1q42-q43, 1p36 respectively), HPC2/ELAC2 – chromosome 17 (17p11), HPC20 – chromosome 20 (20q13) and HPCX – chromosome X (Xq27-q28) [6]. These results are not unambiguous, probably due to the multifactorial etiology of the prostate cancer.

About 90% of prostate cancers are sporadic cases that are probably caused by the combination of environmental factors and several mutations with low penetrance. It has been suggested that attention should be paid to the androgen metabolism, because elevated levels of steroid hormones, especially testosterone, can be associated with prostate cancer development and progression. Although some studies have failed to find such a correlation it cannot be excluded that mutations in the genes encoding key enzymes of steroid metabolism lead to higher risk of prostate cancer.

The precursor for all steroid hormones is cholesterol, which is metabolized to progesterone through cutting off its side chain and oxidation of hydroxylic group from C-3. This metabolite can be used as the substrate for glucocorticoid, mineralocorticoid or sex hormone synthesis. In case of the sex hormones, progesterone is converted to 17α-hydroxyprogesteron by hydroxylation and then to androstendione by lysis reaction. These two steps are catalyzed by cytochrome P450c17α, which mediates both activities: steroid 17α-hydroxylase and 17, 20-lyase. The product of these reactions can be transformed to either testosterone or estron.

Cytochrome P450c17α is encoded by human *CYP17* gene, which has been localized in chromosome 10 (10q24.3). A single base-pair change (T→C) in the promoter region of this gene has been identified and it was suggested that it leads to creation of additional Sp-1 binding site and consequently higher transcription rate [7]. The aim of our work was to examine the possibility that the polymorphism in the promoter region of *CYP17* gene correlates with increased risk of prostate cancer in Polish male population and whether it can be used as a genetic marker of this malignancy.

Material and methods

Probands (71; mean age 69.4±7.6 years) included in this examination were patients of the Clinic of Oncological Urology of the Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw. Diagnosis was set after prostate gland biopsy. The control group consisted of men (117; mean age 61.9±10.8 years) with no previous history of prostate cancer. Among them were patients of the Institute of Cardiology, the Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology and patients from one of the outpatient clinics in Warsaw.

Blood for the DNA extraction was collected on *IsoCode Stix* blots or blotting papers used for standard screening analysis (both *Schleicher&Schuell*) and also from the blood samples prepared for PSA determination. DNA from *IsoCode Stix* blots was extracted according to manufacturer instruction (S&S *IsoCode® Card/Stix* Protocol). SherlockAX or *Mini Blood Kits for DNA Isolation* (A&A Biotechnology) were used for other samples.

The polymorphism in the promoter region of *CYP17* gene was identified using PCR/RFLP technique. The specific fragment of 419bp was amplified with the following primers: CYP17F 5`-cattcgcacctctggagtc-3` and CYP17R 5`-ggctcttggggtacttg-3` (IBB, PAN). The reaction mixture (total volume 25 μl) apart from DNA, contained 15pmol of each primer, all deoxynucleotide triphosphates (final concentration 250 μM, Amersham), PCR buffer with magnesium (Sigma) and 1U of RedTaq Polymerase (Sigma). The reactions were held in GeneAmp PCR System 9600 (Perkin-Elmer) in following conditions: initial denaturation – 5 min at 95°C, then 35 cycles composed of 95°C for 30 sec, 59°C for 30 sec and 72°C for 35 sec, and finally elongation step at 72°C for 10 minutes. The PCR products were digested overnight with MspA1 I restriction enzyme (*New England Biolabs*) according to manufacturers instructions. The resulting fragments were analyzed in 2% agarose gel (*Sigma*) stained with ethidium-bromide (Sigma).

Statistical analysis was conducted with *Microsoft Excel 2000* (mean values and standard deviations) and *SPSS* package v.8.0 for *Windows 98* (frequencies and statistical significance).

Results

As could be expected three possible genotypes were found in our examined population (see Figure 1),

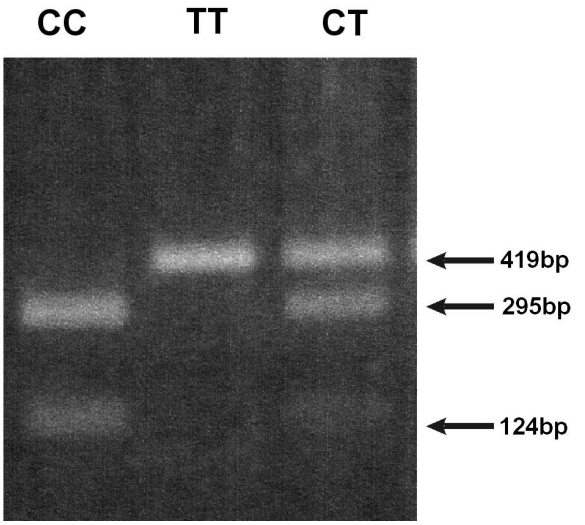

Figure 1. Results of analysis of T→C polymorphism in the promoter region in *CYP17* gene.
DNA was amplified and the resulted fragment was digested with MspA1 I enzyme. The presence of C nucleotide, which is supposed to be a risk factor for prostate cancer development, creates a cleavage site for MspA1 I restriction enzyme (295 and 124bp), which is not observed in case of T allele (419bp)

however their frequencies did not differ between the control and proband groups (Table I). The prevalence of alleles was also similar in both groups (Table II). Therefore, we divided our patient population according to age, where the cut-off value was the mean age in probands group. We observed higher frequency of the CC genotype and the C allele among patients below 70 years of age (31.2% and 0.48 respectively), as compared to an adequate control group (17.3% and 0.37), however the results were not statistically significant. A reversed situation was observed in the elderly group – the CC genotype and the C allele were more common in control group (respectively 30.6% and 0.54), than among prostate cancer patients (12.8% and 0.40). The outcome did not

Table I. Frequencies of genotypes in examined population

group	genotype		
	TT (a1/a1)	TC (a1/a2)	CC (a2/a2)
controls – all	36.8% (43)	41.8% (49)	21.4% (25)
probands – all	33.8% (24)	45.1% (32)	21.1% (15)
controls < 70	43.2% (35)	39.0% (32)	17.3% (14)
probands < 70	34.4% (11)	34.4% (11)	31.2% (10)
controls ≥ 70	22.2% (8)	47.2% (17)	30.6% (11)
probands ≥ 70	33.3% (13)	53.9% (21)	12.8% (5)

Table II. Frequencies of alleles in examined population

group	allel	
	T (a1)	C (a2)
controls – all	0.58	0.42
probands – all	0.56	0.44
controls < 70	0.63	0.37
probands < 70	0.52	0.48
controls ≥ 70	0.46	0.54
probands ≥ 70	0.60	0.40

reach statistical significance. We also observed differences in the genotypes and the alleles frequencies in the control and the proband groups divided according to age (Tables I and II). The difference in the alleles distribution among controls was statistically significant (p=0.015).

Discusion

It is generally known that cytochrome P450c17α plays a key role in steroid metabolism and for this reason any genetic alterations in *CYP17* gene can affect sex-hormone synthesis. A single nucleotide polymorphism (T→C) in the promoter region was described and suggested to create an additional Sp1-binding site similar to this observed in ε-globine gene. Although *in vitro* examination of recombinant Sp-1 binding to changed sequence in gel mobility shift assay did not support this hypothesis, it cannot be excluded that the C variant correlates with altered gene expression and modification in androgen metabolism [8].

The frequency of the CC genotype in Polish population (21.4%) according to our study is slightly higher than its prevalence observed in other white populations such as Swedish – 16% (9) or American Whites – 14% [10]. In the Japanese and related populations, which have the incidence of prostate cancer about 5 times lower, the frequency of CC variant is estimated for about 26% [11]. Consequently the genotype should have a lower frequency in black populations, which have the highest worldwide occurrence of prostate cancer calculated as 2 times higher than in white populations. Such difference was not found when African American or Nigerian populations were compared with a White-American group [12].

We were not able to find a correlation between any variant of *CYP17* gene and prostate cancer risk and therefore we divided our groups according to age (<70 and ≥70). The occurrence of prostate cancer in these groups was different in Polish population. The incidence and mortality rates were as follows: 9/100 000 and 4.5/100 000 for men before their seventies and 252/100 000 and 215/100 000 – men 70 years of age and older (dr J. Lissowska, personal communication). We observed slight differences between probands and control groups divided according to age. Therefore, it seems that the CC genotype can correlate with prostate cancer occurrence in Polish men below 70 years of age. Contrary results obtained in the elder group can be caused by a non-homogenous control population. It is possible that some of these men have developed benign prostatic hyperplasia, which is also suspected to depend on androgen metabolism.

Conflicting results concerning the correlation between *CYP17* variants and prostate cancer were already reported. In Austrian population the CC genotype was more common among patients with prostate cancer (23.8%) than in the control group (9.5%) and the result was statistically significant (p=0.03; [13]). The same tendency was also observed among Americans. In three

independent investigations from North California, Bethesda (majority of participants were of Caucasian origin) and Baltimore (African American population) the CC variant had higher frequency in patients with prostate cancer (14%, 18% and 15%, respectively) as compared to the controls (11%, 14% and 9%, respectively). The analysis made for the first and third center revealed statistically significant results [3, 10, 12]. Moreover, examination according to age was performed in the North Carolina and Bethesda centers. Men younger than 65 years (North Carolina) carrying at least one C allele were more susceptible to prostate cancer development (p=0.03, OR-2.3; [3]). Also in the Japanese population the CC genotype was more prevalent among men with prostate cancer (19%) compared to controls (14%). When the examined group was divided acc. to age (cut-off value 72 years) such a correlation was not found in the elder population, however it was even stronger in the group below 72 years (p=0.04, OR-4.02 [14]).

Contradicting results were reported in another investigation performed on Japanese men. The CC variant, which is supposed to correlate with prostate cancer, was more frequent among controls (28%) than in prostate cancer patients (18%) and the results were statistically significant (p=0.022; [11]) as in the Swedish population, where the TT genotype was more frequent in patients (39%) than among controls (29%; p=0,04; [9]).

Although these results are contradictory it seems that the T→C polymorphism in the promoter region of *CYP17* does affect sex-hormone metabolism. It has been shown that the mutated allele is correlated with higher progesterone and estrogen levels. In women progesterone level on 22nd day and estrogen (E_2) levels on the 11th and 22nd day of the menstrual cycle were respectively about 30%, 57% and 28% higher in premenopausal subjects carrying two C alleles as compared to subjects homogenous with the T variant (p values 0.04, 0.04 and 0.06, respectively). This can be a risk factor for breast cancer development [7]. Such relationships between the genotype and testosterone, estradiol and dihydrotestosterone serum levels were not found [15, 16].

CYP17 is not the only gene, the genetic alterations of *CYP17* can influence androgen metabolism. Also mutations in *SRD5A2* gene encoding 5α-reductase type II, which catalyzes the conversion of testosterone to its more active form – dihydrotestosterone (DHT), can affect the level of circulating sex-hormones [15, 16]. Furthermore, two polymorphic sites with variable number of tri-nucleotide repeats (CAG and GGN) were found in the gene for nuclear androgen receptor on chromosome X. Several studies have confirmed that shorter variants correlated with enhanced prostate cancer risk and poor prognosis of the disease [17, 18].

It seems that polymorphism of the *CYP17* gene can influence prostate cancer development especially in men below 70 years of age (Polish population), however further studies are necessary to obtain statistically significant results. Also the analysis of polymorphisms in other genes correlating with steroid hormones metabolism and their signaling pathways might help to provide evidence for the genetic basis of sporadic prostate cancer incidence.

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