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Source / Izvornik: **Collegium antropologicum, 2014, 38, 141 - 146**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:239:491614

Coll. Antropol. **38** (2014) 1: 141–146
Original scientific paper

Association Study of Cytochrome P450 1A1*2A Polymorphism with Prostate Cancer Risk and Aggressiveness in Croatians

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A B S T R A C T

*Cytochrome P450 1A1 (CYP1A1) is an enzyme participating in the bioactivation of various endogenous and environmental reactive compounds that can bind to DNA and thus induce cancerogenesis. Gene encoding the enzyme is expressed in the prostate tissue and is polymorphic. CYP1A1*2A gene polymorphism is associated with elevated enzyme activity and/or inducibility which can lead to accumulation of genotoxic compounds and consequently to cancerogenesis. We examined the association of this polymorphism with prostate cancer (PCa) risk and aggressiveness. The case-control study consisted of 120 PCa patients and 120 benign prostatic hyperplasia (BPH) controls, in Croatian population. Regarding aggressiveness, PCa patients were grouped according to the Gleason score (GS), tumor stage (T) and existence of distant metastasis (M). The polymorphism was analyzed using real-time polymerase chain reaction (PCR). We did not observe association of mutated allele with PCa risk, neither with PCa aggressiveness. Furthermore, frequency of polymorphic genotype was slightly higher in BPH group (16.6%* vs. *14.2%, respectively) and also in less aggressive form of PCa (20.4%* vs. *9.6% for GS<7; 15.6%* vs. *9.1% for T<3; 16.7%* vs. *10.0% for no distant metastasis). Comparing our findings with other published results, we can assume that the ethnicity influence the genotype distribution and thus may affect the etiology of PCa, even possibly in the way to cause an opposite effect among different ethnic groups. Given the small number of participants, results should be validated on the larger sample size.*

***Key words**: CYPA1A1, single nucleotide polymorphism, prostate cancer, Croatia, polycyclic aromatic hydrocarbons, smoking, DNA adducts, frequency, Gleason score, metastasis*

Introduction

Prostate cancer is a major health problem in developed countries[1,2] and the second cause of death among cancers in men. In Croatians is the second most common cancer in male population and shows increased incidence[3]. Although the etiology of prostate cancer is unknown, there are evidence that environmental factors such as diet and lifestyle, chronic inflammation, and genetic factors are important in the risk of development and progression of the disease[4,5]. This is partly confirmed by the large differences in the incidence among different ethnic groups[6], up to 50-fold[7]. Testing of gene polymorphisms therefore seems to be important area of investigation.

The cytochrome P450 1A1 gene (CYP1A1) is located on chromosome 15q22. It encodes phase I metabolism enzyme with aryl hydrocarbon hydroxylase activity[8]. This enzyme is involved in bioactivation of various environmental carcinogen compounds like polycyclic aromatic hydrocarbons (PAH) and polyaromatic amines. Besides, it participates in oxidative metabolism of estrogens by forming 2-hydroxy catechol estrogens (2-OHCE)[8]. Generated genotoxic products can bind to DNA, forming

Received for publication March 25, 2013

DNA-adducts and damaging the cell[9]. The CYP1A1 enzyme is expressed in prostate tissue[4] and is highly inducible by exposure to PAHs and other substances that bind to aryl hydrocarbon (Ah) receptors[10].

Several polymorphic variants within CYP1A1 have been reported[11]. The CYP1A1*2A (rs4646903) occurs due to T3801C substitution in the 3' flanking region, forming an Msp1 restriction site of the CYP1A1 gene (CYP1A1m1)[12]. The polymorphic variant is associated with elevated enzyme activity and/or inducibility[8,12], which can lead to accumulating of toxic, reactive intermediaries. The incidence of the mutated allele is about 10% in Caucasians[13] and shows marked interethnic variability. Previous studies showed a positive association of CYP1A1*2A polymorphism with risk of lung[14], cervix[15], bladder[16] and gastric cancer[17]. Regarding association with prostate cancer (PCa), results are controversial[12].

In this study we determined frequencies of CYP1A1* 2A polymorphism in patients with PCa and benign prostate hyperplasia (BPH) in Croatian population and estimated the role of allele variants in development and aggressiveness of PCa by themselves and in combination with smoking.

Materials and Methods

Characterization of the sample

This case-control study comprises on 240 men who were examined at the Department of Urology, University Hospital Centre Osijek in 2008–2010. Subjects were divided into two groups: 120 PCa patients and 120 patients with BPH who represented controls. Groups were age matched. Participants were subjected to digital rectal examination (DRE) and blood sampling for prostate specific antigen (PSA) testing. Subjects who had normal DRE and PSA<4 ng/ml, or those with some other site of cancer were excluded from the study. Those with elevated PSA value and/or abnormal DRE, were subjected to transrectal ultrasound (TRUS) – guided needle biopsy. Diagnosis was confirmed by histopathological finding.

PCa patients were stratified according to the Gleason score (GS), tumor stage (T) and existence of distant metastasis (M). According to the GS, patients were grouped as less aggressive form of cancer with GS<7 and highly aggressive form with GS≥7. According to the T, they were divided into T<3 and T≥3. Patients who were suspected for the existence of distant metastasis underwent scintigraphy and computed tomography (CT) and then were classified as those who have distant metastasis (M1) and those without them (M0). 2 of 120 patients did not have data on the GS, T and M, and hence were excluded from the testing of aggressiveness.

Patients fulfilled the questionnaire about clinical characteristics and gave signed informed consent of participation according to the guidelines by the Helsinki Declaration. The study is approved by Ethic Committee of the University Hospital Centre Osijek and by the Ministry of Science, Education and Sports of the Republic of Croatia.

DNA isolation and genotyping

Genomic DNA was isolated from peripheral blood lymphocytes using High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany). Detection of CYP1A1 T3801C polymorphism was performed by real-time PCR and melting curve analysis on a LightCycler 1.5 (Roche, Mannheim, Germany). The PCR primers (5'-CCTGAACCCCATTCTGTGTTG-3', 5'-AAA AAAAAAAAAAAAAAAAGCTGTG-3') were synthesized by Invitrogen (Paisley, UK) and hybridization probes (5'-CACCTCCCIGGCTCACACGATT-FL-3', 5'-LCRed640-CCACCTCAGCCTCTGAGTAGTTGGGG-P-3') by TIB MOLBIOL (Berlin, Germany) according to Harth et al.[18]. PCR was performed in a total volume of 10 μL with

TABLE 1
CHARACTERISTICS OF THE STUDY GROUPS

Characteristics	Cases	Controls
	N (%)	N (%)
Sample size	120	120
Age (mean years±SD)	67.52±7.76	67.57±6.65
PSA (ng/mL)		
Median (range)	12.35 (2.1–3346.0)	6.81 (0.57–28.0)
<4	7 (5.8)	15 (12.5)
4.1–10.0	39 (32.5)	76 (63.3)
10.1–20.0	30 (25.0)	26 (21.7)
>20.0	43 (35.8)	3 (2.5)
Family history of CaP		
Negative	110 (91.7)	113 (94.2)
Positive	10 (8.3)	7 (5.8)
Smoking status		
Non–smokers	94 (78.3)	99 (82.5)
Smokers	26 (21.7)	21 (17.5)
BMI (kg/m^2)		
<25	27 (22.9)	34 (29.1)
25–30	57 (48.3)	66 (56.4)
>30	34 (28.8)	17 (14.5)
TRUS (X±SD)	50.967±28.094	76.925±42.922
GS		
<7	54 (45.8)	–
≥7	64 (54.2)	–
Distant metastasis		
M0	78 (66.1)	–
M1	40 (33.9)	–
Tumor stage		
T<3	96 (81.4)	–
T≥3	22 (18.6)	–

PSA – prostate specific antigen, BMI – body mass index, TRUS – transrectal ultrasound, GS – Gleason score

TABLE 2
CYP1A1*2A GENOTYPE AND ALLELE FREQUENCIES

Variant	Cases	Controls	OR (95%CI)	p
	N (%)	N (%)		
TT	103 (85.5)	100 (83.3)	1.0	–
TC	17 (14.2)	20 (16.6)	0.83 (0.41–1.67)	0.592
CC	0	0	–	–
T allele	223 (92.9)	220 (91.7)	1.0	–
C allele	17 (7.1)	20 (8.3)	0.84 (0.43–1.64)	0.608
Non smokers				
TT	80 (86.0)	82 (82.8)	1.0	–
TC	13 (14.0)	17 (17.2)	0.78 (0.35–1.72)	0.543
CC	0	0	–	–
Smokers				
TT	23 (85.2)	18 (85.7)	1.31 (0.66–2.61)	0.443
TC	4 (14.8)	3 (14.3)	1.37 (0.30–6.30)	0.689
CC	0	0	–	–

OR – odds ratio

30–60 ng of genomic DNA, 0.75 μM primers each, 0.3 μM probes each, 4.0 mM $MgCl_2$ and 1 x LC DNA Master Hybridization Probes Mix (Roche, Mannheim, Germany). The reaction mixtures were subjected to initial 2 min denaturation step at 94 °C followed by 52 cycles of denaturation (94 °C / 3 s), annealing (58 °C / 20 s) and extension (72 °C / 35 s). After amplification melting curve analysis was performed (95 °C / 2 min / 20 °C / s, 48 °C / 1 min / 2 °C / s, 40 °C / 2 min / 2 °C / s, 80 °C / 0 s / 0.1 °C / s). The fluorescence signal plotted against temperature gave peaks at 61 °C and 68.5 °C for the allele T and C, respectively.

Statistical analysis

Fisher exact test was used for genotype and allele comparison between groups. Odds ratio (OR) with 95% confidence intervals (CI) were used for calculating the strength of association of genotypes with cancer risk and aggressiveness. Groups were tested for Hardy-Weinberg equilibrium. To test the differences in body mass index (BMI), PSA level and prostate volume values, t-test and Mann-Whitney Rank Sum Test were used according to the distribution. $p<0.05$ was considered statistically significant. Statistical analysis were performed using MedCalc for Windows, version 12.4.0.0 (MedCalc Software, Mariakerke, Belgium).

Results

In Table 1 are presented relevant demographic and clinical data on CaP patients and BPH controls. Family history of PCa and smoking status were similar between groups, while PSA and TRUS values differed significantly ($p<0.001$) as expected.

CYP1A1 genotype and allele frequencies did not differ significantly between PCa patients and BPH controls. Genotype frequencies for cases and controls were all in Hardy-Weinberg equilibrium. No association between PCa incidence and CYP1A1 polymorphism was found (Table 2). The same table contains genotypes and alleles distributions according to the smoking status, and appropriate OR for PCa risk among smokers. We did not observe any positive association by analyzing polymorphism and smoking together.

The results of testing association between mutated allele and tumor parameters of aggressiveness are presented in Table 3. Obtained results showed lack of association with GS, T and M status.

Discussion and Conclusion

In this study we analyzed prevalence of CYP1A1*2A polymorphism in 120 PCa patients and 120 BPH controls of Croatian population and assessed association of mutated allele with PCa risk and aggressiveness. Besides, we tested does smokers carriers of C allele have a higher risk of PCa development.

Based on epidemiological studies it was confirmed that environmental factors such as precarcinogens from cigarette smoke and diet may be responsible for development of numerous cancers. There are also numerous studies concerning link between smoking and intake of charbroiled meat with the incidence of PCa[8]. Experiments have shown that potentially toxic compounds which derive from such sources can be activated by CYP1A1 into the compounds which are capable of binding to DNA. CYP1A1 is mainly expressed in extra hepatic tissue including prostate, with high catalytic specificity

TABLE 3
GENOTYPE AND ALLELE DISTRIBUTION ACCORDING TO THE GLEASON SCORE, TUMOR STAGE AND THE EXISTENCE OF DISTANT METASTASIS

Variant	N (%)	N (%)	OR (95%CI)	p
	GS<7	GS≥7		
TT	43 (79.6)	58 (90.6)	1	–
TC	11 (20.4)	6 (9.4)	0.40 (0.14–1.18)	0.097
CC	0	0	–	–
T allele	97 (89.8)	122 (95.3)	1	–
C allele	11 (10.2)	6 (4.7)	0.43 (0.15–1.21)	0.434
	T<3	T≥3		
TT	81 (84.4)	20 (90.9)	1	–
TC	15 (15.6)	2 (9.1)	0.54 (0.11–2.56)	0.437
CC	0	0	–	–
T allele	177 (92.2)	42 (95.5)	1	–
C allele	15 (7.8)	2 (4.5)	0.56 (0.12–2.55)	0.455
	M0	M1		
TT	65 (83.3)	36 (90.0)	1	–
TC	13 (16.7)	4 (10.0)	0.56 (0.17–1.83)	0.334
CC	0	0	–	–
T allele	143 (91.7)	76 (95.0)	1	–
C allele	13 (8.3)	4 (5.0)	0.58 (0.18–1.84)	0.354

GS – Gleason score, T – tumor stage, M – distant metastasis, OR – odds ratio

towards PAHs like benzo(a)pyrene (B[a]P) and 7,12- dimethyl benz(a)anthracene (7,12-DMBA) and N-nitrosamines, compounds derived from cigarette smoke[10]. The same enzyme participates in the metabolic activation of heterocyclic amines like 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), product of cooked meat[10]. All this compounds are capable of forming DNA-adducts[10]. Furthermore, smoking can affect the level of steroid hormones in a way that lead the increase level of bioavailable testosterone and thus contribute to the development and progression of PCa[19].

High level of CYP1A1 expression was found in malignant breast tissue, esophageal carcinomas and urinary bladder tumors[10]. Studies have shown that CYP1A1*2A polymorphism is related with different types of tumors. The polymorphism shows large inter-ethnic variability in healthy population, with relatively high proportion among Asians, a bit smaller among African Americans and smallest in Caucasians[8]. Only a few studies were made to asses a potential role of this enzyme in PCa, but results are inconclusive. Our results showed similar genotype and allele distribution between cases and controls. The frequency of C allele was 0.8 in controls, which is in agreement with other studies on Caucasians[13]. The rare CC homozygote was not found in our population. We observed no association with PCa risk. Positive association of CYP1A1*2A polymorphism with PCa was found only among Indian[8] and Chilean[20] population. In study by Chang at al.[21] conducted mostly on Caucasians, the frequency of C allele was significantly lower in PCa cases compared to controls suggesting a protective role of mutated allele. Similar results were observed by Silig at al.[20] on Turkish population, although lacking statistical significance. A frequency of polymorphic genotype in Croatian population was also sligtly higher in controls. Results imply that ethnicity may affect both, polymorphism distribution and the PCa risk.

Regarding connection between the polymorphism and PCa aggressiveness, we obtained lack of association with all three tested parameters. A polymorphic genotype frequency was even higher in less aggressive PCa. Link between CYP1A1*2A and PCa with GS ≥7 and metastatic form of cancer was found only in Japanese[22]. Taking together these results with those of risk testing, ethnic differences can have a significant impact in the cancer etiology even possibly in way to cause opposite effects among ethnicities.

We did not observe any connection between PCa risk and combined effect of smoking and mutated genotype. Although smoking affects the occurrence of some cancers, epidemiologic studies did not support clear causal link between smoking and PCa development. PAH-DNA adducts are increased in prostate tumor cells of ever smokers Caucasians, but not African Americans[23]. High-risk genotype combinations in Caucasians revealed increased adducts level, which confirms the hypothesis of

PAH activation prior to binding to DNA[23]. Such results were not obtained for African Americans, indicating that association between smoking and PAH-DNA adducts differs by race and is under the influence of genetic variants. Besides, PAHs may originate from other sources than smoking like those from grilled meat or ambient air pollutions which imply the importance of the environment and lifestyle as a part of multicausal etiology of PCa.

This is the first such examination in Croatians, conducted on relatively homogenous group. However, the study suffers from some limitations related to small number of participants and lack of information about exposure to other carcinogens than smoking. Additional problem is the low proportion of mutation in Croatians. Furthermore, in respect to relatively small number of smokers, we could not additionally stratify them according to the extent of tobacco use, which seems important in risk assessment. However, taking into consideration our findings with other published results, we can assume that the ethnic and geographical background could affect the genotype distribution and the etiology of the disease and that gene polymorphism itself is not sufficient in the development of multifactorial diseases such as PCa. Although the results of our study are negative, they should be validated with larger sample size in order to obtain more accurate results.

Acknowledgments

The study is financially supported by the Ministry of Science, Education and Sports of the Republic of Croatia as a part of scientific project of university professor Josip Galić »Immunological factors in the development and progression of prostate cancer«.

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ISPITIVANJE POVEZANOSTI POLIMORFIZMA CITOKROMA P450 1A1*2A S RIZIKOM OD KARCINOMA PROSTATE I AGRESIVNOŠĆU U HRVATSKOJ POPULACIJI

SAŽETAK

Citokrom P450 1A1 (CYP1A1) je enzim koji sudjeluje u bioaktivaciji raznih endogenih i egzogenih reaktivnih spojeva koji se mogu vezati na DNA i tako izazvati karcinogenezu. Gen koji kodira taj enzim je eksprimiran u tkivu pros-

tate i polimorfan. CYP1A1*2A genski polimorfizam je povezan s povišenom aktivnošću i/ili inducibilnoću enzima što može dovesti do nakupljanja genotoksičnih spojeva i posljedično do karcinogeneze. Ispitali smo povezanost tog polimorfizma s rizikom od karcinoma prostate i agresivnošću. Studija ispitivanja parova sastojala se od 120 pacijenata s karcinomom prostate (PCa) i 120 kontrola s benignom hiperplazijom prostate (BPH), stanovništva Hrvatske. Obzirom na agresivnost, PCa pacijenti su grupirani prema Gleason zbroju (GS), stadiju tumora (T) i postojanju udaljenih metastaza (M). Polimorfizam je analiziran pomoću lančane reakcije polimeraze (PCR). Nismo uočili povezanost između mutiranog alela i rizika od PCa, niti s bilo kojim ispitivanim parametrom agresivnosti. Štoviše, frekvencija polimorfnog genotipa bila je viša u skupini BPH (16,6% naspram 14,2%) i u manje agresivnom obliku PCa (20,4% naspram 9,6% za GS<7; 15,6% naspram 9,1% za T<3; 16,7% naspram 10,0% za M0). Uspoređujući naše rezultate s drugim objavljenim rezultatima možemo pretpostaviti da etnička pripadnost može utjecati na distribuciju i tako utjecati na etiologiju PCa, čak možda i na način da izazove suprotan učinak kod različitih etničkih skupina. Obzirom na mali broj ispitanika, rezultate bi trebalo potvrditi na većem uzorku.