Polymorphisms in the gene encoding CYP1A2 influence prostate cancer risk and progression

MARTA VILČKOVÁ[1], MÁRIA ŠKEREŇOVÁ[2,3], DUŠAN DOBROTA[1,2], PETER KAPLÁN[1], JANA JUREČEKOVÁ[1], JÁN KLIMENT[4], MÁRK HÍVEŠ[1], RÓBERT DUŠENKA[4], DANIEL EVIN[1,5], MARTINA KNOŠKO BROŽOVÁ[1] and MONIKA KMEŤOVÁ SIVOŇOVÁ[1]

[1]Department of Medical Biochemistry, Jessenius Faculty of Medicine; [2]Department of Clinical Biochemistry, Jessenius Faculty of Medicine and University Hospital Martin, [3]Biomedical Center Martin, Jessenius Faculty of Medicine; Departments of [4]Urology and [5]Nuclear Medicine, Jessenius Faculty of Medicine and University Hospital Martin, Comenius University in Bratislava, 03601 Martin, Slovak Republic

Received October 13, 2022; Accepted December 16, 2022

DOI: 10.3892/ol.2023.13671

Abstract. The role of the cytochrome P450 1A2 (*CYP1A2*) rs2472299, rs2470890 and rs11072508 polymorphisms in prostate cancer risk, disease progression and tumour development remains unclear. The potential associations of these three *CYP1A2* polymorphisms and haplotypes with prostate cancer susceptibility and its clinicopathological characteristics were therefore investigated. The present case-control study consisted of 522 patients with prostate cancer and 554 healthy controls. High-resolution melting analysis was used to determine the *CYP1A2* polymorphisms. No significant association in prostate cancer risk was seen for *CYP1A2* rs2472299 and rs11072508. However, a significantly decreased risk of prostate cancer was found for *CYP1A2* rs2470890 [odds ratio (OR), 0.67; P=0.02] in the recessive model. After analysis of the associations of clinical status and these three *CYP1A2* polymorphisms, the *CYP1A2* rs2470890 and rs11072508 polymorphisms showed a positive association with a higher Gleason score (rs2470890 OR, 1.36, P=0.04 in the allelic model; rs11072508 OR, 1.37, P=0.04 in the allelic model and OR, 1.60, P=0.03 in the dominant model). All three polymorphisms showed a significant positive association with pathological T stage in the additive, allelic and dominant genetic models (P<0.05). Haplotype analysis revealed that the most common haplotypes 'GTT' and 'ACC' were significantly associated with pathological T stages 3 and 4 (OR, 0.62; P=0.02 and OR, 1.54; P=0.03, respectively). A significant association was found between the 'GTT' haplotype and the Gleason score (OR, 0.71; P=0.03). In conclusion, these *CYP1A2* polymorphisms and haplotypes have the potential to predict prostate cancer disease progression.

Introduction

Prostate cancer is a multifactorial disease and the most frequently diagnosed malignancy associated with significant mortality and morbidity in men. The well-established risk factors are age, ethnicity, family history of prostate cancer and genetic predisposition (1). In addition, dietary factors, such as the intake of red meat, and smoking have been considered as potential prostate cancer risk factors (2).

Members of the general population are exposed to a vast number of xenobiotics during the course of their lifetimes, including drugs, industrial chemicals and food procarcinogens (3). The key mechanism for maintaining homeostasis during exposure to various xenobiotics is biotransformation. This process is divided into two phases: Phase I involves oxidation, which introduces a reactive group into the xenobiotics that enter the body or into endogenous compounds; and phase II, which generates water-soluble compounds by the conjugation of the products of phase I reactions or parent compounds with suitable functional groups yielding an excretable product. Biotransformation occasionally results in toxic metabolites (via bioactivation) (4,5).

Among the enzymes that metabolize xenobiotics, the cytochrome P450 (CYP450) enzyme superfamily is the most important and extensively studied group of activation (phase I) enzymes (6). The CYP450 superfamily consists of 57 genes and is divided into 18 families in humans. Only CYP450 families 1, 2 and 3 are responsible for the metabolism of the majority of drugs and other xenobiotics. Moreover, the CYP1 family includes three proteins: CYP1A1, CYP1A2 and CYP1B1 (7). CYP1A2 is expressed mainly in the liver (13-15% of all CYP450), although its expression has also

Correspondence to: Dr Monika Kmet'ová Sivoňová, Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University in Bratislava, 4D Malá Hora, 03601 Martin, Slovak Republic
E-mail: monika.kmetova.sivonova@uniba.sk

Abbreviations: CYP1A2, cytochrome P450 1A2; HRMA, high-resolution melting analysis; PAH, polycyclic aromatic hydrocarbons; HCA, heterocyclic amines; B[a]P, benzo[a]pyrene; SNPs, single nucleotide polymorphisms; CI, confidence interval; OR, odds ratio; PSA, prostatic specific antigen

Key words: CYP1A2, polymorphism, haplotype, prostate cancer, Slovak population

been detected in prostate tissue (8,9). CYP1A2 metabolizes a number of clinical drugs (e.g., analgesics and antipyretics, antipsychotics, antidepressants, anti-inflammatory drugs, the muscle relaxant tizanidine and the lipoxygenase inhibitor zileuton) (10), a number of procarcinogens [e.g., aromatic and heterocyclic amines, polycyclic aromatic hydrocarbons (PAH) and aflatoxin B1] (11,12) and several important endogenous compounds (e.g., oestrogens, melatonin, retinoic acid, arachidonic acid and prostaglandins, bilirubin and uroporphyrinogen) (13-15).

Both environmental and genetic factors affect the activity of CYP1A2. The well-established inducers of CYP1A2 activity are cigarette smoking, habitual heavy coffee consumption, cruciferous vegetables, heavy exercise and grilled meat (16,17). Heterocyclic amines (HCA), derived from meats cooked at high temperature require metabolic activation for the formation of stable HCA-DNA adducts (2). Other mutagens present in cooked meat and formed by the pyrolysis of fat include PAH, e.g., benzo[*a*]pyrene (B[*a*]P), which has been shown to be metabolically activated in prostatic tissues (8). The inhibition of B[*a*]P adduct formation by α-naphthoflavone, an inhibitor of the CYP1 family (18), indicates that CYP1 enzymes are at least partially responsible for the metabolic activation of PAH in human prostatic tissue (8). Similarly, constituents of cigarette smoke, such as PAH, require metabolic activation, evasion of detoxification processes and subsequent binding to DNA to exert their carcinogenic action (19). Some drugs such as phenobarbital, carbamazepine, omeprazole and rifampicin, are important inducers of CYP1A2 (20), whereas other drugs such as fluvoxamine, quinolone antibiotics and oral contraceptives act as CYP1A2 inhibitors (21,22).

Interindividual, sex-related and ethnic differences in CYP1A2 activity have been reported and may cause variation in the activation of some carcinogens and, thereby, in cancer susceptibility (23). Notably, 35-75% of the interindividual variability in CYP1A2 activity has been suggested to be attributable to genetic factors (17).

To date, >177 single nucleotide polymorphisms (SNPs) in the upstream sequence and more than 21 variant alleles (**1B* to **21*) of human *CYP1A2* have been identified (https://www.pharmvar.org/htdocs/archive/*cyp1a2*.htm), although the functional consequences of most of these are unknown (23). Due to the role of CYP1A2 in tumorigenesis, numerous investigations have been undertaken to determine the effects of *CYP1A2* polymorphisms on various diseases, including cancer, and several meta-analyses have been published with conflicting results (24,25). Moreover, to the best of our knowledge, the possible association of *CYP1A2* rs2472299, rs2470890 and rs11072508 polymorphisms, and prostate cancer risk and progression has not yet been studied. The effect of these polymorphisms and haplotypes on prostate cancer susceptibility and the clinicopathological features of the disease was therefore investigated in the present study.

Materials and methods

Study population. The present case-control study was approved by the Ethical Board of Jessenius Faculty of Medicine, Comenius University (Martin, Slovak Republic) and conducted in accordance with The Declaration of Helsinki. Most of the subjects were also enrolled in our previous studies (26,27).

The inclusion and exclusion criteria for the case group were as follows: i) age ≥50 years; ii) Caucasian; and iii) histologically confirmed prostate cancer. The indication for prostate biopsy was either a suspicious finding on digital rectal examination (DRE) or elevated serum levels of prostate specific antigen (PSA), or both. Patients with prior or present evidence of other cancers or other major pathologies and patients who had a first-degree relative (brother or father) with a confirmed diagnosis of prostate cancer were excluded.

The inclusion criteria for the control group were as follows: i) age ≥50 years; ii) Caucasian; iii) no current or previous diagnoses of cancer; iv) no evidence of family history of prostate cancer; and v) negative DRE and negative serum PSA according to age-specific reference values (28).

A one-to-one case-control study was designed with an estimated total number of 408 patients and controls each. This number provided an 80% power to detect a difference in the proportions of at least 0.1 in any direction at a significance level of 0.05 assuming the most conservative prevalence in controls (0.5). To account for the non-probabilistic nature of the data-generating process and potential drop-out, the sample size was increased by 35% (408 to 551). A total of 1,076 Caucasian men were studied, including 522 patients with prostate cancer and 554 healthy controls, at the Department of Urology, Jessenius Faculty of Medicine and University Hospital Martin, Comenius University in Bratislava. Prostate cancer patients and controls were enrolled from May 2005 to December 2019. All subjects agreed to participate in the study and written informed consent was obtained from all. Venous blood (3 ml) from all participants was withdrawn into Vacutainer tubes containing EDTA as the anticoagulant.

For all of the patients with prostate cancer, histological evaluation was performed on specimens collected using prostate needle biopsy or transurethral resection of the prostate. Biopsy materials were immediately fixed in a 10% solution of buffered formaldehyde for 24 h at 25˚C. Fixed material was embedded into paraffin blocks and histological slides of 3-4 μm thickness were cut and stained using a commercial Hematoxylin and Eosin Staining Kit (cat. no. ab245880; Abcam) according to the manufacturer's protocols. The protocol of the International Society of Urological Pathology was used for histological slides analysis and the final biopsy record (29). Histological slides were assessed using a BX45 light microscope (Olympus Corporation). The Gleason score for the histopathological grade (30) was recorded and the patients were divided into two groups as follows: i) low-grade, score ≤7; and ii) high-grade, score >7.

Cases and controls were tested for total serum prostatic specific antigen (PSA) levels using a Beckman Coulter Access® Hybritech® assay (cat. no. 37200, Beckman Coulter, Inc.) according to the manufacturer's protocol.

Genotyping. Genomic DNA was isolated from peripheral blood samples (300 μl) using a Wizard® Genomic DNA Purification Kit (catalogue no. A1125; Promega Corporation) and ethanol precipitation according to the manufacturer's protocol.

Three tagged SNPs, capturing common variability of the *CYP1A2* gene, were adopted according to the approach

Table I. Primer sets and PCR conditions.

CYP1A2 SNP	Primer sequence, 5'-3'	Amplicon, bp	PCR conditions
rs2472299	F: TCACATGTTGAGCTGAGGAGT R: CCCTTATCAATTTCTTCAGGCATTCA	60	Initial denaturation 95˚C/5 min; 95˚C/10 sec, 54˚C/10 sec, 72˚C/10 sec, 40 cycles, final extension 72˚C/4 min.
rs2470890	F: CTGTGAACATGTCCAGGCG R: CCTCAGAATGGTGGTGTCTT	65	Initial denaturation 95˚C/5 min; 95˚C/10 sec, 58˚C/10 sec, 72˚C/10 sec, 40 cycles; final extension 72˚C/4 min.
rs11072508	F: GGCACTTTGTCCCACTTAGTCC R: CCTCTGTCCCCATCTGCCC	79	Initial denaturation 95˚C/5 min; 95˚C/10 sec, 58˚C/10 sec, 72˚C/10 sec, 40 cycles; final extension 72˚C/4 min.

CYPIA1, cytochrome P450 1A2; F, forward; R, reverse; SNP, single nucleotide polymorphism.

described by Matakova *et al* (31). The common variability given by the presence of polymorphic sites with minor allele frequency >10% was studied. Briefly, an algorithm implemented in Haploview 4.2 (32) was used with the 1000 Genomes Project data (for population of Caucasian residents with northern and western European ancestry from UT, USA, CEU from phase 1 release; https://www.internationalgenome.org/home) from an extended genomic region of the *CYP1A2* gene (33). Three SNPs resulted from the analysis: rs2472299 from the 5'UTR of the *CYP1A2* gene (NC_000015.10:g.74741059A>G), the synonymous variant rs2470890 (NC_000015.10:g.74755085T>C), and rs11072508 from the 3'UTR of the *CYP1A2* gene (NC_000015.10:g.74770056C>T).

For the genotyping of selected SNPs, high-resolution melting analysis (HRMA) was carried out on a LightCycler 480 II (Roche Diagnostics). HRMAs were performed with the LightCycler® 480 High Resolution Melting Master Kit (cat. no. 0490963100; Roche Diagnostics GmbH) according to the manufacturer's protocols. Primer3Plus software (34) was used for the selection of primer sequences when designing genotyping reactions. Positive and negative controls were included in all reactions. The positive controls were external DNA samples with all three of the genotypes identified in pilot runs as having their own distinct melting curve. The negative control was a 'no template' control with nuclease-free water as a 'template' without a melting curve at the end of the run analysis. The specific primers and the PCR conditions are shown in Table I.

Statistical analysis. Genetic analysis of the determined genotypes and association analysis between each SNP and the defined groups of study participants were performed using PLINK 1.07 software (35). Five genetic models (allelic, dominant, recessive, genotypic and additive) were constructed to evaluate the association between *CYP1A2* rs2472299, rs2470890 and rs11072508 polymorphisms and prostate cancer risk. If *D* was the minor allele and *d* was the major allele then the models were constructed as follows: allelic, *D* vs. *d*; dominant, *DD*, *Dd* vs. dd; recessive, *DD* vs. *Dd*,*dd*; genotypic, *DD* vs. *Dd* vs. *dd*; and additive, *DD* vs. *Dd* and *Dd* vs. *dd*.

All genetic model associations were analysed using Fisher's exact test, with the exception of the additive model, which was analysed using the Cochran-Armitage trend test as this works with zero, one and two weights for each individual genotype, according to the burden of the minor allele which was required as the additive model assumed that the effect of a heterozygous genotype was halfway between the other two homogroups. The association of haplotypes was analysed using Pearson's χ^2-test. $P<0.05$ was considered to indicate a statistically significant difference.

PLINK calculated haplotypes based on multi-marker predictors using the standard E-M algorithm. Haplotype frequencies were estimated based on the distribution of a probabilistically-inferred set of haplotypes for each individual. To perform haplotype association tests, PLINK used Pearson's χ^2-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

The 522 patients with prostate cancer had a mean ± SD age of 65.8±9.5 years and a serum PSA concentration of 44.3±4.5 ng/ml. Out of 421 patients, 275 (65.3%) had a Gleason score ≤7 and 146 (34.7%) had a Gleason score >7. In the remaining 101 cases, the final pathological grade was not included in the analysis since the grading had been performed using other grading systems. A total of 119 patients (50%) had tumour stages pT1/pT2 and an equal number had tumour stages pT3/pT4. In 284 of the 522 patients the pathological stage was not included as the patients didn't undergo surgical removal of the prostate in the University Hospital Martin. The 554 healthy controls had a mean ± SD age of 64.7±10.3 years and a serum PSA concentration of 3.2±0.5 ng/ml.

The genotypic distribution for all SNPs in the controls and patients with prostate cancer was consistent with the Hardy-Weinberg equilibrium ($P<0.05$, data not shown). For further analyses of the genotype frequencies of all SNPs, five genetic models (genotypic, additive, allelic, dominant and recessive) were used, as shown in Table II. No significant

Table II. Genotype and allele frequencies of *CYP1A2* polymorphisms in patients with prostate cancer and the healthy controls.

CYP1A2	A1	A2	Model	Prostate cancer, n	Healthy controls, n	P-value[a]	OR (95% CI)
rs2472299	A	G	GENO[e]	58/243/221	75/244/235	0.45	NA
	A	G	ADD[f]	359/685	394/714	0.57	NA
	A	G	ALLELIC[b]	359/685	394/714	0.58	0.95 (0.79-1.13)
	A	G	DOM[c]	301/221	319/235	1.00	1.00 (0.79-1.28)
	A	G	REC[d]	58/464	75/479	0.23	0.79 (0.55-1.15)
rs2470890	C	T	GENO[e]	66/267/189	99/248/207	0.03	NA
	C	T	ADD[f]	399/645	446/662	0.33	NA
	C	T	ALLELIC[b]	399/645	446/662	0.35	0.92 (0.77-1.09)
	C	T	DOM[c]	333/189	347/207	0.71	1.05 (0.82-1.35)
	C	T	REC[d]	66/456	99/455	0.02	0.67 (0.48-0.93)
rs11072508	C	T	GENO[e]	66/267/189	88/266/200	0.29	NA
	C	T	ADD[f]	399/645	442/666	0.42	NA
	C	T	ALLELIC[b]	399/645	442/666	0.45	0.93 (0.78-1.11)
	C	T	DOM[c]	333/189	354/200	1.00	0.99 (0.78-1.28)
	C	T	REC[d]	66/456	88/466	0.14	0.77 (0.54-1.08)

[a]P-values calculated using Fisher's exact test, except for ADD where Cochran-Armitage trend test was used; [b]allelic model, *D* vs. *d*; [c]dominant model, *DD*, *Dd* vs. *dd*; [d]recessive model, *DD* vs. *Dd*, *dd*; [e]genotypic model, *DD* vs. *Dd* vs. *dd*; and [f]additive model, *DD* vs. *Dd* and *Dd* vs. *dd*. A1, minor allele; A2, major allele; GENO, genotypic model; ADD, additive model; ALLELIC, allelic model; DOM, dominant model; REC, recessive model; CYPIA1, cytochrome P450 1A2; OR, odds ratio; CI, confidence interval; NA, not available (design of association test does not generate OR); D, the minor allele; d, the major allele.

association with susceptibility for prostate cancer for the *CYP1A2* rs2472299 and rs11072508 polymorphisms was found in any of the genetic models. The genotype frequencies differed significantly between prostate cancer and control groups in terms of the *CYP1A2* rs2470890 polymorphism (P=0.03) in the genotypic model. For rs2470890, a significantly decreased risk of prostate cancer was observed in the recessive model [CC vs. CT + TT; odds ratio (OR), 0.67; 95% confidence interval (CI), 0.48-0.93; P=0.02].

The patients were further divided by PSA levels (<10 and ≥10 ng/ml), Gleason score (≤7 and >7) and pathological T stage (pT1/pT2 and pT3/pT4). As shown in Table III, stratification analysis of the *CYP1A2* rs2472299 and rs2470890 polymorphisms revealed no significant association with PSA levels in any of the genetic models. However, a significant association was observed between the *CYP1A2* rs11072508 polymorphism and serum PSA levels ≥10 ng/ml (OR, 1.51; 95% CI, 1.01-2.26; P=0.05) in the dominant model. The additive genetic model showed a significant association between the *CYP1A2* rs2470890 and rs11072508 polymorphisms with the development of higher grade carcinomas (Gleason score >7; P=0.03 and P=0.03, respectively). Moreover, the *CYP1A2* rs2470890 polymorphism was significantly associated with the development of higher grade carcinomas (Gleason score >7) in the allelic genetic model (OR, 1.36; 95% CI, 1.02-1.82; P=0.04). Furthermore, a significant association was observed between the rs11072508 polymorphism and higher grade carcinomas (Gleason score >7) in the allelic (OR, 1.37; 95% CI, 1.03-1.83; P=0.04) and dominant (OR, 1.60; 95% CI, 1.04-2.45; P=0.03) genetic models. No significant associations were found in any of the genetic models for the rs2472299 polymorphism (P>0.05) with a high Gleason score risk. The associations between *CYP1A2* polymorphisms and pathological T stage were also determined (Table III). *CYP1A2* rs2472299, rs2470890 and rs11072508 showed a significant association with pathological T stage in additive (P=0.02, P=0.007 and P=0.03, respectively), allelic (OR, 1.59, P=0.02; OR, 1.67, P=0.009; and OR, 1.49, P=0.05, respectively) and dominant (OR, 1.79, P=0.03; OR, 2.31, P=0.004; and OR, 1.94, P=0.03, respectively) genetic models.

The linkage disequilibrium, measured using the squared correlation coefficient r^2, between each SNP was in the range 0.683-0.829. Analysis of the *CYP1A2* gene revealed five haplotype groups among which the 'GTT' and 'ACC' haplotypes (order of SNPs in haplotype: rs2472299>rs2470890>rs11072508) were the most common in controls (91.8%) and patients with prostate cancer (92.9%) (Table IV). Haplotype analysis revealed that haplotypes did not alter the prostate cancer risk. The association between the haplotypes of the *CYP1A2* gene and the clinical outcomes were also analysed (Table V). A significant association was found between the 'GTT' haplotype and the Gleason score and the pathological T stage (OR, 0.71, P=0.03; and OR, 0.62, P=0.02, respectively), suggesting the protective effect of the major 'GTT' haplotype in prostate cancer pathology. Moreover, the 'ACC' haplotype was associated with pT3/pT4 (OR, 1.54; 95% CI 1.04-2.26; P=0.03). No associations were detected between the haplotypes and serum PSA values (P>0.05).

Table III. Risk of prostate cancer associated with the *CYP1A2* polymorphisms and clinicopathological characteristics of patients.

				PSA				Gleason score				Pathological T stage			
CYP1A2	A1	A2	Model	<10 ng/ml, n	≥10 ng/ml, n	OR (95% CI)	P-value[a]	≤7, n	>7, n	OR (95% CI)	P-value[a]	pT1/ pT2, n	pT3/ pT4, n	OR (95% CI)	P-value[a]
rs2472299	A	G	GENO[e]	26/ 93/90	24/ 105/85	NA	0.64	28/122/ 125	19/ 70/57	NA	0.39	11/48/ 58	18/53/ 39	NA	0.07
	A	G	ADD[f]	145/ 273	153/ 275	NA	0.74	178/ 372	108/ 184	NA	0.17	70/ 164	89/ 131	NA	0.02
	A	G	ALLELIC[b]	145/ 273	153/ 275	1.05 (0.79-1.39)	0.77	178/ 372	108/ 184	1.23 (0.91-1.65)	0.19	70/ 164	89/ 131	1.59 (1.08-2.35)	0.02
	A	G	DOM[c]	119/ 90	129/ 85	1.15 (0.78-1.69)	0.49	150/ 125	89/ 57	1.30 (0.87-1.96)	0.22	59/ 58	71/ 39	1.79 (1.05-3.05)	0.03
	A	G	REC[d]	26/ 183	24/ 190	0.89 (0.49-1.61)	0.76	28/ 247	19/ 127	1.32 (0.71-2.46)	0.42	11/ 106	18/ 92	1.89 (0.85-4.19)	0.16
rs2470890	C	T	GENO[e]	26/ 103/80	31/115/ 68	NA	0.38	30/135/ 110	24/77/ 45	NA	0.09	14/50/ 53	19/62/ 29	NA	0.01
	C	T	ADD[f]	155/ 263	177 /251	NA	0.18	195/ 355	125/ 167	NA	0.03	78/ 156	100/ 120	NA	0.007
	C	T	ALLELIC[b]	155/ 263	177/ 251	1.19 (0.91-1.57)	0.21	195/ 355	125/ 167	1.36 (1.02-1.82)	0.04	78/ 156	100/ 120	1.67 (1.14-2.44)	0.009
	C	T	DOM[c]	129/ 80	146/ 68	1,33 (0.89-1.99)	0.18	165/ 110	101/ 45	1.49 (0.97-2.91)	0.07	64/ 53	81/ 29	2.31 (1.32-4.05)	0.004
	C	T	REC[d]	26/ 183	31/ 183	1.19 (0.68-2.09)	0.57	30/ 245	24/ 122	1.61 (0.90-2.87)	0.13	14/ 103	19/ 91	1.54 (0.73-3.24)	0.27
rs11072508	C	T	GENO[e]	26/ 100/83	30/ 119/65	NA	0.13	31/ 134/110	23/ 80/43	NA	0.07	15/54/ 48	19/62/ 29	NA	0.07
	C	T	ADD[f]	152/ 266	179/ 249	NA	0.09	196/ 354	126/ 166	NA	0.03	84/ 150	100/ 120	NA	0.03
	C	T	ALLELIC[b]	152/ 266	179/ 249	1.26 (0.95-1.66)	0.11	196/ 354	126/ 166	1.37 (1.03-1.83)	0.04	84/ 150	100/ 120	1.49 (1.02-2.17)	0.05
	C	T	DOM[c]	126 83	149/ 65	1.51 (1.01-2.26)	0.05	165/ 110	103/ 43	1.60 (1.04-2.45)	0.03	69/ 48	81/ 29	1.94 (1.11-3.41)	0.03
	C	T	REC[d]	26/ 183	30/ 184	1.15 (0.65-2.02)	0.67	31/ 244	23/ 123	1.47 (0.82-2.63)	0.22	15/ 102	19/ 91	1.42 (0.68-2.96)	0.36

[a]P-values calculated using Fisher's exact test, except for ADD where Cochran-Armitage trend test was used; [b]allelic model, *D* vs. *d*; [c]dominant model, *DD*, *Dd* vs. *dd*; [d]recessive model, *DD* vs. *Dd*, *dd*; [e]genotypic model, *DD* vs. *Dd* vs. *dd*; and [f]additive model, *DD* vs. *Dd* and *Dd* vs. *dd*. A1, minor allele; A2, major allele; GENO, genotypic model; ADD, additive model; ALLELIC, allelic model; DOM, dominant model; REC, recessive model; CI, confidence interval; OR, odds ratio; PSA, prostatic specific antigen; CYPIA1, cytochrome P450 1A2; NA, not available (design of association test does not generate OR); D, the minor allele; d, the major allele.

Table IV. Frequencies of *CYP1A2* haplotypes (in order: rs2472299>rs2470890>rs11072508) in patients with prostate cancer and healthy controls.

Haplotype	Prostate cancer, n	Healthy controls, n	OR (95% CI)	P-value[a]
GTT	0.605	0.584	1.09 (0.92-1.29)	0.34
ACC	0.324	0.334	0.96 (0.80-1.15)	0.64
GCC	0.046	0.053	0.86 (0.58-1.27)	0.43
GTC	0.012	0.012	0.98 (0.45-2.15)	0.95
ACT	0.014	0.017	0.84 (0.42-1.65)	0.59

[a]P-values calculated using χ^2 test. CI, confidence interval; OR, odds ratio; CYPIA2, cytochrome P450 1A2.

Discussion

To the best of our knowledge, the present study is the first to analyse the association of *CYP1A2* polymorphisms rs2472299, rs2470890 and rs11072508, and haplotypes with the risk and clinicopathological features of prostate cancer in the Slovak population. The lack of significant associations in the present study among the *CYP1A2* rs2472299 and rs11072508 polymorphisms and prostate cancer risk suggests that the direct impact of these polymorphisms on prostate cancer susceptibility is limited. In the case of polymorphism *CYP1A2* rs2470890, an association with significantly reduced prostate cancer risk for CC genotype carriers in the recessive model was observed.

The associations of genetic variations of *CYP1A2* with clinicopathological characteristics (serum PSA, Gleason score, pathological T stage) were further examined in order to evaluate potential associations with the aggressive form of prostate cancer. Notably, all three studied *CYP1A2* polymorphisms were significantly associated with pathological T stage (pT3/pT4) in the additive, allelic and dominant genetic models. Moreover, rs11072508 and rs2470890 polymorphic variants were found to have significant associations with higher grade carcinomas (Gleason score >7) in the additive, allelic and dominant genetic models. However, no significant association was found between these *CYP1A2* polymorphisms and serum PSA levels in patients with prostate cancer.

In case-control studies, statistical power to detect disease susceptibility depends, among other factors, on the selected genetic models. Therefore, it is important to carefully choose the most accurate and representative genotype model (36,37). However, a commonly accepted strategy to select the best model has not been developed (38). Thus, dominant, recessive, and additive models are often used. The dominant model assumes that having one or more copies of a major allele increases the risk of having an altered phenotype in comparison with minor allele. The recessive model assumes that one or more copies of minor allele has the strongest impact on phenotype and, thus, requires a major allele homozygote genotype to alter risk. The additive model ranks genotypes with two allele copies over those that have one or none from highest to lowest impact on risk (37,38).

Only one study has investigated the association of these three *CYP1A2* polymorphisms and haplotypes with cancer risk. Matakova *et al* (31) studied the risk of lung cancer in a case-control study of 105 patients with lung cancer and 189 controls. The study reported a significant association of the *CYP1A2* rs2470890 and rs2422299 polymorphisms with lung cancer risk. Moreover, it was shown that the haplotype 'ACC', which is present at the highest frequency, was associated with an increased lung cancer risk and that the rare haplotype 'GTC' was significantly associated with a decreased lung cancer risk in the Slovak population. These polymorphisms have been studied alone or in combination with other polymorphisms in various types of cancer, including lung cancer (39,40), hepatocellular carcinoma (41), breast cancer (42,43), and colorectal cancer (44), in various population groups.

A number of research groups have investigated the relationship between other *CYP1A2* polymorphisms (rs762551, rs2069514, rs2069525 and rs5762551) and prostate cancer risk. However, the results were inconsistent (42-48). Some of these studies have examined the interaction between the *CYP1A2* polymorphisms and environmental exposure, such as cigarette smoking or the high intake of grilled-smoked meat. For example, Koda *et al* (45) reported that *CYP1A2* rs762551 risk genotypes (AA, CA, and CA + AA) were associated with an increased risk of prostate cancer among Japanese individuals with a high heterocyclic aromatic amine intake. It was speculated that the higher activation of heterocyclic aromatic amines in the liver and/or prostate by CYP1A2 is important for the increased risk of prostate cancer. Furthermore, another study revealed an association between smoking status, the *CYP1A2* rs762551 polymorphism and localized prostate cancer risk among white non-Hispanic individuals (49).

A possible explanation for the inconsistent results between these studies might be that the same polymorphisms serve different roles in cancer susceptibility in different ethnicities and in different tumour types (40). Moreover, prostate cancer is a heterogeneous disease and *CYP1A2* polymorphisms alone might not predispose individuals to prostate cancer. Therefore, gene-gene interactions among several different genes should be investigated in order to explain the pathogenesis of prostate cancer. Factors other than genetic ones, such as lifestyle (smoking, drinking and dietary habits) and environmental exposure, might influence CYP1A2 enzyme activity. High *in vivo* CYP1A2 activity has been suggested to be a susceptibility factor for bladder, colon and rectum cancer in which exposure to aromatic and heterocyclic amines has been implicated in the aetiology of the disease (50). Additionally, the *CYP1A2* rs2069514 and rs762551 polymorphisms are

Table V. Frequencies of *CYP1A2* haplotypes (in order: rs2472299>rs2470890>rs11072508) and clinicopathological characteristics in patients with prostate cancer.

	PSA				Gleason score				Pathological T stage			
Haplotype	<10 ng/ml	≥10 ng/ml	OR (95% CI)	P-value[a]	≤7	>7	OR (95% CI)	P-value[a]	pT1/pT2	pT3/pT4	OR (95% CI)	P-value[a]
GTT	0.626	0.562	0.76 (0.57-1.00)	0.06	0.632	0.552	0.71 (0.54-0.95)	0.03	0.643	0.532	0.62 (0.43-0.91)	0.02
ACC	0.325	0.334	1.05 (0.78-1.41)	0.78	0.303	0.347	1.23 (0.91-1.67)	0.19	0.287	0.385	1.54 (1.04-2.26)	0.03
GCC	0.037	0.062	1.74 (0.91-3.34)	0.09	0.041	0.066	1.67 (0.88-3.15)	0.11	0.052	0.060	1.16 (0.52-2.57)	0.73
GTC	0.000	0.024	NA		0.011	0.017	1.48 (0.49-4.47)	0.47	0.018	0.009	0.61 (0.14-2.79)	0.45
ACT	0.013	0.019	1.58 (0.51-4.93)	0.45	0.013	0.018	1.36 (0.42-4.35)	0.61	0.000	0.014	NA	0.07

[a]P-values calculated using χ^2 test. CI, confidence interval; OR, odds ratio; NA, not applicable; PSA, prostatic specific antigen; CYP1A2, cytochrome P450 1A2.

reported to be associated with increased enzyme activity in smokers (51,52).

Hormones, such as androgens and oestrogens, are known to be contributors to the development and progression of prostate cancer (1,53). Furthermore, the CYP1A2 enzyme is well documented as being the most active in the metabolic conversion of oestradiol to 2-hydroxyoestradiol (54). The increased formation of 2-hydroxylated oestrogens has been shown to contribute to a decreased susceptibility to breast cancer, since 2-hydroxyoestrogens can weakly bind only to the oestrogen receptor (55). Therefore, we hypothesize that the altered CYP1A2 enzyme activity in oestrogen metabolism partially affects prostate cancer development.

In conclusion, the present study demonstrated a significantly decreased risk of prostate cancer only with the *CYP1A2* rs2470890 polymorphism in the recessive genetic model in the Slovak population. The *CYP1A2* rs2472299 and rs11072508 polymorphisms were not shown to be significantly associated with prostate cancer risk in this population. Moreover, the association of the *CYP1A2* rs2470890 and rs11072508 polymorphisms was observed with the development of higher grade carcinomas (Gleason score >7) in the allelic (rs2470890 and rs11072508) and dominant (rs11072508) genetic models. Furthermore, all three polymorphisms were found to have a statistically significant association with pathological T stage in the additive, allelic and dominant genetic models. The haplotype analysis indicated the protective effect of the most frequent haplotype 'GTT' was associated with high-grade carcinomas (Gleason score >7) and pT3/pT4 in patients with prostate cancer. The significant association of the second most common haplotype, 'ACC', with higher pathological T stage (pT3/pT4) was observed. Further research is required to confirm the associations between these *CYP1A2* polymorphisms and oncological outcome, and to interpret their biological significance in disease phenotype. Additional genetic and functional studies are also needed to validate the findings of the present study and to clarify the role of the 'GTT' and 'ACC' haplotypes in CYP1A2 enzyme activity.

Acknowledgements

Not applicable.

Funding

This work was supported by the Agency of Ministry of Education, Science, Research and Sport of the Slovak Republic (grant nos. 1/0014/22 and APVV-15-0181).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MSK and MŠ confirm the authenticity of all the raw data. MKS, MV, JK and DE made substantial contributions to conception and design of the present study. MŠ, DD and JJ performed acquisition of data. PK, MH, MKB and RD

performed analysis and interpretation of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This case-control study was approved by the Ethical Board of Jessenius Faculty of Medicine, Comenius University (Martin, Slovak Republic; approval no. EK 6/2022) and conducted in accordance with The Declaration of Helsinki. Written informed consent was obtained from all subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ziaran S, Varchulova Novakova Z, Bohmer D and Danisovic L: Biomarkers for determination prostate cancer: Implication for diagnosis and prognosis. Neoplasma 62: 683-691, 2015.
2. Joshi AD, Corral R, Catsburg C, Lewinger JP, Koo J, John EM, Ingles SA and Stern MC: Red meat and poultry, cooking practices, genetic susceptibility and risk of prostate cancer: Results from a multiethnic case-control study. Carcinogenesis 33: 2108-2118, 2012.
3. Liska DJ: The detoxification enzyme systems. Altern Med Rev 3: 187-198, 1998.
4. Almazroo OA, Miah MK and Venkataramanan R: Drug metabolism in the liver. Clin Liver Dis 21: 1-20, 2017.
5. Kaur G, Gupta SK, Singh P, Ali V, Kumar V and Verma M: Drug-metabolizing enzymes: Role in drug resistance in cancer. Clin Transl Oncol 22: 1667-1680, 2020.
6. Anzenbacher P and Anzenbacherová E: Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci 58: 737-747, 2001.
7. Go RE, Hwang KA and Choi KC: Cytochrome P450 1 family and cancers. J Steroid Biochem Mol Biol 147: 24-30, 2015.
8. Williams JA, Martin FL, Muir GH, Hewer A, Grover PL and Phillips DH: Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue. Carcinogenesis 21: 1683-1689, 2000.
9. Zhou SF, Wang B, Yang LP and Liu JP: Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. Drug Metab Rev 42: 268-354, 2010.
10. Zanger UM and Schwab M: Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 138: 103-141, 2013.
11. Eaton DL, Gallagher EP, Bammler TK and Kunze KL: Role of cytochrome P4501A2 in chemical carcinogenesis: Implications for human variability in expression and enzyme activity. Pharmacogenetics 5: 259-274, 1995.
12. Gunes A and Dahl ML: Variation in CYP1A2 activity and its clinical implications: Influence of environmental factors and genetic polymorphisms. Pharmacogenomics 9: 625-637, 2008.
13. Nebert DW and Dalton TP: The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat Rev Cancer 6: 947-960, 2006.
14. Zaccaro C, Sweitzer S, Pipino S, Gorman N, Sinclair PR, Sinclair JF, Nebert DW and De Matteis F: Role of cytochrome P450 1A2 in bilirubin degradation studies in Cyp1a2 (-/-) mutant mice. Biochem Pharmacol 61: 843-849, 2001.
15. Lambrecht RW, Jacobs JM, Sinclair PR and Sinclair JF: Inhibition of uroporphyrinogen decarboxylase activity. The role of cytochrome P-450-mediated uroporphyrinogen oxidation. Biochem J 269: 437-441, 1990.
16. Djordjevic N, Ghotbi R, Bertilsson L, Jankovic S and Aklillu E: Induction of CYP1A2 by heavy coffee consumption in Serbs and Swedes. Eur J Clin Pharmacol 64: 381-385, 2008.
17. Rasmussen BB, Brix TH, Kyvik KO and Brøsen K: The interindividual differences in the 3-demthylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. Pharmacogenetics 12: 473-478, 2002.
18. Shimada T, El-Bayoumy K, Upadhyaya P, Sutter TR, Guengerich FP and Yamazaki H: Inhibition of human cytochrome P450-catalyzed oxidations of xenobiotics and procarcinogens by synthetic organoselenium compounds. Cancer Res 57: 4757-4764, 1997.
19. Rodgman A, Smith CJ and Perfetti TA: The composition of cigarette smoke: A retrospective, with emphasis on polycyclic components. Hum Exp Toxicol 19: 573-595, 2000.
20. Hladun O, Papaseit E, Martín S, Barriocanal AM, Poyatos L, Farré M and Pérez-Mañá C: Interaction of energy drinks with prescription medication and drugs of abuse. Pharmaceutics 13: 1532, 2021.
21. Christensen M, Tybring G, Mihara K, Yasui-Furokori N, Carrillo JA, Ramos SI, Andersson K, Dahl ML and Bertilsson L: Low daily 10-mg and 20-mg doses of fluvoxamine inhibit the metabolism of both caffeine (cytochrome P4501A2) and omeprazole (cytochrome P4502C19). Clin Pharmacol Ther 71: 141-152, 2002.
22. Faber MS, Jetter A and Fuhr U: Assessment of CYP1A2 activity in clinical practice: Why, how, and when? Basic Clin Pharmacol Toxicol 97: 125-134, 2005.
23. Zhou SF, Yang LP, Zhou ZW, Liu YH and Chan E: Insights into the substrate specificity, inhibitors, regulation, and polymorphisms and the clinical impact of human cytochrome P450 1A2. AAPS J 11: 481-494, 2009.
24. Daly AK: Polymorphic variants of cytochrome P450: Relevance to cancer and other diseases. Adv Pharmacol 74: 85-111, 2015.
25. Vukovic V, Ianuale C, Leoncini E, Pastorino R, Gualano MR, Amore R and Boccia S: Lack of association between polymorphisms in the CYP1A2 gene and risk of cancer: Evidence from meta-analyses. BMC Cancer 16: 83, 2016.
26. Híveš M, Jurečeková J, Kliment J, Grendár M, Kaplán P, Dušenka R, Evin D, Vilčková M, Holečková KH and Sivoňová MK: Role of genetic variations in CDK2, CCNE1 and p27KIP1 in prostate cancer. Cancer Genomics Proteomics 19: 362-371, 2022.
27. Sivonova MK, Jurecekova J, Kaplan P, Hives M, Grendar M, Tomascova A, Dusenka R, Drobkova H, Evin D and Kliment J: Association of MDM2 T309G (rs2279744) polymorphism and expression changes with risk of prostate cancer in the Slovak population. Anticancer Res 40: 6257-6264, 2020.
28. Pienta KJ: Critical appraisal of prostate-specific antigen in prostate cancer screening: 20 Years later. Urology 73 (5 Suppl): S11-S20, 2009.
29. van Leenders GJLH, van der Kwast TH, Grignon DJ, Evans AJ, Kristiansen G, Kweldam CF, Litjens G, McKenney JK, Melamed J, Mottet N, *et al*: The 2019 international society of urological pathology (ISUP) consensus conference on grading of prostatic carcinoma. Am J Surg Pathol 44: e87-e99, 2020.
30. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR and Humphrey PA; Grading Committee: The 2014 international society of urological pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma: Definition of grading patterns and proposal for a new grading system. Am J Surg Pathol 40: 244-252, 2016.
31. Matakova T, Halasova E, Dzian A, Hruby R, Halasa M, Javorka K and Skerenova M: Associations of CYP1A2 polymorphisms with the risk haplotypes in lung cancer in the Slovak population. Adv Exp Med Biol 911: 23-32, 2016.
32. Barrett JC, Fry B, Maller J and Daly MJ: Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263-265, 2005.
33. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA and Abecasis GR: A global reference for human genetic variation. Nature 526: 68-74, 2015.
34. Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R and Leunissen JAM: Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res 35(Web Server Issue): W71-W74, 2007.
35. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ and Sham PC: PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559-575, 2007.
36. Lewis CM: Genetic association studies: Design, analysis and interpretation. Brief Bioinform 3: 146-153, 2002.

37. Horita N and Kaneko T: Genetic model selection for a case-control study and a meta-analysis. Meta Gene 5: 1-8, 2015.
38. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP and Zondervan KT: Basic statistical analysis in genetic case-control studies. Nat Protoc 6: 121-133, 2011.
39. Aldrich MC, Selvin S, Hansen HM, Barcellos LF, Wrensch MR, Sison JD, Kelsey KT, Buffler PA, Quesenberry CP Jr, Seldin MF and Wiencke JK: CYP1A1/2 haplotypes and lung cancer and assessment of confounding by population stratification. Cancer Res 69: 2340-2348, 2009.
40. Rotunno M, Yu K, Lubin JH, Consonni D, Pesatori AC, Goldstein AM, Goldin LR, Wacholder S, Welch R, Burdette L, *et al*: Phase I metabolic genes and risk of lung cancer: Multiple polymorphisms and mRNA expression. PLoS One 4: e5652, 2009.
41. Chen X, Wang H, Xie W, Liang R, Wei Z, Zhi L, Zhang X, Hao B, Zhong S, Zhou G, *et al*: Association of CYP1A2 genetic polymorphisms with hepatocellular carcinoma susceptibility: A case-control study in a high-risk region of China. Pharmacogenet Genomics 16: 219-227, 2006.
42. Bai X, Xie J, Sun S, Zhang X, Jiang Y and Pang D: The associations of genetic polymorphisms in CYP1A2 and CYP3A4 with clinical outcomes of breast cancer patients in northern China. Oncotarget 8: 38367-38377, 2017.
43. Anderson LN, Cotterchio M, Mirea L, Ozcelik H and Kreiger N: Passive cigarette smoke exposure during various periods of life, genetic variants, and breast cancer risk among never smokers. Am J Epidemiol 175: 289-301, 2012.
44. Wang H, Yamamoto JF, Caberto C, Saltzman B, Decker R, Vogt TM, Yokochi L, Chanock S, Wilkens LR and Le Marchand L: Genetic variation in the bioactivation pathway for polycyclic hydrocarbons and heterocyclic amines in relation to risk of colorectal neoplasia. Carcinogenesis 32: 203-209, 2011.
45. Koda M, Iwasaki M, Yamano Y, Lu X and Katoh T: Association between NAT2, CYP1A1, and CYP1A2 genotypes, heterocyclic aromatic amines, and prostate cancer risk: A case control study in Japan. Environ Health Prev Med 22: 72, 2017.
46. Wei W, Ge JP, Dong J, Gao JP, Zhang ZY and Gong J: Single nucleotide polymorphisms of CYP1A2 and their correlation with prostate cancer. Zhonghua Nan Ke Xue 17: 998-1001, 2011 (In Chinese).
47. Cunningham JM, Hebbring SJ, McDonnell SK, Cicek MS, Christensen GB, Wang L, Jacobsen SJ, Cerhan JR, Blute ML, Schaid DJ and Thibodeau SN: Evaluation of genetic variations in the androgen and estrogen metabolic pathways as risk factors for sporadic and familial prostate cancer. Cancer Epidemiol Biomarkers Prev 16: 969-978, 2007.
48. Murata M, Watanabe M, Yamanaka M, Kubota Y, Ito H, Nagao M, Katoh T, Kamataki T, Kawamura J, Yatani R and Shiraishi T: Genetic polymorphisms in cytochrome P450 (CYP) 1A1, CYP1A2, CYP2E1, glutathione S-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. Cancer Lett 165: 171-177, 2001.
49. Shahabi A, Corral R, Catsburg C, Joshi AD, Kim A, Lewinger JP, Koo J, John EM, Ingles SA and Stern MC: Tobacco smoking, polymorphisms in carcinogen metabolism enzyme genes, and risk of localized and advanced prostate cancer: Results from the California collaborative prostate cancer study. Cancer Med 3: 1644-1655, 2014.
50. Gooderham NJ, Murray S, Lynch AM, Edwards RJ, Yadollahi-Farsani M, Bratt C, Rich KJ, Zhao K, Murray BP, Bhadresa S, *et al*: Heterocyclic amines: Evaluation of their role in diet associated human cancer. Br J Clin Pharmacol 42: 91-98, 1996.
51. Zhenzhen L, Xianghua L, Ning S, Zhan G, Chuanchuan R and Jie L: Current evidence on the relationship between three polymorphisms in the CYP1A2 gene and the risk of cancer. Eur J Cancer Prev 22: 607-619, 2013.
52. Ghotbi R, Christensen M, Roh HK, Ingelman-Sundberg M, Aklillu E and Bertilsson L: Comparisons of CYP1A2 genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. Eur J Clin Pharmacol 63: 537-546, 2007.
53. Wigle DT, Turner MC, Gomes J and Parent ME: Role of hormonal and other factors in human prostate cancer. J Toxicol Environ Health B Crit Rev 11: 242-259, 2008.
54. Tsuchiya Y, Nakajima M and Yokoi T: Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett 227: 115-124, 2005.
55. Samavat H and Kurzer MS: Estrogen metabolism and breast cancer. Cancer Lett 356: 231-243, 2015.