

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

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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

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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: GGCACCTTTGTCCCACTTAGTCC R: CCTCTGTCCCCATCTGCC	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.

CYP1A1, 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 \pm SD age of 65.8 \pm 9.5 years and a serum PSA concentration of 44.3 \pm 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 \pm SD age of 64.7 \pm 10.3 years and a serum PSA concentration of 3.2 \pm 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.

<i>CYP1A2</i>	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)

^aP-values calculated using Fisher's exact test, except for ADD where Cochran-Armitage trend test was used; ^ballelic model, *D* vs. *d*; ^cdominant model, *DD*, *Dd* vs. *dd*; ^drecessive model, *DD* vs. *Dd*, *dd*; ^egenotypic model, *DD* vs. *Dd* vs. *dd*; and ^fadditive 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; *CYP1A1*, 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.

CYP1A2	A1	A2	Model	PSA				Gleason score				Pathological T stage							
				<10 ng/ml, n		≥10 ng/ml, n		≤7, n		>7, n		pT1/ pT2, n		pT3/ pT4, n		OR (95% CI)		P-value ^a	
				OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	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				
	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				
rs11072508	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				
	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					

^aP-values calculated using Fisher's exact test, except for ADD where Cochran-Armitage trend test was used; ^ballelic model, *D* vs. *d*; ^cdominant model, *DD*, *Dd* vs. *dd*; ^drecessive model, *DD* vs. *Dd*, *dd*; ^egenotypic model, *DD* vs. *Dd* vs. *dd*; and ^fadditive 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; CYP1A1, 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

^aP-values calculated using χ^2 test. CI, confidence interval; OR, odds ratio; *CYP1A2*, 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.

Haplotype	PSA			Gleason score			Pathological T stage					
	<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)
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

^aP-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.

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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.

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