Original Article
Genetic polymorphisms in the androgen metabolism pathway and risk of prostate cancer in low incidence Malaysian ethnic groups

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Abstract: Androgens are involved in prostate cancer (PCa) cell growth. Genes involved in androgen metabolism mediate key steps in sex steroid metabolism. This study attempted to investigate whether single nucleotide polymorphisms (SNPs) in the androgen metabolism pathway are associated with PCa risk in low incidence Asian ethnic groups. We genotyped 172 Malaysian subjects for cytochrome P450 family 17 (CYP17A1), steroid-5-alpha-reductase, polypeptide 1 and 2 (SRD5A1 and SRD5A2), and insulin-like growth factor 1 (IGF-1) genes of the androgen metabolism pathway and assessed the testosterone, dihydrotestosterone and IGF-1 levels. SNPs in the CYP17A1, SRD5A1, SRD5A2, and IGF-1 genes were genotyped using real-time polymerase chain reaction. Although we did not find significant association between SNPs analysed in this study with PCa risk, we observed however, significant association between androgen levels and the IGF-1 and several SNPs. Men carrying the GG genotype for SNP rs1004467 (CYP17A1) had significantly elevated testosterone (P = 0.012) and dihydrotestosterone (DHT) levels (P = 0.024) as compared to carriers of the A allele. The rs518673 of the SRD5A1 was associated with prostate specific antigen (PSA) levels. Our findings suggest CYP17A1 rs1004467 SNP is associated with testosterone and DHT levels indicating the importance of this gene in influencing androgen levels in the circulatory system of PCa patients, hence could be used as a potential marker in PCa assessment.

Keywords: Prostate cancer, single nucleotide polymorphism, testosterone, dihydrotestosterone, insulin-like growth factor 1, androgen

Introduction

Prostate cancer (PCa) represents the most commonly diagnosed non-cutaneous malignancy among men in developed countries [1]. The American Society of Cancer indicated that PCa is the second leading cause of death from cancer in men [2]. However, a remarkably 20 times lower incidence has been recorded in the Asian population [3]. According to the latest cancer registry in Malaysia, PCa ranks as the fourth most common cancer among males and the incidence rates has been continuously rising from 821 cases in 2008 [3, 4] to 1186 in 2012 [6]. The highest incidence is among the Chinese (15.8 per 100,000) followed by the Indians (14.8 per 100,000) and Malays (7.7 per 100,000) [7].

The role of the androgen metabolism pathway in the etiology of PCa has been well documented [7, 8]. These findings imply that the polymorphisms in the androgen metabolism pathway might be associated with PCa outcome. Nonetheless, the underlying genetic mechanisms of the androgen metabolism pathway and risk factors of PCa remain largely undefined. PCa is heterogenous in nature, often complex, with multiple risk factors involved [10]. A study has reported that 42 to 57% of all PCa risk factors may be attributed to inherited genetics [11]. Racial variations could influence disease progression and incidence. Many studies have reported on gene polymorphisms conferring risk for PCa including gene variants involved in the androgen metabolism pathway [12]. As an androgen-regulated organ, prostate abnormali-

ties are often associated with levels of androgens such as testosterone (T), dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulphate (DHEA-S) and androstenedione. These hormones are essential for normal development, growth activities and maintenance of physiologic functions of the prostate gland [13]. Androgen signalling pathway is regulated by several crucial candidate genes [14]. Observations that PCa is androgen-regulated have led to suggestions that genetic alterations in the androgen metabolism pathway could potentially confer susceptibility to the disease. Some of the key regulatory genes involved in the androgen metabolism pathway are the *CYP17A1*, *HSD3B2*, *CYP3A*, *AKR1C*, *HSB17B*, and *SRD5A2* genes [15].

In the present study, we examined the association of single nucleotide polymorphisms (SNPs) in the androgen metabolism pathway with risk of PCa in the Malaysian men. While most of the reports are conducted in the Caucasians, there is a paucity of information in the Asian population. Moreover, the South East Asian Chinese population may differ in terms of population structure from other Asians including the Chinese Han in Beijing and the Japanese as indicated from the HapMap (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2790583/). Against this background, we investigate the association between the *CYP17A1*, *SRD5A1*, *SRD5A2*, and *IGF-1* SNPs within the androgen metabolism pathway and the risk of PCa in Malaysian men. We also examined the relationship between these SNPs and levels of DHT, testosterone and IGF-1 hormones related to the androgen metabolism pathway.

Materials and methods

Study population

A total of 172 Malaysian men consisting of 81 prostate cancer cases and 91 benign controls were enrolled. Case and control groups were recruited from the University of Malaya Medical Centre, Malaysia, for conditions unrelated to known or likely risk factors for prostate cancer and benign prostatic hyperplasia which were then histologically-confirmed to be either prostate cancer or benign prostatic hyperplasia. Tumour grade was evaluated in these samples using the Gleason scoring system. The diagnosis were graded as (a) the low grade group, with Gleason scores 2-5, (b) the intermediate group

with Gleason scores 6-7, or (c) the high-grade group, with Gleason scores 8-10. Controls were men with benign prostatic hyperplasia conditions, proven to be negative for prostate cancer after biopsies and digital rectal examinations. Clinical characteristics were obtained from medical records, including Gleason pathological grade, age at diagnosis, and PSA level at presentation in the clinic. All subjects in both groups provided informed consent to participate in this study. Ethics approval (MEC Ref. Num: 805.12) for the study was given by the University of Malaya Medical Centre Ethics Committee and the Medical Research and Ethics Committee, Ministry of Health Malaysia.

SNP selection and genotyping

A volume of 5 ml of blood was collected in EDTA tubes and stored in -80°C until used. DNA was isolated from the participants' whole blood cells with the use of QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). A total of 8 single-nucleotide polymorphisms (SNPs) within the four candidate genes, namely, *CYP17A1* (rs-1004467 and rs10883782), *IGF-1* (rs1242-3791, rs1106381 and rs5742723), *SRD5A1* (rs166050 and rs518673), and *SRD5A2* (rs-523349) were genotyped. Genotyping was performed using predesigned Taqman SNP genotyping assays (Applied Biosystems, Foster City, CA) using StepOne Real-Time PCR systems (Applied Biosystems, Foster City, CA). Two negative controls were included in each batch of samples.

Hormone analysis

Sera separated from whole blood collected in plain tubes were used to determine the levels of hormones. Measurements of testosterone, dihydrotestosterone and IGF-1 serum levels were performed using ELISA kits (*Dihydrotestosterone, ELISA Kit, BLK Spain and IGF-1, Human, Quantikine ELISA kits, R & D Systems, USA*) according to the manufacturer's instruction.

Statistical analysis

Statistical analysis was performed using SPSS software version 20.0 (SPSS, Inc., Chicago, IL). Data were presented as percentage or mean ± standard deviation (SD). To compare the PCa cases and BPH, Analysis of Covariance (ANCOVA) using the general linear model was applied with age as covariate. The genetic as-

Table 1. Clinical characteristics of subjects

Characteristics	Cases, n (%)	Controls, n (%)	P value*
Age at diagnosis, years			
< 50	2 (2.47)	1 (1.10)	
51-60	9 (11.11)	12 (13.18)	
61-70	23 (28.40)	41 (45.05)	
> 70	47 (58.02)	37 (40.66)	
Median ± SD	71.5 ± 7.94	69 ± 6.94	
Mean Age ± SD	70.33 ± 7.96	68.56 ± 7.27	0.153
PSA, ng/mL			
< 4	8 (9.87)	8 (8.79)	
4.1-10.0	13 (16.05)	55 (60.44)	
10.1-20.0	14 (17.20)	16 (17.58)	
> 20	46 (56.70)	8 (8.79)	
Median ± SD	21.61 ± 25.1	6.88 ± 9.79	< 0.0001
Mean PSA ± SD	88.75 ± 173.4	9.98 ±9.63	
Serum Sex Steroids			
DHT, pg/ml			
Median	873.76	763.38	
Mean ± SD	960.26 ± 423.27	794.77 ± 451.39	0.03
T, ng/ml			
Median	4.38	2.81	
Mean ± SD	5.03 ± 2.79	3.53 ± 3.53	0.007
IGF-1, ng/dl			
Median	4.63	4.06	
Mean ± SD	4.67 ± 0.42	4.13 ± 0.46	< 0.0001
Gleason Score			
Low Grade (2-5)	17 (20.89)		
Intermediate Grade (6-7)	31 (38.27)		
High Grade (8-10)	33 (40.75)		

*P values obtained using ANCOVA adjusted for age as covariate. *PSA*, Prostate-Specific Antigen. *DHT*, Dihydrotestosterone. *T*, Testosterone. *IGF-1*, Insulin-like Growth Factor 1.

in **Table 1**. Mean age for cases and controls were 70.33 ± 7.96 and 68.56 ± 7.27 years respectively (**Table 1**). The mean age for the controls and cases were not significantly different (P = 0.153). There was a significant difference in PSA levels between cases and controls (P < 0.0001). PSA is elevated to much higher levels in PCa cases compared to benign prostate disease. Mean PSA was 88.75 ng/ml ± 173.4 and 9.98 ng/ml ± 9.63 in the PCa and control group respectively. Serum sex steroids measured, DHT, testosterone and IGF-1, were all significantly higher in cases, P = 0.03, P = 0.007 and P < 0.0001 respectively. Hardy-Weinberg equilibrium (HWE) was checked for *CYP17A1* (rs-1004467 and rs108837-82), *IGF-1* (rs12423791, rs1106381 and rs57427-23), *SRD5A1* (rs166050 and rs518673), and *SRD-5A2* (rs523349) prior to genetic analysis. All SNPs were in agreement with the Hardy-Weinberg equilibrium. There were no significant differences in genotype frequencies for all SNPs between the PCa group and controls (data not shown).

Allele frequencies of *CYP17A1*, *IGF-1*, *SRD5A1* and *SRD5A2* SNPs showed no significant differences between the PCa group and controls (**Table 2**). Association between the *IGF-1*, *CYP17A1, SRD5A1, SRD5A2* SNPs and clinical characteristics in PCa cases such as Gleason score, serum PSA level, DHT, IGF-1 and testosterone are shown in **Table 3**. We did not find any significant association between the above mentioned SNPs and risk of PCa in our study cohort. However, IGF-1 hormone level was significantly associated with rs10883782 SNP of *CYP17A1* gene (P = 0.018).

We investigated the association of the SNPs with clinical parameters such as serum PSA

sociation tests were performed using binary logistic regression analysis to calculate the odds ratio (ORs) and confidence intervals. The models were adjusted for age and race. Test for normality was done using Kolmogorov-Smirnov test. The clinical characteristics between the PCa cases and BPH controls were compared using Student's *t*-test and Mann-Whitney U test. Genotype distribution for each SNP was tested for departure from Hardy-Weinberg equilibrium (HWE) using a goodness of fit χ^2-test. Analysis of Variance (ANOVA) and Kruskal-Wallis tests were used to compare parameters among genotypes. *P* value of < 0.05 was considered to be statistically significant.

Results

The demographic and clinical characteristics of the study subjects in this study are summarized

Table 2. Allele frequency associations between *IGF-1*, *CYP17A1*, *SRD5A1*, *SRD5A2* and cases and controls

| Gene | SNP ID | Minor Allele Frequency (MAF) | Allele Frequency | | P Value* | (OR, 95% CI) |
			Cases (n = 81)	Controls (n = 91)		
IGF-1	rs12423791 (G > C)	0.235	0.222	0.258	0.472	0.839 (0.520-1.354)
	rs1106381 (T > G)	0.198	0.209	0.187	0.648	1.132 (0.665-1.927)
	rs5742723 (G > T)	0.209	0.222	0.198	0.626	1.033 (0.991-1.077)
CYP17A1	rs1004467 (A > G)	0.247	0.246	0.258	0.739	0.919 (0.560-1.508)
	rs10883782 (A > G)	0.174	0.185	0.165	0.699	1.120 (0.629-1.995)
SRD5A1	rs166050 (A > G)	0.128	0.099	0.099	0.830	0.927 (0.464-1.851)
	rs518673 (G > A)	0.284	0.222	0.247	0.678	0.901 (0.550-1.475)
SRD5A2	rs523349 (G > C)	0.445	0.302	0.247	0.840	0.951 (0.587-1.543)

*P values adjusted for age. *SNP*, Single Nucleotide Polymorphism. *IGF-1*, Insulin-like Growth Factor 1. *CYP17A1*, Cytochrome P450 family 17. *SRD5A1*, Steroid-5-Alpha-Reductase alpha polypeptide 1. *SRD5A2*, Steroid-5-Alpha-Reductase alpha polypeptide 2. *MAF*, Minor Allele Frequency. *OR*, Odds Ratio. *CI*, Confidence Interval.

Table 3. Association of *IGF-1*, *CYP17A1*, *SRD5A1*, *SRD5A2* SNPs and clinical characteristics in PCa cases

| SNP ID | P Value* | | | | | Odds Ratio (95% CI) | P value |
	PSA	Gleason Score	DHT	IGF-1	T		
IGF-1							
rs12423791 (G > C)	0.363	0.881	0.445	0.593	0.452	0.772 (0.496-1.202)	0.252
rs1106381 (T > G)	0.331	0.741	0.446	0.282	0.366	0.946 (0.579-1.547)	0.826
rs5742723 (G > T)	0.336	0.693	0.452	0.268	0.331	0.949 (0.596-1.510)	0.826
CYP17A1							
rs1004467 (A > G)	0.386	0.928	0.631	0.499	0.401	0.761 (0.488-1.187)	0.228
rs10883782 (A > G)	0.586	0.282	0.649	0.018	0.419	0.923 (0.534-1.626)	0.804
SRD5A1							
rs166050 (A > G)	0.725	0.889	0.374	0.843	0.798	0.892 (0.385-2.067)	0.79
rs518673 (G > A)	0.291	0.483	0.521	0.794	0.452	0.976 (0.659-1.445)	0.904
SRD5A2							
rs523349 (G > C)	0.443	0.256	0.498	0.379	0.385	0.851 (0.534-1.355)	0.496

CI, Confidence Interval. *CYP17A1*, Cytochrome P450 family 17. *IGF-1*, Insulin-like Growth Factor 1. *OR*, Odds Ratio. *SNP*, Single Nucleotide Polymorphism. *SRD5A1*, Steroid-5-Alpha-Reductase alpha polypeptide 1. *SRD5A2*, Steroid-5-Alpha-Reductase alpha polypeptide 2.

and androgens DHT and testosterone levels as well as the IGF-1 hormone among cases and controls (**Table 4**). DHT and testosterone levels were significantly associated with rs1004467 SNP of the *CYP17A1*, *P* = 0.024 and *P* = 0.012 respectively. The rs1004467 GG homozygotes exhibited significantly elevated levels of mean circulatory DHT and testosterone compared to AA and AG. Likewise, significantly increased level of PSA was seen in the *SRD5A1* rs518673 GG homozygotes compared to the non-carriers (*P* = 0.029).

Discussion

Genome-wide association studies (GWAS) have been a powerful method in identifying genetic variants that predispose individuals to diseases [16]. However, papers involving GWAS studies tend to report SNPs that achieve significance of P ≤ 10^{-7}. Considering this fact, possibilities exist whereby SNPs that reach significance level for a candidate gene study (P ≤ 10^{-3} or P ≤ 10^{-4}) may be missed out in reporting of susceptible genes [17]. Candidate gene studies are therefore still worthwhile when performing association studies of polymorphisms with low allele frequencies and for conducting post-GWAS phase study.

Many studies have been successful in reporting strong associations of several variants with PCa risk. Our study examined the association

Table 4. Comparison of PSA, androgens and IGF-1 hormone between *IGF-1*, *CYP17A1*, *SRD5A1* and *SRD5A2* genotypes in cases and controls

Genes	SNPs	Genotypes	Characteristics (mean ± SD)				
			Age* (Years)	PSA# (ng/ml)	DHT* (pg/ml)	T# (ng/ml)	IGF-1* (ng/dl)
IGF-1	rs12423791	GG (*n* = 102)	69.7 ± 7.74	43.1 ± 105.06	873.9 ± 458.4	4.09 ± 3.17	4.46 ± 0.46
		CG (*n* = 57)	68.5 ± 6.92	37.0 ± 110.08	929.2 ± 450.8	4.88 ± 3.50	4.45 ± 0.56
		CC (*n* = 13)	70.8 ± 7.77	98.5 ± 243.65	758.4 ± 280.4	3.70 ± 2.11	4.40 ± 0.65
	P-value		0.502	0.481	0.443	0.309	0.932
	rs1106381	TT (*n* = 111)	69.0 ± 7.84	39.9 ± 100.60	880.3 ± 442.9	4.07 ± 2.9	4.47 ± 0.45
		TG (*n* = 54)	70.2 ± 6.99	42.4 ± 114.58	900.9 ± 457.9	4.82 ± 3.8	4.42 ± 0.58
		GG (n = 7)	69.1 ± 4.71	154.4 ± 330.35	807.1 ± 374.1	4.61 ± 3.3	4.44 ± 0.79
	P-value		0.646	0.483	0.867	0.576	0.865
	rs5742723	GG (*n* = 110)	69.4 ± 7.61	40.2 ± 100.98	879.8 ± 442.5	4.03 ± 2.89	4.47 ± 0.46
		GT (*n* = 52)	68.9 ± 7.17	40.6 ± 116.46	915.6 ± 463.5	4.92 ± 3.84	4.44 ± 0.58
		TT (*n* = 10)	71.9 ± 6.91	125.3 ± 275.19	769.2 ± 354.6	4.38 ± 2.96	4.24 ± 0.67
	P-value		0.516	0.438	0.634	0.444	0.468
CYP17A1	rs1004467	AA (*n* = 97)	69.8 ± 7.59	49.8 ± 130.39	860.4 ± 430.0	4.27 ± 2.93	4.44 ± 0.52
		AG (*n* = 63)	68.9 ± 6.58	35.8 ± 114.59	846.5 ± 420.6	3.86 ± 3.37	4.48 ± 0.52
		GG (*n* = 12)	68.2 ± 11.3	32.5 ± 45.78	1231.3 ± 526.4	7.17 ± 3.68	4.42 ± 0.41
	P-value		0.642	0.164	**0.024**	**0.012**	0.89
	rs10883782	AA (*n* = 116)	68.9 ± 7.51	51.9 ± 143.49	884.9 ± 449.4	4.25 ± 3.24	4.49 ± 0.53
		AG (*n* = 52)	70.6 ± 7.54	34.1 ± 62.93	859.7 ± 444.4	4.64 ± 3.32	4.41 ± 0.47
		GG (*n* = 4)	68.3 ± 3.77	11.9 ± 11.32	1132.1 ± 181.9	3.09 ± 1.79	3.94 ± 0.43
	P-value		0.367	0.541	0.501	0.607	0.085
SRD5A1	rs166050	AA (*n* = 141)	68.8 ± 7.67	49.9 ± 133.56	888.6 ± 451.3	4.42 ± 3.13	4.46 ± 0.53
		AG (*n* = 28)	72.4 ± 5.67	26.1 ± 50.76	861.1 ± 425.1	4.06 ± 3.77	4.43 ± 0.41
		GG (*n* = 3)	68.0 ± 7.81	21.8 ± 10.20	878.9 ± 199.6	3.47 ± 2.64	4.20 ± 0.41
	P-value		0.066	0.198	0.961	0.568	0.662
	rs518673	GG (*n* = 102)	70.1 ± 7.41	53.7 ± 127.70	890.5 ± 426.6	4.20 ± 2.96	4.49 ± 0.55
		GA (*n* = 59)	67.9 ± 7.52	36.1 ± 123.10	844.9 ± 428.3	4.26 ± 2.73	4.38 ± 0.44
		AA (*n* = 11)	70.6 ± 7.25	16.2 ± 23.45	1024.8 ± 663.2	6.11 ± 6.81	4.46 ± 0.56
	P-value		0.176	**0.029**	0.524	0.988	0.546
SRD5A2	rs523349	GG (*n* = 44)	70.3 ± 5.51	23.6 ± 34.67	829.8 ± 439.1	4.61 ± 3.87	4.43 ± 0.47
		GC (*n* = 103)	68.9 ± 7.69	42.2 ± 98.22	904.3 ± 421.9	4.03 ± 2.86	4.50 ± 0.55
		CC (*n* = 25)	69.8 ± 9.40	99.7 ± 249.58	907.9 ± 531.3	4.92 ± 3.26	4.33 ± 0.46
	P-value		0.508	0.466	0.657	0.496	0.354

*P Values obtained using ANOVA. #P Values obtained using Kruskal-Wallis test. *n*, number of subjects. *PSA*, Prostate-Specific Antigen. *DHT*, Dihydrotestosterone. *IGF-1*, Insulin-like Growth Factor 1. *T*, Testosterone. *PCa*, Prostate Cancer. *SNP*, Single Nucleotide Polymorphism. Highlighted *P*-values are significant at *P* < 0.05.

of polymorphisms in the *CYP17A1* (rs1004467, rs10883782 and rs1171624), *IGF-1* (rs1242-3791, rs5742723, rs1106381), *SRD5A1 (*rs16-6050 and rs518673) and *SRD5A2* (rs523349), with the risk of PCa and serum androgens as well as PSA and IGF-1 levels in Malaysian subjects. However, our results found no significant association between the above mentioned SNPs and risk of PCa. A study conducted in 456 Slovak patients with PCa also reported no association between rs523349 SNP of the

SRD5A2 gene with risk of PCa and a similar finding was also reported by Schleutker *et al.* [18]. The rs166050C risk variant was found to be positively correlated with greater prostatic exposure to androsterone in a Caucasian population [19]. The same variant was also found be associated with higher risk of biochemical recurrence in cohorts of Caucasian and Asian men after radical prostatectomy whereas rs-518673 was associated with reduced risk of PCa [20]. Association of SNPs of androgen

metabolism and hormonal milieu in patients with PCa in our population revealed significance between *CYP17A1* and the levels of DHT and testosterone while *SRD5A1* was found to be associated with the circulatory PSA levels.

PSA is widely used as a screening test for early detection, diagnosis and monitoring of PCa [21]. The rs3822430 and rs1691053 SNPs of *SRD5A1* have been previously reported to influence PSA levels in Spanish men [22]. The *SRD5A1* and *SRD5A2* genes play crucial role in androgen metabolism [19, 23]. Variants within *SRD5A2* have been associated with circulating sex steroid concentration and progression of disease [24]. A study had reported the role of PSA in increasing bioavailability of insulin-like growth factors that contribute towards prostate growth [25]. The current study shows for the first time that the rs518673 is associated with circulatory PSA levels. Clinical trials have shown that the administration of 5-alpha inhibitors such as finasteride and dutasteride influence the reduction of serum PSA levels [26, 27]. Association of the *SRD5A1* SNP with the PSA levels in our study further strengthens the importance of the *SRD5A* variants' role in androgen metabolism and drug treatment. Pharmacological targets such as thalidomide and flavopiridol that alter PSA expression could also influence tumor growth [28]. Hence, the rs518673 of *SRD5A1* might be an interesting gene marker for PSA and PCa treatment intervention especially in men with asymptomatic PCa.

Androgens are required for normal growth and functional activities of the human prostate [29]. Androgens play a major role in the carcinogenesis of PCa [30]. In the prostate, testosterone is converted to the more potent androgen DHT by the enzyme 5-alpha-reductase type 2 (*SRD5A2*) [31]. Most attention has been focused on DHT which is regulated by the *SRD5A* genes along the androgen metabolism pathway. Some findings suggest that the 5-alpha-reductase activity is reduced in the Asian population consequently leading to reduction of DHT concentration and androgen-mediated stimulation of the prostate gland [32]. Our study indicates that all the SNPs of *SRD5A* gene analysed are not associated with the risk of prostate cancer, which is in agreement with another study carried out in other Asian population [33].

The *CYP17A1* gene encodes the enzyme cytochrome P450 17α-hydroxylase, an enzyme that influences androgen metabolism by converting cholesterol to testosterone and has been reported to be implicated in PCa [34]. A recent study have shown a significant association between the A2 allele of the *CYP17* gene polymorphism (rs743572) and PCa [35]. According to this study, there is 1.2- to 2.8-fold increased risk of PCa involving thymine to cytosine substitution in the 5' untranslated promoter region of rs743572 [36]. The rs1004467 of *CYP17A1* gene was found to be associated with the risk for PCa and disease progression among Japanese men [37]. However we did not observe association between this SNP and risk of the disease in our population. Nevertheless, we found an association between rs1004467 of *CYP17A1* with both DHT and with testosterone levels. In our cohort, DHT level was significantly elevated in the GG allele of rs1004467 of *CYP17A1* compared to AA and AG groups. Our study found that the SNP rs1004467 with GG homozygotes showed a significantly higher concentration of both DHT and testosterone in the serum of this study population. Studies have shown that those with higher levels of DHT were susceptible to the development of castrate resistant PCa in the future [38].

IGF-1 hormone has been linked to carcinogenesis of PCa. Circulating IGF-1 contribute in cell proliferation as well as inhibition of apoptosis [39]. A prospective case-control study revealed that higher plasma IGF-1 level is associated with higher rates of prostate malignancy. Although epidemiology studies from this region are relatively low, there have been several studies which have shown significant associations between genetic markers and the risk of developing PCa. These studies involve short tandem repeats which was carried out in the Malaysian subjects [41], SNPs at chromosome region 8q24 (rs6983561) and 17q12 (rs4430796) polymorphism in Japanese population [42] and rs16901966, rs1447295, rs11986220 and rs-10090154 polymorphisms at 8q24 region in Chinese population [43] were associated with PCa in Asian men. Epidemiological studies have shown an association between high circulating serum IGF-1 levels and the risk of developing advanced PCa [44]. *IGF-1* is known to stimulate the androgen receptors which in turn increase PSA production. These insulin-like

growth factor binding proteins are cleaved by PSA which then causes an increase in the free IGF-1 in the circulatory system [45]. In our study, we investigated the role of *IGF-1* to see if there exists any association between the SNPs as well as the hormone and risk for PCa. Weiss *et al.* reported in a cohort consisting 727 incident PCa cases, and 887 matched controls, that there was no overall association found between IGF-1 and PCa risk [46]. Another case control study involving 210 cases and 224 controls in Swedish subjects reported a significant (P = 0.04) association between serum levels of IGF-1 and risk of PCa [47]. In our study, we found no significant association between *IGF-1* SNPs (rs12423791, rs1106381 and rs5742723) and risk for PCa. There was however, a significant association between *CYP17A1* (rs10883782) and IGF-1 hormone level (P = 0.018) in our population. This could suggest that there exist cross-talks between the androgen receptor signalling pathway and the insulin-like growth factor, which constitutes a pathogenic role in tumour growth and progression of disease.

To the best of our knowledge, this is the first Malaysian study that provides data on association of SNPs with PCa risk, clinical characteristics and circulating androgen levels. A major strength of our study is that a well-defined cohort was used in this study whereby participants in the PCa and control groups were screened for raised PSA, abnormal digital rectal examination, and had their diagnosis confirmed with a transrectal ultrasound (TRUS) guided biopsy of the prostate. Future studies should explore the role of other genes with the risk of PCa and the effect of *CYP17A1* SNP rs1004467 on development of castration resistance of PCa with a larger sample size. We found associations of SNPs with the levels of hormones involved in the clinical manifestation of PCa (testosterone and DHT) which might provide insights that could lead to early detection, diagnosis, management and potential therapeutic targets for PCa.

Conclusions

In conclusion, our analysis showed that the *IGF-1, CYP17A1, SRD5A1* and *SRD5A2* polymorphisms are not associated with the risk of PCa in this cohort. Nevertheless, we found significant associations between *CYP17A1* gene polymorphism with testosterone and DHT. *SRD5A1* gene polymorphism was significantly associated with PSA levels. Our findings warrant future functional studies on androgen expression and extensive analysis of gene-gene interaction, SNP-SNP interaction and gene-environment interactions in a larger sample size.

Disclosure of conflict of interest

None.

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