Role of single nucleotide polymorphisms of the HSD3B1 gene (rs6203 and rs33937873) in the prediction of prostate cancer risk

YASMINE M. AMROUSY[1], HESHAM HAFFEZ[2,3], DOAA M. ABDOU[4] and HANAA B. ATYA[2]

[1]Department of Clinical and Chemical Pathology, Faculty of Medicine; [2]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy; [3]Centre of Scientific Excellence 'Helwan Structural Biology Research', Helwan University, Cairo 11795; [4]Department of Clinical and Chemical Pathology, Kasr Al Ainy, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

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Abstract. 3-β-hydroxysteroid dehydrogenase 1 (HSD3B1) is shown to affect dihydrotestosterone level in prostatic tissue which is a risk factor for prostate cancer (PC). The present study aimed to determine whether rs33937873 (G313A) and rs6203 (C338T) single nucleotide polymorphisms (SNP) in HSD3B1 gene was a potential risk factor for PC susceptibility and can predict the recurrence of PC in Egyptian patients. A total of 186 Egyptian patients were selected with incident primary PC and compared with 180 age healthy controls. The frequencies and the main effect of rs33937873 and rs6203 in HSD3B1 were compared and investigated between the patients and control using genotyping technique and statistical analysis. The mutant GA genotype of G313A in rs33937873 SNP was considered as an independent risk for PC in the multivariate regression analysis [odds ratio (OR)=2.7, 95% confidence intervals (CI): 1.2-5.5, P=0.01] together with positive history of hypertension (HTN) (OR=6.2, 95% CI: 3.2-12.1, P=0.0001) and begin prostatic hyperplasia (BPH; OR=8.9, 95% CI: 4.5-17.5, P=0.0001). Conversely, in rs6203 (C338T), C allele is considered as major risk allele in the development of PC (OR=1.8, 95% CI: 1.3-2.4, P=0.0003). The univariate logistic regression analyses indicated that CC genotype of rs6203 was a PC risk factor (OR=1.9, 95% CI: 1.3-2.9, P=0.002). In addition, the frequency of the A-C haplotype established by rs33937873-rs6203 was also significantly higher for PC (P=0.013). The predication of PC recurrence was associated only with positive family history (OR=7.7, 95% CI: 2.3-25.9, P=0.001) and not for The G313A and C338T SNPs. These results suggested that the two HSD3B1 polymorphisms rs33937873 and rs6203 may modify the risk of PC, particularly among patients with HTN and history of BPH, suggesting them as prominent future markers for prediction of PC risk.

Correspondence to: Dr Hesham Haffez, Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Helwan University, Al Sikka Al Hadid Al Gharbeya, Al Masaken Al Iqtisadeyah, Helwan, Cairo 11795, Egypt
E-mail: hesham.haffez@pharm.helwan.edu.eg

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Introduction

Prostate cancer (PC) is one of the commonest cancer types affecting men. It is the sixth cause of death worldwide with 359,000 cases in 2018 (1). The discovery of the prostate specific antigen (PSA) together with direct rectal examinations allowed the earlier detection of PC (2). Steroids have been reported as a modulating factor that changes the biochemical characteristics of different tissues such as iris/ciliary body, aqueous outflow pathway and sclera in the rabbit eye (3) and prostate tissue in human (3-5). Testosterone and dihydrotestosterone (DHT) are the major classes of sterols and sources for androgens in males. They pose a risk to PC patients with higher levels of 'free' testosterone and a growth hormone in their blood (6). Steroidogenesis enzymes also have been related to modulation in hormonal level and associated with related diseases (7-10). Therefore, the hormonal biosynthesis pathway and their receptors can be altered by genetic variations of the related genes altering and contributing to individual susceptibility to PC (11,12). Androgen deprivation therapy (ADT) is one of the standard care treatments in advanced and metastatic cases, whether through testosterone reduction or antagonism of their mechanism of action (13). However, most patients respond well to the ADT, while some patients still show recurrence and failure to therapy and proceed to castration resistant PC (CRPC) (14). This resistance was referred to as either synthesis of the intratumorally androgen from steroid adrenal precursor or from synthesis of *de novo* cholesterol (15).

A number of studies have focused on different targets for steroid synthesis pathways such as P450 cytochromes of CYP17 and CYP3A4, 5-alpha-reductase type-2 (SRD5A2) (16,17) and 3β-Hydroxysteroid dehydrogenase (3BHSD) genes (18). The 3β-hydroxysteroid dehydrogenases (3β-HSD)/Δ4,5-isomerase is the most important enzyme responsible for catalyzing the 3β-HSD dehydrogenation and Δ4,5-isomerization of the Δ5-steroid precursors into their corresponding Δ4-ketosteroids (19). The activity of this enzyme is important for the synthesis of a number of steroidal hormones including testosterone. 3β-HSD has two key isoenzymes designated as type 1 and type 2 (20). Although these two isoenzymes are

encoded by two different genes, they are 93.5% homologous and located on chromosome 1p13.1 (21). The type 1 gene (HSD3B1) is the most important isoenzyme and a rate-limiting enzyme required for dihydrotestosterone synthesis (18), exclusively expressed in the prostate tissue (20). By contrast, the type 2 gene (HSD3B2) is predominantly expressed as 3β-HSD in the adrenal gland, ovary and testis. HSD3B1 converts DHT to 3-α-diol which is metabolized further by uridine diphosphate-glucuronosyltransferases (22). Excess biosynthesis of testosterone is known to upregulate MMP-2 and/or MMP-9 in a number of tissues including prostate (23,24). A number of studies propose a mechanism of association between androgen level, MMPs and PC progression (25-28). This is confirmed by the association between significantly higher levels of MMP-2 and -9 levels in serum of PC patients compared with control subjects (25,26). MMPs also serve a pivotal role in determining the influence of the extracellular matrix and its structure remodeling with cell phenotype, cell adhesion molecules, a number of cytokines, chemokines and growth factors. Hence, this results in increasing tumor growth, invasion and metastasis in various pathological conditions such as cancer (26,29,30).

Several common forms of polymorphisms are correlated to allele frequencies in HSD3B1 that affect synthesis, activity and stability of DHT such as rs33937873, rs6203, rs33913717, rs6205 and rs1047303 (31,32). rs6203 has been shown to be implicated in several pathogenesis including myopia (33), gastric cancer (34), sex hormone metabolism (35), hypertensive disorders of pregnancy (36) and hypertension (HTN) with left ventricular structure abnormality (37). As to rs6203 and rs33937873, there is no information about their role, effects or relationships with prevalence of PC even in a small-scale study population. Therefore, in the present study, these two SNPs were selected based on their presence in coding region of HSD3B1 gene which may affect the gene product. The genetic variation in HSD3B1 can lead to an elevation in plasma aldosterone with subsequent elevation in HTN and risk of PC (38). The present study investigated the prevalence of rs6203, which is the C/T silent substitution at codon 338 in exon 4 of HSD3B1 (33) and codon 313 of rs33937873 on HSD3B1 gene in Egyptian PC patients. The present study demonstrated the association between the single nucleotide polymorphisms of rs6203 and rs33937873 in HSD3B1 gene and the risk of PC in Egyptian patients. Additionally, the present study investigated the effect of each SNP, alone or in combination, shedding the light on their haplotype effect, disease susceptibility and any associated clinical parameters.

Patients and methods

Patient samples. A total of 366 Egyptian men were incorporated in the study, categorized into 186 clinically diagnosed PC patients with a mean age of 69.7±0.7 years (range, 54-84 years) and 180 healthy controls with a mean age of 62.2±0.9 years (range, 58-79 years). Patients were recruited from the Urology outpatient clinic of Cairo University Hospital and Badr Hospital, Helwan University between June and December 2021. All participants were acknowledged with the study design and risks with written informed consents taken. The present study protocol was performed according to the ethics guidelines and regulations of the Helsinki Declaration. All experimental protocols were approved by the Scientific Research Ethics Committee of the Faculty of Pharmacy, Helwan University (approval number 03H2021). The clinical guidelines for the National Comprehensive Cancer Network (NCCN) were used for proper diagnosis of PC patients (39). The required informed written consents were obtained according to the regulations of the Institutional Ethical Committee (Faculty of Medicine, Cairo University) which govern the nature of the study. The complete history for the patients including (age, family history, history of benign prostatic hyperplasia (BPH), disease onset and treatment) was recorded with essential laboratory assessment including PSA, prostate size, MRI prostate volume and Gleason grading system. A structured questionnaire was administered to collect information on history of illness, occupation, smoking status, and demographic and anthropometric characteristics of the enrolled subjects. Inclusion criteria for control healthy patients included no evidence of prostate cancer or tumor history before or during the study, and patients were randomly selected for matching by geographic region and the expected age distribution of cases. Inclusion criteria for patients with prostate cancer were a clinical diagnosis of primary adenocarcinoma of the prostate by histopathology and a serum prostate-specific antigen level >4 ng/ml (normal range, 2.5-4.0 ng/ml). Exclusion criteria included: i) Receipt of medical therapy known to affect PSA levels (such as betamethasone or testosterone replacement therapy to increase PSA level and aspirin, ibuprofen, naproxen, atorvastatin, simvastatin and thiazide diuretics to decrease PSA level) (40); ii) previous invasive treatment for benign prostatic hyperplasia, with indwelling urethral catheters (40); iii) voided volume on initial uroflowmetry of <150 ml (40); iv) previous prostate surgery, including transurethral resection of the prostate (41); v) any other cancer or metastatic cancer that has been present during the last 3 years (42); vi) a relationship with another participant at the 3rd degree or closer (43); and vii) missing data pertaining to the essential variables (43).

Biochemical analysis. Blood samples (total volume of ~10 ml from each patient) were used for the determination of PSA level (PSA-ELISA Kit; catalog no. MBS590045; MyBioSource, Inc.) and DNA analysis in both groups.

Genotyping assay. Whole blood samples were subjected for genomic DNA extraction using spin-column technique (GeneJET Genomic DNA Purification kit, Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. SNPs genotyping assays were ordered as follows: (rs6203: C_175679504_10 and rs33937873: C__25619111_10; Thermo Fisher Scientific, Inc.). Genotyping of the two SNPs were performed using TaqMan master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). Analysis of data was performed by investigating allele using built-in integrated software for investigating allele frequency in Rotor gene-Q machine (Qiagen GmbH).

Statistical analysis. The χ^2 test was used to assess the association between the investigated polymorphisms and PC. The odds ratio (OR) and confidence intervals (CI) were performed using SPSS 21.00 software (IBM Corp.). Logistic regression

Table I. Clinical and demographic data of patients and controls in the study.

Parameters	Prostate cancer patients (n=186)	Healthy controls (n=180)
Mean age ± SEM, years	69.7±0.7	62.2±0.9
Mean PSA on diagnosis ± SEM, ng/ml	14.9±0.6	2.7±0.2
Family history (prostate cancer), n		
Yes	14	
No	172	
Mean duration ± SEM, years	7.7±0.3	
TMN staging, n		
T1	18	
T2a	28	
T2b	20	
T3	91	
T4	30	
Recurrence, n		
Yes	52	
No	134	
Mean Gleason score ± SEM	6.9±0.16	
Benign prostatic hyperplasia, n		
Yes	94	
No	92	
Mean ADT therapy duration ± SEM, months	7.8±0.15	
Radiotherapy, n		
Yes	48	
No	138	
Diabetes mellitus, n		
Yes	74	14
No	112	166
Hypertension, n		
Yes	96	36
No	90	144

PSA, prostate-specific antigen; TNM, Tumor-Node-Metastasis; ADT, androgen deprivation therapy.

analysis was used for the prediction of risk factors using generalized linear models. Hardy-Weinberg equilibrium and the linkage disequilibrium were both calculated using a goodness-of-fit χ^2 test. The HaploView program (version 4.2; Broad Institute) was applied to estimate the haplotypes (44) using the expectation maximization algorithm. Comparisons were performed using two-tailed unpaired t-test and one-way ANOVA (with Tukey's post hoc test) using GraphPad Prism software (version 5.0; GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Demographic data. All clinical and demographic data of PC patients and control are shown in Table I. It was observed that age was not significantly different between groups ($P>0.05$). The genotypes of both SNPS were in Hardy-Weinberg equilibrium; however, the two investigated polymorphisms were in linkage equilibrium ($D'=0.816$ & $R^2=0.026$). Moreover, demographic data showed that ~50% of PC patients had DM, HTN and BPH in association.

Genotyping analysis. The genotypes distribution and allele frequencies of (rs6203 and rs33937873) among the control subjects and PC patients are shown in Table II and Fig. 1, respectively. Significant differences were observed in the genotype's distribution pattern of rs33937873 between the patients and controls ($P=0.0008$). Furthermore, the difference in the allele frequencies in rs33937873 was significant ($P=0.001$). It was quite noticeable that the AA genotype was rare in both healthy subjects and patients (zero subjects). The odds ratio between mutant GA genotypes and wild-type GG genotype was 2.8 (95% CI: 1.5-5.2; $P=0.001$) in prostate patients compared with controls. OR of A and G alleles was 2.5 (95% CI: 1.4-4.7;

Table II. Distribution of (rs33937873 and rs6203) genotypes and allele frequencies in the study subjects.

HSD3B1 gene variants	Genotypes	Control (n=180), n (%)	Patients (n=186), n (%)	P-value, χ^2 value, df	OR (95% CI), P-value
rs33937873	GG	164 (91.1)	146 (78.5)	0.0008, χ^2=11.2, df=1	2.8 (1.5-5.2), [a]P=0.001
Silent mutation	GA	16 (8.9)	40 (21.5)		
(Pro 313 Pro)	AA	0 (0.0)	0 (0.0)		
	G allele	344 (95.6)	332 (89.2)	0.001, χ^2=10.3, df=1	2.5 (1.4-4.7), [a]P=0.001
	A allele	16 (4.4)	40 (10.8)		
rs6203	CC	66 (36.7)	98 (52.7)	0.0006, χ^2=14.7, df=2	
	CT	90 (50.0)	80 (43.0)		
	TT	24 (13.3)	8 (4.3)		
Silent mutation	CC	66 (36.7)	98 (52.7)	0.002, χ^2=9.4, df=1	1.9 (1.3-2.9), [a]P=0.002
(Leu 338 Leu)	(TT+CT)	114 (63.3)	88 (47.3)		
	T allele	138 (38.3)	222 (61.7)	0.0003, χ^2=13.2, df=1	1.8 (1.3-2.4), [a]P=0.0003
	C allele	96 (25.8)	276 (74.2)		

[a]P-value data obtained from χ^2 test. df, degree of freedom; OR, odds ratio.

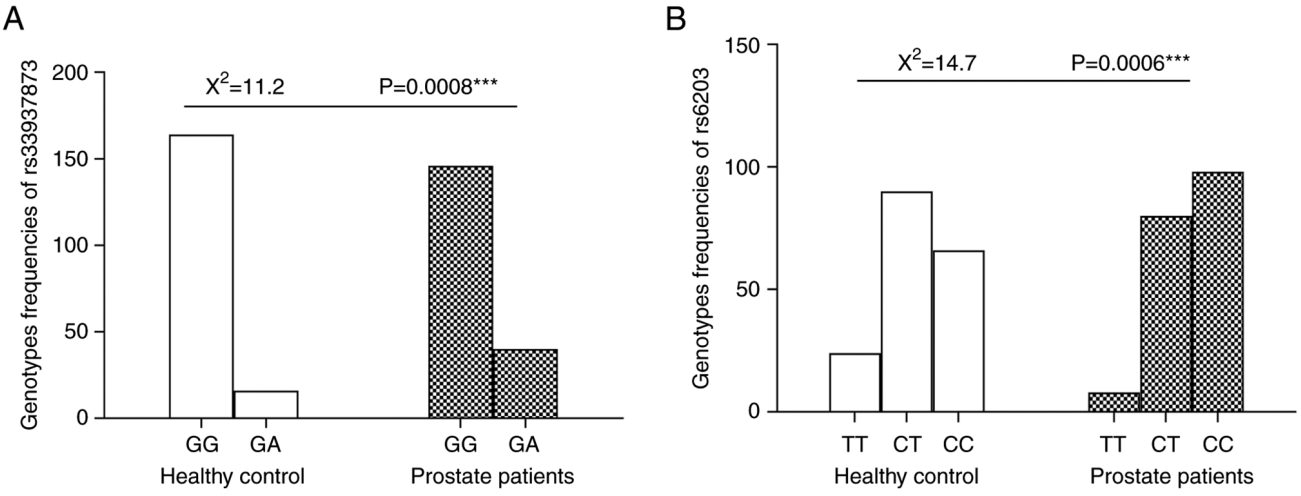

Figure 1. Distribution of 3-β-hydroxysteroid dehydrogenase 1 polymorphisms, (A) rs33937873 and (B) rs6203, in the study subjects. ***P<0.001 using chi-squared test.

P=0.001) in prostate patients compared with controls, suggesting that individuals carrying the A allele are 2.5 times more subjected for developing PC compared with non-carriers.

In the rs6203, The CC genotype elevated the risk of PC incidence (OR=1.9; 95% CI=1.3-2.9; P=0.002) compared with control subjects. OR of C and T alleles was 1.8 (95% CI: 1.3-2.4, P=0.0003) in prostate patients compared with control subjects (Table II).

Haplotype analysis of studied HSD3B1 SNPs. A total of four haplotypes were generated for the two selected SNPs (rs33937873 and rs6203) of HSD3B1 among patients and control; GC was the most frequent, while the AT was the least frequent haplotypes among the studied groups. Higher frequency of AC and lower frequency of GT were significantly associated with prostate cases when compared with control group, as shown in Table III. The prediction of disease risk was weakly correlated with the susceptibility variants. The predictive performance of genetic risk models increases by merging multiple common low-risk loci. Therefore, the haplotype effect between two SNPs on predisposition of PC in Egyptian patients was studied and found that the polymorphism in both genes had an amplified influence on the risk of PC than single locus.

Regression analysis for prediction of PC susceptibility. To investigate the effect of these gene polymorphisms on PC, regression analysis was conducted for prediction of PC susceptibility with examined family history, DM, HTN and BPH as shown in Table IV.

In univariate analysis, it was found that family history, DM, HTN, history of BPH, GA genotype of rs33937873 and CC genotype of rs6203 were associated with risk of PC. However, in multivariable analysis, only patients with history of BPH, HTN and GA genotype of rs33937873 were considered independent predictors of PC susceptibility as shown in Table IV. For the prediction of the PC recurrence regression analysis, this was conducted using age, family history, DM, HTN, BPH,

Table III. Distribution of haplotype analysis in the study cohort.

Haplotype	Control	Patients	P-value
GT	0.375	0.258	0.013
GC	0.581	0.634	0.258
AT	0.009	0.002	0.998
AC	0.036	0.108	0.013

Table IV. Regression analysis for prediction of PC susceptibility.

Regression analysis for susceptibility of PC incidence						
	Univariate regression analysis			Multivariate regression analysis		
Variable	P-value	OR	95% CI	P-value	Adjusted OR	95% CI
Age at diagnosis	0.908	1.002	0.974-1.030	0.211	1.024	0.985-1.064
rs33937873 GA vs. GG	0.001	2.81	1.509-5.228	0.016	2.566	1.191-5.528
rs6203 CC vs. (CT+TT)	0.002	1.924	1.266-2.922	0.158	1.479	0.859-2.546
Family History	0.049	2.849	1.004-8.081	0.583	0.694	0.188-2.56
History of DM	0.0001	7.268	3.97-13.304	0.056	2.195	0.978-4.928
History of HT	0.0001	8.533	4.94-14.74	0.0001	6.251	3.219-12.139
History of BPH	0.0001	10.473	5.815-18.865	0.0001	8.972	4.576-17.591

OR, odd ratio; CI, confidence interval; DM, diabetes mellitus; HT, hypertension; BPH, benign prostatic hyperplasia.

GA genotype of rs33937873 and CC genotype of rs6203 as covariates. Only positive family history was considered a predictor of PC recurrence as represented in Table V.

To investigate the associations of the two selected SNPs and various clinical outcomes in PC patients, patients were stratified according to the type of allelic variant at the polymorphic site of HSD3B1gene. For rs33937873, the statistical analysis was applied to prostate patients carrying the mutant genotype (GA; n=40; 21.5%) who were compared with those carrying the wild-type genotype (GG group; n=146; 78.5%) as the reference group. As for rs6203, the major risk genotype CC (n=98; 52.7%) was compared with the genotype TT (n=8, 4.3%) and heterozygous genotype CT (n=80; 43%; Table VI). In rs33937873, PC patients who carry mutant GA genotypes showed significant increase in prostate volume associated with DM compared with wild ones.

Table V. Regression analysis for prediction of PC recurrence.

Variable	P-Value	OR	95% CI
Age at diagnosis	0.833	0.995	0.952-1.040
rs33937873 GA vs. GG	0.638	0.825	0.371-1.838
rs6203 CC vs. (CT+TT)	0.647	0.861	0.454-1.635
Family History	0.001	7.74	2.306-25.965
History of DM	0.818	0.926	0.48-1.786
History of HT	0.704	1.132	0.596-2.151
History of BPH	0.063	1.858	0.966-3.573

CI, confidence interval; DM, diabetes mellitus; HT, hypertension; BPH, benign prostatic hyperplasia.

Discussion

Prostate cancer is associated with resistance, poor recovery and metastasis. Hence, early diagnosis is essential for improving the outcome (45). PSA was considered as the gold marker in PC; however, the recorded drawbacks, including non-specificity in PC patients leading to misdiagnosis and failure in cancer treatment, limit its clinical applications (46). Therefore, the ideal for prostate tumor is a molecular marker that is highly specific and sensitive to avoid false positive results. The use of genotype information as an aid for selection can be a rapid and accurate way to enhance selection efficiency of PC patient in a cost-effective manner. In the present study, HSD3B1 was a major enzyme of the androgen biosynthetic pathway (47). It catalyzes the conversion of dehydroepiandrosterone to androstenedione in steroidogenic tissues such as the adrenal and prostate tissues (48). HSD3B1 is considered to serve an important role in the production of androgens that fuel PC development with carcinogenesis and resistance later in a castrate environment (49).

A total of two single nucleotide polymorphisms were selected (rs33937873 with codon 313 and rs6203 with codon

Table VI. Influence of the HSD3B1 gene polymorphism (rs33937873 & rs6203) on different biochemical and clinical parameters in prostate cancer patients.

	rs33937873			rs6203			
Parameters	Wild genotype (GG) (n=146)	Mutant genotype (GA) (n=40)	P-value	Wild genotype (TT) (n=8)	Heterozygous mutant genotype (CT) (n=80)	Homozygous mutant genotype (CC) (n=98)	P-value
PSA on diagnosis, ng/ml	14.4±0.5	16.8±1.1	0.06	12.0±1.02	15.2±0.7	15.1±0.6	0.35
Prostate size, cc	0.31±0.01	0.36±0.02	0.07	0.30±0.03	0.31±0.02	0.34±0.01	0.36
MRI prostate volume, cm^3	33.6±0.6	30±1.4	0.02[a]	36.3±2.6	31.4±0.9	33.7±0.8	0.08
BPH, n (%)	72 (49.3)	22 (55.0)	0.6	2 (25.0)	36 (45.0)	56 (57.1)	0.09
Recurrence, n (%)	42 (28.8)	10 (25.0)	0.7	2 (25.0)	24 (30.0)	26 (26.5)	0.8
DM, n (%)	64 (43.8)	10 (25.0)	0.04[a]	2 (25.0)	29 (36.2)	43 (43.9)	0.4
HT, n (%)	76 (52.1)	20 (50.0)	0.9	4 (50.0)	45 (56.2)	47 (48.0)	0.5

[a]P<0.05. Data are presented as mean ± SEM unless otherwise stated. PSA, prostate-specific antigen; MRI, magnetic resonance imaging; BPH, benign prostatic hyperplasia; DM, diabetes mellitus; HT, hypertension.

338), however, the functional impact of these two polymorphisms has not yet been fully elucidated in PC and their ethnic distribution was not studied in Egyptian PC patients. The mutant A allele and GA genotype of HSD3B1 gene (rs33937873) indicated a positive association with PC patients (individuals carrying the minor A allele are 2.5 times more susceptible for developing PC compared with non-carriers). The same situation in HSD3B1 gene (rs6203) was observed with C allele significantly increasing the risk of PC incidence and individuals carrying the C allele are 1.8 times more susceptible for developing PC compared with non-carriers. These results are consistent with a number of findings reported for other polymorphisms in HSD3B1 gene suggesting that polymorphisms in this gene is 'probably damaging'. SNPs can be divided into two main types; non-synonymous SNP or mutation when it presents within the coding region of a gene and this leads to change in amino acid sequence of the resultant protein (50). The other type is synonymous SNPs that affect translation rates or mRNA half-life rather than change the nature of the amino acid (51). SNPs can affect the binding interaction of RNA-protein by modification of secondary structure of RNA (52,53). Additionally, SNPs can affect both gene expression level of the specific protein or its binding with transcription factors (54,55). In all previously mentioned mechanisms, these can lead to modifications in either function or structure of the translated proteins (folding) and related metabolic pathways such as increased cell proliferation, protein dimerization and activation of a number of mediators (56). In the current study, the suggested polymorphisms, either G313A or C338T, may create a new potential protein with different structure and function which induces cellular carcinogenesis, resistance and apoptosis (57). Another suggestion is that the two polymorphisms may render HSD3B1 resistant to ubiquitination and proteasomal degradation, leading to a large amount of protein (DHT) accumulation in the cell, causing prostate tissue carcinogenesis as well as resistance to androgen-deprivation therapy in PC recurrence (57-60). The results suggested that these variants of the HSD3B1 steroidogenic enzyme gene could be a powerful new biomarker capable of identifying patients with aggressive disease who warrant early escalated therapy and in clinical management of the disease. Additionally, the data obtained and suggestions were matched with literature about the role of this enzyme in the degradation of DHT (61) and malfunction in accumulation of DHT in prostatic tissue (62,63).

For the correlation of the contribution of the studied SNPs to PC susceptibility, regression analysis was performed using rs33937873, rs6203 and other variables as covariates. Positive family history, DM, HTN, GA genotype of rs33937873 and CC genotype of rs6203 were associated with risk of PC in univariable analysis. On the other hand, in multivariable analysis, only patients with a history of BPH, HTN and GA genotype of rs33937873 were considered independent predictors of PC susceptibility. Family history was considered the only predictor of PC recurrence in the present study, as a well-known risk factor for developing PC (64). The literature matched this observation and shows that there is a trend of increasing risk of PC incidence in patients with two or three first degree relatives affected to have a five and 11-fold increased risk of developing PC (65-68). In the US, those with a family history of PC should be advised of their significantly increased PC risk to ~9-10% in their lifetime (69). In Africa, some studies found a correlation between PC incidence in different African cultures and their family history reached between 30-70% (69,70). With respect to this biological heterogeneity of PC, the observation is important in understanding PC etiology and incidence risk factor to correctly assess the clinical state of the patient using PSA continuous screening to avoid aggressiveness and high mortality rate.

In addition, the findings of the present study reported significant association between the mutant genotype GA of rs33937873 in PC patients with DM. Although studies mention

that DM and PC have an inverse relationship (71-73), some other studies could not find any evidence of the inverse relationship between DM and PC (74-76). However, some other results in population-based cohort study concur with the findings of the present study suggesting that the relationship between DM and high-grade PC has a positive correlation (77,78). The potential explanation for this hypothesis is related to the activity of the patient such as exercise, body mass index (79,80), glycemic control (78) and ethnicity (81) and further large scale studies are required to understand the proper mechanisms controlling the correlation in each case.

The data presented in the present study shed light on the potential role of these two SNPs in HSD3B1gene as the promising marker for the prediction of PC incidence. The future perspective for is to illustrate the effect of both studied SNPs in different advanced cases of PC such as CRPC through the activity of HSD3B1 and to study their role in susceptibility and resistance of prostate patients to treatments such as abiraterone.

The present study has suggested a potential impact of SNPs in HSD3B1 gene (rs6203 and rs33937873) individually and in combination in relation to the risk of PC in Egyptian patients. These results deserve the trial on a larger study in the context of PC susceptibility to shed the light on the function of HSD3B1 and associated allelic variants in correlation to other androgen-metabolizing enzymes. Moreover, the present study needs to be applied using both *in vitro* and *in vivo* models to confirm the hypothesis and elucidate the role of the gene and its corresponding protein in PC pathogenesis.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HH, HBA, YMA and DMA designed the study, performed the experiments, and wrote and revised the manuscript. HBA, YMA and HH analyzed the datasets. HH searched the literature. HH, HBA YMA and DMA confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their written informed consent to participate in the study. The study was approved by Scientific Research Ethics Committee of the Faculty of Pharmacy, Helwan university (approval number 03H2021).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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