The rs743572 common variant in the promoter of CYP17A1 is not associated with prostate cancer risk or circulating hormonal levels

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Accepted for publication 13 July 2007

Study Type – Prognosis (case control study)
Level of Evidence 3a

OBJECTIVE

To use a large population-based case-control study to test the association between the common genetic variant rs743572 (–34 T to C), prostate cancer risk and circulating levels of several hormones.

SUBJECTS AND METHODS

A previous meta-analysis concluded that reported associations between rs743572 in the promoter of CYP17A1 and prostate cancer risk might reflect publication bias, but a few recent studies reported associations with prostate cancer risk and data suggesting that rs743572 is functional. We genotyped 824 prostate cancer cases and 737 population-based controls, and applied unconditional logistic regression to estimate the association between rs743572 and prostate cancer risk. We also used linear regression of transformed testosterone, androstanediol glucuronide, dehydroepiandrosterone sulphate, androstenedione, sex hormone-binding globulin and oestradiol (circulating levels) measured for controls, to estimate the association between these levels and rs743572. The linear models were adjusted for age and laboratory batch.

RESULTS

Men with different genotypes had similar circulating levels of all the hormones measured (all $P < 0.05$). In the case-control comparison using unconditional unadjusted logistic regression, the odds ratios (95% confidence interval) for prostate cancer were 1.07 (0.87–1.32) and 0.94 (0.71–1.25) for the dominant and recessive models, respectively, and for the co-dominant model, 1.10 (0.88–1.36) and 0.99 (0.73–1.35) for carriers of one or two copies of the C allele, respectively. There was no evidence of heterogeneity in the odds ratios by tumour stage (all $P > 0.3$) and grade (all $P > 0.3$).

CONCLUSION

The results of the present study are consistent with the conclusions of the previous meta-analysis, and suggest that rs743572 has no role in the risk of prostate cancer for men of Caucasian origin.

KEYWORDS

prostate cancer, CYP17, case-control, hormones

INTRODUCTION

The enzyme cytochrome steroid 17α-hydroxylase/17,20 lyase (or p450c17α) catalyses two sequential reactions in the biosynthesis of testosterone in both the gonads and the adrenals. This enzyme is the product of the *CYP17A1* (also known as *CYP17*) gene. The possible association between a common T to C substitution (often called A1 to A2 substitution) in the promoter region of *CYP17A1* 34 nucleotides upstream of initiation of translation (i.e. rs743572, – 34 T to C) and prostate cancer risk has been examined in several studies. In their meta-analysis published in 2003, Ntais *et al.* [1] showed that seven of the 10 studies that examined the association between rs743572 and prostate cancer risk reported odds ratios (ORs) of >1 in carriers of the C allele, compared with non-carriers, but only in one study was the OR significantly >1 (2.2, 95% CI 1.2–4.1) [2]. The pooled OR from the meta-analysis, including in all 2404 cases and 2755 controls, was 1.08 (0.95–1.22), and the authors concluded that previously reported associations might reflect publication bias, and that the rs743572 variant is not associated with the risk of prostate cancer in populations of Caucasian origin. Since then, two further studies reported a significant association between rs743572 and prostate cancer [3,4], and a large study reported that, in organ-confined prostate cancer, rs743572 was associated with moderate- to high-grade tumours [5]. Also, a study in 164 Japanese men showed that carriers of two copies of the C allele in rs743572 had lower circulating levels of androstenedione and free

testosterone than carriers of one or two copies of the T allele [6], suggesting that rs743572 might be functional.

We used an Australian case-control study to estimate and test the association between rs743572, circulating hormone levels, prostate cancer risk, and tumour stage, Gleason score and age at diagnosis.

SUBJECTS AND METHODS

Subjects were participants in the Melbourne and Perth arms of the Risk Factors for Prostate Cancer Study, a population-based case-control study of prostate cancer conducted between 1994 and 1998 and described in detail elsewhere [7,8]. Eligible cases with histopathologically confirmed adenocarcinoma of the prostate diagnosed before 70 years of age were ascertained from the Cancer Registries of Victoria and Western Australia. Tumours that were well-differentiated, those with Gleason scores of <5, and those diagnosed in men aged >69 years were excluded, to focus on prostate cancers that were more likely to be clinically significant and have a major impact on public health. Eligible controls were randomly selected from men on the State Electoral Rolls (registration to vote is compulsory for adult Australian citizens), and were frequency-matched to the expected age distribution of the prostate cancer cases in a ratio of one control per case. In all, 1047 cases and 1058 controls participated in the study (the response rate was 65% and 50%, respectively, of those eligible [9]). Face-to-face interviews were conducted using structured questionnaires to obtain information on potential risk factors, including age, history of prostate cancer in first-degree relatives, country of birth, life-style (including diet), and other potential risk factors for prostate cancer. Tumour stage (stage I to IV according to the American Joint Committee on Cancer [10]) and grade (moderate, Gleason 5–7 or moderately differentiated; high, Gleason 8–10 or poorly differentiated) was recorded from histopathology reports.

Informed consent was obtained from all study participants. Blood samples were available from 831 cases (79% of participants) and 739 controls (70%). A detailed description of participants' characteristics was published [11].

For genotyping, genomic DNA was extracted from buffy coat using spin columns, and genotyped by two independent laboratories, based in Melbourne (A.A.T. and M.C.S.) and Sydney (E.J.D.P. and V.M.H.), while unaware of the participants' characteristics. In the Melbourne laboratory samples were genotyped using the following primer and probe sequences: forward primer 5′-GCCTCCTTGTGCCCTAGAGTT-3′; reverse primer 5′AAATAAGCTAGGGTAAGCAGCAAG A-3′; CYP17A1 C probe 6FAM TACTCCACCGCTGTC-MGB and the CYP17A1 T probe VIC CTACTCCACTGCTGTC-MGB (Applied Biosystems, Foster City, CA, USA). The PCR reaction contained: 2 × Taq Gold universal master mix (Applied Biosystems), 900 nM of each primer, 100 nm each probe and 10 ng of genomic DNA. Cycling conditions were 50 C for 2 min, 95 C for 10 min, followed by 50 cycles of 95 C for 15 s then 66 C for 1 min. The data were collected and analysed on the RotorGene system (Corbett LifeScience, Sydney, Australia). To ensure quality control, 5% of the genotype calls were confirmed by sequencing.

In the Sydney laboratory samples were genotyped using denaturing gradient gel electrophoresis (DGGE). In brief, a 175 bp fragment spanning the *CYP17A1*–34 nucleotide promoter region was screened using DGGE and DGGE-specific primers designed according to previously published criteria for optimum detection of sequence variation [12]. Primer sequences and conditions of amplification are available on request (V.M.H.). The DGGE fragment was electrophoresed in a 9% polyacrylamide gel containing a 40–80% urea and formamide denaturing gradient, and DGGE conditions as previously described [13]. The –34 T to C single nucleotide polymorphism (SNP) was identified by a sequenced-confirmed (Prism 3100 DNA analyser, Applied Biosystems) and uniquely identifiable DGGE banding pattern.

Sixty samples (4%) were genotyped for a second time because they either failed in one of the laboratories or were discordant. Nine samples were still discordant and were excluded from the analysis.

We originally measured hormone levels to assess the possible association with several factors, including genetic variants. We limited this assessment only to controls, because in a case-control design blood samples are collected from cases after diagnosis, and hormone levels might be modified by the presence of the tumour or by treatment. Hormone levels in plasma samples were analysed in the laboratory while unaware of the individuals' characteristics by one author (H.M.). Laboratory quality controls were added to each batch. Plasma dehydroepiandrosterone-sulphate (DHEAS) and sex hormone-binding globulin (SHBG) levels were measured using a competitive immunometric assay (Immulite, DPC, CA, USA). The interassay coefficient of variation (CV) was 12.4% at 2.1 mol/L for DHEAS and 6% at 26 nmol/L for SHBG. Testosterone and oestradiol (E2) levels were measured using electrochemiluminescence immunoassay (Elecsys 2010 analyser, Roche Diagnostics GmbH, Mannheim, Germany). The CV was 1.6% at 36 nmol/L for testosterone and 11.1% at 93 pmol/L for E2. Plasma androstenedione (AS) and 3α-androstanediol glucuronide (3α-diolG) levels were measured using a radioimmunoassay (DSL-4200 and DSL-6000, Diagnostic Systems Lab, Webster, TX, USA). The CV was 10.7% at 3.3 nmol/L for AS and 4.3% at 21.1 nmol/L for 3α-diolG.

Estimates of allele frequencies and tests of deviation from Hardy–Weinberg (H-W) equilibrium were assessed using standard procedures based on asymptotic likelihood theory [14]. Fisher's exact test was used to test for independence between the SNP and age (<55, 55–59, 60–69 years), country of birth (Australia or others), family history of prostate cancer (affected first-degree relatives or not), and tumour stage (stage I–II, III or IV) and grade (Gleason score 5–7 or 8–10). Case-control analyses were conducted using unconditional logistic regression to estimate ORs and their 95% CI [15]. Genotype was included in the model as the indicator variable, and associations between genotype and prostate cancer risk were tested under co-dominant, dominant and recessive models. Polytomous logistic regression models were used to estimate ORs by tumour stage and Gleason score. We investigated whether the ORs varied by age at diagnosis by testing the interaction term between age and genotype using the likelihood ratio test. Potential confounders (i.e. country of birth, age, history of smoking, history of prostate cancer in first-degree relatives and body mass index) were included in the models only if they changed the ORs by ≥5%.

As plasma levels of testosterone, 3α-diolG, DHEAS, AS, SHBG and E2 were skewed, linear

regression of the transformed levels to test the possible association with genotypes was used. Levels of 3α-diolG and E2 were log-10 transformed, while the others were square-root transformed. The linear regression models were adjusted for age and laboratory assay, and were fitted using all the controls. Genotype was included in the models as the indicator variable. The results are presented as the adjusted means and their corresponding 95% CI, derived by transforming the estimates from the fitted regression models into the original scale. We used the likelihood ratio test to assess the relative fits of nested models and the Wald test to assess statistical significance of individual parameters. All tests were two-sided and nominal statistical significance was indicated at $P < 0.05$.

*TABLE 1 rs743572 genotype in CYP17A1 and circulating hormone levels for 737 controls**

	TT (360)	CT (344)	CC (110)	P†
Testosterone, nmol/L	12.4 (11.9–13.0)	12.9 (12.3–13.4)	12.6 (11.7–13.5)	0.6
3α-diolG, nmol/L	14.4 (13.5–15.3)	13.4 (12.7–14.2)	12.5 (11.3–13.9)	0.05
DHEAS, mol/L	2.35 (2.19–2.52)	2.42 (2.27–2.58)	2.12 (1.89–2.38)	0.1
AS, nmol/L	2.14 (2.01–2.28)	2.17 (2.05–2.29)	1.94 (1.76–2.15)	0.2
SHBG, nmol/L	29.2 (27.7–30.8)	28.9 (27.5–30.4)	30.0 (27.5–32.7)	0.8
E2, pmol/L	92.3 (89.5–95.2)	89.3 (86.9–91.8)	88.6 (84.3–93.1)	0.2

**Adjusted back-transformed means and their corresponding 95% CI derived from linear regression of the transformed levels. Levels of 3α-diol G and E2 were log-10 transformed, while the others were square-root transformed. The models were adjusted for age and laboratory batch. †Likelihood ratio test for association between genotype and circulating hormone levels.*

TABLE 2 rs743572 genotype in CYP17A1 and prostate cancer risk

Model	Controls (%)	Cases (%)	OR (95% CI)*	P†
No.	737	824		
Codominant model				0.6
TT	283 (38)	303 (37)	Ref	
TC	344 (47)	404 (49)	1.10 (0.88–1.36)	
CC	110 (15)	117 (14)	0.99 (0.73–1.35)	
$P_{H\text{-}W}$‡	0.7	0.3		
Dominant model				0.5
TT	283 (38)	303 (37)	Ref	
any C	454 (62)	521 (63)	1.07 (0.87–1.32)	
Recessive model				0.7
Any T	627 (85)	707 (86)	Ref	
CC	110 (15)	117 (14)	0.94 (0.71–1.25)	

**From unconditional logistic regression analysis; adjusting for family history of prostate cancer, age, country of birth, baldness, body mass index and smoking history did not materially change the OR estimates. †Test for association between genotype and prostate cancer risk (likelihood ratio test); ‡Exact P value relative to the test for H-W equilibrium.*

RESULTS

Genotyping of the rs743572 variant was successful for 99% of the samples, leaving 824 cases and 737 controls for analysis. More than half of the cases were 60–69 years old at diagnosis (514, 62.4%), 197 (23.9%) were 55–59 years and 113 (13.7%) were <55 years old. Altogether, 253 cases (31%) had stage III or stage IV disease and 222 tumours (27%) had a Gleason score of ≥8.

The distribution of genotypes was consistent with H-W equilibrium for cases, for controls, and for cases and controls combined (all $P > 0.6$). There were no significant associations between genotype and country of birth, age at diagnosis, or family history of prostate cancer for either cases or controls (all $P = 0.5$).

Adjusted means of circulating levels of testosterone, 3α-diolG, DHEAS, AS, SHBG or E2 are presented by genotype in Table 1. The levels of 3α-diol G were 14.4, 13.4 and 12.5 nmol/L in controls with none one or two copies of the C allele, respectively, but the test of association between rs743572 and 3α-diolG levels was not statistically significant ($P = 0.05$). We found no evidence of an association between circulating levels of the other hormones and rs743572 (all $P \geq 0.1$).

The frequency of the C allele was 38% for controls and 39% for cases. The ORs for prostate cancer were all 0.94–1.10, and all the CIs were narrow and included 1.0 (Table 2, all $P > 0.5$). For the dominant model, the ORs for stage I-II, stage III and stage IV prostate cancer were similar (Table 3, P heterogeneity = 0.9), as were those for high- and moderate-grade prostate cancer (P heterogeneity = 0.7), and age at diagnosis <55 years, 55–59 and 60–69 (P heterogeneity = 0.5). The results from the co-dominant and recessive models were similar (data not shown).

DISCUSSION

In this case-control study the common variant rs743572 in *CYP17A1* was not associated with circulating hormone levels in the controls, or with prostate cancer risk, and the CIs were not consistent with ORs of >1.36. Also, there were no variations in associations between the rs743572 variant and tumour stage or grade.

A major strength of our study is that we examined both the association with prostate cancer risk and the circulating hormone levels. Also, the study is larger than any of the studies included in the meta-analysis by Ntais *et al.* [1] and similar in size to the recent Finnish study reporting that, in organ-confined prostate cancer, the C allele in rs743572 was associated with a greater risk of moderate- to high-grade tumours (OR 1.42, 95% CI 1.09–1.83) [5]. The Risk Factors for Prostate Cancer study focused on early onset and moderate- (Gleason score 5–7) to high-grade (Gleason score 8–10) tumours. It was therefore an appropriate setting in which to test the association with tumour grade and stage. The allelic frequency in our control population was virtually identical to that

TABLE 3 rs743572 genotype in CYP17A1 and prostate cancer risk by tumour stage, grade and age at diagnosis (dominant model)

Variable (n)	C-allele carriers N (%) cases	OR (95% CI)*	P†	P heterogeneity‡
Tumour stage				0.7
I–II (567)	354 (62)	1.04 (0.83–1.30)	0.8	
III (199)	131 (66)	1.20 (0.86–1.67)	0.3	
IV (54)	34 (63)	1.06 (0.60–1.88)	0.8	
Tumour grade¶				0.9
Moderate (602)	381 (63)	1.07 (0.86–1.34)	0.5	
High (222)	140 (63)	1.06 (0.78–1.45)	0.7	
Age at diagnosis, years				0.5
<55 (113)	77 (68)	1.30 (0.77–2.21)	0.3	
55–59 (197)	118 (60)	1.26 (0.79–1.99)	0.3	
60–69 (514)	326 (63)	1.01 (0.78–1.30)	0.9	

**For the dominant model. For tumour stage and grade the estimates are from polytomous logistic regression. For age at diagnosis the estimates are from logistic regression including an interaction term between genotype and age. †Test for association between genotype and prostate cancer risk (Wald test). ‡Test for homogeneity of ORs across tumour stage, grade, and age at diagnosis/selection. For tumour stage and grade we used a likelihood ratio test to compare the models with models in which the ORs were constrained to be the same in all the stage and grade categories. For age at diagnosis we used the likelihood ratio test to examine the significance of the interaction term between genotype and age. ¶Moderate, Gleason score 5–7 or moderately differentiated tumours; high, Gleason score 8–10 or poorly differentiated or undifferentiated.*

reported in the two largest studies included in the meta-analysis [16,17].

The response rates were suboptimal and were higher for cases than for controls. Similarly, the proportion of men with blood samples available was higher for cases than for controls. However, these differences between cases and controls are unlikely to be related to *CYP17A1* genotype and therefore to affect risk estimates. Although the present study was larger than previous studies, we cannot exclude small effects of rs743572. Also, almost all men in the present study were of Caucasian origin and therefore we were not able to test whether the association with prostate cancer differed by ethnic origin. In their meta-analysis, Ntais *et al.* [1] indicated that it is possible that rs743572 might be important for men of African descent.

One study speculated that the C allele in rs743572 might change the transcription-factor binding characteristics of the *CYP17A1* promoter region (via the inclusion of an SP-1 site) resulting in increased gene transcription of *CYPA1* mRNA [18]. If this is true, the biosynthesis of testosterone would be increased for carriers of the C allele. The same study showed that rs743572 was associated with polycystic ovaries and premature male-pattern baldness, two conditions related to elevated androgen levels [18]. Two relatively small studies found contradictory results for rs743572 and androgen levels [6,19]. Carriers of two copies of the C allele had higher levels of bioavailable testosterone than the others in a study of 294 white Americans [19], while they had lower levels of AS and free testosterone in a study of 164 Japanese men [6]. Contrary to these findings, in larger studies like the present and one conducted in 621 British men [20], there was no evidence of an association between rs743572 and androgen levels. This is consistent with two studies showing that the presence of the C allele does not affect transcription factor binding [21] and gene expression [22].

The common view that high levels of circulating androgens increase the risk of developing prostate cancer (the 'androgen hypothesis') led to several studies to test whether the C allele in rs743572 is associated with an increased risk of prostate cancer. However, recent evidence contradicts the 'androgen hypothesis' and shows that high levels of androgens are associated with decreased risk of aggressive prostate cancer [23,24]. In conclusion, our study suggests that rs743572 has no major role in increasing the risk of prostate cancer, either overall or in its aggressive form (i.e. Gleason score 8–10).

ACKNOWLEDGEMENTS

We express our gratitude to the study participants, and the many urologists, nurses, and histopathologists who kindly facilitated in the recruitment and collection of patient information and pathology reports. The study was supported by grants from the National Health and Medical Research Council (251533, 940394, 991129, 299955, 396407, 450104) and the Cancer Institute New South Wales (NSW). Also supported by grants from Tattersall's, The Whitten Foundation, Armati Family Fellowship, Australia and by infrastructure provided by The Cancer Council Victoria and the Australian Cancer Research Foundation. VMH is a Cancer Institute NSW Fellow. JLH is an Australia Fellow.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **OR**, odds ratio; **DGGE**, denaturing gradient gel electrophoresis; **SNP**, single nucleotide polymorphism; **3α-diolG**, 3α-diol glucuronide; **DHEAS**, dehydroepiandrosterone-sulphate; **AS**, androstenedione; **SHBG**, sex hormone-binding globulin; **E2**, 17β-oestradiol; **CV**, coefficient of variation; **H–W**, Hardy–Weinberg.