

CYP17 POLYMORPHISMS IN RELATION TO RISKS OF PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA: A POPULATION-BASED STUDY IN CHINA

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Because androgens likely play a key role in prostate growth and prostate cancer development, variants of genes involved in androgen biosynthesis may be related to prostate cancer risk. The enzyme P450c17 α , encoded by the CYP17 gene, catalyzes the conversion of progesterone and pregnenolone into precursors of potent androgens. In the 5' promoter region of the CYP17 gene, a T (A1 allele) to C substitution (A2 allele) has been hypothesized to increase CYP17 gene expression, resulting in higher levels of androgens. To investigate a possible role of CYP17 in prostate diseases, we evaluated the risk of prostate cancer and benign prostatic hyperplasia (BPH) in relation to variation in CYP17 genotype in a population-based case-control study conducted in Shanghai, China. The study included 174 prostate cancer cases, 182 BPH cases and 274 population controls. We observed no statistically significant overall associations of CYP17 genotypes with prostate cancer risk, although associations of the A1/A1 (odds ratio (OR) = 1.42, 95% confidence interval (CI) 0.83–2.48) and A1/A2 (OR 1.41, 95% CI 0.91–2.17) genotypes with prostate cancer were suggested. A similar association of the A1/A1 genotype with BPH was suggested. We found no associations of CYP17 genotypes with serum sex hormone levels or other biomarkers after correction for multiple comparisons. Large population-based studies are needed to clarify whether CYP17 plays a role in prostate cancer risk and whether genotype effects vary in different racial/ethnic and other subgroups.

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Because androgens likely play a key role in prostate growth and prostate cancer development, variants of genes involved in androgen metabolism may be related to prostate disease risk.^{1,2} The enzyme P450c17 α , encoded by the CYP17 gene on chromosome 10, catalyzes the conversion of progesterone and pregnenolone into the androgens androstenedione and dehydroepiandrosterone (DHEA), respectively, which are precursors of potent androgens.³

In the 5' promoter region of the CYP17 gene, a T to C substitution (A2 allele) has been hypothesized to increase CYP17 gene expression, resulting in higher levels of androgens, relative to those associated with the A1 allele.⁴ Results from molecular epidemiologic studies of CYP17 and prostate cancer have been mixed (Table I): while one study reported an increased risk for the A1/A1 genotype,⁵ results from other studies suggest either an increased risk for the A2/A2 genotype^{6–9} or no association in homozygous genotype comparisons.^{10–13} One recent study found that in men with BMI under 24 kg/m², an association with the A1/A1 genotype was suggested, while among men with BMI of 30 kg/m² or greater, an association with the A2/A2 genotype was observed.¹⁴ Additionally, a recent study in Japanese men found an increased risk of benign prostatic hyperplasia (BPH) in men with the A1/A1 genotype.⁵ To study further a possible role of CYP17 in prostate disease, we evaluated the risk of prostate cancer and BPH in

relation to this variation in the CYP17 gene in a population-based case-control study conducted in Shanghai, China.

MATERIAL AND METHODS

Prostate cancer cases

Details of the study have been reported elsewhere.^{15–19} All study subjects were born in China. Briefly, cases of primary prostate cancer (ICD9 185) that were newly diagnosed between 1993 and 1995 were identified through a rapid reporting system that was established between the Shanghai Cancer Institute and 28 collaborating hospitals in urban Shanghai. Cases were permanent residents in 10 urban districts of Shanghai who did not have a history of any other cancer. A total of 268 eligible cases were identified, representing 95% of the cases diagnosed in urban Shanghai during this time period. Of the 268 eligible cases, 243 (91%) were interviewed. Since prostate cancer screening is not widespread in China, most of the identified cancer cases were symptomatic and clinically significant.

After consensus review by U.S. and Shanghai pathology teams, 4 cancer cases were classified as having BPH and excluded from the study. Whenever possible, clinical stage and histologic grade were assessed. Localized cancer is defined as organ-confined cancer (clinical stage A and B), and advanced cancer is defined as regional or remote cancer (stage C and D).

Benign prostatic hyperplasia patients

Upon identification of a prostate cancer case, the next BPH patient admitted to the same hospital as the index cancer case for either transurethral resection of the prostate or prostatectomy was invited to participate in the study. BPH cases underwent digital rectal exam, prostate specific antigen levels measurement and transurethral resection of the prostate. Pathology slides were reviewed to confirm BPH status and assess whether there was histologic evidence of cancer. The study was limited to BPH cases who were permanent residents of Shanghai and who had no history of any cancer. In total, 206 (97%) of the 213 eligible BPH subjects were interviewed.

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TABLE I – STUDIES OF PROSTATE CANCER AND *CYP17* POLYMORPHISMS

Author	Country	Cases/controls	Suggested risk allele	Odds ratio (95% CI)
Lunn <i>et al.</i> 1999	US	108 cases 167 urology controls	A2	A1/A1: 1.0 A1/A2: 1.7 (1.0–3.2) A2/A2: 1.7 (1.0–3.2)
Wadelius <i>et al.</i> 1999	Sweden	178 cases 160 population controls	A1 as reported, but neither in homozygote comparison	A2/A2 or A1/A2: 1.0 A1/A1: 1.6 (1.0–2.5) (homozygotes: A2/ A2: 1.0 A1/A1: 1.2)
Gsur <i>et al.</i> 2000	Austria	63 cases 126 BPH controls	A2	A1/A1: 1.0 A1/A2: 0.9 (0.4–1.9) A2/A2: 2.8 (1.0–7.8)
Yamada <i>et al.</i> 2001	Japan	101 cases 200 BPH controls	A2	A1/A1: 1.0 A1/A2: 2.1 (1.1–4.0) A2/A2: 2.4 (1.0–5.5)
Habuchi <i>et al.</i> 2000	Japan	252 cases 131 hospital controls	A1	A2/A2: 1.0 A1/A2: 1.5 (0.8–2.5) A1/A1: 2.6 (1.4–4.8)
Kittles <i>et al.</i> 2000	US African-American	71 cases 111 urology or screening program controls	A2	A1/A1: 1.0 A1/A2: 2.0 (1.0–3.9) A2/A2: 2.8 (1.0–7.4)
Chang <i>et al.</i> 2001	US	133 hereditary cases 225 sporadic cases 182 screening program controls	Neither	A1/A1: 1.0 A1/A2: 1.3 (0.9–1.9) A2/A2: 1.3 (0.7–2.2)
Haiman <i>et al.</i> 2001	US	590 cases 782 cohort controls	Neither	A1/A1: 1.0 A1/A2: 1.3 (1.0–1.6) A2/A2: 1.2 (0.9–1.6)
Latil <i>et al.</i> 2001	France	268 cases 156 cohort controls	Neither	A1/A1: 1.0 A1/A2: 1.0 (0.6–1.5) A2/A2: 0.9 (0.5–1.8)
Stanford <i>et al.</i> 2002	US	590 cases 538 population controls	Neither, but significant interactions	A1/A1: 1.0 A1/A2: 0.8 (0.6–1.1) A2/A2: 0.9 (0.6–1.3)

Population controls

Based on a population register of all adult residents in urban Shanghai, healthy male subjects were randomly selected from the general population as the comparison group. Those who were deceased, had a history of cancer or had moved out of the area before the sampling of controls were not eligible for the study. Of the 495 potential controls selected from among 6.5 million permanent residents of Shanghai and frequency-matched to the expected age distribution (in 5-year age categories) of prostate cancer cases, 472 (95%) were interviewed. To screen for prostate-related disorders, 314 controls underwent digital rectal examination (DRE), transrectal ultrasound and prostate specific antigen (PSA) testing. One prostate cancer case among the 472 interviewed potential control subjects was identified, confirmed histologically and excluded from the study, leaving 471 population controls in the study. No other control subjects were excluded.

Blood collection and DNA extraction

Two hundred cases (84% of those interviewed), 200 BPH patients (97%) and 330 controls (70%) provided overnight fasting blood samples for the study. Blood samples were processed and separated within 2 hr of collection at a central laboratory in Shanghai. The buffy coat samples were stored at -70°C in Shanghai, and then shipped to the U.S. on dry ice for DNA extraction at the American Type Culture Collection (Manassas, Virginia). Samples from 174 cases, 182 BPH patients and 274 controls had sufficient DNA for *CYP17* genotyping; the numbers with sufficient DNA are reduced because DNA was used for other studies.^{15,18,19} Laboratory personnel were masked to case-control status, and to minimize potential biases due to batch-to-batch laboratory variation, DNA samples were physically arranged such that each assay batch included the same proportion of total cases, BPH patients

and controls. The study was approved by the National Cancer Institute and Shanghai Cancer Institute Institutional Review Boards.

CYP17 genotyping

The single nucleotide polymorphism (SNP) of the T to C transition in *CYP17* was detected using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis as described previously.¹⁰ In addition to the original 174 genotyped cases, DNA samples from 158 prostate cancer cases recruited in 2001 were genotyped to increase the statistical power of genetic analyses. Since we did not have information on age at diagnosis for these 158 cases, all 332 cases were included in a separate computation of a crude odds ratio.

As part of the quality control procedure, 21 split samples from a single individual were spaced at intervals among the study samples to assess the reproducibility of genotyping. All of the 21 samples had identical genotyping results.

Serum hormone and other biomarker assays

Serum samples from study subjects were analyzed for levels of sex hormones, including testosterone (T), dihydrotestosterone (DHT) and 5α -androstane- $3\alpha,17\beta$ -diol glucuronide (3α -diol G), and sex hormone-binding globulin (SHBG), by radioimmunoassay (RIA). Prior to RIA, T and DHT were extracted with hexane:ethyl acetate (3:2) and purified by Celite column partition chromatography as described previously.^{10,20,21} 3α -diol G and SHBG were measured directly in serum using commercial kits [Diagnostic Systems Laboratories (DSL), Webster, TX]. The intra- and inter-assay coefficients of variation ranged from 4 to 8% and 10 to 13%, respectively. In addition, serum insulin and leptin were measured by RIA kits (Linco Research, St. Charles, MO),¹⁷ and plasma insulin-like growth factor (IGF)-I, IGF-II, IGF binding protein

(IGFBP)-1 and IGFBP-3 were measured using ELISA assays as described previously.²²

Statistical analysis

Unconditional logistic regression analyses were used to compute the odds ratios (ORs) and 95% confidence intervals (CIs)²³ to assess the associations of the 3 *CYP17* genotypes (A1/A1, A1/A2, and A2/A2) with prostate cancer and with BPH. Because not all BPH cases are symptomatic and diagnosed clinically, for the BPH analysis, to minimize the extent of misclassification of disease among population controls, we estimated the risk of BPH in relation to *CYP17* in 3 analyses by sequentially excluding controls having BPH by the following criteria: 1) those who reported a history of BPH, 2) those who had BPH detected through the medical examination (DRE and transrectal ultrasound) conducted for our study, and 3) those whose PSA measurements were greater than 4 ng/ml.

To assess the possibility that the *CYP17* genotype may affect prostate cancer risk through an effect on circulating levels of sex hormones, we also examined serum levels of these biomarkers among population controls in relation to *CYP17* genotype using multiway analysis of variance (ANOVA) to adjust for age in separate analyses for each biomarker.²⁴ Because a study of rhesus monkeys treated with androgens reported increased ovarian IGF-I mRNA,²⁵ and because IGF-I and other correlated biomarkers have been associated with prostate cancer,^{17,22} we also examined whether *CYP17* genotype might influence levels of IGFs, IGFBPs, insulin and leptin, perhaps due to subtle differences in androgen levels by genotype. In addition, because the possibility of linkage of the *CYP17* polymorphism with the genetics of insulin resistance has been suggested as a basis for an association of A1/A1 with endometrial cancer and with insulin and C-peptide levels in cases,²⁶ and because insulin resistance was associated with prostate cancer in this study,²⁷ we examined 2 indicators of insulin resistance, namely, the fasting insulin to glucose ratio (I_0/G_0), and the homeostasis model assessment for insulin resistance index [$HOMA-IR = I_0 * G_0 / 22.5$], among controls by genotype, to assess whether these relationships provide support for the observed *CYP17* genotype/prostate cancer relationship. Thus, we conducted separate age-adjusted ANOVA analyses for 15 biomarkers by genotype among the population controls. We used the Bonferroni correction for alpha of 0.05 to assess whether any comparisons were statistically significant after considering that we had conducted multiple ($n=15$) homozygote genotype comparisons among controls.²⁸ In this instance, alpha of 0.05 was divided by 15, indicating a required p value less than 0.003 for statistical significance. Hormone and biomarker serum concentrations were logarithm-transformed for cross-sectional analyses; results are presented as geometric means.

RESULTS

Age at diagnosis ranged from 50 to 94 years (median 73) for the 174 cancer cases in the main analyses. About 2/3 of the cases were diagnosed as having advanced cancer (regional/metastatic stages), and most tumors were moderately or poorly differentiated.

Table II shows the frequencies of the *CYP17* genotypes by subject type; these distributions were in agreement with Hardy-Weinberg equilibrium. Among the population controls, A1/A2 was

the most common genotype (44.2%), followed by A2/A2 (38.7%) and A1/A1 (17.1%). The A1 allele occurred in 61.3%, and the A2 in 82.9% of controls.

Relative to men with the A2/A2 genotype, those with the A1/A1 genotype had a nonsignificant 42% excess prostate cancer risk (OR=1.42, 95% CI 0.83–2.48), and those with the heterozygous A1/A2 genotype had a similar prostate cancer relative risk (OR=1.41, 95% CI 0.91–2.17). The suggested main effect of the A1/A1 genotype did not vary greatly when stratified by age or stage (data not shown). When the 158 additional prostate cancer cases ascertained during 2001 were included in a crude odds ratio for both time periods, the genotype prevalences and effects of the A1/A1 (OR = 1.30, 95% CI 0.82–2.09) and A1/A2 genotypes (OR=1.47, 95% CI 1.03–2.10) were fairly similar to the original crude and age-adjusted results. These 158 cases are not included in the main analyses or Table II.

In examining BPH as an outcome, relative to men with the A2/A2 genotype, those with the A1/A1 genotype had a nonsignificant 44% excess risk (95% CI 0.83–2.51), and those with the heterozygous A1/A2 genotype had an OR of 1.28 (95% CI 0.82–1.99). Risk estimates for BPH were materially unchanged when the following groups of control subjects were sequentially excluded from the analysis: 1) those who reported a history of BPH ($n=29$), 2) those who had BPH detected through the medical examination conducted for our study ($n=69$), and 3) those whose PSA measurements were greater than 4 ng/ml, suggestive of BPH ($n=97$) (data not shown).

Table III presents age-adjusted mean levels of hormones and biomarkers by *CYP17* genotype among the 274 control subjects. There were no statistically significant differences in age-adjusted means of most of these biomarkers by genotype. Although we observed significantly lower mean IGF-I levels by pairwise F test in men having the A1/A1 genotype compared to men having A1/A2 or A2/A2, the differences were not statistically significant when the Bonferroni correction for multiple comparisons was applied. Consistent with this possible difference in IGF-I levels by genotype, when we excluded 4 control men with testosterone measurements less than 100 ng/dL, we observed suggestions of similarly directed differences between homozygous genotypes in mean 3α -diol G (11.3%), DHT (8.6%) and T (7.4%).

DISCUSSION

Results from this population-based study suggest that men with the A1/A1 or A1/A2 *CYP17* genotypes may have an increased risk of prostate cancer, although the results were not statistically significant. Findings from our study of these men with relatively low BMIs (median = 21.5 kg/m²) are consistent with one⁵ but not all⁹ studies in Japanese men, and with results observed in US men with low BMIs (<24 kg/m²).¹⁴ However, other studies, most of which were in Western populations, suggested an increased risk for the A2/A2 genotype,^{6–9} or found no associations in comparisons of homozygous genotypes.^{10–13} A recent meta-analysis reported that the A2 allele increased susceptibility to prostate cancer in subjects of African but not European descent.²⁹

Differences in population characteristics may contribute to inconsistency in results among studies. For example, several studies that suggested an association of A2/A2 with prostate cancer risk

TABLE II – ODDS RATIOS (ORs)¹ AND 95% CONFIDENCE INTERVALS (CIs) FOR PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA (BPH) IN RELATION TO *CYP17* GENOTYPE, SHANGHAI, CHINA

Genotype	Controls ($n = 274$)		Prostate cancer ($n = 174$)			BPH ($n = 182$)		
	N	(%)	N	(%)	OR ² (95% CI)	N	(%)	OR ³ (95% CI)
A2/A2	106	(38.7)	54	(31.0)	1.0	57	(31.3)	1.0
A1/A1	47	(17.1)	34	(19.5)	1.42 (0.83–2.48)	39	(21.4)	1.44 (0.83–2.51)
A1/A2	121	(44.2)	86	(49.4)	1.41 (0.91–2.17)	86	(47.3)	1.28 (0.82–1.99)

¹Adjusted for age. –²Prostate cancer cases vs. population controls. –³BPH vs. population controls.

TABLE III – GEOMETRIC MEAN SERUM OR PLASMA LEVELS OF HORMONES AND BIOMARKERS, AND LEAST-SQUARES MEANS¹ OF OTHER CHARACTERISTICS IN RELATION TO *CYP17* GENOTYPE AMONG 274 POPULATION CONTROLS, SHANGHAI, CHINA²

Hormones and Biomarkers	A1/A1		A1/A2		A2/A2		<i>p</i> -value ³
	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)	
Testosterone (T) (ng/dL)	595	(511–692)	596	(538–659)	589	(530–655)	0.98
DHT (ng/dL)	60.8	(52.9–69.9)	61.1	(55.7–67.0)	65.3	(59.3–71.9)	0.47
3 α -diol G (ng/ml)	4.70	(3.80–5.82)	4.85	(4.21–5.59)	5.19	(4.48–6.02)	0.65
Estradiol (ng/dL)	4.8	(4.4–5.3)	4.8	(4.5–5.1)	4.8	(4.5–5.2)	0.99
SHBG (nmol/L)	33	(28–38)	33	(30–37)	34	(31–38)	0.91
T/SHBG	0.630	(0.535–0.740)	0.622	(0.559–0.694)	0.597	(0.533–0.668)	0.78
E2/SHBG	0.0054	(.0046–.0064)	0.0053	(.0047–.0060)	0.0052	(.0047–.0059)	0.94
Insulin (uU/ml)	8.00	(6.84–9.36)	8.05	(7.25–8.93)	7.67	(6.88–8.54)	0.47
Leptin (ng/ml)	2.7	(2.2–3.3)	3.2	(2.8–3.6)	3.3	(2.9–3.7)	0.20
IGF-I (ng/ml)	106	(95–119)	127	(118–137)	127	(117–137)	0.01 ³
IGF-II (ng/ml)	418	(382–457)	449	(423–476)	457	(430–486)	0.20
IGFBP-1 (ng/ml)	100	(82–123)	81	(71–92)	80	(69–92)	0.12
IGFBP-3 (ug/ml)	2.78	(2.54–3.04)	2.76	(2.60–2.93)	2.82	(2.65–3.01)	0.85
I ₀ /G ₀	0.10	(0.09–0.12)	0.11	(0.09–0.12)	0.10	(0.09–0.12)	0.98
HOMA-IR	1.53	(1.26–1.87)	1.52	(1.34–1.74)	1.40	(1.22–1.60)	0.52
	Least-squares means		Least-squares means		Least-square means		
Age (unadjusted)	71.8		71.6		72.2		0.88
BMI (kg/m ²)	23.0		23.0		22.8		0.93
WHR	0.885		0.887		0.869		0.16

¹Age-adjusted except where indicated. ²Frequencies of subjects missing measurements: DHT (n = 4); 3 α -diol G, T, T/SHBG, and IGFBP-3 (n = 1); insulin (n = 3); leptin (n = 2), IGFBP-1 (N = 6); HOMA-IR and I₀/G₀ (n = 8). ³*p* value for F test. However, none of the differences were statistically significant when the Bonferroni correction for multiple comparisons was applied.

compared cases with BPH or other urology clinic patients, men being screened, or some combination thereof.^{6–9} Our study and another in Asians, which suggested or reported associations of A1/A1 with prostate cancer, compared cases, respectively, with population controls and hospital controls with no prostatic enlargement.⁵ However, 3 studies using cohort controls¹¹ and/or controls participating in prostate cancer screening programs,^{10,12} 1 of which excluded men with abnormal DRE or PSA > =4 ng/ml,¹⁰ observed no strong overall associations of prostate cancer in comparing homozygous genotypes.

It is also possible that some of the differences among studies are due to differences in case characteristics. Two studies suggested¹⁰ or reported¹⁴ associations of A2/A2 with hereditary prostate cancer. While no consistent strong associations with stage,^{5,6,9,10} grade^{8–10} or stage/grade,^{7,11} were observed in prior studies, our cases generally presented at later stages than those in other studies, due to the lack of screening in China. However, our data suggested an association of A1/A1 with both localized and advanced cases.

Although the possibility of linkage of the *CYP17* polymorphism with the genetics of insulin resistance has been hypothesized to provide a basis for an association of A1/A1 with endometrial cancer (EC) and with insulin and C-peptide levels in EC cases,²⁶ we did not find significant differences in HOMA-IR or I₀/G₀ among controls by *CYP17* genotype.

A possible association of the A1/A1 genotype was not reflected in increased hormone or IGF-I levels among controls with the A1/A1 genotype compared to A2/A2; in fact we observed a lower IGF-I level among men with the A1/A1 genotype compared to A1/A2 and A2/A2, but this was not statistically significant after correction for multiple comparisons. No statistically significant overall differences in testosterone and 3 α -diol G by genotype were observed in our study and 2 others;^{12,30,11} however, herein, when several men with very low testosterone measurements were excluded, we noted small differences by genotype consistent in direction with that observed for IGF-I for these 2 hormones and DHT. If there is subtle variation in androgen production by *CYP17* genotype, increased androgen levels could possibly result in increased IGF-I production in the liver, which could explain our observation of possible differences in IGF-I levels by genotype. Increases in ovarian IGF-I mRNA have been reported when rhesus

monkeys were treated with androgens.²⁵ However, if the A2/A2 genotype is associated with higher androgen levels, this does not explain a possible excess risk conferred by A1/A1 in Asian men.

Our study had several strengths, including its population basis, near complete case ascertainment, high response rate and high quality genotyping. Although bias or limitations in generalizability could result from blood sampling refusals, cancer stage in *CYP17* genotyped cases was fairly similar to interviewed cases (36% vs. 34% localized, 64% vs. 66% regional/metastatic), and genotyped cases and controls were similar to those interviewed regarding age, education, BMI, smoking and alcohol consumption, suggesting that the genotyped groups adequately represent the population. Misclassification of prostate cancer among controls should be minimal in our study of a low-risk population, as supported by largely negative results of digital rectal examination and PSA testing conducted among controls. This is reflected in a much lower median level of PSA among controls than that in cases (1.3 ng/ml vs. 87 ng/ml). A weakness of our study is limited statistical power to detect modest effects.

In summary, we observed no statistically significant overall associations of *CYP17* polymorphisms with prostate cancer risk or BPH, although moderate associations of the A1/A1 and A1/A2 genotypes were suggested. We found no associations of *CYP17* with sex hormones. Large population-based studies are needed to clarify whether *CYP17* plays a role in prostate cancer, whether genotype effects vary in different racial/ethnic groups, and whether anthropometric factors modulate the association between *CYP17* and prostate cancer.

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