Ann Surg Oncol (2010) 17:312–322
DOI 10.1245/s10434-009-0698-8

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Association Analysis of Wnt Pathway Genes on Prostate-Specific Antigen Recurrence After Radical Prostatectomy

Shu-Pin Huang, MD, PhD[1,2,3], Wen-Chien Ting, MD[4], Lu-Min Chen, MD[5], Li-Chia Huang, MD[5], Chia-Chu Liu, MD[1], Chien-Wei Chen, PhD[6], Chi-Jeng Hsieh, MS[7,8], Wen-Hui Yang, PhD[9], Ta-Yuan Chang, PhD[10], Hong-Zin Lee, PhD[11], and Bo-Ying Bao, PhD[11]

[1]Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [2]Department of Urology, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan; [3]Department of Urology, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [4]Department of Colorectal Surgery, China Medical University Hospital, Taichung, Taiwan; [5]Department of Obstetrics and Gynecology, China Medical University Hospital, Taichung, Taiwan; [6]Clinical Research and Development, Novartis Pharmaceuticals Corporation, East Hanover, NJ; [7]Department of Health Care Administration, Oriental Institute of Technology, Taipei, Taiwan; [8]Graduate Institute of Health Care Organization Administration, College of Public Health, National Taiwan University, Taipei, Taiwan; [9]Department of Health Services Administration, China Medical University, Taichung, Taiwan; [10]Department of Occupational Safety and Health, China Medical University, Taichung, Taiwan; [11]Department of Pharmacy, China Medical University, Taichung, Taiwan

ABSTRACT

Background. Approximately one-third of prostate cancer (PCa) patients show biochemical failure after radical prostatectomy (RP) and are prone to develop metastasis with significant mortality. Although aberrant Wnt/β-catenin (CTNNB1) signaling has been observed in numerous types of human cancers, including PCa, to our knowledge there is currently no information on the role of Wnt signaling gene polymorphisms in PCa.

Methods. We comprehensively studied the contribution of genetic variations in *CTNNB1* and *adenomatous polyposis coli* (*APC*), one of the key genes encoding the CTNNB1 destruction complex, to PCa risk and prognosis after RP using a hospital-based case–control study. We selected and genotyped 13 tagged single-nucleotide polymorphisms (tSNP) to predict common variants across entire *APC* and *CTNNB1* genes in 307 patients with clinically localized PCa who received RP and 371 unaffected controls.

Results. Four tSNPs (rs3846716, rs2431238, rs41115, and rs565453) and a specific haplotype (GTAAGA) in the *APC* tumor suppressor gene were associated with a 0.57- to 0.71-fold lower risk of localized PCa. The association of tSNPs with prostate-specific antigen (PSA) recurrence in PCa patients was then analyzed by Kaplan–Meier analysis and Cox regression model. Interestingly, we found that the *APC* rs3846716 GA/AA genotypes were also significantly associated with poorer PSA-free survival (log-rank test, $P = 0.037$) compared with the GG genotype.

Conclusions. This is the first report documenting the potential prognostic role of the *APC* rs3846716 GA/AA genotype on PSA recurrence after RP.

S.-P. Huang and B.-Y. Bao contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1245/s10434-009-0698-8) contains supplementary material, which is available to authorized users.

First Received: 22 May 2009;
Published Online: 24 September 2009

B.-Y. Bao, PhD
e-mail: bao@mail.cmu.edu.tw

Patients diagnosed with localized prostate cancer (PCa) are commonly treated with radical prostatectomy (RP), but approximately 35% of patients experience biochemical failure, as manifested by prostate-specific antigen (PSA) relapse, within 10 years.[1,2] Although several clinicopathological indicators, such as PSA, Gleason score, pathologic stage, and surgical margin status, have been currently used for outcome prediction following intended curative RP of primary PCa, finding new biomarkers to improve prediction

of disease recurrence may be beneficial for selecting adjuvant therapy for high-risk patients.[3]

Accumulated evidence has demonstrated significant roles of the Wnt pathway in the development and the progression of human PCa.[4–6] *Adenomatous polyposis coli* (*APC*) was originally identified as a tumor suppressor gene in colon cancer. Loss of APC interaction or activation of Wnt signaling leads to accumulation of nuclear β-catenin (CTNNB1).[7] This activates the T-cell factor (TCF) and lymphoid enhancer factor (LEF) transcription factors, which then activates the targets controlling cell growth and differentiation. Therefore, the interaction of APC with CTNNB1 has been considered to be essential for its tumor suppressor activity.[8] Although mutations in APC, CTNNB1, and other components of the CTNNB1 destruction complex are rare in PCa, an increase in cellular levels of CTNNB1 is frequently observed.[9–11] Genetic studies using mutant mouse models have also demonstrated that specific expression of a mutant CTNNB1, lacking exon 3, in prostate tissues results in the development of prostate intraepithelial neoplasia and hyperplasia of the prostate.[4,6] Taken together, these data provide a solid link between APC/CTNNB1 signaling and the pathogenesis of PCa.

Despite previously conducted studies, genetic variations in *APC* and *CTNNB1* have not been systematically analyzed with respect to prognosis of and susceptibility to PCa.[12] To address this, we conducted a hospital-based case–control study to comprehensively examine the relation of tagged single-nucleotide polymorphisms (tSNP) and haplotypes in two key genes in the Wnt pathway, *APC* and *CTNNB1*, to localized PCa risk, clinicopathological features, and biochemical failure after RP.

MATERIALS AND METHODS

Study Population

The study subjects were expanded from our hospital-based PCa case–control study that has previously been described.[13–17] Briefly, patients with diagnosed and pathologically confirmed PCa were actively recruited from the Kaohsiung Medical University Hospital, Kaohsiung Veterans General Hospital, and National Taiwan University Hospital. Male controls were selected from a population of men receiving general health examinations at these medical centers and Kaohsiung Municipal United Hospital during the same period. Controls did not have significant voiding symptoms (American Urological Association symptom score < 8), their PSA levels were within the normal limits (<4 ng/ml), they had no history of prostate surgery, and no clinical signs of prostate hyperplasia or prostate cancer during digital rectal examination.[13–18] Those with other known malignancies were excluded.

Disease stage was determined by pathologic findings, pelvic computed tomography or magnetic resonance imaging, and radionucleotide bone scans, based on criteria outlined by the American Joint Committee on Cancer (AJCC) tumor–node–metastasis (TNM) classification system (AJCC Cancer Staging Manual, 5th edition, 1997). Pathologic grade was recorded as Gleason score and classified into two groups, with Gleason scores of 2–6 and 7–10.[19] Pathology analyses were done on the whole specimen with step sections (2–3 mm), and positive surgical margin was defined as tumor cells present at the inked margin. The PCa patients who underwent RP were followed up prospectively to investigate the potential role of genetic variants in the progression of PCa (defined by recurrence of PSA). PSA recurrence was defined as two consecutive PSA measurements >0.2 ng/ml at an interval of >3 months, and the first follow-up with PSA level >0.2 ng/ml was considered the date of recurrence.[20] For more precise analysis of the effect of disease recurrence after RP, patients who received adjuvant hormone therapy or radiotherapy and those without sufficient follow-up time were excluded, leaving 307 PCa cases and 371 male controls in the final analysis. This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital, and informed consent was obtained from each participant.

SNP Selection and Genotyping

SNPs that captured the genetic variability in the *APC* and *CTNNB1* genes were selected using public data from the HapMap consortium from the regions that comprised the *APC* and *CTNNB1* locus, plus 20 kb of the 5′ upstream and 10 kb of the 3′ downstream (chromosome 5: 112081483–112219834 for *APC*, chromosome 3: 41196016–41266938 for *CTNNB1*).[21] Haploview was used to generate pairwise linkage disequilibrium (LD) estimates and define haplotype blocks using criteria defined by Gabriel and colleagues,[22,23] as shown in Supplementary Fig. 1. According to HapMap Han Chinese in Beijing, China (CHB) population data, which comprised a total of 86 SNPs with a minor allele frequency (MAF) greater than 0.05, the *APC* gene exhibited two haplotype blocks. Block 1 covered 47 kb and block 2 covered 86 kb. *CTNNB1* also exhibited two haplotype blocks, comprising a total of 39 SNPs with a MAF greater than 0.05. Block 1 covered 53 kb and block 2 covered 11 kb. tSNPs were selected from each gene and tagged to capture the unmeasured variants by Tagger ($r^2 > 0.8$).[24] A total of 15 tSNPs were then selected (2 tSNPs excluded from the following data analysis are not shown in the Supplementary Fig. 1).

Genotyping was carried out by Sequenom iPLEX matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass-spectrometry technology at the National Genotyping Center, Academia Sinica, Taiwan. Briefly, primers for locus-specific polymerase chain reaction (PCR) and allele-specific extension were designed by MassARRAY AssayDesign 3.0 software (Sequenom, San Diego, CA). The sample DNAs were amplified by primers flanking the targeted sequence, followed by dephosphorylation and allele-specific primer extension. The extension products were purified, loaded into a 384-format SpectroChip, and subjected to MALDI-TOF mass spectrometry. The resultant data were analyzed by the Sequenom MassARRAY TYPER software. Quality control included genotyping of 13 blind duplicate samples, which revealed 99% agreement on genotyping calls across all tSNPs assayed. Any tSNPs that did not conform to Hardy–Weinberg equilibrium ($P < 0.01$) and below a genotyping call rate of 85% were removed from analysis ($n = 2$). Thus, a total of 13 tSNPs were included for statistical analysis.

Statistical Analysis

Distribution of demographic characteristics between patients and controls was assessed by Student *t* tests. χ^2 or Fisher exact test were used to compare allele frequencies between patients and controls, followed by permutation test (100,000 permutations) using Haploview.[22] Logistic regression analysis was used to compute odds ratio (OR) and 95% confidence interval (CI) for estimating the associations of genotypes/haplotypes to the risk of localized PCa, clinicopathological features, and PSA recurrence, while adjusting for age in all these variables [age-adjusted OR (aOR)]. The linear regression model was used to estimate the effects of genotypes on age at time of first diagnosis and age-adjusted preoperative PSA levels. The Kaplan–Meier method was used to compare the influence of genotypes in the PSA-free survival interval, and significance was determined using the log-rank test. Univariate and multivariate analyses to determine the interdependency of tSNP genotypes/haplotypes and the risk parameters of age, preoperative PSA, pathologic stage, positive surgical margin, and Gleason score were carried out using Cox proportional hazards regression. Statistical Package for the Social Sciences software version 16.0.1 (SPSS Inc., Chicago, IL) was used for statistical analyses. All haplotype risks were assessed using HPlus, and only haplotypes with estimated frequencies of >0.01 were included.[25,26] A two-sided *P* value of <0.05 was considered statistically significant.

RESULTS

Characteristics of the Study Population

The demographic characteristics of the study population are summarized in Supplementary Table 1. The mean ages (±standard deviation, SD) were 65.7 (±6.5) years for PCa patients who received RP and 61.9 (±9.3) years for the controls. There were no statistical differences in height, weight or body mass index between the groups. Among the 307 RP cases, the majority of PCas were at pathological localized stage (83.1%); however, 111 (36.2%) experienced PSA recurrence during the 38.5 months (mean) and 30.8 months (median) follow-up periods.

Association Between 13 tSNPs of APC and CTNNB1 and Clinically Localized PCa Risk

The tSNP ID, locations, the amino acid substitution, haplotype blocks, and the allele frequencies in the HapMap database and in our study population are shown in Table 1. Among the 13 tSNPs of *APC* and *CTNNB1* genes, two were significantly different in allelic frequency between RP cases and controls. The MAF of rs2707765 and rs2431238 (both in intron 6 of the *APC* gene) were significantly lower in RP cases than in the controls (30.4 vs. 35.7% for rs2707765; 7.4 vs. 12.4% for rs2431238). After permutation test for testing the probability of false-positive findings, we still cannot rule out that the association of rs2431238 is falsely positive.

Genotype frequencies of the tSNPs between RP cases and controls and their associations with risk of clinical localized PCa are shown in Table 2. Four *APC* tSNPs showed lower relative risks of localized PCa for the heterozygous variant carriers versus homozygous wild-type carriers (aOR 0.64, 95% CI 0.45–0.90, for rs3846716; 0.57, 0.36–0.91, for rs2431238; 0.70, 0.49–0.98, for rs41115; and 0.71, 0.50–1.00, for rs565453). Several tSNPs within the *APC* gene were associated with the risk of localized PCa and therefore a haplotype analysis was performed. The haplotypes of *APC* and *CTNNB1* genes with frequency >0.01 and their association with the risk of localized PCa are summarized in Table 3. The *APC* (GTAAGA) haplotype, which contains the minor allele of rs2707765, rs2431238, rs41115, and rs565453, was significantly associated with a decreased risk of localized PCa (aOR 0.58, 95% CI 0.39–0.85). This was consistent with the protective effect of minor alleles of several *APC* tSNPs (rs2431238, rs41115, and rs565453) shown in Table 2. Since APC interacts with CTNNB1 to exert its tumor suppressor activity, a combined haplotype analysis for interactions between *APC* and *CTNNB1* was performed (Supplementary Table 2). We found that the

TABLE 1 Allelic frequencies for APC and CTNNB1 polymorphisms in clinically localized PCa patients compared with controls

tSNP ID	Chromosome position	Location	Block	Allele	HapMap Han MAF	Patient/control MAF	*P* value[a]	Empirical *P* value[b]
APC								
rs3846716	Chr5:112087493	5′ upstream	1	G > A	0.189	0.164, 0.197	0.125	0.431
rs2707765	Chr5:112150431	Intron 6	2	C > G	0.330	0.304, 0.357	**0.040**	0.175
rs2431238	Chr5:112152268	Intron 6	2	C > T	0.089	0.074, 0.124	**0.004**	**0.018**
rs17134945	Chr5:112167921	Intron 8	2	A > G	0.070	0.083, 0.065	0.196	0.596
rs2707761	Chr5:112177826	Intron 8	2	A > G	0.148	0.146, 0.151	0.792	0.999
rs41115	Chr5:112203669	Thr1493Thr	2	A > G	0.189	0.167, 0.198	0.137	0.463
rs565453	Chr5:112213292	3′ downstream	2	C > A	0.189	0.168, 0.196	0.183	0.560
CTNNB1								
rs4016435	Chr3:41225133	Intron 1	1	G > T	0.056	0.064, 0.053	0.388	0.899
rs1798802	Chr3:41236983	Intron 1	1	G > A	0.311	0.301, 0.275	0.294	0.807
rs11564459	Chr3:41251411	Intron 10	-	A > G	0.092	0.033, 0.035	0.803	1.000
rs11564465	Chr3:41252044	Intron 10	2	C > T	0.244	0.231, 0.208	0.296	0.808
rs11564475	Chr3:41255037	Intron 14	2	A > G	0.133	0.144, 0.151	0.723	0.998
rs2293303	Chr3:41255831	Asp780Asp	2	C > T	0.102	0.145, 0.129	0.406	0.923

MAF minor allele frequency

$P \leq 0.05$ are in boldface

[a] χ^2

[b] After 100,000 permutations

combined haplotype GCCAAACTAATAC, which contains the minor allele of *CTNNB1* rs4016435, rs1798802, and rs11564465, was associated with an increased risk of localized PCa (aOR 1.71, 95% CI 1.01–2.90), reflecting the potential interactive effect between *APC* and *CTNNB1* on localized PCa risk.

Association Between 13 tSNPs of APC and CTNNB1 and Clinicopathological Features Among PCa Patients Who Received RP

To explore the potential prognostic role of *APC* and *CTNNB1* tSNPs on the recurrence of PSA after RP, we compared the genotype distributions with the various clinicopathological features among RP cases (Table 4). We found no genotype association with patient age at diagnosis. The mean level of preoperative PSA was 11.9 ± 5.58 ng/ml higher in individuals with *CTNNB1* rs11564459 AG genotype than in those with the AA genotype. *APC* rs3846716 GA + AA genotypes were significantly associated with advanced pathologic stage (aOR 1.80, 95% CI 1.07–3.01), positive surgical margin (aOR 2.07, 95% CI 1.17–3.66), and PSA recurrence after RP (aOR 1.80, 95% CI 1.09–2.99). Two tSNPs (*CTNNB1* rs1798802 and rs11564465) were associated with slightly increased risk of high Gleason score (≥7) when the homozygote/heterozygote frequencies of the minor allele were compared with the homozygote frequencies of the major allele (aOR 1.68, 95% CI 1.04–2.70 for rs1798802; 1.73, 1.05–2.84 for rs11564465). Several *APC* tSNPs, in addition to rs3846716, were also associated with the risk of positive surgical margin, and *APC* rs41115 was associated with higher relative risks of PSA recurrence for the heterozygous variant carriers versus homozygous wild-type carriers (aOR 1.70, 95% CI 1.01–2.88). Haplotype analyses were also performed to determine whether regions of genes or combinations of tSNPs in genes were associated with clinicopathological features (Supplementary Table 3). In *CTNNB1* block 1, the haplotype GA containing the minor allele of rs1798802 was significantly associated with increased risk of high Gleason score (≥7) (aOR 1.65, 95% CI 1.08–2.53). This was consistent with the observation that the minor allele of rs1798802 was associated with an increased risk of high Gleason score (Table 4). However, we found no differences in risk estimates for any of haplotypes on other clinicopathological features (data not shown). The combined haplotype analysis for interactions between *APC* and *CTNNB1* revealed that the ACTGGGCGGACAC haplotype, which contains the minor allele of *APC* rs3846716, rs2431238, rs17134945, rs2707761, and rs41115, was associated with an increased risk of PSA recurrence after RP (aOR 3.18, 95% CI 1.26–8.00) (Supplementary Table 2), reflecting the potential interactive effect between *APC* and *CTNNB1* on PSA recurrence.

TABLE 2 Genotype distribution for the association between tSNPs in APC and CTNNB1 genes and clinically localized PCa risk

tSNP ID	Genotype	Controls (*N*, %)	Patients (*N*, %)	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
APC							
rs3846716	GG	233 (62.8)	218 (71.0)	1.00		1.00	
	GA	130 (35.0)	77 (25.1)	**0.63 (0.45–0.89)**	**0.008**	**0.64 (0.45–0.90)**	**0.010**
	AA	8 (2.2)	12 (3.9)	1.60 (0.64–4.00)	0.311	1.85 (0.73–4.71)	0.199
	GA + AA	138 (37.2)	89 (29.0)	**0.69 (0.50–0.95)**	**0.024**	**0.70 (0.50–0.98)**	**0.036**
rs2707765	CC	151 (40.8)	146 (47.7)	1.00		1.00	
	CG	174 (47.0)	134 (43.8)	0.80 (0.58–1.10)	0.164	0.81 (0.58–1.12)	0.205
	GG	45 (12.2)	26 (8.5)	0.60 (0.35–1.02)	0.059	0.65 (0.38–1.12)	0.120
	CG + GG	219 (59.2)	160 (52.3)	0.76 (0.56–1.03)	0.072	0.78 (0.57–1.06)	0.114
rs2431238	CC	229 (76.6)	257 (86.0)	1.00		1.00	
	CT	66 (22.1)	40 (13.4)	**0.54 (0.35–0.83)**	**0.005**	**0.57 (0.36–0.91)**	**0.017**
	TT	4 (1.3)	2 (0.7)	0.45 (0.08–2.46)	0.353	0.63 (0.11–3.80)	0.614
	CT + TT	70 (23.4)	42 (14.0)	**0.54 (0.35–0.82)**	**0.004**	**0.57 (0.36–0.90)**	**0.016**
rs17134945	AA	323 (87.1)	259 (84.4)	1.00		1.00	
	AG	48 (12.9)	45 (14.7)	1.17 (0.75–1.81)	0.485	1.16 (0.37–1.81)	0.529
	GG	0	3 (1.0)	–	–	–	–
	AG + GG	48 (12.9)	48 (15.7)	1.25 (0.81–1.92)	0.317	1.24 (0.80–1.93)	0.345
rs2707761	AA	265 (73.4)	215 (72.9)	1.00		1.00	
	AG	83 (23.0)	74 (25.1)	1.10 (0.77–1.58)	0.609	1.17 (0.81–1.70)	0.407
	GG	13 (3.6)	6 (2.0)	0.57 (0.21–1.52)	0.261	0.67 (0.24–1.86)	0.439
	AG + GG	96 (26.6)	80 (27.1)	1.03 (0.73–1.45)	0.880	1.11 (0.77–1.59)	0.577
rs41115	AA	234 (63.1)	215 (70.3)	1.00		1.00	
	AG	127 (34.2)	80 (26.1)	**0.69 (0.49–0.96)**	**0.027**	**0.70 (0.49–0.98)**	**0.039**
	GG	10 (2.7)	11 (3.6)	1.20 (0.50–2.88)	0.687	1.33 (0.54–3.24)	0.536
	AG + GG	137 (36.9)	91 (29.7)	**0.72 (0.52–1.00)**	**0.049**	0.74 (0.53–1.03)	0.076
rs565453	CC	234 (63.4)	215 (70.3)	1.00		1.00	
	CA	125 (33.9)	79 (25.8)	**0.69 (0.49–0.96)**	**0.030**	**0.71 (0.50–1.00)**	**0.050**
	AA	10 (2.7)	12 (3.9)	1.31 (0.55–3.09)	0.543	1.44 (0.60–3.45)	0.418
	CA + AA	135 (36.6)	91 (29.7)	0.73 (0.53–1.01)	0.061	0.76 (0.55–1.06)	0.105
CTNNB1							
rs4016435	GG	333 (89.8)	269 (87.6)	1.00		1.00	
	GT	37 (10.0)	37 (12.1)	1.24 (0.76–2.01)	0.387	1.27 (0.77–2.10)	0.348
	TT	1 (0.3)	1 (0.3)	1.24 (0.08–19.9)	0.880	0.78 (0.05–12.6)	0.863
	GT + TT	38 (10.2)	38 (12.4)	1.24 (0.77–2.00)	0.381	1.25 (0.77–2.06)	0.370
rs1798802	GG	196 (54.1)	148 (48.7)	1.00		1.00	
	GA	133 (36.7)	129 (42.4)	1.28 (0.93–1.77)	0.129	1.32 (0.75–1.85)	0.100
	AA	33 (9.1)	27 (8.9)	1.08 (0.62–1.88)	0.776	1.11 (0.63–1.96)	0.714
	GA + AA	166 (45.9)	156 (51.3)	1.25 (0.92–1.69)	0.160	1.28 (0.93–1.76)	0.125
rs11564459	AA	345 (93.0)	287 (93.5)	1.00		1.00	
	AG	26 (7.0)	20 (6.5)	0.93 (0.51–1.69)	0.799	0.98 (0.53–1.82)	0.955
rs11564465	CC	232 (62.5)	184 (60.3)	1.00		1.00	
	CT	124 (33.4)	101 (33.1)	1.03 (0.74–1.42)	0.873	1.06 (0.76–1.48)	0.751
	TT	15 (4.0)	20 (6.6)	1.68 (0.84–3.38)	0.144	1.76 (0.86–3.61)	0.123
	CT + TT	139 (37.5)	121 (39.7)	1.10 (0.80–1.50)	0.558	1.13 (0.82–1.56)	0.453
rs11564475	AA	266 (71.7)	220 (72.8)	1.00		1.00	
	AG	98 (26.4)	77 (25.5)	0.95 (0.67–1.35)	0.773	0.93 (0.65–1.34)	0.705
	GG	7 (1.9)	5 (1.7)	0.86 (0.27–2.76)	0.805	0.88 (0.27–2.87)	0.834
	AG + GG	105 (28.3)	82 (27.2)	0.94 (0.67–1.33)	0.741	0.93 (0.66–1.32)	0.682

TABLE 2 continued

tSNP ID	Genotype	Controls (*N*, %)	Patients (*N*, %)	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
rs2293303	CC	277 (74.7)	222 (72.3)	1.00		1.00	
	CT	92 (24.8)	81 (26.4)	1.10 (0.78–1.55)	0.595	1.10 (0.77–1.57)	0.603
	TT	2 (0.5)	4 (1.3)	2.50 (0.45–13.8)	0.294	2.54 (0.44–14.5)	0.295
	CT + TT	94 (25.3)	85 (27.7)	1.13 (0.80–1.59)	0.490	1.13 (0.79–1.61)	0.497

Variable numbers of controls and patients reflect instances of failed genotyping

OR unadjusted odds ratio, *aOR* age-adjusted odds ratio, 95% *CI* 95% confidence interval

ORs with a $P \leq 0.05$ are in boldface

TABLE 3 Association between APC and CTNNB1 haplotypes and clinically localized PCa risk

tSNP haplotypes	Haplotype distribution			OR (95% CI)		*P* value	aOR (95% CI)		*P* value
	HapMap Han	Controls	Patients						
APC									
Block 2									
CCAAAC	0.633	0.640	0.688	1.00			1.00		
GCAGAC	0.126	0.155	0.130	0.80	(0.59–1.08)	0.141	0.80	(0.59–1.10)	0.170
GTAAGA	0.097	0.123	0.076	**0.57**	(**0.38–0.84**)	**0.004**	**0.58**	(**0.39–0.85**)	**0.006**
GCGAGA	0.108	0.062	0.082	1.24	(0.81–1.90)	0.313	1.23	(0.81–1.87)	0.332
GCAAGA	0.002	0.010	0.007	0.63	(0.18–2.27)	0.481	0.63	(0.17–2.37)	0.496
Rare	0.034	0.010	0.017	1.59	(0.59–4.29)	0.358	1.79	(0.59–5.42)	0.305
CTNNB1									
Block 1									
GG	0.722	0.726	0.698	1.00			1.00		
GA	0.234	0.221	0.238	1.11	(0.87–1.43)	0.402	1.11	(0.87–1.43)	0.403
TA	0.044	0.053	0.064	1.26	(0.79–2.01)	0.333	1.29	(0.80–2.08)	0.295
Block 2									
CAC	0.605	0.637	0.623	1.00			1.00		
CGC	0.145	0.151	0.144	0.98	(0.71–1.35)	0.902	0.98	(0.90–1.35)	0.879
TAT	0.134	0.125	0.144	1.19	(0.85–1.67)	0.304	1.17	(0.83–1.64)	0.368
TAC	0.115	0.083	0.087	1.07	(0.74–1.55)	0.713	1.09	(0.76–1.58)	0.638

OR unadjusted odds ratio, *aOR* age-adjusted odds ratio

ORs with a $P \leq 0.05$ are in boldface

Association of the APC rs3846716 with Recurrence of PSA After RP

During follow-up, 46.6% (41 of the 88) of *APC* rs3846716 GA + AA patients experienced PSA recurrence, which was higher than the incidence rate of 31.8% (69 of 217) in GG cases. Kaplan–Meier survival curves and log-rank test showed that the *APC* rs3846716 genotype was associated with PSA-free survival (Fig. 1). Median estimated cumulative survival was significantly lower in GA/AA carriers than in those homozygous for GG (30 vs. 64 months; $P = 0.037$), showing an earlier recurrence of PCa following RP.

The more aggressive disease in patients with *APC* rs3846716 GA/AA genotypes might be linked to a more unfavorable risk profile of tumor cells. Therefore, we evaluated whether the *APC* rs3846716 genotype is a predictor for PSA-free survival independently of other clinical or molecular risk factors. In a univariate analysis, the *APC* rs3846716 genotype, high preoperative PSA level, advanced pathologic stage, positive surgical margin, and Gleason score ≥ 7 significantly influenced post-RP PSA-free survival time (Table 5). The *APC* rs3846716 genotype did not reach significance when all other risk factors were included in the multivariate analysis ($P = 0.160$). However, *APC* rs3846716 GA/AA genotypes showed a weak

TABLE 4 Association between APC and CTNNB1 polymorphisms and clinicopathological features among PCa patients who received RP

tSNP ID	Genotype	Age β (Seβ)	*P* value	Preoperative PSA β (Seβ)	*P* value	Pathologic stage aOR (95% CI)	*P* value	Gleason score aOR (95% CI)	*P* value	Surgical margin aOR (95% CI)	*P* value	PSA recurrence aOR (95% CI)	*P* value
APC													
rs3846716	GG	0.00		0.00		1.00		1.00		1.00		1.00	
	GA	0.92 (0.87)	0.287	−0.60 (3.04)	0.844	1.69 (0.98–2.91)	0.059	1.50 (0.85–2.66)	0.164	**2.10 (1.15–3.83)**	**0.015**	**2.06 (1.21–3.50)**	**0.008**
	AA	−2.03 (1.94)	0.296	2.82 (6.99)	0.687	2.71 (0.80–9.20)	0.11	1.38 (0.40–4.75)	0.610	1.88 (0.51–6.94)	0.342	0.71 (0.19–2.71)	0.617
	GA + AA	0.53 (0.82)	0.524	−0.16 (2.89)	0.956	**1.80 (1.07–3.01)**	**0.026**	1.48 (0.87–2.54)	0.152	**2.07 (1.17–3.66)**	**0.012**	**1.80 (1.09–2.99)**	**0.023**
rs2707765	CC	0.00		0.00		1.00		1.00		1.00		1.00	
	CG	−0.62 (0.79)	0.433	−0.40 (2.76)	0.886	1.12 (0.68–1.85)	0.669	1.23 (0.75–2.01)	0.415	1.73 (0.99–3.02)	0.054	1.25 (0.76–2.05)	0.373
	GG	−0.18 (1.40)	0.899	3.84 (5.08)	0.451	1.63 (0.68–3.86)	0.271	1.88 (0.74–4.78)	0.182	1.84 (0.73–4.60)	0.195	1.25 (0.53–2.97)	0.609
	CG + GG	−0.55 (0.75)	0.468	0.24 (2.65)	0.928	1.19 (0.74–1.92)	0.482	1.32 (0.82–2.11)	0.257	**1.75 (1.03–2.98)**	**0.040**	1.25 (0.78–2.01)	0.352
rs2431238	CC	0.00		0.00		1.00		1.00		1.00		1.00	
	CT	0.40 (1.11)	0.356	−1.52 (3.91)	0.698	1.54 (0.78–3.03)	0.217	1.55 (0.74–3.26)	0.25	**2.15 (1.02–4.54)**	**0.044**	0.95 (0.47–1.91)	0.888
	TT	0.90 (4.64)	0.193	−6.51 (16.1)	0.687	2.08 (0.13–33.8)	0.605	–	–	–	–	–	–
	CT + TT	0.42 (1.09)	0.699	−1.76 (3.82)	0.645	1.56 (0.80–3.04)	0.192	1.31 (0.65–2.65)	0.456	1.94 (0.93–4.02)	0.076	0.88 (0.44–1.76)	0.724
rs17134945	AA	0.00		0.00		1.00		1.00		1.00		1.00	
	AG	1.03 (1.06)	0.329	1.00 (3.73)	0.788	1.23 (0.64–2.39)	0.536	1.58 (0.77–3.24)	0.211	1.42 (0.68–2.99)	0.354	1.66 (0.88–3.15)	0.121
	GG	−2.30 (3.80)	0.545	7.43 (13.1)	0.571	0.97 (0.09–10.9)	0.981	1.32 (0.12–14.8)	0.820	1.20 (0.11–13.5)	0.883	0.97 (0.09–10.8)	0.979
	AG + GG	0.83 (1.03)	0.422	1.43 (3.62)	0.694	1.21 (0.64–2.31)	0.555	1.56 (0.78–3.12)	0.208	1.40 (0.68–2.88)	0.356	1.61 (0.86–3.00)	0.136
rs2707761	AA	0.00		0.00		1.00		1.00		1.00		1.00	
	AG	−1.26 (0.89)	0.157	0.82 (3.13)	0.794	0.74 (0.41–1.33)	0.311	0.88 (0.51–1.53)	0.653	1.20 (0.65–2.19)	0.564	1.10 (0.64–1.91)	0.729
	GG	−1.84 (2.72)	0.498	−3.41 (9.38)	0.716	2.83 (0.46–17.3)	0.261	–	–	1.67 (0.27–10.3)	0.582	0.91 (0.16–5.09)	0.914
	AG + GG	−1.30 (0.86)	0.132	0.49 (3.03)	0.872	0.82 (0.47–1.43)	0.478	1.01 (0.59–1.72)	0.986	1.23 (0.68–2.21)	0.499	1.09 (0.64–1.86)	0.761
rs41115	AA	0.00		0.00		1.00		1.00		1.00		1.00	
	AG	0.77 (0.85)	0.365	0.01 (3.01)	0.999	1.50 (0.88–2.57)	0.139	1.57 (0.89–2.78)	0.119	**2.08 (1.15–3.76)**	**0.016**	**1.70 (1.01–2.88)**	**0.047**
	GG	−0.34 (2.01)	0.866	6.13 (7.31)	0.402	1.79 (0.53–6.08)	0.351	0.79 (0.23–2.69)	0.71	1.19 (0.30–4.76)	0.81	0.44 (0.09–2.10)	0.304
	AG + GG	0.64 (0.82)	0.433	0.71 (2.88)	0.807	1.54 (0.92–2.57)	0.102	1.43 (0.84–2.44)	0.19	**1.93 (1.10–3.40)**	**0.023**	1.49 (0.90–2.46)	0.124
rs565453	CC	0.00		0.00		1.00		1.00		1.00		1.00	
	CA	0.64 (0.86)	0.458	−0.75 (3.01)	0.803	1.45 (0.84–2.49)	0.178	1.45 (0.82–2.55)	0.199	**1.87 (1.03–3.41)**	**0.041**	1.66 (0.98–2.80)	0.061
	AA	−0.24 (1.94)	0.901	10.30 (6.96)	0.14	2.15 (0.67–6.92)	0.199	0.93 (0.28–3.02)	0.898	1.58 (0.44–5.63)	0.479	0.66 (0.17–2.53)	0.547
	CA + AA	0.52 (0.82)	0.523	0.64 (2.88)	0.825	1.53 (0.92–2.56)	0.103	1.36 (0.80–2.30)	0.260	**1.83 (1.03–3.22)**	**0.038**	1.48 (0.90–2.46)	0.125
CTNNB1													
rs4016435	GG	0.00		0.00		1.00		1.00		1.00		1.00	
	GT	−0.15 (1.16)	0.898	−2.29 (4.11)	0.578	0.93 (0.45–1.95)	0.856	0.88 (0.43–1.82)	0.732	1.29 (0.59–2.83)	0.528	1.01 (0.49–2.08)	0.983
	TT	6.24 (6.55)	0.342	–12.40 (22.6)	0.583	–	–	–	–	–	–	–	–
	GT + TT	0.02 (1.15)	0.984	–2.58 (4.05)	0.524	0.90 (0.43–1.87)	0.771	0.82 (0.40–1.67)	0.584	1.23 (0.56–2.68)	0.608	1.08 (0.53–2.21)	0.824

TABLE 4 continued

tSNP ID	Genotype	Age β (Seβ)	*P* value	Preoperative PSA β (Seβ)	*P* value	Pathologic stage aOR (95% CI)	*P* value	Gleason score aOR (95% CI)	*P* value	Surgical margin aOR (95% CI)	*P* value	PSA recurrence aOR (95% CI)	*P* value
rs1798802	GG	0.00		0.00		1.00		1.00		1.00		1.00	
	GA	–0.08 (0.80)	0.916	–1.01 (2.80)	0.720	0.93 (0.56–1.54)	0.778	1.65 (1.00–2.73)	0.051	1.24 (0.71–2.15)	0.446	0.96 (0.58–1.57)	0.859
	AA	1.08 (1.38)	0.435	–3.36 (4.75)	0.480	0.99 (0.41–2.38)	0.975	1.80 (0.74–4.38)	0.197	1.03 (0.40–2.70)	0.946	0.84 (0.35–2.01)	0.702
	GA + AA	0.12 (0.76)	0.876	–1.43 (2.65)	0.589	0.94 (0.58–1.52)	0.799	**1.68 (1.04–2.70)**	**0.034**	1.20 (0.71–2.03)	0.496	0.94 (0.59–1.50)	0.783
rs11564459	AA	0.00		0.00		1.00		1.00		1.00		1.00	
	AG	–1.35 (1.51)	0.372	**11.90 (5.58)**	**0.034**	0.61 (0.22–1.73)	0.353	0.91 (0.36–2.31)	0.846	0.47 (0.13–1.68)	0.245	0.96 (0.37–2.48)	0.926
rs11564465	CC	0.00		0.00		1.00		1.00		1.00		1.00	
	CT	0 (0.81)	0.996	0.83 (2.87)	0.773	1.17 (0.70–1.96)	0.540	1.65 (0.98–2.78)	0.062	1 (0.56–1.76)	0.986	0.94 (0.57–1.56)	0.818
	TT	–0.32 (1.54)	0.837	–3.85 (5.33)	0.471	0.91 (0.33–2.51)	0.853	2.20 (0.76–6.34)	0.144	0.79 (0.27–2.32)	0.672	0.92 (0.35–2.42)	0.863
	CT + TT	–0.05 (0.77)	0.949	0.02 (2.70)	0.994	1.13 (0.69–1.84)	0.629	**1.73 (1.05–2.84)**	**0.031**	0.96 (0.56–1.64)	0.869	0.94 (0.58–1.52)	0.795
rs11564475	AA	0.00		0.00		1.00		1.00		1.00		1.00	
	AG	0.09 (0.87)	0.916	5.51 (3.07)	0.074	0.99 (0.57–1.72)	0.972	0.80 (0.46–1.37)	0.416	1.05 (0.58–1.90)	0.875	1.01 (0.59–1.73)	0.984
	GG	–4.20 (2.96)	0.156	–1.93 (10.3)	0.851	0.47 (0.05–4.30)	0.503	0.39 (0.06–2.41)	0.312	–	–	1.19 (0.19–7.33)	0.85
	AG + GG	–0.17 (0.85)	0.839	5.03 (2.99)	0.094	0.95 (0.55–1.64)	0.857	0.76 (0.45–1.29)	0.314	0.95 (0.53–1.71)	0.86	1.02 (0.60–1.72)	0.953
rs2293303	CC	0.00		0.00		1.00		1.00		1.00		1.00	
	CT	–0.89 (0.85)	0.296	−1.21 (3.00)	0.687	1.07 (0.62–1.83)	0.819	1.68 (0.96–2.93)	0.07	0.82 (0.44–1.51)	0.519	0.98 (0.57–1.66)	0.929
	TT	2.79 (3.30)	0.398	−7.92 (11.4)	0.487	1.96 (0.27–14.2)	0.507	0.65 (0.09–4.69)	0.665	2.21 (0.30–16.1)	0.434	0.57 (0.06–5.55)	0.625
	CT + TT	–0.72 (0.84)	0.391	−1.54 (2.94)	0.600	1.10 (0.65–1.87)	0.725	1.59 (0.92–2.74)	0.095	0.87 (0.48–1.57)	0.648	0.95 (0.56–1.61)	0.857

Comparison of: pathologic stage between locally advanced versus localized PCa, Gleason score between 7–10 versus 2–6, surgical margin between positive versus negative, and of PSA recurrence between recurrence versus no recurrence

Dash indicates OR not calculated because of lack of this genotype in patients

β β estimates, *Se* standard error, *OR* unadjusted odds ratio, *aOR* age-adjusted odds ratio. Units: Age, years; Preoperative PSA, ng/ml

$P \leq 0.05$ are in boldface

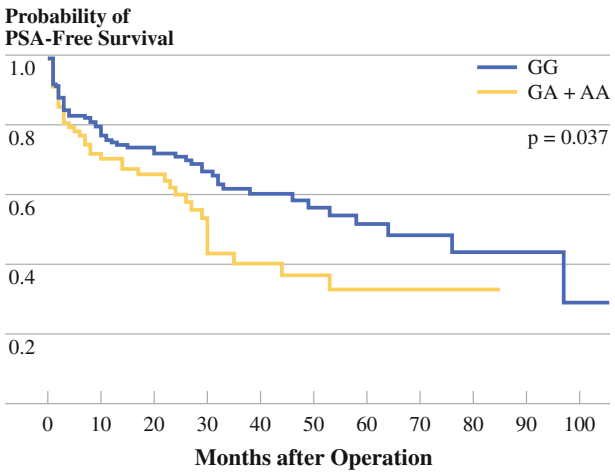

FIG. 1 Kaplan–Meier analysis revealed that APC rs3846716 GA and AA genotypes were associated with a significantly poorer PSA-free survival after RP than the GG genotype

association ($P = 0.063$) with poorer PSA-free survival without adjusting for pathologic stage and surgical margin status because of the strong correlations between *APC* rs3846716 and these two clinicopathological features (Tables 4, 5).

DISCUSSION

Aberrant activation of the Wnt signaling pathway has been found in many tumors, including those of the prostate, where CTNNB1 accumulates in cell nuclei and acts as a transcriptional co-regulator for both TCF/LEF transcription factors and androgen receptor.[27] APC is an important tumor suppressor that functions in the Wnt signaling pathway by regulating CTNNB1 degradation and nuclear export. These observations led to the hypothesis that genetic variations influencing the expression and/or function of APC and CTNNB1 might influence PCa risk and prognosis.

In this study, we successfully genotyped 13 tSNPs from *APC* and *CTNNB1* genes in 307 localized PCa cases and 371 unaffected controls. After adjusting for age, we found that four *APC* tSNPs (rs3846716, rs2431238, rs41115, and rs565453) and a common *APC* haplotype (GTAAGA) displayed significant associations with a decreased risk of localized PCa (Tables 2, 3).

Biochemical failure (PSA recurrence) is an important predictor of disease recurrence after RP, and many PSA recurrent patients are prone to develop metastatic lesions accompanied with significant mortality.[28,29] Therefore, finding new biochemical predictors of PSA recurrence would be beneficial for early detection, treatment selection, and possibly clarifying the mechanism of PSA recurrence. In our investigation on the relationship between 13 tSNPs in *APC*/*CTNNB1* genes and PSA recurrence, we found that PSA-free survival after RP was nominally poorer in *APC* rs3846716 GA + AA genotype carriers than in GG carriers in Kaplan–Meier survival analysis and in the univariate Cox proportional hazard model (Fig. 1; Table 5). However, the effect was attenuated after adjusting for other covariates, suggesting that the mechanisms of *APC* rs3846716 action might be dependent on pathologic stage and surgical margin status (Table 4).

SNPs in *APC* and *CTNNB1* have also been evaluated for associations with PCa risk in the Cancer Genetic Markers

TABLE 5 Cox proportional hazards analysis of factors associated with PSA recurrence after radical prostatectomy

Variables	Univariate analysis		Multivariate analysis			
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (years)	0.99 (0.97–1.02)	0.578	0.99 (0.95–1.02)	0.403	1.00 (0.97–1.03)	0.787
Preoperative PSA (ng/ml)	**1.02 (1.01–1.03)**	**<0.001**	**1.02 (1.01–1.03)**	**<0.001**	**1.03 (1.02–1.04)**	**<0.001**
Pathologic stage						
Localized	1.00		1.00			
Locally advanced	**3.38 (2.29–4.99)**	**<0.001**	**1.94 (1.17–3.22)**	**0.010**		
Surgical margin						
Negative	1.00		1.00			
Positive	**2.89 (1.91–4.36)**	**<0.001**	1.58 (0.96–2.60)	0.070		
Gleason score						
2–6	1.00		1.00		1.00	
7–10	**2.12 (1.38–3.24)**	**0.001**	1.49 (0.88–2.54)	0.140	**1.67 (1.06–2.63)**	**0.027**
rs3846716						
GG	1.00		1.00		1.00	
GA + AA	**1.50 (1.02–2.21)**	**0.041**	1.39 (0.88–2.21)	0.160	1.48 (0.98–2.25)	0.063

Hazard ratios with significance at $P \leq 0.05$ are in boldface

of Susceptibility (CGEMS) genome-wide association study.[30] Our findings that *APC* rs3846716 heterozygous variant carriers showed lower relative risks of localized PCa, but increased risks for PSA recurrence after RP (Table 4), are compatible with the results of the CGEMS study ($P = 0.007$, CGEMS nonaggressive tumors versus controls heterozygote risk OR, 0.84; aggressive tumors versus controls heterozygote risk OR, 1.31). However, further investigation is required to determine the detailed mechanisms.

PCa tends to occur more frequently, to be more aggressive, and to have worse outcome in African Americans and Caucasians than in Chinese.[31–33] Differences in *APC* rs3846716 genotype distributions among different ethnic groups can be observed in the HapMap database (G/G 22.6%, G/A 47.2%, and A/A 30.2% for African ancestry in Southwest USA; G/G 25.7%, G/A 56.6%, and A/A 17.7% for Utah residents with Northern and Western European ancestry; G/G 60.7%, G/A 33.3%, and A/A 6.0% for Chinese in Metropolitan Denver, Colorado).[21] These data are in accordance with our finding that indicates a poorer PSA-free survival after RP in *APC* rs3846716 GA + AA carriers (Fig. 1). Thus, genetic polymorphisms in *APC* rs3846716 might partly explain variations in PCa progression among ethnic groups.

Since the *APC* rs3846716 polymorphism is located in the 5′ upstream region of the *APC* gene, the risk allele, A, creates a putative transcription factor binding site for sex-determining region Y (SRY) product, testis-determining factor, according to the prediction of SNP Function Portal.[34] In mammals, male sex determination results from a cascade of events that are controlled by a master regulator SRY.[35] SRY binds to DNA minor groove via AT-rich sequences and induces a sharp bend in DNA.[36] However, the lack of a potential transcriptional activation domain in human SRY suggests that it may function through interaction with additional transcriptional factors.[37] It has been recently demonstrated that SRY is expressed in prostate and negatively regulates androgen receptor transcriptional activity through direct protein–protein interaction.[38] The loss of SRY and other Y-chromosome-specific genes has been more frequently found in higher stages and grades of PCa.[39] These findings suggest possible roles for SRY in regulation of APC expression and development of disease progression, although further functional study is required to investigate this.

In our study, the most significant tSNPs and haplotypes were in the gene encoding APC, which is involved in the regulation of CTNNB1 levels. Except for some clinicopathological features, we have found no evidence that common variants in *CTNNB1* are associated with localized PCa risk and PSA recurrence, probably because of the relatively small sample size of our study. When we analyzed the tSNPs in relation to the risk of clinical localized PCa and the various clinicopathological features, some associations were only significant for heterozygote carriers, but not homozygote carriers of the minor allele. This might also reflect low power to detect associations due to the limited number of minor allele homozygote carriers. Furthermore, the panel of tSNPs used in this study does not capture all of the common variation in the genes of interest. It is possible that some of the known SNPs were poorly tagged or that unknown common variations in these genes are associated with the disease. Therefore, we cannot rule out any weak associations since this study was only powered to observe moderate effect sizes.

Our study has several strengths. First, the genes selected for the study encoded protein components that form a well-characterized complex that has been directly implicated in many cancers. Second, genotyping of tSNPs (MAF > 0.05) ensured detailed and comprehensive coverage of susceptibility alleles across the entire gene region. Moreover, adequate follow-up clinical information allows for stratification of data by clinical features and recurrence of the disease.

Although the nonsynonymous SNP *APC* I1307K has been shown unlikely to play a significant role in susceptibility to PCa in Jewish Caucasians, to our knowledge, no reports have systematically evaluated the prognostic value of common variants in the Wnt pathway in patients with PCa.[12] In conclusion, we selected tSNPs of *APC* and *CTNNB1* to comprehensively evaluate their possible clinical implications, and provided the first evidence for association of variants in *APC* gene with localized PCa risk and disease progression. However, we were limited by sample size in analyses of outcomes and in subset analyses. In addition, the effects of these genetic variants on PSA nadir and doubling time during disease progression deserve further investigations. Furthermore, our homogeneous Chinese Han population may make our findings less generalizable to other ethnic groups. Thus, subsequent functional analysis and large independent studies are required to establish the relevance of the observed associations to PCa progression and guide future investigations.

ACKNOWLEDGMENT We would like to thank National Genotyping Center of National Research Program for Genomic Medicine, National Science Council (NSC), Taiwan, for their technical support. This work was supported by NSC Grant NSC-96-2321-B-039-005-MY2, NSC-96-2314-B-037-012-MY3, and China Medical University grant CMU97-183.

REFERENCES

1. Han M, Partin AW, Pound CR, Epstein JI, Walsh PC. Long-term biochemical disease-free and cancer-specific survival following anatomic radical retropubic prostatectomy. The 15-year Johns Hopkins experience. *Urol Clin North Am*. 2001;28:555–65.

2. Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. *J Urol*. 2004;172:910–4.
3. Humphrey PA. Gleason grading and prognostic factors in carcinoma of the prostate. *Mod Pathol*. 2004;17:292–306.
4. Bierie B, Nozawa M, Renou JP, Shillingford JM, Morgan F, Oka T, et al. Activation of beta-catenin in prostate epithelium induces hyperplasias and squamous transdifferentiation. *Oncogene*. 2003; 22:3875–87.
5. Chesire DR, Ewing CM, Gage WR, Isaacs WB. In vitro evidence for complex modes of nuclear beta-catenin signaling during prostate growth and tumorigenesis. *Oncogene*. 2002;21:2679–94.
6. Gounari F, Signoretti S, Bronson R, Klein L, Sellers WR, Kum J, et al. Stabilization of beta-catenin induces lesions reminiscent of prostatic intraepithelial neoplasia, but terminal squamous transdifferentiation of other secretory epithelia. *Oncogene*. 2002;21: 4099–107.
7. Beroud C, Soussi T. APC gene: database of germline and somatic mutations in human tumors and cell lines. *Nucleic Acids Res*. 1996;24:121–4.
8. Barker N, Clevers H. Mining the Wnt pathway for cancer therapeutics. *Nat Rev Drug Discov*. 2006;5:997–1014.
9. Chesire DR, Ewing CM, Sauvageot J, Bova GS, Isaacs WB. Detection and analysis of beta-catenin mutations in prostate cancer. *Prostate*. 2000;45:323–34.
10. Gerstein AV, Almeida TA, Zhao G, Chess E, Shih Ie M, Buhler K, et al. APC/CTNNB1 (beta-catenin) pathway alterations in human prostate cancers. *Genes Chromosomes Cancer*. 2002;34:9-16.
11. Voeller HJ, Truica CI, Gelmann EP. Beta-catenin mutations in human prostate cancer. *Cancer Res*. 1998;58:2520–3.
12. Poynter JN, Cooney KA, Bonner JD, White KA, Tomsho LP, Rennert G, et al. APC I1307K and the risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2006;15:468–73.
13. Huang SP, Chou YH, Chang WS, Wu MT, Yu CC, Wu T, et al. Androgen receptor gene polymorphism and prostate cancer in Taiwan. *J Formos Med Assoc*. 2003;102:680–6.
14. Huang SP, Chou YH, Wayne Chang WS, Wu MT, Chen YY, Yu CC, et al. Association between vitamin D receptor polymorphisms and prostate cancer risk in a Taiwanese population. *Cancer Lett*. 2004;207:69–77.
15. Huang SP, Huang CY, Wang JS, Liu CC, Pu YS, Yu HJ, et al. Prognostic significance of p53 and X-ray repair cross-complementing group 1 polymorphisms on prostate-specific antigen recurrence in prostate cancer post radical prostatectomy. *Clin Cancer Res*. 2007; 13:6632–8.
16. Huang SP, Huang CY, Wu WJ, Pu YS, Chen J, Chen YY, et al. Association of vitamin D receptor FokI polymorphism with prostate cancer risk, clinicopathological features and recurrence of prostate specific antigen after radical prostatectomy. *Int J Cancer*. 2006;119:1902–7.
17. Huang SP, Wu WJ, Chang WS, Wu MT, Chen YY, Chen YJ, et al. p53 Codon 72 and p21 codon 31 polymorphisms in prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2004;13:2217–24.
18. Barry MJ, Fowler FJ, Jr, O'Leary MP, Bruskewitz RC, Holtgrewe HL, Mebust WK, et al. The American Urological Association symptom index for benign prostatic hyperplasia. The Measurement Committee of the American Urological Association. *J Urol*. 1992; 148:1549–57 (discussion 1564).
19. Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol*. 1974;111:58–64.
20. Freedland SJ, Sutter ME, Dorey F, Aronson WJ. Defining the ideal cutpoint for determining PSA recurrence after radical prostatectomy. Prostate-specific antigen. *Urology*. 2003;61:365–9.
21. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. *Nature*. 2007;449:851–61.
22. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21: 263–5.
23. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. *Science*. 2002;296:2225–9.
24. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet*. 2005;37:1217–23.
25. Li SS, Khalid N, Carlson C, Zhao LP. Estimating haplotype frequencies and standard errors for multiple single nucleotide polymorphisms. *Biostatistics*. 2003;4:513–22.
26. Zhao LP, Li SS, Khalid N. A method for the assessment of disease associations with single-nucleotide polymorphism haplotypes and environmental variables in case-control studies. *Am J Hum Genet*. 2003;72:1231–50.
27. Truica CI, Byers S, Gelmann EP. Beta-catenin affects androgen receptor transcriptional activity and ligand specificity. *Cancer Res*. 2000;60:4709–13.
28. Freedland SJ, Humphreys EB, Mangold LA, Eisenberger M, Dorey FJ, Walsh PC, et al. Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy. *JAMA*. 2005;294:433–9.
29. Freedland SJ, Humphreys EB, Mangold LA, Eisenberger M, Partin AW. Time to prostate specific antigen recurrence after radical prostatectomy and risk of prostate cancer specific mortality. *J Urol*. 2006;176:1404–8.
30. Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet*. 2007;39:645–9.
31. Bostwick DG, Burke HB, Djakiew D, Euling S, Ho SM, Landolph J, et al. Human prostate cancer risk factors. *Cancer*. 2004;101: 2371–490.
32. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin*. 2007;57:43–66.
33. Pu YS. Prostate cancer in Taiwan: epidemiology and risk factors. *Int J Androl*. 2000;23 Suppl 2:34–6.
34. Wang P, Dai M, Xuan W, McEachin RC, Jackson AU, Scott LJ, et al. SNP Function Portal: a web database for exploring the function implication of SNP alleles. *Bioinformatics*. 2006;22: e523–9.
35. Berta P, Hawkins JR, Sinclair AH, Taylor A, Griffiths BL, Goodfellow PN, et al. Genetic evidence equating SRY and the testis-determining factor. *Nature*. 1990;348:448–50.
36. Werner MH, Huth JR, Gronenborn AM, Clore GM. Molecular basis of human 46X,Y sex reversal revealed from the three-dimensional solution structure of the human SRY-DNA complex. *Cell*. 1995;81:705–14.
37. Dubin RA, Ostrer H. Sry is a transcriptional activator. *Mol Endocrinol*. 1994;8:1182–92.
38. Yuan X, Lu ML, Li T, Balk SP. SRY interacts with and negatively regulates androgen receptor transcriptional activity. *J Biol Chem*. 2001;276:46647–54.
39. Perinchery G, Sasaki M, Angan A, Kumar V, Carroll P, Dahiya R. Deletion of Y-chromosome specific genes in human prostate cancer. *J Urol*. 2000;163:1339–42.