Significant Association of Caveolin-1 (*CAV1*) Genotypes with Prostate Cancer Susceptibility in Taiwan

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Abstract. *Background: Multiple lines of evidence have implicated the Caveolin-1 (CAV1) gene in prostate cancer progression. CAV1 is located within the locus at 7q31-33 associated with prostate cancer aggressiveness, and was identified as being overexpressed in prostate tumors. Therefore, this study evaluated the relationship between the polymorphism of CAV1 and the risk of prostate cancer in Taiwan. Patients and Methods: Two hundred and fifty patients with prostate cancer and five hundred age-matched healthy controls recruited were genotyped. Results: There were significant differences between prostate cancer and control groups in the distributions of their genotypes ($p=0.0004$) and allelic frequencies ($p=4.9\times10^{-5}$) in the CAV1 T29107A (rs7804372) polymorphisms. Conclusion: This study provides evidence for the relationship of this variant of CAV1 and risk of prostate cancer which might merit further study as a genomic marker for early detection of prostate cancer.*

Prostate cancer has become the most frequently diagnosed malignancy among men and one of the most common causes of cancer death in men in recent years. The etiology of prostate cancer is largely unknown, with both genetic and environmental factors likely to be involved (1). Nevertheless, confirmed risk factors for prostate cancer include age, ethnicity, country of origin, and family history.

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In tumors, several genetic alterations have been associated with allelotyping and chromosome deletion. Loss of heterozygosity studies of specific chromosomal regions were performed to identify genomic sites harboring tumor suppressor genes (2-8). The region of 7q31 appears to play a critical role for the clinical aggressiveness and progression of prostate tumors (9), and several potential tumor suppressor genes have been suggested (10).

Caveolin-1 (*CAV1*) is located within this critical region and has been implicated in prostate cancer progression. *CAV1* consists of three exons and is located at 7q31.1 telomeric of the microsatellite marker D7S522. Down-regulation or loss of *CAV1* expression has been reported in many types of human cancer and cancer cell lines (11-17). CAV1 is the major structural and functional protein component of caveolae and the marker protein for this organelle (18). It plays an important role in many signaling pathways, molecular transport, and cellular proliferation and differentiation. The specific functions of the CAV1 protein/caveolae are highly cell- and context-specific (19). Biochemical and molecular analyses of prostate cancer tissues and cell lines identified CAV1 as being mostly up-regulated in metastatic prostate cancer (20, 21). It has also been shown that CAV1 expression is increased in metastatic human prostate cancer and that CAV1 cellular protein expression is predictive of recurrence of the disease after radical prostatectomy (22).

The emerging evidence pointing to the role of CAV1 in carcinogenesis led us to investigate whether different alleles of this gene are associated with prostate cancer. Thus, the aims of the current study were to determine the genotypic frequency of six polymorphisms of the *CAV1* gene at C239A (rs1997623), G14713A (rs3807987), G21985A (12672038), T28608A (rs3757733), T29107A (rs7804372), and G32124A (rs3807992), and find their associations with prostate cancer susceptibility. To the best of our knowledge, this is the most valuable study carried out to evaluate the contribution of *CAV1* polymorphisms in prostate oncology in Taiwan.

Patients and Methods

Study population and sample collection. The study population consisted of 250 prostate cancer patients and 500 cancer-free control volunteers. The patients were recruited at the outpatient clinics of general surgery between 2004 and 2010 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of the patients, including their histological details, were all graded and defined by expert surgeons (Dr. Wu and Chang's team). All the patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. The controls were selected from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastases from known or unknown primary cancer and any familial or genetic diseases, and those whose genotypes could not be identified in our system. The study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all the participants.

Genotyping conditions. Genomic DNA was prepared from peripheral blood leucocytes using a QIAmp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to our previous methods (23-30). In brief, the following primers were used: for *CAV1* C239A (rs1997623), 5'-GTGTCCGCTTCTGCTATCTG-3' and 5'-GCCAAGATGCAGAAGGAGTT-3'; for *CAV1* G14713A (rs3807987), 5'-CCTTCCAGTAAGCAAGCTGT-3' and 5'-CCTC TCAATCTTGCCATAGT-3'; for *CAV1* G21985A (12672038), 5'-GGTGTCAGCAAGGCTATGCT-3' and 5'-CCAGACACTCAGAA TGTGAC-3'; for *CAV1* T28608A (rs3757733), 5'-GCTCAA CCTCATCTGAGGCA-3' and 5'-GGCCTATTGT TGAGTGGATG-3'; for *CAV1* T29107A (rs7804372), 5'-GCCTGAATTG CAATCCTGTG-3' and 5'-ACGGTGTGAAC ACGGACATT-3'; and for *CAV1* G32124A (rs3807992), 5'-GGTGTCTTGCAGTTGAATG-3' and 5'-ACGGAG CTACTCAGTGCCAA-3'. The following cycling conditions were performed: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s; and a final extension at 72˚C for 10 min. The PCR products were studied after digestion with *Avr II, Bfa I, Hae III, Tsp509 I, Sau3AI* and *Nla III*, restriction enzymes for *CAV1* C239A (cut from 485 bp C type into 170+315 bp T type), *CAV1* G14713A (cut from 268 bp A type into 66+202 bp G type), *CAV1* G21985A (cut from 251+43 bp A type into 153+98+43 bp G type), *CAV1* T28608A (cut from 298 bp T type into 100+198 bp A type), *CAV1* T29107A (cut from 336 bp A type into 172+164 bp T type), and *CAV1* G32124A (cut from 213+142+67 bp A type into 142+118+95+67 bp T type), respectively.

Statistical analyses. Only those with all the SNPs data (case/control=250/500) were selected for the final analysis. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *CAV1* single nucleotide polymorphisms (SNP) in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *CAV1* genotypes between cases and controls. The data was recognized as significant when the statistical *p*-value was less than 0.05. All statistical tests were performed using SAS, Version 9.1.3 (SAS Institute Inc., Cary, NC, USA) on two-sided probabilities.

Results

The frequencies of the genotypes for the *CAV1* C239A, G14713A, G21985A, T28608A, T29107A and G32124A between controls and prostate cancer patients are presented in Table I. Genotype distributions of various genetic polymorphisms of *CAV1* T29107A were significantly different between prostate cancer and control groups (p=0.0004), while those for *CAV1* C239A, G14713A, G21985A, T28608A and G32124A were not statistically significant (p>0.05) (Table II).

The frequencies of the alleles for the *CAV1* C239A, G14713A, G21985A, T28608A, T29107A and G32124A between controls and prostate cancer patients are shown in Table II. The *CAV1* T29107A polymorphism was found to be significantly differently distributed between patients with prostate cancer and controls (Table I). It was also found to be associated with prostate cancer susceptibility in the allele frequency analysis model ($p=4.9\times10^{-5}$), confirming the previous finding. In particular, the frequency of the T allele was higher in prostate cancer patients. As for the other five SNPs, the distributions of their allele frequencies were not significantly different in controls and prostate cancer patients (Table II).

Discussion

Expression of the caveolin gene family, particularly CAV1, has been assessed in relationship to several human types of cancer, but few data consider *CAV1* for genetic predisposition to these cancer types (31, 32). Williams and Lisanti interbred *CAV1*$^{-/-}$ null mice with TRAMP mice, which spontaneously develop advanced prostate cancer (33). They found that the loss of *CAV1* reduced progression to metastasis. In order to determine the role of CAV1 and to find potential biomarkers of prostate cancer, six SNPs of the *CAV1* gene were selected from the NCBI website and their associations with the susceptibility for prostate cancer were investigated in a population in central Taiwan. In the present study, we found that the *CAV1* T29107A (rs7804372) SNP was closely associated with the susceptibility to prostate cancer (Table I and II), while the other five polymorphisms were not.

The *CAV1* gene is on the long arm of chromosome 7, in a region associated with tumor suppression and with loss of heterozygosity in several types of cancer (34). In a recent study, among the eleven SNPs of the *CAV1* gene evaluated (35), only one in *CAV1* (rs9920, chr7:115987328; OR_{CT+CC}=1.37, 95% CI=1.12, 1.68) was found to be associated with prostate cancer risk among Caucasians (from a sample of 1458 cases and 1351 controls). Compared with their results, we found the SNP in *CAV1* (rs7804372, chr7:116194228; p=0.0004) was associated with prostate cancer risk in Taiwanese. There is a long

Table I. *Distribution of CAV1 genotypes among prostate cancer patients and controls.*

Genotype	Controls		Patients		*P*-Value
	n	%	n	%	
C239A rs1997623					0.3477
CC	484	96.8%	245	98.0%	
AC	16	3.2%	5	2.0%	
AA	0	0.0%	0	0.0%	
G14713A rs3807987					0.2702
GG	330	66.0%	151	60.4%	
AG	129	25.8%	72	28.8%	
AA	41	8.2%	27	10.8%	
G21985A rs12672038					0.8445
GG	301	60.2%	148	59.2%	
AG	161	32.2%	85	34.0%	
AA	38	7.6%	17	6.8%	
T28608A rs3757733					0.5965
TT	280	56.0%	147	58.8%	
AT	165	33.0%	81	32.4%	
AA	55	11.0%	22	8.8%	
T29107A rs7804372					**0.0004**
TT	254	50.8%	163	65.2%	
AT	196	39.2%	75	30.0%	
AA	50	10.0%	12	4.8%	
G32124A rs3807992					0.5633
GG	251	50.2%	133	53.2%	
AG	198	39.6%	89	35.6%	
AA	51	10.2%	28	11.2%	

[a]Based on Chi-square test.

Table II. *Distribution of CAV1 alleles among prostate cancer patients and controls.*

Allele	Controls		Patients		*P*-value[a]
	n	%	n	%	
C239A rs1997623					0.3511
Allele C	984	98.4%	495	99.0%	
Allele A	16	1.6%	5	1.0%	
G14713A rs3807987					0.0729
Allele G	789	78.9%	374	74.8%	
Allele A	211	21.1%	126	25.2%	
G21985A rs12672038					0.9658
Allele G	763	76.3%	381	76.2%	
Allele A	237	23.7%	119	23.8%	
T28608A rs3757733					0.3020
Allele T	725	72.5%	375	75.0%	
Allele A	275	27.5%	125	25.0%	
T29107A rs7804372					**4.9×10^{-5}**
Allele T	704	70.4%	401	80.2%	
Allele A	296	29.6%	99	19.8%	
G32124A rs3807992					0.6894
Allele G	700	70.0%	355	71.0%	
Allele A	300	30.0%	145	29.0%	

[a]Based on Chi-square test.

genetic distance between these two SNP sites. This may indicate that the rs7804372 in *CAV1* is associated with prostate cancer risk specifically of Taiwanese. However, Langeberg *et al*. also found that two SNPs (rs3807986 and rs3907989) of *CAV1* were not associated with prostate cancer risk (35). This result was similarly to ours for the two SNPs rs3807987 and rs3807992 which lie very close to these two. As for this region in *CAV1*, the results are coincident between Caucasians and Taiwanese. To sum up these findings, we suggest that *CAV1* variant distribution might depend on the ethnic type, and the genetic etiology of prostate cancer may be different among ethnicities.

The overexpression of *CAV1* was reported in various malignancies, including prostate cancer (20, 36). The molecular basis for the initiation of *CAV1* expression in prostate cancer is not clear. The *CAV1* gene promoter has multiple CpG sites, and alterations in gene methylation have been shown in prostate cancer (37). However, patterns of *CAV1* gene methylation have not, thus far, provided a convincing argument for the up-regulation of *CAV1* in prostate cancer. In general, CAV1 has been associated with the stimulatory effects of steroid receptors, including the androgen receptor, suggesting a point of convergence for further mechanistic studies (38).

In conclusion, this is the first study focused in the case–control comparison of *CAV1* SNPs and prostate cancer in Taiwan, and individuals carrying the T allele of T29107A appear to be at a higher risk of prostate cancer. In the future, the T allele of T29107A might be a useful marker in prostate oncology.

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References

1 Brawley OW, Giovannucci E and Kramer BS: Epidemiology of prostate cancer. *In*: Comprehensive Textbook of Genitourinary Oncology. Vogelzang SP, Shipley WU and Coffey DS (eds.). Philadelphia: Lippincott Williams and Wilkins, pp. 533-544, 2000.

2 Alers JC, Krijtenburg PJ, Rosenberg C, Hop WC, Verkerk AM, Schroder FH, van der Kwast TH, Bosman FT and van Dekken H: Interphase cytogenetics of prostatic tumor progression: specific chromosomal abnormalities are involved in metastasis to the bone. Lab Invest *77*: 437-448, 1997.

3 Alers JC, Rochat J, Krijtenburg PJ, Hop WC, Kranse R, Rosenberg C, Tanke HJ, Schroder FH and van Dekken H: Identification of genetic markers for prostatic cancer progression. Lab Invest *80*: 931-942, 2000.

4 Cui J, Deubler DA, Rohr LR, Zhu XL, Maxwell TM, Changus JE and Brothman AR: Chromosome 7 abnormalities in prostate cancer detected by dual-color fluorescence *in situ* hybridization. Cancer Genet Cytogenet *107*: 51-60, 1998.

5 Latil A, Cussenot O, Fournier G, Baron JC and Lidereau R: Loss of heterozygosity at 7q31 is a frequent and early event in prostate cancer. Clin Cancer Res *1*: 1385-1389, 1995.

6 Nupponen NN, Kakkola L, Koivisto P and Visakorpi T: Genetic alterations in hormone-refractory recurrent prostate carcinomas. Am J Pathol *153*: 141-148, 1998.

7 Tobias ES, Hurlstone AF, MacKenzie E, McFarlane R and Black DM: The TES gene at 7q31.1 is methylated in tumours and encodes a novel growth-suppressing LIM domain protein. Oncogene *20*: 2844-2853, 2001.

8 Zenklusen JC, Conti CJ and Green ED: Mutational and functional analyses reveal that ST7 is a highly conserved tumor-suppressor gene on human chromosome 7q31. Nat Genet *27*: 392-398, 2001.

9 Takahashi S, Shan AL, Ritland SR, Delacey KA, Bostwick DG, Lieber MM, Thibodeau SN and Jenkins RB: Frequent loss of heterozygosity at 7q31.1 in primary prostate cancer is associated with tumor aggressiveness and progression. Cancer Res *55*: 4114-4119, 1995.

10 Chene L, Giroud C, Desgrandchamps F, Boccon-Gibod L, Cussenot O, Berthon P and Latil A: Extensive analysis of the 7q31 region in human prostate tumors supports *TES* as the best candidate tumor suppressor gene. Int J Cancer *111*: 798-804, 2004.

11 Hatanaka M, Maeda T, Ikemoto T, Mori H, Seya T and Shimizu A: Expression of caveolin-1 in human T-cell leukemia cell lines. Biochem Biophys Res Commun *253*: 382-387, 1998.

12 Lee SW, Reimer CL, Oh P, Campbell DB and Schnitzer JE: Tumor cell growth inhibition by caveolin re-expression in human breast cancer cells. Oncogene *16*: 1391-1397, 1998.

13 Wiechen K, Sers C, Agoulnik A, Arlt K, Dietel M, Schlag PM and Schneider U: Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. Am J Pathol *158*: 833-839, 2001.

14 Bender FC, Reymond MA, Bron C and Quest AF: Caveolin-1 levels are down-regulated in human colon tumors, and ectopic expression of caveolin-1 in colon carcinoma cell lines reduces cell tumorigenicity. Cancer Res *60*: 5870-5878, 2000.

15 Bagnoli M, Tomassetti A, Figini M, Flati S, Dolo V, Canevari S and Miotti S: Downmodulation of caveolin-1 expression in human ovarian carcinoma is directly related to alpha-folate receptor overexpression. Oncogene *19*: 4754-4763, 2000.

16 Davidson B, Nesland JM, Goldberg I, Kopolovic J, Gotlieb WH, Bryne M, Ben-Baruch G, Berner A and Reich R: Caveolin-1 expression in advanced-stage ovarian carcinoma – a clinicopathologic study. Gynecol Oncol *81*: 166-171, 2001.

17 Racine C, Belanger M, Hirabayashi H, Boucher M, Chakir J and Couet J: Reduction of *caveolin 1* gene expression in lung carcinoma cell lines. Biochem Biophys Res Commun *255*: 580-586, 1999.

18 Glenney JR Jr. and Soppet D: Sequence and expression of caveolin, a protein component of caveolae plasma membrane domains phosphorylated on tyrosine in Rous sarcoma virus-transformed fibroblasts. Proc Natl Acad Sci USA *89*: 10517-10521, 1992.

19 Li WP, Liu P, Pilcher BK and Anderson RG: Cell-specific targeting of caveolin-1 to caveolae, secretory vesicles, cytoplasm or mitochondria. J Cell Sci *114*: 1397-1408, 2001.

20 Nasu Y, Timme TL, Yang G, Bangma CH, Li L, Ren C, Park SH, DeLeon M, Wang J and Thompson TC: Suppression of caveolin expression induces androgen sensitivity in metastatic androgen-insensitive mouse prostate cancer cells. Nat Med *4*: 1062-1064, 1998.

21 Yang G, Truong LD, Timme TL, Ren C, Wheeler TM, Park SH, Nasu Y, Bangma CH, Kattan MW, Scardino PT and Thompson TC: Elevated expression of caveolin is associated with prostate and breast cancer. Clin Cancer Res *4*: 1873-1880, 1998.

22 Yang G, Truong LD, Wheeler TM and Thompson TC: Caveolin-1 expression in clinically confined human prostate cancer: a novel prognostic marker. Cancer Res *59*: 5719-5723, 1999.

23 Bau DT, Mau YC, Ding SL, Wu PE and Shen CY: DNA double-strand break repair capacity and risk of breast cancer. Carcinogenesis *28*: 1726-1730, 2007.

24 Bau DT, Tseng HC, Wang CH, Chiu CF, Hua CH, Wu CN, Liang SY, Wang CL, Tsai CW and Tsai MH: Oral cancer and genetic polymorphism of DNA double-strand break gene *Ku70* in Taiwan. Oral Oncol *44*: 1047-1051, 2008.

25 Bau DT, Wu HC, Chiu CF, Lin CC, Hsu CM, Wang CL, Wang RF and Tsai FJ: Association of *XPD* polymorphisms with prostate cancer in Taiwanese patients. Anticancer Res *27*: 2893-2896, 2007.

26 Chang CH, Chang CL, Tsai CW, Wu HC, Chiu CF, Wang RF, Liu CS, Lin CC and Bau DT: Significant association of an *XRCC4* single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 1777-1782, 2009.

27 Chang CH, Chiu CF, Wang HC, Wu HC, Tsai RY, Tsai CW, Wang RF, Wang CH, Tsou YA and Bau DT: Significant association of *ERCC6* single nucleotide polymorphisms with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 5121-5124, 2009.

28 Chang CH, Wang RF, Tsai RY, Wu HC, Wang CH, Tsai CW, Chang CL, Tsou YA, Liu CS and Bau DT: Significant association of *XPD* codon 312 single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 3903-3907, 2009.

29 Liu CJ, Hsia TC, Wang RF, Tsai CW, Chu CC, Hang LW, Wang CH, Lee HZ, Tsai RY and Bau DT: Interaction of *cyclooxygenase 2* genotype and smoking habit in Taiwanese lung cancer patients. Anticancer Res *30*: 1195-1199, 2010.

30 Wang HC, Liu CS, Chiu CF, Chiang SY, Wang CH, Wang RF, Lin CC, Tsai RY and Bau DT: Significant association of DNA repair gene *Ku80* genotypes with breast cancer susceptibility in Taiwan. Anticancer Res *29*: 5251-5254, 2009.

31 Conde MC, Ramirez-Lorca R, Lopez-Jamar JM, Molero E, Ramirez-Armengol JA, Moreno Nogueira JA, Pascual MH, Ruiz A, Martin-Cordova CG, Real LM and Royo JL: Genetic analysis of *caveolin-1* and *eNOS* genes in colorectal cancer. Oncol Rep *16*: 353-359, 2006.

32 Han SE, Park KH, Lee G, Huh YJ and Min BM: Mutation and aberrant expression of caveolin-1 in human oral squamous cell carcinomas and oral cancer cell lines. Int J Oncol *24*: 435-440, 2004.
33 Williams TM and Lisanti MP: Caveolin-1 in oncogenic transformation, cancer, and metastasis. Am J Physiol Cell Physiol *288*: C494-506, 2005.
34 Cohen AW, Hnasko R, Schubert W and Lisanti MP: Role of caveolae and caveolins in health and disease. Physiol Rev *84*: 1341-1379, 2004.
35 Langeberg WJ, Tahir SA, Feng Z, Kwon EM, Ostrander EA, Thompson TC and Stanford JL: Association of *caveolin-1* and -*2* genetic variants and post-treatment serum caveolin-1 with prostate cancer risk and outcomes. Prostate *70*: 1020-1035, 2010.
36 Tahir SA, Yang G, Ebara S, Timme TL, Satoh T, Li L, Goltsov A, Ittmann M, Morrisett JD and Thompson TC: Secreted caveolin-1 stimulates cell survival/clonal growth and contributes to metastasis in androgen-insensitive prostate cancer. Cancer Res *61*: 3882-3885, 2001.
37 Cui J, Rohr LR, Swanson G, Speights VO, Maxwell T and Brothman AR: Hypermethylation of the *caveolin-1* gene promoter in prostate cancer. Prostate *46*: 249-256, 2001.
38 Pflug BR, Reiter RE and Nelson JB: Caveolin expression is decreased following androgen deprivation in human prostate cancer cell lines. Prostate *40*: 269-273, 1999.

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