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

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Abstract. *Aim: Japanese and American groups reported that single nucleotide variation of caveolin-1 gene (CAV1) plays an important role in breast cancer risk. The aim of this study was to evaluate the association of six polymorphic genotypes of CAV1, which is reported to be overexpressed in tumors, with breast cancer within a Taiwanese population. Patients and Methods: A total of 1232 patients with breast cancer and equal number of healthy controls in central Taiwan were genotyped via polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) and six polymorphic variants of CAV1 were analyzed for their association with breast cancer susceptibility. Results: The distribution of genotypes of CAV1 G14713A and T29107A were significantly different between breast cancer and control groups ($p=5.6\times10^{-5}$ and 1.9×10^{-4}, respectively), while those for CAV1 C239A, G21985A, T28608A and G32124A were not significant ($p>0.05$). The percentages of AG genotype of G14713A and TT genotype of T29107A are higher in the cancer group than in the control group. The two single nucleotide polymorphisms were chosen for haplotype analysis and the data showed that compared with GG/TT haplotype of CAV1 G14713A/T29107A, the GG/AT and GG/AA groups have a lower risk of breast cancer (odds ratio, OR=0.69, 95% confidence interval, CI=0.57-0.92). On the contrary, the AG/TT haplotype confers a higher risk of breast cancer (OR=1.50, 95% CI=1.14-2.12). Conclusion: Our results provide evidence for CAV1 genotypes being involved in predisposition to breast cancer. The association of the potential risk haplotype agrees well with a role of CAV1 genotype in breast cancer risk and the association with tumor progression needs further investigation.*

Breast cancer is about twice as common in the first-degree relatives of women with the disease as in the general population, consistent with variation in genetic susceptibility to the disease (1). Inherited mutations in *BRCA1* and *BRCA2* genes lead to a high risk of breast and other types of cancer, however, the majority of many familial cases do not segregate mutations in the two genes (2). This observation has led to the concept that breast cancer susceptibility is largely 'polygenic', that is, susceptibility is conferred by multiple loci, each with a small effect on breast cancer risk (3).

Multiple independent lines of experimental evidence suggest that the gene for caveolin1 (*CAV1*) functions as a mammary gland tumor suppressor gene (4, 5). Firstly, *CAV1* mRNA and protein levels are down-regulated in various cancer cell lines, including in oncogene-transformed NIH 3T3 cells, in many human and mouse breast cancer cell lines, in primary human mammary gland tumors, and in transgenic breast cancer mouse cells (6-9). Secondly, *CAV1* re-expression in breast cancer cell lines inhibits anchorage-dependent growth in soft agar and reduces their invasive potential (8, 10). Finally, *CAV1* expression also reduces the migratory and invasive potential of MTLn3 cells, a metastatic mammary carcinoma line, by preventing epidermal growth factor (EGF)-induced lamellipodia formation and reducing cell migration (11). In the human genome, *CAV1* gene consists of three exons and is located at 7q31.1. *CAV1* is the major structural and functional protein component of caveolae, which plays an important role in many signaling pathways, molecular transport, and cellular proliferation and differentiation. The specific functions of the *CAV1* protein are highly cell- and context-dependent (12). The genomic

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Key Words: Caveolin-1, breast cancer, polymorphic genotypes.

Table I. *Characteristics among breast cancer patients and controls.*

Characteristic	Controls (n=1232)			Patients (n=1232)			*P*-value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age at onset (years)							
<40	359	29.1%		362	29.4%		0.89[a]
40-55	558	45.3%		547	44.4%		
>55	315	25.6%		323	26.2%		
Age at menarche (years)			12.4 (0.7)			12.1 (0.6)	0.79[b]
Age at first birth of child (years)			29.4 (1.2)			29.8 (1.4)	0.63[b]
Age at menopause (years)			48.8 (1.8)			49.3 (2.0)	0.59[b]
Site							
Unilateral				1198	97.2%		
Bilateral				34	2.8%		
Family history							
First degree (Mother, sister and daughter)				55	4.5%		
Second degree				6	0.5%		
No history				1171	95%		
Habit							
Cigarette smokers	86	7.0%		170	13.8%		<0.0001[a]
Alcohol drinkers	91	7.4%		162	13.1%		<0.0001[a]

Statistical results based on [a]Chi-square and [b]unpaired Student's *t*-test.

association of *CAV1* with all types of cancer is not well understood and the analyzes are often focused on the genetic mutations not polymorphisms. In 2006, a study reported that some mutations of *CAV1* were associated with breast cancer risk (13). The above evidence pointing to the role of *CAV1* in carcinogenesis led us to study whether different alleles of this gene are associated with breast 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 their association with breast cancer susceptibility.

Patients and Methods

Study population and sample collection. The study population consisted of 1232 breast cancer patients and 1232 age-matched cancer-free control volunteers. The patients diagnosed with breast cancer were recruited at the outpatient clinics of general surgery between 2004 and 2008 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. Wang, Liu and Su). All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. The same number of age-matched non-breast cancer, healthy volunteers as controls were selected after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria for the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups completed a short questionnaire which included habits.

Genotyping conditions. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to our previous methods (14-20). Briefly, the following primers were used for *CAV1* genotyping: for C239A (rs1997623), 5'-GTGTCCGCTTCTGC TATCTG-3' and 5'-GCCAAGATGCAGAAGGAG TT-3'; for G14713A (rs3807987), 5'-CCTTCCAGTAAGCAAGCTGT-3' and 5'-CCTCTCAATCTTGCCATAGT-3'; for G21985A (12672038), 5'-GGTGTCAGCAAGGCTATGCT-3' and 5'-CCAGACACTCAGAA TGTGAC-3'; for T28608A (rs3757733), 5'-GCTCAACCTCATCTG AGGCA-3' and 5'-GGCCTATTGTTGAGTGGATG-3'; for T29107A (rs7804372), 5'-GCCTGAATTGCAATCCTGTG-3' and 5'-ACGGTGTGAACACGGACATT-3'; and for G32124A (rs3807992), 5'-GGTGTCTTGCAGTTGAATG-3' and 5'-ACGGAGCTACTCAG TGCCAA-3'. The following cycling conditions were used: 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, *Sau3A*I and *Nla*III, restriction enzymes for *CAV1* C239A (cut from 485 bp C type into 170+315 bp T type), G14713A (cut from 268 bp A type into 66+202 bp G type), G21985A (cut from 251+43 bp A type into 153+98+43 bp G type), T28608A (cut from 298 bp T type into 100+198 bp A type), T29107A (cut from 336 bp A type into 172+164 bp T type), and G32124A (cut from 213+142+67 bp A type into 142+118+95+67 bp T type), respectively.

Statistical analyses. Only those matches with all the single nucleotide polymorphism data (case/control=1232/1232) 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 polymorphism in the controls

Table II. *Distribution of CAV1 genotypes among breast cancer patients and controls.*

Genotype	Controls		Patients		*P*-value[a]
	n	%	n	%	
239A rs1997623					0.3486
CC	1190	96.6%	1179	95.6%	
AC	39	3.2%	52	4.2%	
AA	3	0.2%	2	0.2%	
G14713A rs3807987					0.000056
GG	801	65.0%	704	57.1%	
AG	311	25.2%	409	33.2%	
AA	120	9.7%	119	9.7%	
G21985A rs12672038					0.2442
GG	742	60.2%	718	58.3%	
AG	401	32.5%	403	32.7%	
AA	89	7.3%	111	9.0%	
T28608A rs3757733					0.9522
TT	718	58.3%	713	58.4%	
AT	409	33.2%	401	32.8%	
AA	105	8.5%	108	8.8%	
T29107A rs7804372					0.00019
TT	649	52.7%	745	60.5%	
AT	472	38.3%	410	33.2%	
AA	111	9.0%	77	6.3%	
G32124A rs3807992					0.4962
GG	604	49.0%	588	47.7%	
AG	493	40.0%	520	42.2%	
AA	135	11.0%	124	10.1%	

[a]*P*-value based on Chi-square test.

Table III. *Distribution of CAV1 alleles among breast cancer patients and controls.*

Allele	Controls		Patients		*P*-value[a]
	n	%	n	%	
C239A rs1997623					0.2705
Allele C	2419	98.2%	2410	97.7%	
Allele A	45	1.8%	56	2.3%	
G14713A rs3807987					0.0014
Allele G	1913	77.6%	1817	73.7%	
Allele A	551	22.4%	647	26.2%	
G21985A rs12672038					0.1273
Allele G	1885	76.5%	1839	74.6%	
Allele A	579	23.5%	625	25.4%	
T28608A rs3757733					0.9205
Allele T	1845	74.9%	1827	74.8%	
Allele A	619	25.1%	617	25.2%	
T29107A rs7804372					0.00022
Allele T	1770	71.8%	1900	77.1%	
Allele A	694	28.2%	564	22.9%	
G32124A rs3807992					0.8777
Allele G	1701	69.0%	1696	68.8%	
Allele A	763	31.0%	768	31.2%	

[a]*P*-value based on Chi-square test.

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. Cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. The data was recognized as significant when the statistical *p*-value was less than 0.05. To evaluate effect modification by smoking, stratified analyses were conducted for chosen SNPs to compare the association across exposure categories of smoking status (never-smokers and smokers). All statistical tests were performed using SAS, Version 9.1.3 (SAS Institute Inc., Cary, NC, USA) on two-sided probabilities.

Results

The frequency distributions of selected characteristics of 1232 breast cancer patients and 1232 non-cancer controls are shown in Table I. These characteristics of patients and controls were all well matched. None of the differences between groups were statistically significant ($p>0.05$) (Table I). As for the individual behaviors, cigarette smoking and alcoholism were significantly more frequent in the breast cancer group in this population ($p<0.05$) (Table I).

The frequencies of the genotypes for *CAV1* C239A (rs1997623), G14713A (rs3807987), G21985A (12672038), T28608A (rs3757733), T29107A (rs7804372), and G32124A (rs3807992) in controls and breast cancer patients are summarized in Table II. The distribution of genotypes of *CAV1* G14713A and T29107A were significantly different between breast cancer and control groups ($p=0.000056$ and 0.00019, respectively) with a higher proportion of AG carriers and TT carriers, respectively, in the breast cancer group. Differences for *CAV1* C239A, G21985A, T28608A and G32124A were not significant ($p>0.05$) (Table II). We also performed allelic frequency analysis, and the frequencies of the alleles for *CAV1* C239A, G14713A, G21985A, T28608A, T29107A and G32124A in controls and breast cancer cases are shown in Table III. The two SNPs of *CAV1* found to be associated with breast cancer risk in Table II, G14713A and T29107A, were again found to be associated with higher breast cancer susceptibility, with greater frequency of the A allele and T allele, respectively, in their allele in the breast cancer group (Table III; $p=0.00014$ and 0.000022, respectively). As for the other four SNPs, the distributions of their allele frequencies were not significantly different in controls and breast cancer patients (Table III).

Considering potential interactions between the two significant SNPs of the *CAV1* gene and breast cancer susceptibility, the risk of breast cancer as related to haplotypic distributions of *CAV1* G14713A and T29107A were further analyzed (Table IV). Compared with the GG/TT haplotype of

Table IV. *Distribution of CAV1 G14713A/T29107A haplotypes among breast cancer patients and controls.*

G14713A/ T29107A	Controls		Patients		Odds ratio	Adjusted odds ratio	*P*-value[b]
haplotype	n	%	n	%	(95% CI)	(95% CI)[a]	
GG/TT	422	34.3%	425	34.5%	1.00 (Reference)	1.00 (Reference)	
GG/AT or GG/AA	379	30.8%	279	22.6%	0.73 (0.60-0.90)	0.69 (0.57-0.92)	0.0025
AG/TT	164	13.3%	247	20.0%	1.50 (1.18-1.90)	1.48 (1.14-2.12)	0.0011
AG/AT or AG/AA	147	11.9%	162	13.1%	1.09 (0.84-1.42)	1.08 (0.85-1.44)	0.5069
AA/TT	63	5.1%	72	5.8%	1.13 (0.79-1.63)	1.10 (0.76-1.65)	0.5174
AA/AT or AA/AA	57	4.6%	47	3.8%	0.82 (0.54-1.23)	0.86 (0.59-1.24)	0.3513

95% CI, 95% confidence interval. [a]Adjusted for age, smoking, and alcohol drinking behaviors. [b]Based on Fisher's exact two-tailed test.

CAV1 G14713A/T29107A, the GG/AT and GG/AA groups have a lower risk of breast cancer (OR=0.69, 95% CI=0.57-0.92). On the contrary, the AG/TT haplotype conferred a higher risk of breast cancer (OR=1.50, 95% CI=1.14-2.12). After adjusting for age, smoking and alcohol drinking behaviors, these differences remained significant. Other combinations of AG/AT or AG/AA, AA/TT, and AA/AT or AA/AA did not confer significantly altered cancer risk compared to the wild-type GG/TT haplotype (Table IV). The results showed that individuals with the heterozygous AG genotype, but not homozygous AA genotype, of *CAV1* G14713A were at higher risk of breast cancer. As for *CAV1* T29107A, those with TT genotype were at higher breast cancer risk.

Discussion

In 2001, a Japanese group reported that up to 16% of breast cancer samples harbored a P132L mutation in the *CAV1* gene (21). Furthermore, in the population of the USA, it was reported that the *CAV1* P132L mutation is present in about 19% of estrogen receptor α (ER α)-positive breast cancer cases but not in ER α-negative breast cancer cases. (13). All the data suggested that the single nucleotide variations played an important role in breast carcinogenesis. Thus, we aimed at investigating the association of *CAV1* polymorphism with breast cancer.

In this study, the genotypes of 1232 breast cancer patients and the same number of age-matched cancer-free controls were examined. We found that the genotypes of *CAV1* single nucleotide polymorphisms, G14713A and T29107A were differentially distributed between the breast cancer and healthy control groups (Table II). In addition, the allelic frequencies of the two SNPs were also differentially distributed between the two groups (Table III). The results showed that the A allele of G14713A and T allele of T29107A were both risk factors for breast cancer susceptibility. Furthermore, the combination haplotypes of *CAV1* G14713A and T29107A were also examined (Table IV). The results suggest that individuals with the GG/AT or GG/AA G14713A/T29107A haplotype were at lower risk of breast cancer, and those with AG/TT haplotype were at higher risk, compared with the GG/TT haplotype (Table IV). It is interesting to note that those with the AA/TT haplotype did not have the highest odds ratio of breast cancer.

The sample size and similar trends of significant data after age and behavior adjustments (Table IV) strengthen the accuracy and reliability of our findings. In addition, the frequencies of *CAV1* polymorphic variant alleles were similar to those reported in the NCBI website for other Asian population studies. For instance, the minor A allele frequency of *CAV1* G14713A was 22.4% in our control group, close to those of 16.7% for Beijing and 22.2% for Tokyo populations in NCBI, which strongly suggests no selection bias for participant enrolments in terms of genotypes.

In conclusion, this is the first report to provide evidence for *CAV1* single nucleotide polymorphisms G14713A and T29107A, but not C239A, G21985A, T28608A, or G32124A, being associated with breast cancer risk. The A allele of *CAV1* G14713A and T allele of *CAV1* T29107A might become potential biomarkers for the early screening detection and prediction of breast cancer and integrative cancer therapy.

Acknowledgements

We thank Judy Wang, Tzu-Shin Weng and the Tissue Bank at the China Medical University for their technical assistance. This study was supported by research grants from the Terry Fox Cancer Research Foundation, and China Medical University and Hospital (DMR-100-179).

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Received June 20, 2011
Revised August 26, 2011
Accepted August 29, 2011