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The functional promoter polymorphism (–842G>C) in the *PIN1* gene is associated with decreased risk of breast cancer in non-Hispanic white women 55 years and younger

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Abstract

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PIN1, an isomerase that causes conformational changes in proteins, plays an important role in mammary epithelial cell growth both physiologically and pathologically. Thus, genetic variants in the *PIN1* gene may alter protein function and cancer risk. We have previously demonstrated an association between a *PIN1* promoter variant (−842G>C; rs2233678) and risk of squamous cell carcinoma of the head and neck, a finding supported by additional functional data. In the present study, we genotyped two promoter single nucleotide polymorphisms (SNPs) (−842G>C, rs2233678 and −667T>C, rs2233679) and one synonymous SNP (Gln33Gln; G>A, rs2233682) in exon 2 to evaluate their associations with risk of sporadic breast cancer in non-Hispanic white women 55 years and younger. We found that the carriers of −842C variant alleles had decreased risk of breast cancer with an adjusted odd ratio (OR) of 0.67 and 95% confidence interval (CI) of 0.50–0.90. This reduced risk was more evident in women after reproductive age of 45 (OR = 0.63, 95% CI = 0.42–0.93), ever-smokers (OR = 0.56, 95% CI = 0.36–0.88), and ever-drinkers (OR = 0.67, 95% CI = 0.45–0.99). No such associations were observed for *PIN1* −667T>C and *PIN1* Gln33Gln. However, the haplotypes of these three SNPs were also associated with reduced risk of breast cancer. In conclusion, the *PIN1* polymorphisms may contribute to the etiology of sporadic breast cancer in non-Hispanic white women 55 years and younger. Further validation in large population-based studies is needed.

Keywords

Genetic susceptibility; Molecular epidemiology; PIN1; Breast cancer

Introduction

Phosphorylation of proteins on serine/threonine residues preceding proline (Ser/Thr-Pro), also called pro-directed phosphorylation, is a key signaling mechanism in various cellular mechanism. Deregulation of this mechanism can result in cell transformation and oncogenesis [1–4]. Studies have shown that proteins containing phosphorylated Ser/Thr-Pro motifs are isomerized specifically by the peptidyl-prolyl cis/trans isomerase PIN1, revealing PIN1 involvement in a novel post-phosphorylation regulatory mechanism [5,6]. PIN1 binds to and isomerizes specific Ser/Thr-Pro motifs after phosphorylation and changes conformation of proteins, affecting enzymatic activity, phosphorylation status, protein–protein interactions, subcellular location, or protein stability [7–10].

Aberrant overexpression of PIN1 in some common cancers, such as lung, colon, prostate, and breast cancers, has been reported previously [11–14]. In breast cancer studies [12–14], the expression of PIN1 was significantly correlated with that of cyclin D1, Her-2/Neu and β -catenin that are strong prognostic factors for human breast cancer, indicating that PIN1 overexpression might be associated with tumor cell growth. In addition, the *PIN1* knockout mice study showed that *PIN1* $-/-$ female mice had defective mammary epithelial duct development during pregnancy [15]. These indicate that PIN1 plays an important role in mammary epithelial cell growth both physiologically and pathologically.

The association between *PIN1* promoter polymorphisms and risk of diseases has been evaluated only in a few studies [16–20]. Three studies reported some controversial associations on the *PIN1* promoter polymorphisms with the risk of Alzheimer's disease in different study populations [16,18,19]. One study investigated the roles of *PIN1* −842G>C (rs2233678) and −667T>C (rs2233679) single nucleotide polymorphism (SNPs) in hepatocellular carcinoma (HCC) and found that the increased risk of HCC was associated with −667T allele in hepatitis B and C co-infected patients [17]. In a recent study of squamous cell carcinoma of the head and neck (SCCHN) in non-Hispanic whites, we reported that the common *PIN1* SNP −842G>C (rs2233678) in the promoter region was associated with decreased risk of SCCHN and also demonstrated that this SNP was functional, because the variant C led to diminished promoter

activity [20]. However, no reported studies have investigated the association between the *PIN1* polymorphisms and risk of breast cancer.

In the present study, we genotyped the two common promoter SNPs (−842G>C (rs2233678) and −667T>C (rs2233679) and one synonymous SNP (Gln33Gln; G>A (rs2233682) in exon 2 to test the hypothesis that the *PIN1* polymorphisms are associated with risk of sporadic breast cancer in non-Hispanic white women 55 years and younger, a unique study population in which we previously evaluated the role of other genetic variations in risk of developing breast cancer [21–23].

Materials and methods

Study population

As described previously [22], all subjects in this study population were non-Hispanic white women 55 years and younger. The 467 patients were diagnosed with primary breast cancer and recruited at The University of Texas M.D. Anderson Cancer Center between 1998 and 2006. These cases were histopathologically confirmed and untreated before the blood drawing. Patients with a previous cancer history or multiple primary neoplasms at the time of diagnosis were excluded from this study. During the same time period, we recruited 488 cancer-free controls from hospital visitors who were genetically unrelated to the cases. Controls were frequency-matched to the cases by age (± 5 years). Participants who had smoked at least 100 cigarettes in their lifetime were assigned as ever-smokers and the remainder as never-smokers. Participants who had drunk alcoholic beverage at least once a week for ≥ 1 year in their lifetime were assigned as ever-drinkers and the remaining as never-drinkers. Additional information including expression status of estrogen receptor (ER)/progesterone receptor (PR), menopausal status, age at first full-term pregnancy, number of live births, use of hormone replacement therapy or oral contraceptives, and body mass index was only available for the cases. Thirty-milliliter of blood sample was collected after the informed consent was signed from each participant. The study protocol was approved by M. D. Anderson Cancer Center institutional review board.

SNP selection and genotyping

We selected the only reported two common (minor allele frequency >0.05) SNPs (−842G>C, rs2233678 and −667T>C, rs2233679) and one common synonymous SNP in exon 2 (Gln33Gln; G>A, rs2233682) among 77 SNPs listed in the dbSNP database, because no non-synonymous SNPs have been reported for the *PIN1* gene (<http://www.ncbi.nlm.nih.gov/projects/SNP/> as of 11/2009). The three *PIN1* SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Since the −842G>C and −667T>C are close in distance, we used same forward primer (5'-CGGGCTCT GCAGACTCTATT-3') and reverse primer (5'-AAATT TGGCTCCTCCATCCT-3') to amplify the fragment, but two different enzymes to identify the genotypes; *Ban*II (New England BioLabs, Beverly, MA) for −842G>C and *Sac*I (New England BioLabs) for −667T>C. A pair of primers 5'-GGAGCACAACCCTAGCTGAA-3' (forward) and 5'-GGCTGTGCTTCACCAGCA-3' (reverse) and enzyme *Bsr*I (New England BioLabs) were used to genotype Gln33Gln; G>A, using experimental conditions previously reported [20]. The amplified and digested fragments were separated in 3% MetaPhor agarose gel, and the genotype results were evaluated by two people who were blinded to the subject's case or control status as previously reported [20]. Genotyping of 10% of samples were randomly performed twice and no discrepancy was observed.

Statistical analysis

The distribution of *PIN1* genotypes and selected risk factors between cases and controls was compared by using χ^2 test. To compare the observed with expected genotype frequencies, Hardy–Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$, where p is the frequency of the variant allele and $q = 1 - p$) was tested by a goodness-of-fit χ^2 test in cancer-free controls. The association between *PIN1* genotypes and breast cancer risk was evaluated by calculating the odds ratios (ORs) and 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analyses. We detected linkage disequilibrium (LD) of any pair of SNPs, constructed haplotypes with three variants based on the observed genotypes by the SAS PROC ALLELE and PROC HAPLOTYPE procedures in SAS/Genetics software, and evaluated the association between the haplotypes and breast cancer risk by logistic regression models. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant by using the SAS/Genetics software (version 9.1.3; SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the study population

This study included 467 breast cancer and 488 cancer-free controls. Since we used frequency matching by age, neither there was significant difference in the distribution of age ($P = 0.796$), nor was there any difference in smoking and drinking status between cases and controls ($P = 0.117$, and 0.140 , respectively) (Table 1). These variables were also used in multivariate logistic regression models to further adjust for any possible residual confounding effect on the associations between selected *PIN1* polymorphisms and risk of breast cancer.

Distribution of *PIN1* genotypes and risk of breast cancer

The genotype and allele distributions of *PIN1* –842G>C, –667T>C, and Gln33Gln are presented in Table 2. The observed genotype frequencies of these SNPs were all in agreement with the Hardy–Weinberg equilibrium in the controls ($P = 0.160$ for –842G>C, $P = 0.229$ for –667 T>C, and $P = 0.183$ for Gln33Gln). The LD analysis showed that all polymorphisms were not in LD in the controls (LD for *PIN1* –842G>C and *PIN1* –667T>C: $D' = 0.673$ and $r^2 = 0.159$; LD for *PIN1* –842G>C and *PIN1* Gln33Gln, G>A: $D' = 0.106$ and $r^2 = 0.001$; and LD for *PIN1* –667T>C and *PIN1* Gln33Gln, G>A: $D' = 0.544$ and $r^2 = 0.013$, Fig. 1). These suggest that each SNP may, if any, have an independent effect.

As shown in Table 2, the genotype and allele frequencies of *PIN1* –842G>C were significantly different between cases and controls ($P = 0.023$ and 0.016 , respectively). Compared with the –842GG homozygotes, –842GC heterozygotes had a significantly decreased risk of breast cancer (OR = 0.66, 95% CI = 0.49–0.89) after adjustment for age, smoking, and alcohol status; –842CC homozygotes had only a non-significant decreased risk of breast cancer (OR = 0.79, 95% CI = 0.30–2.07), possibly because of the small number of this subgroup. Therefore, we combined the –842GC and –842CC and found that the –842C allele carriers (i.e., GC + CC genotypes) had 0.67-fold decreased risk of breast cancer (95% CI = 0.50–0.90), compared with –842GG carriers. However, there were no associations between genotypes of *PIN1* –667T>C or *PIN1* Gln33Gln and breast cancer risk (Table 2).

Stratification analysis of *PIN1* –842G>C genotypes and risk of breast cancer

When we further performed stratification analysis of –842G>C genotypes and risk of breast cancer by age, smoking status, and alcohol use, the decreased risk of breast cancer associated with the –842C variant allele was more evident among women of 46–55 years (OR = 0.63, 95% CI = 0.42–0.93), ever-smokers (OR = 0.56, 95% CI = 0.36–0.88), and ever-drinkers (OR = 0.67, 95% CI = 0.45–0.99) (Table 3). We also stratified the *PIN1* genotypes by reproductive

characteristics (e.g., menopausal status, age at first full-term pregnancy, number of live births, and use of hormone replacement or oral contraceptives), ER/PR status, tumor histology, and stages in the cases. We did not find differences in the genotype distribution of three SNPs between strata of each variable except for menopausal status. Among patients with menopause, 49.3% had -667TT, 38.2% had -667TC and 12.5% had -667CC; in non-menopause patients, 37.3% had -667TT, 51.9% had -667TC, and 10.8% had -667CC. The P value for the χ^2 test was 0.032.

***PIN1* haplotypes and risk of breast cancer**

Finally, we evaluated the association between the risk of breast cancer and haplotypes constructed from the three *PIN1* polymorphisms (-842G>C, -677T>C, and Gln33 Gln, G>A). Eight possible haplotypes were obtained, five of which had a frequency less than 5%; therefore, we grouped them together as shown in Table 4. The distribution of haplotypes was significantly different between the cases and controls ($P = 0.048$, Table 4). Compared to the most common haplotype with major alleles of three SNPs (G-T-G), all other haplotypes G-C-G, C-C-G, showed reduced risk of breast cancer, especially the combined rare (frequency <0.05) haplotype group that included C-C-A, C-T-A, C-T-G, G-C-A, and G-T-A (OR = 0.51, 95% CI = 0.31–0.84) (Table 4).

Discussion

To the best of our knowledge, this is the first report on the role of genetic variants in *PIN1* in breast cancer susceptibility. In this case-control study, we investigated the association between *PIN1* polymorphisms and risk of sporadic breast cancer in non-Hispanic white women who were ≤ 55 years, and found that the -842C variant allele was significantly associated with a decreased risk of breast cancer. The results are consistent with our recent report on the association study of *PIN1* polymorphisms and risk of SCCHN [20]. Therefore, our findings expand the role of the *PIN1* promoter SNP -842G>C in the carcinogenesis of different types of organs, including the head and neck and breast.

PIN1 overexpression has been reportedly prevalent in human cancers [24], including breast cancer [13]. Complex and heterogeneous genetic alterations are involved in the development of breast cancer [25], including amplification of oncogenes, such as myc, cyclin D1, and Her-2/Neu [26]. Several studies have reported that increased expression of *PIN1* in breast cancer was strongly correlated with that of cyclin D1, β -catenin, Her-2, and Ras [12–14,27]. For example, *PIN1* was overexpressed by more than 80% in cyclin D1-overexpressing breast tumors, and the average *PIN1* level in cyclin D-overexpressing breast tumor was about twice of those in cyclin D1-negative breast tumors [13]. β -catenin levels were increased and correlated with *PIN1* overexpression but decreased in *PIN1*-deficient tissues [12]. The transcription factor E2F-mediated *PIN1* expression and c-Neu and Ha-Ras enhance *PIN1* expression via E2F, suggesting that *PIN1* may be a requisite translator and an enhancer of oncogenic signal transduction for Ras- and Neu-induced mammary epithelial cell transformation [27]. It was also reported that *PIN1* expression was repressed by BRCA1 [28].

In previous functional analyses of the *PIN1* -842G>C polymorphism, the -842C variant allele showed functional relevance by diminishing the promoter activity [16,20], and the -842CC genotype was associated with low levels of the *PIN1* protein in peripheral mononuclear cells [16]. Therefore, -842C variant alleles would have low expression of *PIN1* and be associated with a reduced risk of breast cancer. In this study, the decreased risk of breast cancer associated with the -842C variant allele was more evident in women older than 45 years than women in reproductive age. It has been reported that *PIN1* maintains cell proliferation and that mice with *PIN1* knockout developed defective mammary epithelial ducts during pregnancy and exhibited

age-dependent disorders in adults [15,29]. This suggests that *PIN1* may have interactions with female hormones in addition to regulating the expression of oncogenes.

The etiology of breast cancer is a complex combination of genetic, environmental, and reproductive factors, such as earlier menarche, nulliparity, later age at first birth, and later menopause [30–32]. Among these, hormonal factor has been implicated as a major etiological factor in the development of breast cancer. Several studies have shown close link between estrogen action and c-myc and cyclin D1, which are rapidly upregulated in response to estrogen and induce cell cycle progression [33–36]. We have also found that *PIN1* –677T>C SNP is associated with menopausal status, which may implicate the correlation of *PIN1* and hormone levels. Women's fecundity decline starts at age of 35, accelerates between 35 and 40 years of age, and approaches zero by age 45 [37]. In that point, we could speculate that the changes in hormonal status with aging may have affected the role of *PIN1* or its interaction with other oncogenes, attributing to our study results; however, this speculation needs to be tested in future studies.

Interestingly, ever-smokers or ever-drinkers in our study had more pronounced decreased risk of breast cancer when carrying the –842C variant allele. It has been reported that nicotine can promote proliferation of endothelial cells [38], and alcohol interferes with the retinoic acid and mitogen-activated protein kinase (MAPK) signaling, which further impair normal cell proliferation and apoptosis [39]; therefore, the –842C allele associated with relatively lower *PIN1* expression, compared with the –842G allele, may lead to a stronger protective effect on cell proliferation among ever-smokers or ever-drinkers. However, these trends were not found in our SCCHN study [20], suggesting the findings in the present study could be either by chance or due to an interaction between *PIN1* and female hormones [40], which needs to be validated in larger studies.

Our study has several limitations. First is that our study is a hospital-based case–control study with a relative small sample size, which might have a selection bias. Since the controls were selected in the hospital setting, they might not represent the general population. Another limitation is the lack of reproductive information for the controls, which led to exclusion of these parameters in further analysis of risk association with *PIN1* polymorphisms.

In conclusion, in this hospital-based case–control study of sporadic breast cancer, we found a significant association between the *PIN1* –842C allele and decreased risk of breast cancer in non-Hispanic white women aged ≤55 years. The decreased risk was more evident in women after reproductive age and women exposed to tobacco smoking and alcohol drinking. Further validation of our findings in large population-based studies is needed.

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Fig. 1. Linkage disequilibrium (LD) for the three SNPs of *PIN1*; (A) D' and (B) r^2 values

Table 1
Frequency distributions of selected variables in breast cancer cases and controls

Variable	Cases (<i>n</i> = 467)		Controls (<i>n</i> = 488)		<i>p</i> ^a
	<i>n</i>	%	<i>n</i>	%	
Age (years)					
≤45	199	42.6	212	43.4	0.796
46–55	268	57.4	276	56.6	
Smoking status					
Never	278	59.5	266	54.5	0.117
Ever	189	40.5	222	45.5	
Drinking status					
Never	215	46.0	248	50.8	0.140
Ever	252	54.0	240	49.2	
Histology					
Carcinomas in situ	75	16.1			
Invasive carcinomas	392	83.9			
Stage					
0	75	16.1			
I	183	39.2			
II	158	33.8			
III	46	9.8			
IV	5	1.1			
ER ^b					
Negative	129	27.6			
Positive	293	62.8			
Unknown	45	9.6			
PR ^b					
Negative	176	37.7			
Positive	246	52.7			
Unknown	45	9.6			

^aTwo-sided χ^2 test

^bThe ER and PR expression in tumor tissues of breast cancer were only available in 422 cases

Table 2
Distribution of *PIN1* polymorphisms and logistic regression analysis for association with risk of breast cancer

Genotypes	Cases (n = 467) n (%)	Controls (n = 488) n (%)	<i>P</i> ^a	Crude OR (95% CI)	Adjusted OR (95% CI) ^b
<i>PIN1</i> -842G>C					
GG	358 (76.7)	336 (68.9)	0.023	1.00 (ref.)	1.00 (ref.)
GC	101 (21.6)	143 (29.3)		0.66 (0.49–0.89)	0.66 (0.49–0.89)
CC	8 (1.7)	9 (1.8)		0.83 (0.32–2.19)	0.79 (0.30–2.07)
GC + CC	109 (23.3)	152 (31.1)		0.68 (0.51–0.90)	0.67 (0.50–0.90)
C allele	0.125	0.165	0.016		
<i>PIN1</i> -667T>C					
TT	189 (40.5)	194 (39.8)	0.968	1.00 (ref.)	1.00 (ref.)
TC	223 (47.8)	237 (48.6)		0.97 (0.74–1.27)	0.96 (0.73–1.26)
CC	55 (11.8)	57 (11.7)		0.99 (0.65–1.51)	0.97 (0.63–1.47)
TC + CC	278 (59.5)	294 (60.25)		0.97 (0.75–1.26)	0.96 (0.74–1.25)
C allele	0.357	0.360	0.924		
<i>PIN1</i> Gln33Gln, G>A					
GG	445 (95.3)	465 (95.3)	0.816	1.00 (ref.)	1.00 (ref.)
GA	20 (4.3)	22 (4.5)		0.95 (0.51–1.77)	0.92 (0.49–1.71)
AA	2 (0.4)	1 (0.2)		2.09 (0.19–23.13)	2.12 (0.19–23.53)
GA + AA	22 (4.7)	23 (4.7)		1.00 (0.55–1.82)	0.97 (0.53–1.77)
A allele	0.026	0.025	0.885		

The significance levels are $P < 0.05$ for all the bold values

^aTwo-sided χ^2 test for either genotype distribution or allele frequency

^bAdjusted for age, smoking status, and alcohol use in a logistic regression model

Stratification analysis of the *PIN1* -842G>C genotypes by selected variables in breast cancer cases and controls

Table 3

Cases (n = 467)				Controls (n = 488)		Crude OR (95% CI)		Adjusted OR (95% CI) ^a	
GG n (%)	GC + CC n (%)	GG n (%)	GC + CC n (%)	GG n (%)	GC + CC n (%)	GC + CC vs. GG	GC + CC vs. GG	GC + CC vs. GG	GC + CC vs. GG
Age (years)									
≤45	148 (74.4)	51 (25.6)		143 (67.5)	69 (32.6)	0.71 (0.47–1.10)	0.71 (0.47–1.10)	0.72 (0.47–1.10)	
46–55	210 (78.4)	58 (21.6)		193 (69.9)	83 (30.4)	0.64 (0.44–0.95)	0.64 (0.44–0.95)	0.63 (0.42–0.93)	
Smoking status									
Never	211 (75.9)	67 (24.1)		188 (70.7)	78 (29.3)	0.77 (0.52–1.12)	0.77 (0.53–1.13)	0.77 (0.53–1.13)	
Ever	147 (77.8)	42 (22.2)		148 (66.7)	74 (33.3)	0.57 (0.37–0.89)	0.56 (0.36–0.88)	0.56 (0.36–0.88)	
Drinking status									
Never	168 (78.1)	47 (21.9)		176 (71.0)	72 (29.0)	0.68 (0.45–1.05)	0.68 (0.45–1.04)	0.68 (0.45–1.04)	
Ever	190 (75.4)	62 (24.6)		160 (66.7)	80 (33.3)	0.65 (0.44–0.97)	0.67 (0.45–0.99)	0.67 (0.45–0.99)	

The significance levels are *P* < 0.05 for all the bold values

^a ORs were adjusted for age, smoking status, and alcohol use in logistic regression models within the strata

Table 4

Associations between haplotypes of three *PIN1* variants of -842G>C, *PIN1* -667T>C, *PIN1* Gln33Gln G>A, and breast cancer risk

Haplotypes	Cases (934 Alleles) n (%)	Controls (976 Alleles) n (%)	OR (95% CI) ^a	P
G-T-G	592 (63.4)	595 (61.0)	1.00 (ref.)	
G-C-G	202 (21.6)	201 (20.6)	0.98 (0.77–1.23)	0.837
C-C-G	115 (12.3)	131 (13.4)	0.86 (0.65–1.14)	0.303
Others ^b	25 (2.7)	49 (5.0)	0.51 (0.31–0.84)	0.008
<i>P</i> = 0.048 ^c				

^a Adjusted for age, smoking status, and alcohol use

^b Frequency less than 0.05 (C-C-A, C-T-A, C-T-G, G-C-A, G-T-A)

^c Global test