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Effect of *EME1* exon variant Ile350Thr on risk and early onset of breast cancer in southern Chinese women

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Abstract

Essential meiotic endonuclease 1 homolog 1 (EME1) is a key DNA repair protein that participates in the recognition and repair of DNA double-strand breaks. Deficiency of the *EME1* gene can lead to spontaneous genomic instability and thus contribute to tumorgenesis. We hypothesized that the exon variants of *EME1* confer genetic susceptibility to breast cancer. In a case-control study of 748 breast cancer patients and 778 normal controls, we analyzed the association between two exon variants of *EME1* (i.e.,Ile350Thr: rs12450550T > C and Glu69Asp: rs3760413T > G) and breast cancer risk. We found that compared to the common Ile/Ile genotype, the Thr variant genotypes (Thr/Ile + Thr/Thr) conferred a 1.47-fold increased risk of breast cancer (OR=1.47, 95% CI=1.13-1.92). The variant Ile350Thr was also associated with early onset of breast cancer (r = -0.116, P = 0.002). The mean age of onset was 44.4 years for Thr/Thr genotype carriers and 46.5 years for Thr/Ile genotype carriers, which was significantly lower than that (49.4 years) for Ile/Ile genotype carriers (P = 0.006). Moreover, no significant association was observed between the Glu69Asp variant and breast cancer risk. Our findings suggest that the *EME1* variant Ile350Thr contributes to an increased risk and early onset of breast cancer.

Keywords: *EME1*, exon variant, breast cancer, early onset

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INTRODUCTION

Breast cancer is the most common cancer diagnosed in females worldwide[1,2]. In China, data from the National Central Cancer Registry showed that the incidence rate of breast cancer was 21.6/100,000, ranking second among all cancers, and the mortality was 5.7/100, 000, which ranks the sixth among all cancers[3]. However, lacking effective early diagnostic markers, patients are more likely to be diagnosed at advanced tumor stages in China[4], and it was also reported that the age of breast cancer onset among Chinese women is younger than other populations[4].

The pathogenesis of breast cancer is complex, involving environmental factors and genetic alterations. Epidemiological studies have identified many etiologic factors, including reactive oxygen species[5], radiation[6,7], UV light[8] and various carcinogens[9]. These factors can damage DNA including formation of double-strand break (DSB). DSB is one of the most critical type of DNA damages and contributes to increased risk of breast cancer[10-14]. Unrepaired DSBs can easily lead to chromosomal aberrations, increased genetic instability and ultimately lead to cancer development[15,16]. In response to this damage, there are two major pathways, the homologous recombination (HR) and non-homologous end-joining (NHEJ) pathway, involving in DSB repair in humans[17-19]. HR is a fundamental process that typically repairs DSBs caused by the replication machinery, which attempts to synthesize across a single-strand break or unrepaired lesion, causing collapse of the replication fork through several DNA repair molecules, such as the MRN complex and essential meiotic endonuclease 1 homolog 1 (EME1)[20]. These DNA repair molecules are very important in maintaining genetic stability, and many human diseases are genetically determined by them, corresponding with somatic mutations or genetic variants in these genes[21-24].

EME1 is an essential participator of the HR pathway by being part of the structure-specific endonuclease complex methyl methanesulfonate-sensitive UV-sensitive 81-EME1 (MUS81-EME1)[25]. It plays an important role in perturbed replication fork processing and DNA repair by HR, and maintains genome integrity in collaboration with multiple checkpoint pathways[26]. EME1 has activity on a number of branched DNA structures: nicked Holliday junctions[27], aberrant replication fork structures, D-loops and 3´-flap structure[28,29]. Moreover, *EME1* deficiency can lead to spontaneous genomic instability as haplo-insufficiency of *EME1* spontaneously promotes chromosome damage such as breaks and activates the intra-S-phase checkpoint through the ATM-Chk1/Chk2 pathways. Furthermore, haplo-insufficiency of *EME1* activates the G2/M checkpoint through the ATM-Chk2 pathway and promotes DNA re-replication[25,30]. Haplo-insufficiency of *EME1* can also render cells more sensitive to cisplatin-induced DNA damage[31]. Thus, the dysfunction of *EME1* can lead to genomic instability and tumorgenesis.

Human *EME1* gene (MIM: 610885) is located on 17q21.33, containing 9 exons and encoding a 570-amino acid protein (***Fig. 1A***). Genetic variants in *EME1* coding region causes changes in amino acids, which may affect the ability of EME1 for DNA repair by virtue of ectopic DNA binding or abnormal interaction with MUS81, and thus contribute to genetic susceptibility of cancer[32]. Previous study has reported that one exon variant in *EME1* is significantly associated with glioblastoma multiforme susceptibility in humans[33]. However, no study has tested the effect of these exon variants on breast cancer. Therefore, we hypothesized that single nucleotide polymorphism (SNP) in the exons of *EME1* may contribute to the susceptibility of breast cancer. To test this hypothesis, a hospital-based case-control study was employed to examine the role of two exon variants (Ile350Thr: rs12450550T > C and Glu69Asp: rs3760413T > G) of *EME1* in determining breast cancer susceptibility among 748 breast cancer patients and 778 cancer-free controls.

SUBJECTS AND METHODS

Study subjects

We conducted a case-control study in a southern Chinese population, which has been described previously[34,35]. In brief, 748 newly diagnosed breast cancer patients, who were histopathologically confirmed, were consecutively recruited between March 2007 and March 2011 from four urban hospitals (i.e., the First, the Second and the Tumor Hospitals affiliated to Guangzhou Medical University, and Guangzhou Chest Hospital) and one suburban hospital, Panyu People´s Hospital, with a response rate of 91%. Seven hundred and seventy-eight age (± 5)-matched cancer-free controls were randomly selected from a subject pool of more than 10,000 individuals. When the cases were recruited, these individuals participated in the healthy checkup programs in community health stations in Guangzhou during the same period with a response rate of about 85%. All subjects were ethnic Han Chinese. A questionnaire was used for collecting information of demographic features, including age, age at menarche, menstrual history and family history

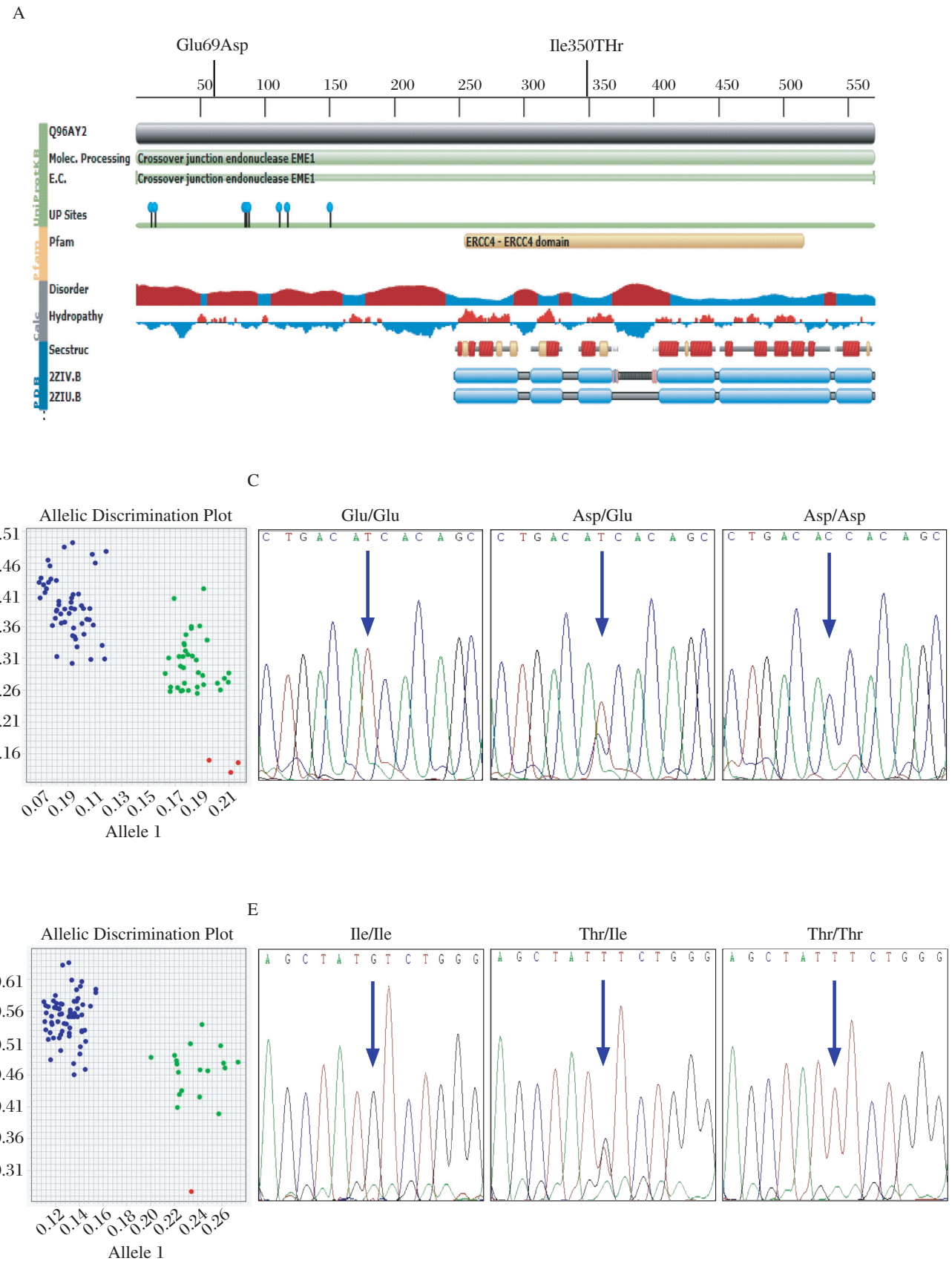

***Fig. 1* Protein structure of EME1 and EME1 variants genotyping.** A: EME1 protein structure; B and C: Genotyping of Glu69Asp by Taq–man assay and direct sequencing, Glu69Asp, genotypes of Asp/Asp were blue, Asp/Glu green, and Glu/Glu red; D and E: Genotyping of Ile350Thr by Taqman assay and direct sequencing, Ile350Thr, genotypes of Ile/Ile were blue, Ile/Thr green, and Thr/Thr red.

of cancer. The definitions of these factors were described in previous studies[36-39]. Additional information was available only for the cases, including tumor stage and the expression status of estrogen receptor (ER)/progesterone receptor (PR). Each subject was asked to donate 5 mL blood after having given their informed consent. The study protocol was approved by the institutional review board of Guangzhou Medical University.

SNP selection and genotyping

According to the dbSNP database (http://www.ncbi.nlm.nih.gov/), two common SNPs with minor allele frequency (MAF > 5%) located in the exons of *EME1* were found and selected. They are the variant rs3760413T > G, which causes an amino acid change from glutamic acid (Glu) to aspartic acid (Asp) at codon 69, and rs12450550T > C, which causes an amino acid change from isoleucine (Ile) to threonine (Thr) in EME1.

The two SNPs were genotyped by using Taqman assay on the ABI PRISM 7500 Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA). For Glu69Asp, the following two primers were used: 5′-TGT TTG TGT GAC AGT TTC AGC T -3′ (forward) and 5′-TGT CCT CCA GCA CCA GAG TTA TT -3′ (reverse), and two probes: FAM-AT T TCT GGG ACA GGT GGT G for the T allele and HEX- AT G TCT GGG ACA GGT GGT G for the G allele were performed. For Ile350Thr, the following primers were used: 5′-CAC TAT GAA AGG GAA GGA AAC GC -3′ (forward) and 5′ -TCA CCA GGG CAA ATC CAA AC-3′ (reverse), and probes: FAM-TAA CTG ACA T CAC AGC AA for the T allele and HEX- TAACTGACAC CACAGCA for the C allele. The genotypes were automatically determined by the ABI 7500 Sequence Detection Systems software 2.0.1 (***Fig. 1***). We randomly selected 10% of the samples for repeated assays and 30 samples for re-sequencing, and the results were 100% concordant (***Fig. 1***).

Statistical analysis

Chi-square (χ^2) test was performed to assess differences in the frequency distributions of age, age at menarche, menstrual history, family history of cancer and *EME1* genotypes as well as alleles between cases and controls. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit chi-square test in cancer-free controls. The unconditional logistic regression models with or without adjustment for age, age at menarche, menstrual history and family history of cancer were used for calculating crude and adjusted odd ratios (OR) and their 95% confidence intervals (95% CI) for each variant. The PROC ALLELE statistical procedure in SAS/Genetics (SAS Institute Inc., Cary, NC, USA) software was performed to assess the LD of two SNPs. Homogeneity test between stratum-ORs was tested by the Breslow-day test. A multiplicative interaction was suggested when OR 11 > OR 10 × OR 01, in which OR 11 = the OR when both factors were present, OR 01 = the OR when only factor 1 was present, OR 10 = the OR when only factor 2 was present. The study power was calculated by using the PS Software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). The FPRP test was applied to detect false-positive association findings[40]. All tests were analyzed by using the SAS software (version9.3; the SAS Institute, Cary, NC, USA). All statistical tests were 2-sided and $P < 0.05$ was considered statistically significant.

RESULTS

Demographics of the study subjects

The distributions of demographic characteristics of cases and controls are shown in ***Table 1***. There were more subjects who were premenopausal and had a family history of cancer in cases than controls ($P < 0.05$ for both), while there was no significant difference in the distributions of age and age at menarche between cases and controls ($P > 0.05$ for both). Moreover, there were 43 (57.9%) and 441 (59.0%) of cases with positive expression of ER and PR in tumors. According to the American Joint Committee on Cancer (AJCC) staging classifications (2002), 158 (21.1%) cases were in stage Ⅰ, 425 (56.8%) were in stage Ⅱ, 102 (13.7%) were in stage Ⅲ and 63 (8.4%) were in stage Ⅳ.

EME1 genotypes and breast cancer risk

The genotype and allele distributions of the *EME1* variants Glu69Asp and Ile350Thr among the cases and controls are summarized in Table 2. The observed genotype frequencies of them were both in accordance with the Hardy-Weinberg equilibrium ($\chi^2 = 1.924$, $P = 0.165$ for Glu69Asp and $\chi^2 = 0.043$, $P = 0.835$ for Ile-350Thr). Compared with the common Ile/Ile genotype, the Thr/Ile variant genotype had an increased risk of breast cancer (OR = 1.41, 95% CI = 1.07-1.85, $P = 0.016$), and the Thr/Thr variant genotype conferred a much higher risk (OR = 2.70, 95% CI = 1.03-7.09, $P = 0.044$). The increased risk caused by the variant was on a Thr allele-dependent dose-response manner ($P_{trend} = 0.002$). After the two variant genotypes were combined, the Thr genotypes (Thr/Ile + Thr/Thr) contributed to a 1.47-fold risk of breast cancer (OR =

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

Variables	Patients (n = 748)		Controls (n = 778)		P^a	OR (95% CI)
	n	%	*n*	%		
Age (years)						
Mean±SD	48.4±11.3		48.0±11.4	0.491		
< 50	415	55.5	430	55.3	0.934	1.00(ref.)
⩾ 50	333	44.5	348	44.7		0.99(0.81-1.21)
Age at menarche (years)						
Mean±SD	14.7±1.88		14.8±2.07	0.324		
⩽ 14	396	52.9	399	51.3	0.518	1.00(ref.)
> 14	352	47.1	379	48.7		0.94(0.77-1.14)
Menstrual history						
Premenopause	390	52.1	363	46.7	0.033	1.00(ref.)
Menopause	358	47.9	415	53.3		0.80(0.66-0.98)
Family history of cancer						
No	673	90.0	727	93.4	0.014	1.00(ref.)
Yes	75	10.0	51	6.6		1.59(1.10-2.30)
Estrogen receptor status						
Positive	433	57.9				
Negative	315	42.1				
Progesterone receptor status						
Positive	441	59.0				
Negative	307	41.0				
Stages						
I	158	21.1				
II	425	56.8				
III	102	13.7				
IV	63	8.4				

[a]*P* values for a χ^2 test for categorical data and Student´s t test for continues data.

Table 2 **Frequency distribution of genotypes in *EME1* and results of logistic regression analysis for associations with breast cancer risk**

Genotypes	Cases, *n* (%)	Controls[a], *n* (%)	P^b	Crude OR (95% CI)	Adjusted OR (95% CI)[c]
Total number of subjects	748	778			
Total number of alleles	1,496	1,556			
Glu69Asp (rs3760413T > G)					
Glu/Glu	423 (56.5)	454 (58.4)	0.769	1.00 (reference)	1.00 (reference)
Asp/Glu	284 (38.0)	282 (36.3)		1.08 (0.88-1.34)	1.11 (0.90-1.38)
Asp/Asp	41 (5.5)	42 (5.4)		1.05 (0.67-1.64)	1.01 (0.64-1.59)
Trend test *P* value				0.476	0.364
Asp/Glu + Glu/Glu	325 (43.5)	324 (41.6)		1.08 (0.88-1.32)	1.10 (0.90-1.35)
Glu allele	0.245	0.235	0.542		
Ile350Thr (rs12450550T > C)					
Ile/Ile	593 (79.3)	656 (84.3)	0.016	1.00 (reference)	1.00 (reference)
Thr/Ile	141 (18.8)	116 (14.9)		1.35 (1.03-1.76)	1.41 (1.07-1.85)
Thr/Thr	14 (1.9)	6 (0.8)		2.58 (0.99-6.76)	2.70 (1.03-7.09)
Trend test *P* value				0.005	0.002
Thr/Ile + Thr/Thr	155 (20.7)	122 (15.7)		1.41 (1.08-1.83)	1.47 (1.13-1.92)
Thr allele	0.113	0.082	0.004		

[a]The observed genotype frequencies of the two SNPs among the control subjects were both in agreement with the Hardy-Weinberg equilibrium ($P^2+2pq+q^2 = 1$) ($\chi^2 = 1.924$, $P = 0.165$ for Glu69Asp and $\chi^2 = 0.043$, $P = 0.835$ for Ile350Thr).

[b]A two-sided χ^2 test for differences in distribution of genotype frequencies between cases and controls.

[c]Adjusted in a logistic regression model that included age, age at menarche, menstrual history, and family history of cancer.

1.47, 95% CI = 1.13-1.92, $P = 0.005$). However, for the Glu69Asp variant, both genotype and allele frequencies did not differ significantly between cases and controls ($P = 0.769$ and 0.542, respectively). Moreover, there was no significant difference in LD between the two SNPs (Glu69Asp and Ile350Thr).

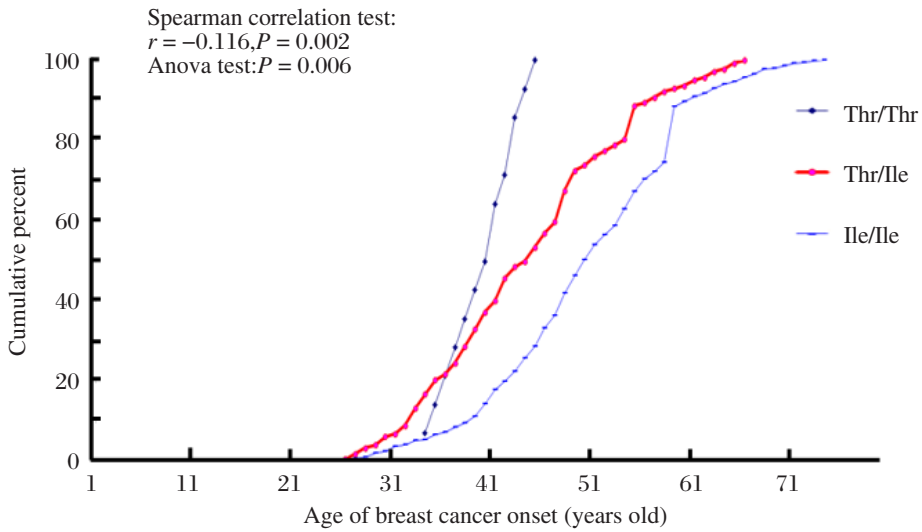

Fig. 2 **Age at onset of breast cancer and the Ile350Thr genotypes.**

Table 3 **Stratification analysis of the *EME1* Ile350Thr genotypes by selected variables in cases and controls**

Variable	Cases (n = 748)		Controls (n = 778)		Crude OR (95%CI)	Adjusted OR (95% CI)[a]	P_{homo}[b]	P_{inter}[c]
	Ile/Ile	Ile/Thr+Thr/Thr	Ile/Ile	Ile/Thr+Thr/Thr	Ile/Thr+Thr/Thr	Ile/Thr+Thr/Thr		
	n	n	n	n	vs. Ile/Ile	vs. Ile/Ile		
Age (years)								
< 50	321	94	364	66	1.62 (1.14-2.29)	1.67 (1.17-2.40)	0.232	0.032
⩾ 50	272	61	292	56	1.17 (0.79-1.74)	1.14 (0.74-1.76)		
Age at menarche (years)								
⩽ 14	315	81	331	68	1.25 (0.88-1.79)	1.36 (0.94-1.97)	0.357	0.421
> 14	278	74	325	54	1.60 (1.09-2.36)	1.60 (1.08-2.37)		
Menstrual history								
Premenopause	313	77	308	55	1.38 (0.94-2.02)	1.58 (1.04-2.41)	0.855	0.552
Menopause	280	78	348	67	1.45 (1.01-2.08)	1.30 (0.89-1.92)		
Family history of cancer								
Yes	60	15	41	10	1.03 (0.42-2.50)	1.08 (0.42-2.75)	0.473	0.356
No	533	140	615	112	1.44 (1.07-1.90)	1.52 (1.15-2.01)		
Estrogen receptor status								
Positive	344	89	656	122	1.39 (1.03-1.88)	1.47 (1.08-2.00)	0.916	
Negative	249	66			1.42 (1.02-1.99)	1.47 (1.05-2.06)		
Progesterone receptor status								
Positive	349	92	656	122	1.42 (1.05-1.92)	1.48 (1.09-2.01)´	0.928	
Negative	244	63			1.39 (0.99-1.95)	1.45 (1.03-2.04)		
Stage								
I	133	25	656	122	1.01 (0.63-1.62)	1.00 (0.62-1.62)	0.398	
II	328	97			1.59 (1.18-2.14)	1.67 (1.23-2.26)		
III	80	22			1.48 (0.89-2.46)	1.51 (0.91-2.53)		
IV	52	11			1.14 (0.58-2.24)	1.18 (0.60-2.33)		

[a]ORs were adjusted for age, age at menarche, menstrual history, and family history of cancer in a logistic regression model.

[b]*P* value of homogeneity test between strata for the related ORs of *EME1* (Ile/Thr+Thr/Thr vs. Ile/Ile).

[c]*P* value of test for the multiplicative interaction between Ile350Thr and selected variables on cancer risk in logistic regression models.

Stratification analysis of *EME1* genotypes and risk of breast cancer

Only the data for the Ile350Thr variant are presented in ***Table 3*** as the Glu69Asp variant had no further significant findings (data not shown). The increased risk of breast cancer caused by Thr variant genotypes were more obvious in younger individuals (aged less than 50 years) than those whose age of menarche were less than 14 years, who were premenopausal and who had no family history of cancer. However, the differences between these stratum-ORs

were not significant (Breslow-day test: $P > 0.05$ for all). Additionally, there was no significant difference in the strata of ER status, PR status and clinical stages. Moreover, there was a significantly negative interaction between the Thr adverse genotypes and age on breast cancer risk ($P = 0.032$).

EME1 variants and early onset of breast cancer

As the association between the Ile350Thr genotypes and breast cancer risk was more obvious in younger individuals, and there was a significant interaction between the Thr variant genotypes and age, we determined the correlation between age at onset of breast cancer and the number of adverse alleles in the cases. The mean age of breast cancer onset was 44.4 years for Thr/Thr genotype carriers and 46.5 years old for Thr/Ile genotype carriers, which was significantly lower than that (49.4 years) for Ile/Ile carriers (***Fig 2***, $P = 0.006$).

DISCUSSION

In the current hospital-based case-control study of 748 patients and 778 controls, we found that the Thr variant genotypes of *EME1* conferred an increased risk and earlier onset of breast cancer in Chinese based a Thr allele-dependent dose-response manner. However, no significant association was observed for Glu69Asp variant and breast cancer risk. To the best of our knowledge, this is the first genotype association study of *EME1* variants and breast cancer risk. The EME1 protein consists of a central nuclease domain, two repeats of the helix-hairpin-helix (HhH) motif at the C-terminal region, a linker helix and a flexible intradomain linker. The C-terminal region is essential for the function of EME1 on DNA repair such as the recognition of DNA and binding to MUS81[32]. The variant Ile350Thr is located in the second HhH region (residues 345-352), which is required for DNA-binding and nuclease activity[32]. To support the biological plausibility of the variant, we performed bioinformatics analysis with the Snpinfo software (http://snpinfo.niehs.nih.gov/snpfunc.html) and found that the Ile350Thr variant belong to exon splicing enhancers (ESEs). This suggested that this variant may influence selective splicing of EME1 and cause the abnormal transcripts of EME1. Consistently, several transcripts of EME1 were observed (http://www.ensembl.org/index.html). Moreover, the SNPs3D software showed that the Ile350Thr variation is unlikely to be tolerable, indicating that the Thr variation is of an unknown biological function, which may be destructive[41]. Therefore, it is biologically conceivable that the Ile350Thr variant conferred an increased risk of breast cancer. However, functional assays are warranted.

Only one study has tested the genetic variants in *EME1*, and reported that the variant Ile350Thr was associated with an increased risk of glioblastoma multiforme[33]. This is consistent with our finding. We also found that the variant was associated with the early onset of breast cancer. Deficient *EME1* can lead to genomic instability and promote tumorigenesis in the early stage of tumor initiation[25]. Thus, it is possible that the variant had effect on the onset of breast cancer. The young age of breast cancer onset among Chinese women is a big challenge of cancer prevention[4]; these findings might provide a valuable genetic marker to predict risk of breast cancer and the early onset of breast cancer in females.

The current study has some advantages. With a relative large sample size, we have achieved a more than 90% study power (two-sided test, $\alpha = 0.10$) to detect an OR of 1.47 for the Thr variant genotypes (which occurred at a frequency of 15.7% in the controls) compared with the Ile/Ile genotype. Additionally, we performed the false-positive report probability (FPRP) analysis and found that under the assumption of a prior probability of 0.01 and a prior OR of 1.50 as suggested by Wacholder et al.[42], the FPRP for the observed association between the Ile350Thr polymorphism and the risk of breast cancer yielded a value of 0.13. It is lower than the pre-set FPRP-level criterion 0.20, suggesting that this finding is noteworthy. Moreover, bioinformatics analysis presented a functional possibility of the variant. All these suggested that our findings of association between the variant Ile350Thr and breast cancer risk as well as early onset is unlikely to be occasional. However, as a hospital-based case-control study, some limitations such as selection bias cannot be avoidable.

In conclusion, our study found that the exon variant Ile350Thr of *EME1* was significantly associated with an increased risk of early onset of breast cancer in Southern Chinese females. It suggested that the variant Ile350Thr may be a genetic marker for susceptibility and the early onset of breast cancer. Validations with larger population-based studies in the different ethnic groups are needed.

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