Combined Effects of Peroxisome Proliferator–Activated Receptor Alpha and Apolipoprotein E Polymorphisms on Risk of Breast Cancer in a Taiwanese Population

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Background: The peroxisome proliferator–activated receptor alpha (PPARA) and apolipoprotein E (APOE) proteins are reported to be correlated with lipid metabolism, cardiovascular disease, and breast cancer.

Methods: We screened *APOE* and *PPARA* (*S24F* and *V227A*) polymorphisms in 306 breast cancer patients and 300 noncancer controls and determined the relationship between their genetic polymorphisms and breast cancer risk. Interactions with clinical characteristics were also examined.

Results: We found that the risk of breast cancer was associated with *APOE* genotypes ($P = 0.014$) but not with *PPARA S24F* or *V227A* genotypes. The combined effects of *F24*/*APOE* genotypes ($P = 0.003$) on breast cancer risk were more significant than the individual effect of *APOE* genotypes ($P = 0.014$). *F24*/*ε4* carriers had a higher tendency to develop breast cancer than *F24*/*ε3* carriers ($P = 0.013$), and this effect is stronger than with individual *ε4* carriers ($P = 0.029$). In addition, both *F24*/*ε4* and *V227*/*ε4* carriers were significantly enriched in the human epidermal growth factor receptor 2/neu negative status.

Conclusions: These findings suggest that the *APOE ε4* genotype plays a major role in the prediction of breast cancer, but the *PPARA F24* mutation enhances this outcome. The combined effects of *F24*/*ε4* genotypes are positively associated with risk of breast cancer.

Key Words: PPAR alpha, Apolipoprotein E, genetic polymorphism, breast cancer

(*J Investig Med* 2012;60: 1209–1213)

Epidemiological studies have shown an association between high-fat diets and high incidence of breast cancer.[1] Previous reports suggest that some lipid-related genes, including estrogen receptor α (*ERα*), apolipoprotein E (*APOE*), and peroxisome proliferator–activated receptor alpha (*PPARA*) are associated with breast cancer risk.[2,3] Apolipoprotein E has 3 functionally distinct isoforms of the protein (E2, E3, and E4) encoded by the corresponding alleles *ε2*, *ε3*, and *ε4* in humans.[4] Our previous study showed that the *ε4* allele was associated with the breast cancer risk and human epidermal growth factor receptor 2 (HER2)/neu negative status in patients with breast cancer.[2] However, different studies show that the presence of an *ε4* allele had neither association with breast cancer risk nor influence on tumor cell proliferation.[5,6] Therefore, the presence of additional genetic variants is suggested to concomitantly influence the risk of breast cancer. Recently, the *PPARA* polymorphism (rs4253760) in intron 6 was suggested to be a candidate for increasing breast cancer risk.[7] However, except for the *L162V* mutation in exon 5, the correlation between *PPARA* variants in other exons and breast cancer risk is still unclear.[7]

The *PPARA* gene is located on chromosome 22 and consists of 8 exons that encode the PPARα protein. PPARα is a ligand-activated transcription factor that regulates lipid metabolism by controlling the gene expression of β-oxidation enzymes, apolipoproteins, and fatty acid transport proteins.[8] In rodent breast cancer models, activation of PPARα by long-chain fatty acids and synthetic ligands has been reported to reduce tumor incidence and progression.[9] In breast cancer cell models, activation of PPARα by arachidonic acid has been reported to stimulate[8] or inhibit[10] breast cancer cell proliferation. Genetic variants of *PPARA* were found to be correlated with lipoprotein levels, cardiovascular disease, obesity, type 2 diabetes, and cancers.[11] In particular, the *L162V* and *V227A* variants of *PPARA* were associated with dyslipidemia.[12,13]

We preliminarily screened the *S24F*, *L162V*, and *V227A* polymorphisms in the *PPARA* gene in 306 patients with breast cancer and 300 cancer-free subjects from previous data collection before 2004.[2] No significant correlation between the *S24F*, *L162V*, and *V227A* variants of *PPARA* and breast cancer risk was found (unpublished data). However, a positive association between PPARα and breast tumorigenesis was reported in isolated rat mammary gland epithelial cells[14] and breast cancer cell lines.[8,15] Therefore, we suggest that the *PPARA* gene may play a synergistic role in the development of breast cancer. In this study, we ask whether there is a possible combined effect of *APOE* and *PPARA* polymorphisms on the risk of breast cancer. To test this hypothesis, the combined effects of *APOE* and *PPARA* polymorphisms (*S24F* and *V227A*) on breast cancer risk were studied. Interactions with clinical characteristics were also examined.

MATERIALS AND METHODS

Study Population

The study population was composed of female Taiwanese ranging in age from 25 to 73 years. A total of 606 subjects were recruited between 2001 and 2004; details of the retrospective study population and data collection methods have been

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Received June 1, 2012, and in revised form July 23, 2012.
Accepted for publication July 24, 2012.

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Supported by research grants from the China Medical University (CMU100-TS-01) and the Taiwan Department of Health, China Medical University Hospital Cancer Research Center of Excellence (DOH101-TD-C-111-005).

Supplemental digital content is available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.jinvestigativemed.com).

ISSN: 1081-5589
DOI: 10.231/JIM.0b013e31826be86a

TABLE 1. Correlation Between Genetic Polymorphisms of *APOE* and *PPARA* and Breast Cancer Risk

	Control (n = 300)	Cancer (n = 306)	X^2 Test (Control vs Cancer)	Logistic Regression Analysis (Adjusted for Age)		
Genotype	Genotype Frequency n (%)		*P*	OR	95% CI	*P*
*APOE**						
ε3 carrier	183 (61.0)	151 (49.0)	0.014	1.0 (ref.)		
ε2 carrier	40 (13.3)	49 (16.0)		1.605	0.957–2.691	0.073
ε4 carrier	77 (25.7)	106 (35.0)		1.559	1.047–2.321	0.029
PPARA S24F (TCT→TTT)†						
S carrier	27 (9.0)	23 (7.5)	0.507	1.0 (ref.)		
F carrier	273 (91.0)	283 (92.5)		0.989	0.529–1.851	0.973
PPARA V227A (GTC→GCC)‡						
V carrier	277 (92.3)	285 (93.1)	0.703	1.0 (ref.)		
A carrier	23 (7.7)	21 (6.7)		0.890	0.453–1.751	0.736

*The *APOE* polymorphism was classified as an *ε2* carrier (2/2, 2/3, and 2/4), an *ε3* carrier (3/3), or an *ε4* carrier (3/4 and 4/4).

†The *PPARA S24F* polymorphism was classified as an *S* carrier (C/C) or an *F* carrier (C/T and T/T).

‡The *PPARA V227A* polymorphism was classified as a *V* carrier (T/T) or an *A* carrier (T/C and C/C).

published previously.[2] Patients with breast cancer patients (n = 306; mean [SD] age, 48.5 [11.3]) were recruited from the China Medical University Hospital and Fong Yuan Hospital. Noncancer controls (n = 300; mean [SD] age, 41.3 [10.5]) were recruited from the Taichung Blood Center and China Medical University Hospital.[2] This study was approved by the Institutional Review Board of the China Medical University Hospital.

Polymorphism Analysis

Genomic DNA was extracted from whole blood by using the Viogene isolation kit (Viogene, Taiwan). *APOE* genotyping was performed according to the previous report.[2] The polymorphism *V227A* (rs1800234), which is located on exon 6 of *PPARA,* was analyzed using polymerase chain reaction (PCR) restriction fragment length polymorphism analysis. The amplicon was generated with the following PCR primers: forward primer (5′-TCCATAGTGGAAAGCCGA-3′) and reverse primer (5′-TTTCCATCTTCGCGGTCCTT-3′). The PCR product was digested by the *Sau*96I (New England Biolabs, Ipswich, MA) restriction enzyme. Restriction fragments were separated on a 3% agarose gel. The *S24F* polymorphism (TCT/serine to TTT/phenylalanine) is caused by a genetic mutation at exon 3 and does not alter any restriction site; a mismatch PCR restriction fragment length polymorphism method was therefore used to genotype all the individuals. The amplicon was generated with the following PCR primers: forward primer (5′-GATCTAGA GAGCCCGTGAT-3′) and reverse primer (5′-GATGGAGACCA TCCTGGCTA-3′). The mismatched site, with G substituted for T, is located on the nucleotide 17 of forward primer. Accordingly, the PCR products generated from the mutants cannot be digested by the *Dpn*II (New England Biolabs) restriction enzyme. Restriction fragments were separated on a 3% agarose gel.

Statistical Analysis

All data were analyzed using SPSS version 15.0 for Windows. The genotypes of patients with breast cancer and noncancer controls were compared using the χ^2 test and the Fisher exact test. Effects of the *PPARA* and *APOE* interaction on clinical characteristics in patients with breast cancer were analyzed using the χ^2 test and the Fisher exact test. Logistic regression analysis was performed to test the association of gene polymorphisms with both breast cancer risk and measures of clinical characteristics after adjustment for age. $P < 0.05$ was considered statistically significant.

RESULTS

Correlation Between Genetic Polymorphisms of *APOE* and *PPARA* and Breast Cancer Risk

The genotypes of *PPARA* and *APOE* are shown in Table 1. The *S24F* polymorphism of *PPARA* was classified as an *S24* carrier (C/C) or an *F24* carrier (C/T and T/T). The *V227A* polymorphism was classified as a *V227* carrier (T/T) or an *A227* carrier (T/C and C/C). The *APOE* polymorphism was classified as an *ε2* carrier (2/2, 2/3, or 2/4), an *ε3* carrier (3/3), or an *ε4* carrier (3/4 or 4/4). First, we evaluated whether only one gene variant was correlated with breast cancer risk (Table 1). We found that neither the *S24F* ($P = 0.507$) nor the *V227A* ($P = 0.703$) polymorphism was associated with breast cancer risk. A significant difference in *APOE* genotype levels between the patients with breast cancer and the noncancer controls was observed ($P = 0.014$). We then used logistic regression to explore the correlation between the *APOE* polymorphisms and the breast cancer risk after adjustment for age (Table 1). Because the *APOE* mutation types are classified as either wild type (*ε3*) or mutation type (*ε2* and *ε4*), all logistic regression analyses compare the mutation type with the wild type. The results showed that *ε4* carriers had a higher risk of breast cancer in comparison to *ε3* carriers ($P = 0.029$).

Combined Genetic Effects of *S24F, V227A,* and *APOE* Polymorphisms on Breast Cancer Risk

The combined genetic effects of 2 gene variants (*PPARA*/*APOE*) on the risk for breast cancer were measured (Table 2). The combined effect of *S24*/*APOE* carriers showed no significant association with breast cancer ($P = 0.493$), whereas that of *F24*/*APOE* carriers had a significant effect on breast cancer ($P = 0.003$). This suggested that there was a statistically significant interaction effect of *S24F* and *APOE* on breast cancer. A similar result was also found in the interaction between *V227A* and *APOE*. A significant correlation between *V227*/*APOE* carriers and breast cancer was found ($P = 0.012$), whereas no significant correlation between *A227*/*APOE* and breast cancer was observed ($P = 0.772$). These findings suggested that both *F24* and *V227* carriers enhanced the correlation between *APOE*

TABLE 2. Combined Genetic Effects of *PPARA*/*APOE* Polymorphisms on Breast Cancer Risk

Genotype	n (%)		X^2 (Control vs Cancer)	Logistic Regression Analysis (Adjusted for Age)		
*S24F**/*APOE*† Genotypes						
S Carrier	Control	Cancer	*P*	OR	95% CI	*P*
S24/ε3	14 (51.9)	15 (65.2)	0.493	1.0 (ref.)		
S24/ε2	7 (25.9)	3 (13.0)		0.517	0.095–2.817	0.446
S24/ε4	6 (22.2)	5 (21.7)		0.801	0.152–4.237	0.794
F Carrier						
F24/ε3	169 (61.9)	135 (47.7)	0.003	1.0 (ref.)		
F24/ε2	33 (12.1)	46 (16.3)		1.785	1.029–3.095	0.039
F24/ε4	71 (26.0)	102 (36.0)		1.683	1.116–2.537	0.013
V227A‡/*APOE* Genotypes						
V Carrier						
V227/ε3	173 (62.5)	143 (50.2)	0.012		1.0 (ref.)	
V227/ε2	34 (12.3)	42 (14.7)		1.579	0.902–2.763	0.110
V227/ε4	70 (25.3)	100 (35.1)		1.648	1.090–2.492	0.018
A Carrier						
A227/ε3	10 (43.5)	7 (33.3)	0.772	1.0 (ref.)		
A227/ε2	6 (26.1)	7 (33.3)		0.852	0.192–3.786	0.833
A227/ε4	7 (30.4)	7 (33.3)		1.191	0.246–5.764	0.828
F24/V227/APOE Genotypes						
F24/V227/ε3	160 (63.5)	130 (49.2)	0.005	1.0 (ref.)		
F24/V227/ε2	28 (11.1)	40 (15.2)		1.793	0.992–3.239	0.503
F24/V227/ε4	64 (25.4)	94 (35.6)		1.704	1.110–2.616	0.015

*The *S24F* polymorphism of *PPARA* was classified as an *S* carrier (C/C) or an *F* carrier (C/T and T/T).

†The *APOE* polymorphism was classified as an *ε2* carrier (2/2, 2/3, and 2/4), an *ε3* carrier (3/3), or an *ε4* carrier (3/4 and 4/4).

‡The *V227A* polymorphism of *PPARA* was classified as a *V* carrier (T/T) or an *A* carrier (T/C and C/C).

genotypes and breast cancer. The *F24*/*APOE* carriers in particular showed an increased risk of breast cancer with a *P* value changing from 0.014 to 0.003. Logistic regression analysis showed that *F24*/*ε4* genotypes were associated with an increased risk of breast cancer ($P = 0.013$) in comparison to *F24*/*ε3* genotypes. Additionally, *F24*/*ε2* carriers showed an increased risk of breast cancer ($P = 0.039$). Patients with *V227*/*ε4* genotypes also had a significant influence on the risk of breast cancer ($P = 0.018$, *V227*/*ε4* vs *V227*/*ε3*). In addition, when the *F24*/*V227* genotypes and *APOE* polymorphisms were combined, an additive effect on breast cancer risk was observed in *F24*/*V227*/*ε4* genotypes ($P = 0.015$, *F24*/*V227*/*ε4* vs *F24*/*V227*/*ε3*).

Association of *F24*/*APOE* and *V227*/*APOE* Carriers With Clinical Characteristics

The associations of *F24*/*APOE* and *V227*/*APOE* genotypes with clinical characteristics are shown in Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/JIM/A11). No statistically significant relationship was found between *F24*/*APOE* genotypes and clinical characteristics including menopause, tumor grade, TNM classification, estrogen receptor, progesterone receptor, associated ductal carcinoma in situ, and lymphatic invasion ($P > 0.05$). However, the presence or absence of HER2/neu was associated with *F24*/*APOE* ($P = 0.006$) and *V227*/*APOE* ($P = 0.005$) polymorphisms in patients with breast cancer. Logistic regression analysis showed that patients with *F24*/*ε4* genotypes were also significantly enriched in HER2/neu negative status ($P = 0.007$, *F24*/*ε4* vs *F24*/*ε3*; Table 3). Similar results were also found in patients with *V227*/*ε4* genotypes ($P = 0.005$, *V227*/*ε4* vs *V227*/*ε3*). However, 2 gene polymorphisms (*F24*/*APOE*, $P = 0.005$; *V227*/*APOE*, $P = 0.007$) had no additive effect on HER2/neu status over one gene polymorphism (*APOE*, $P = 0.005$).

DISCUSSION

In this study, we found that the *F24*/*ε4* and *V227*/*ε4* genotypes were associated with an increased risk of breast cancer. According to χ^2 test, the combined effects of the *F24*/*APOE* polymorphisms on breast cancer risk showed more statistical significance ($P = 0.003$; Table 2) than the single effect of *APOE* polymorphisms ($P = 0.014$; Table 1). Logistic regression analysis showed that subjects with *F24*/*ε4* genotypes ($P = 0.013$; Table 2) had a higher tendency to develop breast cancer than subjects with only an *ε4* genotype ($P = 0.029$; Table 1). A higher risk tendency was also found in *F24*/*ε2* carriers ($P = 0.039$; Table 2) than in only *ε2* carriers ($P = 0.073$; Table 1). Notably, no significant relationship between *S24F* polymorphisms and breast cancer risk was found with any single mutation of *PPARA* codon 24. This indicated that *S24F* polymorphisms did not directly link to breast cancer. These findings suggested that the *APOE* mutation plays a major role in the prediction of breast cancer and the *PPARA F24* mutation enhances this outcome.

We are the first team to study the *S24F* polymorphism (TCT/serine to TTT/phenylalanine) in the *PPARA* gene. The T-allele frequency occurred in 79.1% of the breast cancer cases and 77.5% of the noncancer controls. The genotype frequency of C/T and T/T (F carrier) occurred in 92.5% of the breast cancer cases and 91.0% of the noncancer controls (Table 1). The *S24F*

TABLE 3. Correlation Between Genetic Polymorphisms of *PPARA*/*APOE* and HER2/neu Status

	Total Number	HER2/neu, n (%)		Logistic Regression Analysis		
Genotype	**n**	**Negative**	**Positive**	**OR**	**95% CI**	***P***
*APOE**						
ε3	87	53 (49.5)	34 (65.4)	1.0 (ref.)		
ε2	25	14 (13.1)	11 (21.1)	1.225	0.498–3.011	0.695
ε4	47	40 (37.4)	7 (13.5)	0.273	0.110–0.679	0.005
F24/*APOE*†						
F24/*ε3*	77	47 (48.0)	30 (62.5)	1.0 (ref.)		
F24/*ε2*	23	12 (12.2)	11 (22.9)	1.436	0.562–3.668	0.449
F24/*ε4*	46	39 (39.8)	7 (14.6)	0.281	0.111–0.710	0.007
V227/*APOE*‡						
V227/*ε3*	87	52 (51.5)	34 (68.0)	1.0 (ref.)		
V227/*ε2*	22	12 (11.9)	10 (20.0)	1.295	0.496–3.276	0.615
V227/*ε4*	43	37 (36.6)	6 (12.0)	0.248	0.095–0.651	0.005

*The *APOE* polymorphism was classified as an *ε2* carrier (2/2, 2/3, and 2/4), an *ε3* carrier (3/3), or an *ε4* carrier (3/4 and 4/4).

†*F24* polymorphism of *PPARA* was classified as C/T and T/T carriers.

‡*V227* polymorphism of *PPARA* was classified as a T/T carrier.

mutation is located on the A/B region of the PPARα protein, which has a ligand-independent activating function (AF-1).[16] Deletion of the first 25 amino acid residues from the N-terminal side was reported to completely remove the AF-1 activity.[16] Therefore, we hypothesized that the *F24*/*ε4* mutation correlated with an increased risk of breast cancer might link to change AF-1 activity. It is possible that Ser24 mutating to Phe24 might cause a structure change in the α-helix in the A/B region or a change in the AF-1 activity. However, we need more evidence to prove this hypothesis in subsequent studies.

Furthermore, *V227*/*ε4* genotypes were also associated with an increased risk of breast cancer (Table 2). The *V227A* polymorphism is located on the hinge region of *PPARA*, which can enhance recruitment of the nuclear receptor corepressor and attenuate transactivation functions.[13] Most studies reported that the *V227A* polymorphism was associated with dyslipidemia,[12,13] and few reports focused on the prediction of breast cancer. In this study, we found that the *V227A* polymorphism was not correlated with the risk of breast cancer (Table 1). In addition, the statistical results between the *V227*/*APOE* carriers ($P = 0.012$) and the *APOE* carriers ($P = 0.014$) showed only a small change in *P* value. Therefore, we suggested that *V227A* polymorphism might not be a direct cause of breast cancer.

No statistically significant relationship was found between *F24*/*APOE* carriers and any clinical characteristic except the absence of HER2/neu, which showed a positive correlation ($P = 0.006$; Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/JIM/A11). A similar result was also found in *V227*/*APOE* carriers ($P = 0.006$; Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/JIM/A11). HER2/neu negative status in patients with breast cancer may favorably influence response to adjuvant tamoxifen therapy.[16] *F24*/*ε4* carriers enriched in the HER2/neu negative status might provide useful information in predicting the response to hormone therapy or HER2/neu–targeted therapy in patients with breast cancer.

CONCLUSIONS

This study indicates that the combined effects of the *F24*/*APOE* polymorphisms on breast cancer risk show more statistical significance than the single effect of *APOE* polymorphisms. Subjects with *F24*/*ε4* genotypes present an especially high tendency for breast cancer risk.

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