

# 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

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Epidemiological studies have shown an association between high-fat diets and high incidence of breast cancer.<sup>1</sup> Previous reports suggest that some lipid-related genes, including estrogen receptor  $\alpha$  (*ER $\alpha$ ), apolipoprotein E (*APOE*), and peroxisome proliferator–activated receptor alpha (*PPARA*) are associated with breast cancer risk.<sup>2,3</sup> Apolipoprotein E has 3 functionally*

distinct isoforms of the protein (E2, E3, and E4) encoded by the corresponding alleles  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  in humans.<sup>4</sup> Our previous study showed that the  $\epsilon 4$  allele was associated with the breast cancer risk and human epidermal growth factor receptor 2 (HER2)/neu negative status in patients with breast cancer.<sup>2</sup> However, different studies show that the presence of an  $\epsilon 4$  allele had neither association with breast cancer risk nor influence on tumor cell proliferation.<sup>5,6</sup> 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.<sup>7</sup> However, except for the *L162V* mutation in exon 5, the correlation between *PPARA* variants in other exons and breast cancer risk is still unclear.<sup>7</sup>

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

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.<sup>2</sup> 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 PPAR $\alpha$  and breast tumorigenesis was reported in isolated rat mammary gland epithelial cells<sup>14</sup> and breast cancer cell lines.<sup>8,15</sup> 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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**TABLE 1.** Correlation Between Genetic Polymorphisms of *APOE* and *PPARA* and Breast Cancer Risk

Genotype	Control (n = 300) Genotype Frequency n (%)	Cancer (n = 306) Genotype Frequency n (%)	X <sup>2</sup> Test (Control vs Cancer) <i>P</i>	Logistic Regression Analysis (Adjusted for Age)		
				OR	95% CI	<i>P</i>
<i>APOE</i> *						
$\epsilon 3$ carrier	183 (61.0)	151 (49.0)	0.014	1.0 (ref.)		
$\epsilon 2$ carrier	40 (13.3)	49 (16.0)		1.605	0.957–2.691	0.073
$\epsilon 4$ carrier	77 (25.7)	106 (35.0)		1.559	1.047–2.321	0.029
<i>PPARA S24F</i> (TCT→TTT)†						
<i>S</i> carrier	27 (9.0)	23 (7.5)	0.507	1.0 (ref.)		
<i>F</i> carrier	273 (91.0)	283 (92.5)		0.989	0.529–1.851	0.973
<i>PPARA V227A</i> (GTC→GCC)‡						
<i>V</i> carrier	277 (92.3)	285 (93.1)	0.703	1.0 (ref.)		
<i>A</i> carrier	23 (7.7)	21 (6.7)		0.890	0.453–1.751	0.736

\*The *APOE* polymorphism was classified as an  $\epsilon 2$  carrier (2/2, 2/3, and 2/4), an  $\epsilon 3$  carrier (3/3), or an  $\epsilon 4$  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.<sup>2</sup> 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.<sup>2</sup> 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.<sup>2</sup> 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'-TTTCCATCTTCGCGTCTT-3'). The PCR product was digested by the *Sau96I* (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 *DpnII* (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  $\chi^2$  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  $\chi^2$  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  $\epsilon 2$  carrier (2/2, 2/3, or 2/4), an  $\epsilon 3$  carrier (3/3), or an  $\epsilon 4$  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 ( $\epsilon 3$ ) or mutation type ( $\epsilon 2$  and  $\epsilon 4$ ), all logistic regression analyses compare the mutation type with the wild type. The results showed that  $\epsilon 4$  carriers had a higher risk of breast cancer in comparison to  $\epsilon 3$  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 <sup>2</sup> (Control vs Cancer)	Logistic Regression Analysis (Adjusted for Age)		
				OR	95% CI	P
<i>S24F</i> */ <i>APOE</i> † Genotypes						
<i>S</i> Carrier	Control	Cancer	<i>P</i>	OR	95% CI	<i>P</i>
<i>S24/ε3</i>	14 (51.9)	15 (65.2)	0.493	1.0 (ref.)		
<i>S24/ε2</i>	7 (25.9)	3 (13.0)		0.517	0.095–2.817	0.446
<i>S24/ε4</i>	6 (22.2)	5 (21.7)		0.801	0.152–4.237	0.794
<i>F</i> Carrier						
<i>F24/ε3</i>	169 (61.9)	135 (47.7)	0.003	1.0 (ref.)		
<i>F24/ε2</i>	33 (12.1)	46 (16.3)		1.785	1.029–3.095	0.039
<i>F24/ε4</i>	71 (26.0)	102 (36.0)		1.683	1.116–2.537	0.013
<i>V227A</i> ‡/ <i>APOE</i> Genotypes						
<i>V</i> Carrier						
<i>V227/ε3</i>	173 (62.5)	143 (50.2)	0.012		1.0 (ref.)	
<i>V227/ε2</i>	34 (12.3)	42 (14.7)		1.579	0.902–2.763	0.110
<i>V227/ε4</i>	70 (25.3)	100 (35.1)		1.648	1.090–2.492	0.018
<i>A</i> Carrier						
<i>A227/ε3</i>	10 (43.5)	7 (33.3)	0.772	1.0 (ref.)		
<i>A227/ε2</i>	6 (26.1)	7 (33.3)		0.852	0.192–3.786	0.833
<i>A227/ε4</i>	7 (30.4)	7 (33.3)		1.191	0.246–5.764	0.828
<i>F24/V227/APOE</i> Genotypes						
<i>F24/V227/ε3</i>	160 (63.5)	130 (49.2)	0.005	1.0 (ref.)		
<i>F24/V227/ε2</i>	28 (11.1)	40 (15.2)		1.793	0.992–3.239	0.503
<i>F24/V227/ε4</i>	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  $\epsilon 2$  carrier (2/2, 2/3, and 2/4), an  $\epsilon 3$  carrier (3/3), or an  $\epsilon 4$  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 Supplemental 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  $\chi^2$  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  $\epsilon 4$  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  $\epsilon 2$  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

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

\*The *APOE* polymorphism was classified as an  $\epsilon 2$  carrier (2/2, 2/3, and 2/4), an  $\epsilon 3$  carrier (3/3), or an  $\epsilon 4$  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 *PPAR $\alpha$*  protein, which has a ligand-independent activating function (AF-1).<sup>16</sup> Deletion of the first 25 amino acid residues from the N-terminal side was reported to completely remove the AF-1 activity.<sup>16</sup> 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  $\alpha$ -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.<sup>13</sup> Most studies reported that the *V227A* polymorphism was associated with dyslipidemia,<sup>12,13</sup> 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.<sup>16</sup> *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.

## REFERENCES

- Brewster A, Helzlsouer K. Breast cancer epidemiology, prevention, and early detection. *Curr Opin Oncol*. 2001;13:420–425.
- Chang NW, Chen DR, Wu CT, et al. Influences of apolipoprotein E polymorphism on the risk for breast cancer and *HER2/neu* status in Taiwan. *Breast Cancer Res Treat*. 2005;90:257–261.
- Galliechio L, McSorley MA, Newschaffer CJ, et al. Body mass, polymorphisms in obesity-related genes, and the risk of developing breast cancer among women with benign breast disease. *Cancer Detect Prev*. 2007;31:95–101.
- Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis*. 1988;8:1–21.
- Zunarelli E, Nicoll JA, Migaldi M, et al. Apolipoprotein E polymorphism and breast carcinoma: correlation with cell proliferation indices and clinical outcome. *Breast Cancer Res Treat*. 2000;63:193–198.
- Chang SJ, Hou MF, Tsai SM, et al. Association between the apolipoprotein E genotypes and breast cancer patients in Taiwanese. *Breast Cancer Res Treat*. 2006;98:109–113.
- Golembesky AK, Gammon MD, North KE, et al. Peroxisome proliferator-activated receptor- $\alpha$  (*PPARA*) genetic polymorphisms and breast cancer risk: a Long Island ancillary study. *Carcinogenesis*. 2008;29:1944–1949.
- Suchanek KM, May FJ, Robinson JA, et al. Peroxisome proliferator-activated receptor  $\alpha$  in the human breast cancer cell lines MCF-7 and MDA-MB-231. *Mol Carcinog*. 2002;34:165–171.
- Gimble JM, Pighetti GM, Lerner MR, et al. Expression of peroxisome proliferator activated receptor mRNA in normal and tumorigenic rodent mammary glands. *Biochem Biophys Res Commun*. 1998;253:813–817.
- Bocca C, Bozzo F, Martinasso G, et al. Involvement of *PPA*  $\alpha$  in the growth inhibitory effect of arachidonic acid on breast cancer cells. *Br J Nutr*. 2008;100:739–750.

11. Fidaleo M. Human health risk assessment for peroxisome proliferators: more than 30 years of research. *Exp Toxicol Pathol.* 2009;61:215–221.
12. Chan E, Tan CS, Deurenberg-Yap M, et al. The V227A polymorphism at the PPARA locus is associated with serum lipid concentrations and modulates the association between dietary polyunsaturated fatty acid intake and serum high density lipoprotein concentrations in Chinese women. *Atherosclerosis.* 2006;187:309–315.
13. Liu MH, Li J, Shen P, et al. A natural polymorphism in peroxisome proliferator-activated receptor-alpha hinge region attenuates transcription due to defective release of nuclear receptor corepressor from chromatin. *Mol Endocrinol.* 2008;22:1078–1092.
14. Roberts-Thomson SJ, Snyderwine EG. Characterization of peroxisome proliferator-activated receptor alpha in normal rat mammary gland and 2-amino-1-methyl-6-phenylimidazo[4, 5-b]pyridine-induced mammary gland tumors from rats fed high and low fat diets. *Toxicol Lett.* 2000;118:79–86.
15. Razanamahefa L, Prouff S, Bardon S. Stimulatory effect of arachidonic acid on T-47D human breast cancer cell growth is associated with enhancement of cyclin D1 mRNA expression. *Nutr Cancer.* 2000;38:274–280.
16. Hi R, Osada S, Yumoto N, et al. Characterization of the amino-terminal activation domain of peroxisome proliferator-activated receptor alpha. Importance of alpha-helical structure in the transactivating function. *J Biol Chem.* 1999;274:35152–35158.