RESEARCH ARTICLE

Lack of Effects of Peroxisome Proliferator-Activated Receptor Gamma Genetic Polymorphisms on Breast Cancer Risk: a Case-Control Study and Pooled Analysis

Boyoung Park[1], Aesun Shin[2], Kyee-Zu Kim[2],Yeon-Su Lee[3], Jung-Ah Hwang[3], Yeonju Kim[4], Joohon Sung[5], Keun-Young Yoo[2], Eun-Sook Lee[6]*

Abstract

A growing body of evidence suggests that the peroxisome proliferator-activated receptor-gamma (PPARγ) gene may harbor targets for the chemoprevention of breast cancer. However, it is unclear whether polymorphisms in the PPARγ gene are associated with the susceptibility of breast cancer. We performed a candidate gene association study between PPARγ polymorphisms and breast cancer and a meta-analysis on the association of breast cancer with selected PPARγ variants. Six single nucleotide polymorphisms (SNPs) in the PPARγ gene were analyzed among 456 breast cancer patients and 461 controls from the National Cancer Center in Korea. Association between the polymorphisms and breast cancer risk were assessed using the Cochrane-Armitage test for trend and a multivariate logistic regression model. Two SNPs, rs3856806 and rs1801282, had been previously analyzed, thus enabling us to perform pooled analyses on their associations with breast cancer susceptibility. Our findings from the candidate gene association study showed no association between the PPARγ gene polymorphisms and breast cancer risk. A meta-analysis combining existing studies and our current study also refuted an association of the PPARγ gene with breast cancer. Our findings suggest that the PPARγ gene may not harbor variants that alter breast cancer susceptibility, although a moderate sample size might have precluded a decisive conclusion.

Keywords: PPARγ - single nucleotide polymorphisms - breast cancer - susceptibility

Asian Pac J Cancer Prev, **15 (21)**, 9093-9099

Introduction

Breast cancer is the most frequently occurring cancer and the most common cause of cancer death among women worldwide; breast cancer accounted for 23% of new cancer cases and 14% of cancer deaths in 2008 (Jemal et al., 2011). It has been suggested that environmental risk factors such as lifestyle, hormonal and reproductive factors, and exposure to chemical carcinogens explain 30-50% of cases; hereditary factors such as high-penetrance susceptibility BRCA 1/2 mutations cause 5-10% of cases; and the other 40-65% can be attributed to unknown factors, such as gene-environment infteractions (Yoo et al., 2006; Park et al., 2009; Yanhua et al., 2012; Mahdi et al., 2013).

Obesity is an alleged risk factor for the development of postmenopausal breast cancer (Calle and Kaaks, 2004; Carmichael and Bates, 2004; Ronco et al., 2012; Sangrajrang et al., 2013). Hypotheses to explain this association include the increased production of estrogen in adipose tissue, increased circulating insulin and insulin-like growth factor related to metabolic syndrome, and the tumorigenesis function of adipokines from fat tissue (Lorincz and Sukumar, 2006). Additionally, type 2 diabetes has been suggested to be associated with breast cancer risk (Ronco et al., 2012; Abbastabar et al., 2013). A recent meta-analysis showed that women with type 2 diabetes were 27% more likely to develop breast cancer than other women; even after adjustment for body mass index, a 16% increased risk was still observed (Boyle et al., 2012). These results suggest that the hyperinsulinemia associated with obesity and insulin resistance might be carcinogenic to breast tissue (Minatoya et al., 2013).

Peroxisome proliferator-activated receptor-gamma (PPARγ), a member of the nuclear hormone receptor superfamily, is a transcription factor that plays a major role in lipogenesis, adipogenesis, glucose homeostasis, insulin sensitization, and inflammatory cytokine production (Spiegelman, 1998; He, 2009). PPARγ has been found in

*[1]National Cancer Control Institute, [6]Center for Breast Cancer, [3]Cancer Genomics Branch, National Cancer Center, Gyeonggi-do, [5]Department of Epidemiology and Institute of Health and Environment, School of Public Health, [2]Department of Preventive Medicine, College of Medicine, South Korea, Seoul National University, Korea, [4]Cancer Epidemiology Program, University of Hawai'i Cancer Center, Honolulu, Hawaii, USA *For correspondence: eslee@ncc.re.kr*

various tissues, including endothelial tissue (Kaplan et al., 2007) and normal and malignant epithelium (Mueller et al., 1998), but is expressed at the highest levels in adipose tissues (Auwerx, 1999). Given the role of obesity and insulin insensitivity in breast carcinogenesis, it has been suggested that polymorphisms in the PPARγ gene might be related to the development of breast cancer (Kotta-Loizou et al., 2012). Previous studies showed that PPARγ inhibited the development of preneoplastic lesions in mouse mammary tissue (Mehta et al., 2000), and application of a PPARγ agonist reduced the rate of tumor growth and reversed the malignant phenotype in animal mammary models (Mueller et al., 1998). In human breast carcinoma tissues, a PPARγ activator inhibited the estrogen-mediated proliferation of cancer cells, suggesting PPARγ modulates estrogenic action in human breast cancer cells (Suzuki et al., 2006). In addition, modest efficacy of PPARγ as a chemopreventive target was observed in some cancers, such as prostate cancer and thyroid cancer, and PPARγ agonists inhibited tumor progression in a variety of cancer patients, including breast cancer, colon cancer, or prostate cancer patients, in clinical trials (Grommes et al., 2004; Peters et al., 2012).

The PPARγ polymorphism that has been examined most often in epidemiological studies is PPARγ Pro12 Ala (rs1801282); however, the study populations were restricted to Caucasians, and the results were inconsistent. One study showed an inverse association between PPARγ Pro12 Ala and breast cancer risk (Vogel et al., 2007), while another study showed an increased risk of breast cancer if the patient harbored the PPARγ Pro12 Ala polymorphism (Fratiglioni and Wang, 2007). However, most studies presented no significant association between breast cancer risk and the PPARγ Pro12 Ala polymorphism (Memisoglu et al., 2002; Gallicchio et al., 2007; Justenhoven et al., 2008). A study conducted in East Asia investigated three polymorphisms in the PPARγ gene and suggested that there was no significant effect of the individual PPARγ polymorphisms on breast cancer risk, whereas haplotype analysis showed a significant result (Wu et al., 2011). These discrepancies might be caused by insufficient statistical power due to small sample sizes. Meta-analysis may be a useful way to increase the statistical power by combining the sample sizes of individual studies.

Therefore, we investigated the association between PPARγ genetic polymorphisms and breast cancer risk in an East Asian population and performed a pooled analysis using our results and the results from other studies to analyze the association.

Materials and Methods

1) Case-control study

Study participants

The study participants were recruited from the National Cancer Center in Korea. Subjects consisted of 456 female breast cancer patients who were diagnosed at the Center for Breast Cancer between September 2001 and December 2005 and 461 female control patients who participated in a cancer screening program between August 2002 and December 2005. Controls were matched to the age distribution of the cases in 5-year age groups. Information on menstrual factors such as age at menarche, menopause, and age at menopause, as well as the patient's reproductive history, was obtained using a self-administered questionnaire. Height and weight were measured, and body mass index (BMI) was calculated. All participants provided written informed consent, and the study protocol was approved by the Institutional Review Board of the National Cancer Center.

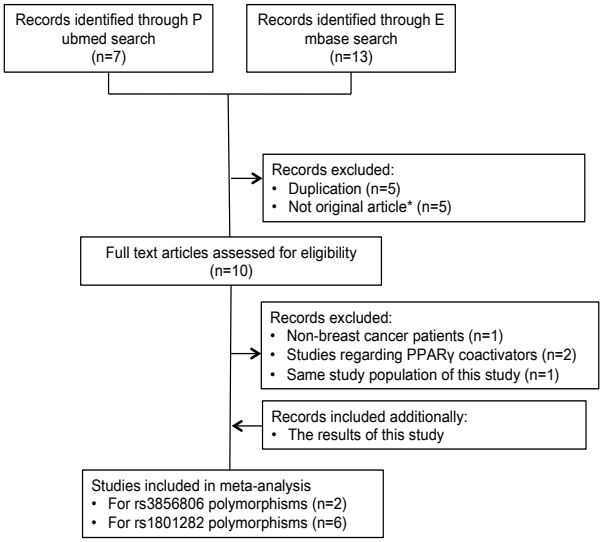

Figure 1. Flow Chart of Meta-analysis for Exclusion/ Inclusion of the Studies. *Including 1 review Article, 2 Abstracts, 1 Conference Scene, and 1 Meeting Report

Genotyping

Six functional single nucleotide polymorphisms (SNPs) in the PPARγ gene (rs4684846, rs1801282, rs2120825, rs2938395, rs1175540, and rs3856806) were selected from previous studies (Paynter et al., 2004; Koh et al., 2006; Vogel et al., 2007). We designed the multiplex PCR and extended primers using the MassARRAY Assay Design software version 3.0 (Sequenom, CA, USA). Genomic DNA was isolated from buffy coats using a DNA Blood Midi M48 Kit (Qiagen, Inc., CA, USA), and 10 ng DNA from each sample was used in the genotyping reaction. The iPLEX Gold assay on the MassARRAY platform (Sequenom), which is based on MALDI-TOF spectrometry, was used for genotyping. The conditions of the PCR and single base extension were the same as previously described (Yoo et al., 2012). To assess the quality of the genotyping assay, 10% of the total samples were run as duplicates, and a concordance test was performed. To check the fitness, genotype clusters were examined manually. Genotype results were collected using the Typer software (Sequenom, version 4.0).

Statistical analysis

The characteristics of the breast cancer cases and controls were compared using a t-test or a chi-square test. The genotype frequencies between the case group and the control group for each of the 2 x 3 contingency tables in the additive model were compared using the Cochrane-Armitage trend test. For the dominant and recessive model, a chi-square test was applied. To estimate

Table 1. Characteristics of the Study Subjects in a Breast Cancer Case-control Study in Korea

Variables	Cases (N=456)	Controls (N=461)	P-value[1]
Age (≤49 years) (N, %)	294(64.5)	296(64.2)	0.93
Age at menarche (years) (Mean±SD)	15.2±1.6	14.9±1.9	0.02
Menopausal status (N, %)	160(35.1)	194(42.1)	0.06
Age at menopause (years) (Mean±SD)	48.1±5.6	47.9±6.3	0.82
Nulliparous (N, %)	48 (10.5)	33 (7.2)	0.05
Number of children (≥3) (N, %)	103 (22.6)	133 (28.9)	0.11
Body mass index (<25.0 kg/m2) (N, %)	305(66.9)	353 (76.6)	<0.01

[1] Frequency was compared using Chi-square test, and means were compared using T-test.

the risk of each SNP in the PPARγ gene, multiple logistic regressions were applied, and the results were presented with odds ratio (OR) and 95% confidence intervals (CI). In the minimally adjusted model, the only variable in this model that was adjusted for was age. In addition to age, the variables with a statistical significance value less than 0.1 in the univariate analysis were adjusted. These included age at menarche, menopause status, hormone replacement therapy, history of being pregnant, number of children, and BMI. We applied dominant, recessive, and codominant models. We used the SAS software version 9.1 (SAS Institute Inc., Cary, NC) for the statistical analyses of the case-control study.

2) Meta-analysis

Literature search and data extraction

Literature databases, including PubMed Central and Embase, were searched comprehensively with combinations of the following keywords: "peroxisome proliferator-activated receptor gamma" or "PPARγ", "genotype", "polymorphism", "variant" or "variation", "breast", and "cancer" or "carcinoma". The search was limited to human studies, articles related to breast cancer, and articles written in English. If multiple articles based on the same study population were identified, the study that contained the larger sample size was selected. After removing duplicated articles from the two databases, 15 articles remained.

The studies included in the meta-analysis met all of the following inclusion criteria: (1) independent case-control or cohort studies that evaluated the associations between SNPs in the PPARγ gene and the risk of breast cancer; (2) sufficient data for the calculation of crude OR with CI; (3) breast cancer cases regardless of stage, hormone receptor status, menopausal status, and histological type; and (4) articles written in English. Exclusion criteria included the following: (1) not an original article (n=5); (2) non-breast cancer patients (n=1); (3) studies regarding PPARγ coactivators (n=2); and (4) a duplicated population of the current case-control study (n=1) (Kim et al., 2012). Additionally, the results from this case-control study were included. Therefore, two articles including this study were analyzed for rs3856806, and six articles including this study were analyzed for rs1801282 (Figure 1). The following information was extracted from each study: the first author's name, year of publication, country, ethnicity, source of controls, menopausal status, and the number of subjects in each genotype in the cases and controls. Two authors independently assessed the articles for compliance with the inclusion/exclusion criteria and reached a consistent decision.

Statistical analysis

The associations between polymorphisms in the PPARγ gene and the risk of breast cancer were assessed by calculating the pooled OR and 95% CI. Associations under three different types of ORs were calculated using the codominant model, the dominant model, and the recessive model. Q statistics were used to investigate the heterogeneity between studies. A p-value greater than 0.05 indicated that there was no significant heterogeneity, allowing for the applicability of the fixed effects model (Mantel–Haenszel method) (Mantel and Haenszel, 1959). A Begg's funnel plot was generated for rs1801282 to detect bias or systematic heterogeneity (Begg and Mazumdar, 1994), and an Egger's test was used to estimate the publication bias. A p-value of 0.05 or lower was considered a statistically significant publication bias (Egger et al., 1997). Sensitivity analysis was performed by excluding each study in turn for the rs1801282 polymorphism. All statistical tests in the meta-analysis were performed using the STATA software version 12.0 (StataCorp LP, College Station, TX, USA).

Results

1) Case-control study

The basic characteristics of the study participants are shown in Table 1. The patient's age at menarche, the proportion of nullipara, and the proportion of participants with a BMI <25 kg/m2 were significantly different between the cases and controls, with a p-value ≤ 0.05.

The genotype distributions of the 6 SNPs in the PPARγ gene between the cases and the controls are presented in Table 2. None of the 6 PPARγ polymorphisms had a significantly different distribution between the cases and the controls (p-values range from 0.29-0.90). Both in the codominant and the recessive models, the TT genotype of rs3856806 was related to a decreased risk of breast cancer with marginal significance after adjustment (OR: 0.43, 95% CI: 0.17-1.10 compared to the CC genotype; OR: 0.42, 95% CI: 0.16-1.07 compared to the CC+CT genotype). No significant associations were observed with any of the other five SNPs (rs4684846, rs1801282, rs2120825, rs2938395, and rs1175540).

2) Meta-analysis results

The characteristics of the studies included in the meta-analysis are summarized in Table 3. All studies regarding the rs3856806 SNP were hospital-based case-control studies and included East Asians. Among the six studies that investigated rs1801282, five studies included mostly Caucasians and were all population-based case-control studies. Only one study that examined the rs1801282 SNP and breast cancer risk included East Asians, and this study was a hospital-based case-control study.

The results of the meta-analysis including the current case-control study are presented in Table 4. Significant heterogeneities were not found between the studies, according to the Q statistic results. Therefore, a fixed effects model was employed. When examining the

rs3856806 polymorphism, the results of the analysis including 2 studies consisting of 747 cases and 1,030 controls showed that there was no significant association between the rs3856806 polymorphism and breast cancer risk. For the rs1801282 polymorphism, 6 studies with 2,668 cases and 3,764 controls were examined, and the results suggested that there was no significant association between the rs1801282 polymorphism and breast cancer risk (CG vs. CC: OR: 0.91, 95% CI: 0.79-1.04; CG+GG vs. CC: OR: 0.92, 95% CI: 0.81-1.05). These pooled estimates for all the genetic models were insensitive to the exclusion of individual studies, demonstrating the statistical robustness of the results (data not shown). No publication bias was observed according to the Begg's

Table 2. PPARγ Genotype Frequency and Odds Ratios for Breast Cancer Risk, according to PPARγ Genetic Polymorphisms in a Case-control Study among Korean Women

	Genetic Frequency (%) [1]			Model 1 [2]			Model 2 [3]		
rs number and genotype	Cases	Controls	P-value	OR	95% CI	P-value	OR	95% CI	P-value
rs4684846									
GG	120 (26.4)	123 (27.6)	0.49	1			1		
GA	231 (50.9)	215 (48.3)		1.1	(0.81-1.51)	0.54	1.09	(0.77-1.52)	0.64
AA	103 (22.7)	107 (24.0)		0.99	(0.68-1.43)	0.95	0.97	(0.65-1.45)	0.88
GA+AA vs. GG			0.34	1.06	(0.79-1.43)	0.68	1.05	(0.76-1.44)	0.78
AA vs. GG+GA			0.63	0.93	(0.68-1.26)	0.63	0.92	(0.66-1.29)	0.63
rs1801282									
CC	413 (90.8)	412 (90.6)	0.50	1			1		
CG	40 (8.8)	42 (9.2)		0.95	(0.60-1.50)	0.82	0.87	(0.53-1.44)	0.59
GG	2 (0.4)	1 (0.2)		2	(0.18-22.09)	0.57	1.71	(0.13-23.13)	0.69
CG+GG vs. CC			0.90	0.97	(0.62-1.52)	0.91	0.89	(0.54-1.47)	0.65
GG vs. CC+CG			0.56	2.01	(0.18-22.19)	0.51	1.73	(0.13-23.25)	0.68
rs2120825									
TT	416 (93.3)	421 (91.9)	0.26	1			1		
GT	29 (6.5)	37 (8.1)		0.79	(0.48-1.31)	0.37	0.77	(0.45-1.34)	0.36
GG	1 (0.2)	0 (0.0)		-	-	-	-	-	-
GT+GG vs. TT			0.44	0.82	(0.50-1.35)	0.44	0.79	(0.46-1.36)	0.4
rs2938395									
AA	143 (31.4)	150 (32.7)	0.33	1			1		
GA	225 (49.5)	225 (49.0)		1.05	(0.78-1.41)	0.75	0.97	(0.71-1.33)	0.86
GG	87 (19.1)	84 (18.3)		1.09	(0.75-1.59)	0.67	1.13	(0.75-1.70)	0.57
GA+GG vs. AA			0.69	1.06	(0.80-1.40)	0.69	1.01	(0.75-1.36)	0.94
GG vs. AA+GA			0.75	1.06	(0.76-1.47)	0.75	1.15	(0.80-1.65)	0.46
rs1175540									
CC	151 (33.3)	159 (34.7)	0.33	1			1		
CA	219 (48.4)	218 (47.6)		1.06	(0.79-1.42)	0.7	1	(0.73-1.37)	0.99
AA	83 (18.3)	81 (17.7)		1.08	(0.74-1.58)	0.7	1.12	(0.74-1.68)	0.59
CA+AA vs. CC			0.66	1.06	(0.81-1.40)	0.66	1.03	(0.77-1.38)	0.84
AA vs. CC+CA			0.80	1.04	(0.74-1.46)	0.81	1.12	(0.78-1.62)	0.55
rs3856806									
CC	320 (70.5)	311 (70.2)	0.29	1			1		
CT	126 (27.7)	117 (26.4)		1.05	(0.78-1.41)	0.76	1.12	(0.81-1.54)	0.51
TT	8 (1.8)	15 (3.4)		0.52	(0.22-1.24)	0.14	0.43	(0.17-1.10)	0.08
CT+TT vs. CC			0.93	0.99	(0.74-1.31)	0.93	1.03	(0.75-1.40)	0.87
TT vs. CC+CT			0.12	0.51	(0.22-1.22)	0.13	0.42	(0.16-1.07)	0.06

CI, confidence interval; OR, odds ratio; PPARγ, peroxisome proliferator-activated receptor-gamma; [1] Cochrane-Armitage test for trend was applied for additive model and chi-square test was applied for dominant and recessive model; [2] Adjusted for age; [3] Adjusted for age, age at menarche, menopause status, hormone replacement therapy, pregnancy, number of children, and body mass index

Table 3. Characteristics of the Studies Included in the Meta-analysis

Study, Year	Country	Ethnicity	Source of controls	Menopausal status (% of postmenopause for cases)	Distribution of genotypes					
					Cases			Controls		
rs3856806					CC	CT	TT	CC	CT	TT
Wu, 2011	Taiwan	East Asian	HB	Mixed (42.2% for cases, 32.9% for controls)	162	110	19	328	219	40
Park, 2013	Korea	East Asian	HB	Mixed (35.1% for cases, 42.1% for controls)	320	128	8	311	117	15
rs1801282					CC	CG	GG	CC	CG	GG
Justenhoven, 2008	Germany	Caucasian	PB	Mixed (75.7% for cases, 75.9% for controls)	452	135	6	462	145	15
Gallicchio, 2007	USA	Caucasian	PB	Post-menopaused (100%)	48	7	1	689	188	18
Wang, 2007	USA	Mixed (Caucasian 99.6%)	PB	Post-menopaused (100%)	376	87	15	375	98	5
Vogel, 2007	Demark	Caucasian	PB	Post-menopaused (100%)	283	71	7	258	93	10
Memisoglu, 2002	USA	Caucasian	PB	Mixed (50.0% for cases, 50.0% for controls)	563	148	14	752	190	11
Park, 2013	Korea	East Asian	HB	Mixed (35.1% for cases, 42.1% for controls)	413	40	2	412	42	1

HB, hospital-based; PB, population-based.

Table 4. Summary Odd Ratios and 95% Confidence Intervals of the Association between the PPARγ Polymorphisms and Breast Cancer Risk Using a Fixed Model

Polymorphisms	Number of studies	Odds ratio	95% CI	Statistical model	Phetero
rs3856806	2				
CT vs. CC		1.03	0.84-1.27	Fixed	0.89
TT vs. CC		0.79	0.49-1.28	Fixed	0.25
CT+TT vs. CC		1.00	0.82-1.22	Fixed	0.92
TT vs. CC+CT		0.79	0.49-1.27	Fixed	0.24
rs1801282	6				
CG vs. CC		0.91	0.79-1.04	Fixed	0.39
GG vs. CC		1.11	0.73-1.68	Fixed	0.06
CG+GG vs. CC		0.92	0.81-1.05	Fixed	0.27
GG vs. CC+CG		1.14	0.75-1.72	Fixed	0.07

Phetero, P value for heterogeneity of Q statistics.

funnel plot and the Egger's test, which had a p-value of 0.21.

Discussion

This hospital based case-control study and meta-analysis suggest there is no association between PPARγ polymorphisms and breast cancer risk. We examined the association between the rs1801282 polymorphism, which is the most studied PPARγ polymorphism, and breast cancer risk for the first time in Asian females in a case-control study. Additionally, this is the first systematic review and pooled meta-analysis regarding PPARγ polymorphisms, although several previous meta-analyses were conducted for other adiposity related genes, such as leptin or leptin receptor polymorphisms (Liu and Liu, 2011; Wang et al., 2012).

Obesity and type 2 diabetes are important risk factors for breast cancer and are correlated with a worse prognosis (Carmichael and Bates, 2004; Barone et al., 2008). These two risk factors share biological mechanisms that affect breast cancer tissue. These mechanisms include the direct effect of insulin on the proliferation of breast cancer cells, increased estrogen production and bioavailability, and changes in adipokines (Vona-Davis and Rose, 2012). The functional variants of the PPARγ gene are related to both lipid metabolism and insulin sensitivity and can affect obesity, type 2 diabetes, and diabetic complications; these effects have been confirmed by several epidemiological studies, including meta-analysis (Masud and Ye, 2003; Gouda et al., 2010). Although the biological functions of PPARγ in cancer development, such as promoting terminal differentiation, inhibiting cell growth, increasing apoptosis in human cancer cell lines, and inhibiting tumorigenesis (Peters et al., 2012), have been well documented, epidemiological studies regarding the associations between PPARγ polymorphisms and breast cancer risk, including the case-control study presented here, have shown inconclusive results with relatively small sample sizes. In this pooled analysis that combined the sample sizes of individual epidemiological studies, we did not find a significant association between two PPARγ polymorphisms (rs3856806 and rs1801282) and breast cancer risk.

Several limitations of this study should be mentioned. In this case-control study, we obtained information on the adjusted factors, such as menstrual and reproductive factors, from self-administered questionnaires, and the possibility of information bias cannot be ruled out. However, this is a non-differential misclassification, and the effects of misclassification on the results are expected to be minimal. Because correction for multiple comparisons was not performed in this study, the estimates should be interpreted cautiously. Additionally, we conducted a candidate gene association study and did not consider linkage disequilibrium or the function of combined SNPs, as previous studies have done (Li et al., 2011; Wu et al., 2011). Considering the linkage disequilibrium across the population, the selected SNPs in this study were positional candidates, rather than functional candidates.

There are also several limitations to the meta-analysis. First, the control populations were not uniformly defined. Although the study populations of the two studies included in the meta-analysis of rs3856806 were East Asian populations, there might be differences in the genetic distribution, gene effects, or gene-environmental interactions between countries. Additionally, for meta-analysis of rs1801282, Caucasians, East Asians, and other ethnicities were mixed. Although we did not find different results in the sensitivity analysis, there might be different effects of ethnicity on the genetic predisposition to human diseases, as many previous studies have shown (Pan et al., 2005). For the rs1801282 polymorphism, although most studies selected controls from healthy populations, one study used participants with benign breast diseases as controls (Gallicchio et al., 2007), and this case-control study used participants in a cancer screening program that did not have abnormal findings during the screening. Therefore, non-differential misclassification was possible because those with benign breast diseases may have risks of developing breast cancer. Second, because we could not confirm the menopausal status or BMI of the study populations included in the meta-analysis, this meta-analysis was unable to address gene-environmental interactions that could be important factors in the association of PPARγ polymorphisms and breast cancer risk. Additionally, the pooled analysis was performed only on the basis of the number of patients with each type of polymorphism, and unadjusted estimates were calculated. Therefore, a more precise analysis should be conducted if the confounding factors of the individuals are available. Third, because there were only two studies for the rs3856806 polymorphism, we could not assess the publication bias or perform sensitivity analysis. Fourth, although we combined all available data from the literature, the number of populations included in the pooled analysis was not enough to obtain a high statistical power. Fifth, in the meta-analysis, we did not perform corrections for multiple comparisons.

In conclusion, this case-control study and meta-analysis suggest there is no significant association between PPARγ polymorphisms and the risk of developing breast cancer despite the biological effects that PPARγ has on

breast carcinogenesis.

Acknowledgements

This study was supported by grant from the National Cancer Center of Korea (#1410692-1).

References

Abbastabar H, Hamidifard P, Roustazadeh A, et al (2013). Relationships between breast cancer and common non-communicable disease risk factors: an ecological study. *Asian Pac J Cancer Prev*, **14**, 5123-5.

Auwerx J (1999). PPARgamma, the ultimate thrifty gene. *Diabetologia*, **42**, 1033-49.

Barone BB, Yeh HC, Snyder CF, et al (2008). Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis. *JAMA*, **300**, 2754-64.

Begg CB, Mazumdar M (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**, 1088-101.

Boyle P, Boniol M, Koechlin A, et al (2012). Diabetes and breast cancer risk: a meta-analysis. *Br J Cancer*, **107**, 1608-17.

Calle EE, Kaaks R (2004). Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer*, **4**, 579-91.

Carmichael AR, Bates T (2004). Obesity and breast cancer: a review of the literature. *Breast*, **13**, 85-92.

Egger M, Davey Smith G, Schneider M, et al (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, **315**, 629-34.

Fratiglioni L, Wang HX (2007). Brain reserve hypothesis in dementia. *J Alzheimers Dis*, **12**, 11-22.

Gallicchio L, McSorley MA, Newschaffer CJ, et al (2007). Body mass, polymorphisms in obesity-related genes, and the risk of developing breast cancer among women with benign breast disease. *Cancer Detect Prev*, **31**, 95-101.

Gouda HN, Sagoo GS, Harding AH, et al (2010). The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: a HuGE review and meta-analysis. *Am J Epidemiol*, **171**, 645-55.

Grommes C, Landreth GE, Heneka MT (2004). Antineoplastic effects of peroxisome proliferator-activated receptor gamma agonists. *Lancet Oncol*, **5**, 419-29.

He W (2009). PPARgamma2 polymorphism and human health. *PPAR Res*, **2009**, 849538.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Justenhoven C, Hamann U, Schubert F, et al (2008). Breast cancer: a candidate gene approach across the estrogen metabolic pathway. *Breast Cancer Res Treat*, **108**, 137-49.

Kaplan J, Cook JA, O'Connor M, et al (2007). Peroxisome proliferator-activated receptor gamma is required for the inhibitory effect of ciglitazone but not 15-deoxy-Delta 12,14-prostaglandin J2 on the NFkappaB pathway in human endothelial cells. *Shock*, **28**, 722-6.

Kim KZ, Shin A, Lee YS, et al (2012). Polymorphisms in adiposity-related genes are associated with age at menarche and menopause in breast cancer patients and healthy women. *Hum Reprod*, **27**, 2193-200.

Koh WP, Yuan JM, Van Den Berg D, et al (2006). Peroxisome proliferator-activated receptor (PPAR) gamma gene polymorphisms and colorectal cancer risk among Chinese in Singapore. *Carcinogenesis*, **27**, 1797-802.

Kotta-Loizou I, Giaginis C, Theocharis S (2012). The role of peroxisome proliferator-activated receptor-gamma in breast cancer. *Anticancer Agents Med Chem*, **12**, 1025-44.

Li Y, Wedren S, Li G, et al (2011). Genetic variation of ESR1 and its co-activator PPARGC1B is synergistic in augmenting the risk of estrogen receptor-positive breast cancer. *Breast Cancer Res*, **13**, 10.

Liu C, Liu L (2011). Polymorphisms in three obesity-related genes (LEP, LEPR, and PON1) and breast cancer risk: a meta-analysis. *Tumour Biol*, **32**, 1233-40.

Lorincz AM, Sukumar S (2006). Molecular links between obesity and breast cancer. *Endocr Relat Cancer*, **13**, 279-92.

Mahdi KM, Nassiri MR, Nasiri K (2013). Hereditary genes and SNPs associated with breast cancer. *Asian Pac J Cancer Prev*, **14**, 3403-9.

Mantel N, Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*, **22**, 719-48.

Masud S, Ye S (2003). Effect of the peroxisome proliferator activated receptor-gamma gene Pro12Ala variant on body mass index: a meta-analysis. *J Med Genet*, **40**, 773-80.

Mehta RG, Williamson E, Patel MK, et al (2000). A ligand of peroxisome proliferator-activated receptor gamma, retinoids, and prevention of preneoplastic mammary lesions. *J Natl Cancer Inst*, **92**, 418-23.

Memisoglu A, Hankinson SE, Manson JE, et al (2002). Lack of association of the codon 12 polymorphism of the peroxisome proliferator-activated receptor gamma gene with breast cancer and body mass. *Pharmacogenetics*, **12**, 597-603.

Minatoya M, Kutomi G, Asakura S, et al (2013). Equol, adiponectin, insulin levels and risk of breast cancer. *Asian Pac J Cancer Prev*, **14**, 2191-9.

Mueller E, Sarraf P, Tontonoz P, et al (1998). Terminal differentiation of human breast cancer through PPAR gamma. *Mol Cell*, **1**, 465-70.

Pan Z, Trikalinos TA, Kavvoura FK, et al (2005). Local literature bias in genetic epidemiology: an empirical evaluation of the Chinese literature. *PLoS Med*, **2**, 334.

Park SK, Kang D, Kim Y, et al (2009). Epidemiologic characteristics of the breast cancer in Korea. *J Korean Med Assoc AID*, **52**, 937-45.

Paynter RA, Hankinson SE, Colditz GA, et al (2004). No evidence of a role for PPARgamma Pro12Ala polymorphism in endometrial cancer susceptibility. *Pharmacogenetics*, **14**, 851-6.

Peters JM, Shah YM, Gonzalez FJ (2012). The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. *Nat Rev Cancer*, **12**, 181-95.

Ronco AL, De Stefani E, Deneo-Pellegrini H, et al (2012). Diabetes, overweight and risk of postmenopausal breast cancer: a case-control study in Uruguay. *Asian Pac J Cancer Prev*, **13**, 139-46.

Sangrajrang S, Chaiwerawattana A, Ploysawang P, et al (2013). Obesity, diet and physical inactivity and risk of breast cancer in Thai women. *Asian Pac J Cancer Prev*, **14**, 7023-7.

Spiegelman BM (1998). PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes*, **47**, 507-14.

Suzuki T, Hayashi S, Miki Y, et al (2006). Peroxisome proliferator-activated receptor gamma in human breast carcinoma: a modulator of estrogenic actions. *Endocr Relat Cancer*, **13**, 233-50.

Vogel U, Christensen J, Nexo BA, et al (2007). Peroxisome proliferator-activated receptor-gamma2 Pro12Ala, interaction with alcohol intake and NSAID use, in relation to risk of breast cancer in a prospective study of Danes. *Carcinogenesis*, **28**, 427-34.

Vona-Davis L, Rose DP (2012). Type 2 diabetes and obesity

metabolic interactions: common factors for breast cancer risk and novel approaches to prevention and therapy. *Curr Diabetes Rev*, **8**, 116-30.

Wang LQ, Shen W, Xu L, et al (2012). The association between polymorphisms in the leptin receptor gene and risk of breast cancer: a systematic review and pooled analysis. *Breast Cancer Res Treat*, **136**, 231-9.

Wu MH, Chu CH, Chou YC, et al (2011). Joint effect of peroxisome proliferator-activated receptor gamma genetic polymorphisms and estrogen-related risk factors on breast cancer risk: results from a case-control study in Taiwan. *Breast Cancer Res Treat*, **127**, 777-84.

Yanhua C, Geater A, You J, et al (2012). Reproductive variables and risk of breast malignant and benign tumours in Yunnan province, China. *Asian Pac J Cancer Prev*, **13**, 2179-84.

Yoo JY, Kim SY, Hwang JA, et al (2012). Association study between folate pathway gene single nucleotide polymorphisms and gastric cancer in Koreans. *Genomics Inform*, **10**, 184-93.

Yoo KY, Kim Y, Park SK, et al (2006). Lifestyle, genetic susceptibility and future trends of breast cancer in Korea. *Asian Pac J Cancer Prev*, **7**, 679-82.