RESEARCH COMMUNICATION

Prostate Stem Cell Antigen Single Nucleotide Polymorphisms Influence Risk of Estrogen Receptor Negative Breast Cancer in Korean Females

Sook-Young Kim[1&], Jae-Young Yoo[1&], Aesun Shin[2], Yeonju Kim[3,5], Eun-Sook Lee[4], Yeon-Su Lee[1*]

Abstract

Introduction: Breast cancer is the second leading cancer in Korean women. To assess potential genetic associations between the prostate stem cell antigen (PSCA) gene in the chromosome 8q24 locus and breast cancer risk in Korean women, 13 SNPs were selected and associations with breast cancer risk were analyzed with reference to hormone receptor (HR) and menopausal status. Methods: We analyzed DNA extracted from buffy coat from 456 patients and 461 control samples, using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) based upon region-specific PCR followed by allele-specific single base primer extension reactions. Risks associated with PSCA genotypes and haplotypes were estimated with chi-square test (χ^2 -test), and polytomous logistic regression models using odds ratios (OR) and 95% confidence intervals (CIs), by HR and menopausal status. Results: In case-control analysis, odds ratios (OR) of rs2294009, rs2294008, rs2978981, rs2920298, rs2976395, and rs2976396 were statistically significant only among women with estrogen receptor (ER) negative cancers, and those of rs2294008, rs2978981, rs2294010, rs2920298, rs2976394, rs10216533, and rs2976396 were statistically significant only in pre-menopausal women, and not in postmenopausal women. Risk with the TTGGCAA haplotype was significantly elevated in ER (-) status (OR= 1.48, 95% CI= 1.03~2.12, p<0.05). Especially risk of allele T of rs2294008 is significantly low in pre-menopausal breast cancer patients and AA genotype of rs2976395 in ER (-) status represents the increase of OR value. Conclusion: This report indicated for the first time that associations exist between PSCA SNPs and breast cancer susceptibility in Korean women, particularly those who are pre-menopausal with an estrogen receptor negative tumor status.

Key words: PSCA - breast cancer - 8q24 - polymorphism - biomarker

Asian Pacific J Cancer Prev, **12**, 41-48

Introduction

Breast cancer is the most common cancer in females worldwide and the second leading type in Korean women (Ferlay et al., 2010; Jung et al., 2011). According to the cancer registration report issued by the National Cancer Center (NCC) in South Korea, breast cancer accounted for about 14.7% of all cancers in Korean women in the year 2008 (Jung et al., 2011). In 2008, 12,584 cases were newly diagnosed and the age-standardized incidence rate was 42.1 per 100,000 populations (Jung et al., 2011). Traditional breast cancer screening methods are mammography and ultrasound imaging (Benson et al., 2009). However, the patient age distribution is relatively young, so that many patients have dense breasts so that lesions are difficult to detect and a palpable abnormality combined with a negative mammogram can pose a great dilemma for the breast cancer specialist (Smith, 2007). In addition Asian women's breasts tend to be smaller and the tissue is denser than in Americans or other Caucasians (Habel et al., 2007). Therefore there is a need for alternative screening approaches, perhaps with molecular markers. Furthermore, patients showed the five-year relative survival rates in 2005, for stage I, II, III, and IV Korean breast cancers were 98.4%, 91.6%, 69.7% and 30.2% respectively (Ahn, 2004; Ferlay et al., 2010), so that diagnostic methods which are sensitive to early stage breast cancer and economical for population screening are a high priority.

Development of molecular diagnostic methods using novel clinical tools to detect breast cancer at all stages is an area of intense research activity and recent efforts have been directed at the identification of biomarkers that may have diagnostic, prognostic and/or therapeutic

*[1]Cancer Genomics Branch, [2]Molecular Epidemiology Branch, [3]Cancer Early Detection Branch, [4]Convergence Technology Division, National Cancer Center, Goyang, South Korea, [5]Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI, USA [&]These authors contribute equally to this paper *For correspondence: yslee2@ncc.re.kr*

applications (Raff et al., 2009). Biomarker research concerned with single nucleotide polymorphisms (SNPs) is expected to open up a new era in the field of cancer research and cancer early diagnostics (Kim et al., 2009). To date, well known breast cancer susceptibility genes such as the breast cancer 1, early on set (BRCA1) and breast cancer 2, early on set (BRCA2) are known to be only responsible for less than 5% of all breast cancer patients (Onay et al., 2006; Yang and Lippman, 1999). Breast cancer is a multifactorial disease, and less than 10% are considered caused by defects in single genes (monogenic) (Wiechec and Hansen, 2009). Many studies have shown that cancer risks associated with individual but commonly occurring SNPs are incremental (Imyanitov, 2009), so recent work through large consortial studies have focused on cancer susceptibility loci in genic (CASP8, FGFR2, TNRC9, MAP3K1, LSP1) and non-genic regions (8q24, 2q25, 5q12) (Garcia-Closas and Chanock, 2008). Various other genes related to breast cancer (CYP2D6, CYP19A1, CYP2B6, CYP1B1, FGFR4, GSTP1, TGFβ1) are linked with hormone and drug responses (Wiechec and Hansen, 2009).

SNPs may be associated not only with one specific cancer but rather with several cancer types (Onay et al., 2006). For example, genetic variation on chromosome 8q24 has been related to prostate, breast, gastric, and colorectal cancer (Berndt et al., 2008; Fletcher et al., 2008). Furthermore, several prostate cancer susceptibility loci have been identified as candidate cancer risk genes for other epithelial cancers, including lymphocyte antigen 6 complex locus K (LY6K) and the Prostate Stem Cell Antigen (PSCA) on chromosome 8q24 (Sakamoto et al., 2008; Wu et al., 2009; Yeager et al., 2008).

The PSCA gene, encoding a 123 amino acid cell surface protein with 30% homology to stem cell antigen type 2 (SCA-2), an immature lymphocyte cell surface marker (Raff et al., 2009; Yeager et al., 2008), is more highly expressed in human prostate cancer than normal tissues, and also in placenta, so it is an ideal target for cancer diagnosis and therapy (Reiter et al., 1998). Several studies have revealed a correlation between upregulation of PSCA and relevant clinical benchmarks, such as the Gleason score and metastasis in prostate cancer, while others have demonstrated the efficacy of PSCA targeting in treatment through various modalities (Raff et al., 2009). Moreover, recently a viral vector PSCA vaccine demonstrated efficacy in a mouse model (Ahmad et al., 2009). If any association existed between PSCA and breast cancer, transfer of vaccination know-how developed for prostate cancer might be possible.

Clearly there are similarities between prostate and breast cancer, with reference to epidemiological, genetic, and biochemical characteristics (Lopez-Otin and Diamandis, 1998) and especially regarding influences of androgens and estrogens (Coffey, 2001). The incidence of contralateral breast cancer was reported to be 2.7% in male breast and/or prostate cancer patients, similar to that in woman (Ozet et al., 2000).

Given this background we decided to focus on possible interactions between PSCA SNPs and breast cancer risk, selecting 13 SNP sites in the coding region of PSCA for study. In addition to overall assessment, division was made into pre- and post-menopausal and hormone receptor (HR) positive and negative groups.

Materials and Methods

Study Population

Buffy coat samples of 456 patients (49.7%) who had undergone surgery at the Breast Cancer Center, National Cancer Center of Korea between September 2001 and August 2005 were the subjects. A total of 461 archival control (50.3%) buffy coat samples from cancer screening examinees at the National Cancer Center of Korea between August 2002 and December 2005 were included for comparison. Cases were more likely to be nulliparous and have no history of breast feeding than controls, with earlier menarche. All samples were from Koreans. This study was approved by the Institutional Review Board (IRB) of the National Cancer Center in Korea with written informed consent from the patients.

Genotyping

Genomic DNA was isolated from the 75*ul* buffy coat using a MagAttract DNA Blood Midi M48 Kit (Qiagen, Inc., Valencia, CA, USA) on a Qiagen BioRobot M48 workstation. The quantity and quality of isolated genomic DNA were measured with a Nanodrop® ND-1000 spectrophotometer (Nanodrop Technologies, DE, USA) and a Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Inc., USA). The multiplex PCR primers and extended primers for candidate SNPs selected from the previous study (Sakamoto et al., 2008) were designed by two reaction groups using MassARRAY Assay Design software version 3.0 (Sequenom, CA, USA) . PCR amplification were performed in a total volume of 5 *ul* with 10 ng of genomic DNA, 1.625 mM $MgCl_2$, 0.1 units of HotStarTaq polymerase (Qiagen, Valencia, CA, USA), 0.5 mM dNTP, and 100 nM primers. The PCR amplification started at 94 °C for 15 min, followed by 45 cycles of 94 °C for 20 s, 50 °C for 30 s, and 72 °C for 1 min, with final extension of 72 °C for 3 min. After dephosphorylation of PCR products, PCR products were input to the allele-specific single base primer extension reactions. The extension mixture consisted of 0.222X iPLEX buffer, 0.5X iPLEX termination mix, 0.5X iPLEX enzyme (iPLEX Gold Reaction Kit; Sequenom) and 625 nM to 1.25 μM extension primers in a total 9 *ul* volume. The extension reaction was performed using 2-step 200 short-cycle programs. The sample was denatured at 94 °C for 5 s, and strands were annealed at 52 °C for 5 s and extended at 80 °C for 5 s. The annealing and extension cycle was repeated 4 more times for a total of 5 cycles, looped back to a 94 °C denaturing step for 5 s, and then entered the 5 cycle annealing and extension loop again. The 5 annealing and extension steps with the single

denaturing step were repeated an additional 39 times for a total of 40 cycles. A final extension was done at 72 °C for 3 min. After allele-specific single base primer extension reactions, polymorphic sites were determined by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, SpectroREADER, Sequenom). Resulting genotype data was collected by MassArray Typer software version 4.0 (Sequenom, CA, USA).

Statistical Analysis

The chi-square test (χ^2-test) was used to test differences in genotype frequencies of PSCA polymorphisms between normal and patient samples, overall and divided into pre- and post- menopausal and receptor positive and negative groups. The genotype specific risks and allele

Table 1. Association between the PSCA Polymorphisms and Breast Cancer Risk with Reference to Menopausal Status with OR Values (95% confidence intervals)

	Cases		Controls		All participants		Pre-menopause		Post-menopause	
	N	%	N	%	Co-dominant	Dom/Rec	Co-dominant	Dom/Rec	Co-dominant	Dom/Rec
rs13262164 (n=450) (n=459)										
CC	268	59.6	294	64.0	1		1		1	
CT	160	35.5	144	31.4	1.29 (0.96-1.74)	1.26 (0.95-1.68)	1.12 (0.76-1.65)	1.08 (0.75-1.57)	1.48 (0.90-2.42)	1.49 (0.92-2.40)
rs6471587 (n=457) (n=453)										
CC	357	78.1	346	76.4	1		1		1	
CG	94	20.6	102	22.5	1.09	1.09 (0.77-1.53)	0.92 (0.60-1.43)	0.95 (0.62-1.45)	1.47 (0.84-2.56)	1.39 (0.80-2.41)
GG	6	1.3	5	1.1	1.09 (0.31-3.79)	1.07 (0.31-3.71)	1.34 (0.28-6.39)	1.36 (0.29-6.48)	0.50 (0.05-5.22)	0.45 (0.04-4.72)
rs13262164 (n=450) (n=459)										
CC	268	59.6	294	64.0	1	1		1		
CT	160	35.5	144	31.4	1.29 (0.96-1.74)	1.26 (0.95-1.68)	1.12 (0.76-1.65)	1.08 (0.75-1.57)	1.48 (0.90-2.42)	1.49 (0.92-2.40)
TT	22	4.9	21	4.6	1.09 (0.56-2.13)	0.99 (0.51-1.93)	0.87 (0.38-1.97)	0.84 (0.37-1.88)	1.57 (0.46-5.30)	1.37 (0.41-4.57)
rs2294008 (n=451) (n=459)										
CC	119	26.4	113	24.6	1		1		1	
CT	216	47.9	240	52.3	0.90 (0.64-1.26)	0.95 (0.69-1.32)	0.61* (0.39-0.96)	0.65 (0.42-1.01)	1.47 (0.84-2.56)	1.53 (0.91-2.57)
TT	116	25.7	106	23.1	1.08 (0.72-1.60)	1.16 (0.84-1.60)	0.76 (0.44-1.29)	1.07 (0.70-1.64)	1.66 (0.88-3.12)	1.31 (0.77-2.21)
rs2978981 (n=441) (n=451)										
CC	119	27	115	25.5	1		1		1	
CT	206	46.7	229	50.8	0.92 (0.65-1.30)	0.97 (0.70-1.34)	0.60* (0.38-0.95)	0.65 (0.42-1.00)	1.61 (0.92-2.84)	1.65 (0.98-2.78)
TT	116	26.3	107	23.7	1.08 (0.73-1.60)	1.14 (0.82-1.57)	0.75 (0.44-1.27)	1.06 (0.70-1.62)	1.71 (0.90-3.23)	1.27 (0.75-2.15)
rs2294009 (n=453) (n=460)										
GG	448	98.9	456	99.1	1		1		1	
GA	5	1.1	4	0.9	2.22 (0.50-9.86)	2.22 (0.50-9.86)	-	-	1.24 (0.22-6.87)	1.24 (0.22-6.87)
rs2294010 (n=453) (n=460)										
CC	120	26.5	114	24.8	1		1		1	
AG	216	47.7	240	52.2	0.89 (0.63-1.25)	0.95 (0.69-1.31)	0.61* (0.39-0.96)	0.66 (0.43-1.01)	1.45 (0.83-2.53)	1.52 (0.91-2.56)
GG	117	25.8	106	23.0	1.08 (0.73-1.61)	1.17 (0.85-1.62)	0.77 (0.45-1.31)	1.09 (0.72-1.66)	1.66 (0.88-3.13)	1.32 (0.78-2.23)
rs3736001 (n=452) (n=460)										
GG	353	78.1	363	78.9	1		1		1	
GA	94	20.8	92	20	0.99 (0.70-1.41)	1.00 (0.72-1.41)	0.88 (0.57-1.37)	0.91 (0.60-1.39)	1.28 (0.72-2.29)	1.24 (0.70-2.20)
AA	5	1.1	5	1.1	1.16 (0.32-4.15)	1.16 (0.32-4.15)	1.34 (0.28-6.41)	1.38 (0.29-6.55)	0.63 (0.05-7.40)	0.59 (0.05-6.97)
rs2976392(n=453) (n=460)										
AA	115	25.4	106	23	1		1		1	
AG	217	47.9	239	52	0.84 (0.60-1.19)	0.88 (0.63-1.21)	0.80 (0.52-1.25)	0.94 (0.62-1.43)	0.90 (0.51-1.58)	0.77 (0.45-1.30)
GG	121	26.7	115	25	0.95 (0.64-1.40)	1.06 (0.77-1.46)	1.35 (0.80-2.28)	1.56 (1.02-2.41)	0.60 (0.32-1.13)	0.64 (0.38-1.08)
rs2920298(n=454) (n=460)										
TT	119	26.2	115	25	1		1		1	
TC	218	48	238	51.7	0.92 (0.66-1.30)	0.97 (0.70-1.34)	0.62 (0.39-0.97)*	0.66 (0.43-1.01)	1.55 (0.89-2.71)	1.61 (0.96-2.70)
CC	117	25.8	107	23.3	1.08 (0.73-1.60)	1.14 (0.82-1.57)	0.75 (0.44-1.27)	1.05 (0.69-1.60)	1.71 (0.91-3.24)	1.30 (0.77-2.21)
rs2976394 (n=453) (n=459)										
CC	121	26.7	114	24.9	1		1		1	
CT	217	47.9	237	51.6	0.90 (0.64-1.26)	0.94 (0.68-1.29)	0.60 (0.38-0.94)*	0.64 (0.42-0.98)*	1.51 (0.86-2.63)	1.55 (0.92-2.59)
TT	115	25.4	108	23.5	1.03 (0.70-1.53)	1.11 (0.80-1.53)	0.73 (0.43-1.23)	1.04 (0.69-1.58)	1.62 (0.86-3.04)	1.25 (0.74-2.12)
rs10216533(n=453) (n=460)										
GG	121	26.7	114	24.8	1		1		1	
GA	218	48.1	240	52.2	0.89 (0.63-1.25)	0.94 (0.68-1.29)	0.60 (0.38-0.94)*	0.64(0.42-0.98)*	1.49 (0.86-2.60)	1.53 (0.91-2.57)
AA	114	25.2	106	23	1.04 (0.70-1.54)	1.12 (0.81-1.55)	0.74 (0.44-1.27)	1.06 (0.70-1.62)	1.61 (0.85-3.05)	1.26 (0.74-2.13)
rs2976395 (n=449) (n=460)										
GG	333	74.2	353	76.7	1		1		1	
AA	116	25.8	107	23.3	1.15 (0.83-1.59)	1.15 (0.83-1.59)	1.15 (0.83-1.59)	1.06 (0.70-1.61)	1.06 (0.70-1.61)	1.06 (0.70-1.61)
					1.32 (0.78-2.24)	1.32 (0.78-2.24)	1.32 (0.78-2.24)			
rs2976396 (n=453) (n=460)										
GG	118	26.1	114	24.8	1		1		1	
GA	218	48.1	239	52	0.92 (0.65-1.29)	0.97 (0.70-1.34)	0.62 (0.39-0.97)*	0.66 (0.43-1.02)	1.55 (0.88-2.70)	1.60 (0.95-2.69)
AA	117	25.8	107	23.2	1.08 (0.73-1.61)	1.15 (0.83-1.58)	0.76 (0.45-1.30)	1.07 (0.70-1.63)	1.70 (0.90-3.21)	1.29 (0.76-2.19)

OR, odds ratio, adjusted for education attainment, body mass index, age at menarche, age at the first live birth, and menopausal status; Dom/Rec, dominant for heterzygotes, recessive for homozygotes; *p<0.05

Table 2. Association between the PSCA Polymorphisms and Breast Cancer Risk by Estrogen Receptor (ER) Status

		ER (+), OR (95% CI)			ER (-), OR (95% CI)		
		Co-dominant	Dominant	Recessive	Co-dominant	Dominant	Recessive
rs6471587	CC	1			1		
	CG	1.06 (0.74-1.54)	1.07 (0.74-1.53)		1.19 (0.74-1.96)	1.18 (0.72-1.93)	
	GG	1.14 (0.30-4.31)		1.12 (0.30-4.23)	0.98 (0.11-8.49)		0.94 (0.11-8.12)
rs13262164	CC	1			1		
	CT	1.28 (0.90-1.78)	1.25 (0.92-1.71)		1.36 (0.87-2.13)	1.36 (0.89-2.09)	
	TT	1.04 (0.50-2.16)		0.95 (0.46-1.96)	1.32 (0.51-3.39)		1.18 (0.46-2.99)
rs2294008	CC	1			1		
	CT	0.90 (0.63-1.30)	0.92 (0.65-1.30)	0.89 (0.53-1.51)	1.09 (0.67-1.77)		
	TT	0.96 (0.62-1.47)		1.02 (0.72-1.46)	1.51 (0.85-2.68)		**1.63 (1.03-2.59)***
rs2978981	CC	1			1		
	CT	0.92 (0.64-1.33)	0.93 (0.66-1.31)	0.94 (0.55-1.61)	1.14 (0.70-1.87)		
	TT	0.95 (0.62-1.45)		1.00 (0.70-1.43)	1.56 (0.88-2.78)		**1.62 (1.02-2.58)***
rs2294009	GG	1			1		
	GA	1.17 (0.18-7.41)	1.17 (0.18-7.41)		**5.53 (1.02-29.9)***	**5.53 (1.02-29.9)***	
rs2294010	AA	1			1		
	AG	0.89 (0.62-1.29)	0.91 (0.65-1.29)		0.89 (0.52-1.51)	1.09 (0.67-1.77)	
	GG	0.96 (0.63-1.48)		1.04 (0.73-1.48)	1.52 (0.86-2.70)		1.64 (1.04-2.60)
rs3736001	GG	1			1		
	GA	0.99 (0.68-1.45)	1.00 (0.70-1.45)		1.04 (0.62-1.74)	1.04 (0.62-1.73)	
	AA	1.21 (0.31-4.72)		1.21 (0.31-4.71)	1.03 (0.12-9.06)		1.02 (0.12-8.96)
rs2976392	AA	1			1		
	AG	0.93 (0.64-1.36)	0.98 (0.68-1.39)		0.63 (0.38-1.04)	0.64 (0.40-1.02)	
	GG	1.06 (0.69-1.62)		1.11 (0.79-1.56)	0.67 (0.37-1.19)		0.90 (0.55-1.47)
rs2920298	TT	1			1		
	TC	0.92 (0.64-1.32)	0.93 (0.66-1.31)		0.95 (0.56-1.62)	1.14 (0.70-1.87)	
	CC	0.95 (0.62-1.46)		1.01 (0.71-1.43)	1.56 (0.87-2.76)		1.61 (1.01-2.54) *
rs2976394	CC	1			1		
	CT	0.89 (0.62-1.27)	0.90 (0.64-1.27)		0.95 (0.56-1.61)	1.11 (0.68-1.82)	
	TT	0.92 (0.60-1.41)		1.00 (0.70-1.42)	1.46 (0.82-2.61)		1.51 (0.95-2.41)
rs10216533	GG	1			1		
	GA	0.88 (0.61-1.26)	0.90 (0.64-1.26)		0.95 (0.56-1.62)	1.11 (0.68-1.81)	
	AA	0.94 (0.61-1.43)		1.02 (0.72-1.45)	1.45 (0.81-2.60)		1.50 (0.94-2.39)
rs2976395	GG	1			1		
	AA	1.02 (0.71-1.45)	1.02 (0.71-1.45)	1.02 (0.71-1.45)	**1.61 (1.02-2.55)***	**1.61 (1.02-2.55)***	**1.61 (1.02-2.55)***
rs2976396	GG	1			1		
	GA	0.92 (0.64-1.32)	0.93 (0.66-1.32)		0.94 (0.55-1.60)	1.13 (0.69-1.85)	
	AA	0.96 (0.63-1.47)		1.02 (0.71-1.45)	1.55 (0.87-2.76)		**1.61 (1.02-2.55)***

OR, odds ratio, adjusted for education attainment, body mass index, age at menarche, age at the first live birth, and menopausal status; *p<0.05

frequencies of PSCA haplotypes in breast cancer patients and controls were estimated as OR and 95% confidence intervals (CI), after adjustment for BMI, education attainment (data not shown), age at menarche, age at the first live birth, and menopausal status for the risk related to ER and/or PR (negative or positive) status. Risks associated with PSCA genotypes & haplotypes were estimated with χ^2-test, and polytomous logistic regression models using odds ratios (OR) and 95% confidence intervals (CIs). Statistical analyses were performed using SAS (Ver. 9.0, SAS Institute Inc., Cary, NC, USA).

Results

Association between PSCA Gene and Menopausal Status

We calculated p-value of χ^2-test for 13 SNPs, and detected the statistical significance in the variables such as pre-menopause and post-menopause (p<0.05) (Table 1). In rs6471587, rs13262164, and rs3736001, the frequency of minor homologous genotypes was less than 5% in all groups. Minor homologous genotype was not detected in rs2294009. In rs2294008, rs2978981, rs2294010, rs2920298, rs2976394, rs10216533, and rs2976396, every codominant model and some dominant model of pre-menopause group, ORs for minor alleles were less than 1 and statistically significant (p<0.05). In minor genotype of these SNPs, breast cancer risk was decreased, but the direction of the associations was opposite in post-menopause group.

Association between PSCA Gene and ER, PR Status

Association between the 13 SNPs of PSCA gene and breast cancer risk by ER status and PR status was summarized (Tables 2 and 3). We have found that significant associations between genotypes of PSCA SNPs and the risk of breast cancer were only observed for ER (-) tumors (p<0.05). We have found similarity between OR values by ER positive (+) group (p>0.05). In TT genotype of rs2294008 and rs2978981, OR values were significantly high the same as rs2920298, rs2976395 and rs2976396 at recessive model in ER (-) group (p<0.05). Whereas, OR values of minor homologous genotype in rs2294010, rs2976392, and rs2920298 were low (OR<1.000) in ER (+) group, breast

Table 3. Association between the PSCA Polymorphisms and Breast Cancer Risk by Progesterone Receptor (ER) Status

		PR (+), OR (95% CI)			ER (-), OR (95% CI)		
		Co-dominant	Dominant	Recessive	Co-dominant	Dominant	Recessive
rs6471587	CC	1			1		
	CG	1.02 (0.70-1.50)	1.02 (0.70-1.49)		1.20 (0.76-1.88)	1.21 (0.77-1.88)	
	GG	0.95 (0.22-4.07)		0.95 (0.22-4.04)	1.44 (0.28-7.50)		1.38 (0.26-7.15)
rs13262164	CC	1			1		
	CT	1.32 (0.94-1.85)	1.28 (0.92-1.76)		1.29 (0.86-1.93)	1.30 (0.88-1.91)	
	TT	1.00 (0.46-2.14)		1.23 (0.52-2.88)	1.35 (0.57-3.20)		1.23 (0.52-2.88)
rs2294008	CC	1			1		
	CT	0.88 (0.60-1.28)	0.90 (0.63-1.29)		0.95 (0.59-1.51)	1.07 (0.69-1.66)	
	TT	0.95 (0.61-1.48)		1.03 (0.72-1.50)	1.34 (0.79-2.26)		1.39 (0.91-2.13)
rs2978981	CC	1			1		
	CT	0.88 (0.60-1.30)	0.90 (0.63-1.29)		1.01 (0.63-1.61)	1.13 (0.72-1.75)	
	TT	0.94 (0.60-1.46)		1.02 (0.70-1.47)	1.37 (0.81-2.32)		1.37 (0.89-2.09)
rs2294009	GG	1			1		
	GA	0.73 (0.07-7.41)	0.73 (0.07-7.41)	-	4.58 (0.94-22.26)	4.58 (0.94-22.26)	-
rs2294010	AA	1			1		
	AG	0.87 (0.59-1.27)	0.89 (0.63-1.28)		0.95 (0.59-1.51)	1.07 (0.69-1.66)	
	GG	0.96 (0.61-1.49)		1.05 (0.73-1.52)	1.35 (0.80-2.28)		1.40 (0.91-2.14)
rs3736001	GG	1			1		
	GA	0.98 (0.66-1.44)	0.98 (0.67-1.44)		1.02 (0.64-1.63)	1.04 (0.66-1.65)	
	AA	1.01 (0.23-4.46)		1.02 (0.23-4.47)	1.50 (0.28-8.02)		1.49 (0.28-7.95)
rs2976392	AA	1			1		
	AG	0.91 (0.61-1.34)	0.96 (0.67-1.39)		0.75 (0.48-1.19)	0.75 (0.49-1.15)	
	GG	1.07 (0.69-1.67)		1.15 (0.80-1.63)	0.75 (0.44-1.27)		0.90 (0.58-1.40)
rs2920298	TT	1			1		
	TC	0.88 (0.60-1.29)	0.90 (0.63-1.29)		1.01 (0.63-1.62)	1.13 (0.73-1.75)	
	CC	0.94 (0.60-1.47)		1.02 (0.71-1.47)	1.37 (0.81-2.31)		1.36 (0.89-2.07)
rs2976394	CC	1			1		
	CT	0.85 (0.58-1.24)	0.87 (0.61-1.24)		1.01 (0.63-1.61)	1.10 (0.71-1.71)	
	TT	0.91 (0.59-1.41)		1.01 (0.70-1.46)	1.30 (0.77-2.20)		1.29 (0.84-1.98)
rs10216533	GG	1			1		
	GA	0.85 (0.58-1.23)	0.87 (0.61-1.24)		0.89 (0.62-1.59)	1.10 (0.71-1.70)	
	AA	0.91 (0.58-1.42)		1.02 (0.70-1.47)	1.32 (0.78-2.24)		1.33 (0.87-2.04)
rs2976395	GG	1			1		
	AA	1.03 (0.71-1.49)	1.03 (0.71-1.49)	1.03 (0.71-1.49)	1.37 (0.90-2.10)	1.37 (0.90-2.10)	1.37 (0.90-2.10)
rs2976396	GG	1			1		
	GA	0.89 (0.61-1.30)	0.91 (0.63-1.30)		1.00 (0.62-1.60)	1.11 (0.72-1.72)	
	AA	0.96 (0.61-1.49)		1.03 (0.72-1.49)	1.36 (0.80-2.29)		1.36 (0.89-2.07)

OR, odds ratio, adjusted for education attainment, body mass index, age at menarche, age at the first live birth, and menopausal status

cancer risk was not significantly decreased (p>0.05). Especially, rs2294009 in the ER (-) group, OR value of GA genotype was 5.53 (95% CI= 1.02~29.93) (p<0.05). We analyzed the correlation with PR status and genotypes of PSCA SNPs, however, did not find any significance association (p>0.05).

Association between PSCA Haplotype and ER, PR Status

When we analyzed the allele frequencies of PSCA haplotypes in breast cancer patient and control group, we have not detected significantly difference in the frequency of PSCA haplotypes between the breast cancer patients and controls. Haplotype analysis was done with most common type of 12 PSCA polymorphisms in breast cancer patients and controls, but we didn't find significant difference related to haplotype frequency. In case of other haplotypes, we could find the difference even though very low frequency each 0.0101 and 0.0093 in control and patient groups. Additional haplotype analysis was done with most common type of 7 PSCA polymorphisms in breast cancer patient by HR status, we didn't find significant different. However in case of TTGGCAA haplotype, frequency (0.3060) of ER (-) group was higher than ER (+) group's and OR value also significantly high (OR= 1.48, 95% CI= 1.03~2.12, p<0.05) (Table 4).

Association between PSCA Gene and Breast Cancer Risk by ER and PR Status

In association among ER (+), PR (+), ER (+) PR (+), and genotypes of PSCA SNPs in control group, we did not find any statistically significance association. In rs2294009, increasing pattern of OR value was shown in the ER (-) & PR (-) (OR = 4.08 CI 0.85-19.6) but not significant (p>0.05). We have detected no significant difference in the allele frequencies of PSCA haplotypes between the breast cancer ER or PR (+) status (p>0.05). Also, there was no significant difference in haplotype and OR analysis with most common type of 7 PSCA polymorphisms in ER or PR (+) breast cancer patient (p>0.05). Through these analysis, we could confirm that significant difference of breast tumorigenesis risk depend on the SNP variation of PSCA gene affect to occurrence of breast cancer with related to hormone secretion variation and/or receptor retention in patient

Table 4. Allele Frequencies and Odds Ratios of PSCA Haplotypes by ER and PR Status

Haplotypes[1)]	Controls	All cases	ER(+)	OR	ER(-)	OR	PR(+)	OR	PR(-)	OR
CCGATGG	0.504	0.502	0.514	1	0.466	1	0.519	1	0.484	1
TTGGCAA	0.231	0.258	0.242	1.02	0.306	**1.48***	0.247	1.03	0.276	1.28
				(0.78-1.33)		**(1.03-2.12)**		(0.78-1.36)		(0.92-1.77)
TTGGCGA	0.252	0.232	0.239	0.95	0.211	0.94	0.236	0.94	0.224	0.97
				(0.73-1.24)		(0.64-1.39)		(0.71-1.23)		(0.69-1.37)
Others	0.012	0.008	0.005	0.58	0.017	2.32	0.004	0.47	0.016	1.92
				(0.15-2.25)		(0.67-8.09)		(0.09-2.25)		(0.60-6.13)

[1)] Composed of two polymorphic sites: rs2294008-rs2978981-rs2294009-rs2294010-rs2920298-rs2976395-rs2976396; OR: odds ratio, adjusted for body mass index, age at menarche, age at the first live birth, and menopausal status; *p<0.05

groups at pre-menopause and ER (-) groups.

At the result of this research, we can conclude that variation related to the PSCA gene and ER (-) status affect to the breast cancer patients.

Discussion

The present study, for the first time to our knowledge, demonstrated associations between the PSCA gene in the 8q24 cancer risk region and breast cancer susceptibility in Korean women, and associations were observed especially for the pre-menopausal case with the rs2294008, rs2978981, rs2294010, rs2920298, rs2976394, rs10216533, and rs2976396 SNPs, as well as for ER (-) with rs2294009, rs2294008, rs297898, rs2920298, rs2976395, and rs2976396.

At the present there is very little information available on the functional consequencies of the various polymorphisms in the PSCA gene investigated here, despite the fact that many results related to cancer risk have been reported (Chu et al., 2010; Matsuo et al., 2009; Sakamoto et al., 2008; Wu et al., 2009). In rs2294008, for diffuse type of gastric cancer in Japanese (925 cases, 1,396 controls), the overall p-value was $2.2x10^{-15}$ with an allelic OR of 1.67 (95% CI= 1.47–1.90) and in Korean (454 cases, 390 controls), the overall p-value was $6.3x10^{-11}$ with an allelic OR of 1.91 (95% CI= 1.57–2.33) (Sakamoto et al., 2008). Regarding rs2294008, slightly different results were shown among projects for bladder cancer, the overall p-value in US validation subjects (1,713 patients, 3,871 controls), being $3.53x10^{-5}$ with an allelic OR of 1.19 (95% CI= 1.10–1.30), whereas in European validation subjects (3,985 patients, 34,762 controls), the p-value was $9.83x10^{-5}$ and the allelic OR was 1.12 (95% CI= 1.06–1.18) (Wu et al., 2009). However, consistent associations with cancer were shown for a missense variation of the PSCA gene across nations (Wu et al., 2009). The rs2294008 of PSCA SNP may alter start codon (first methionine); a polymorphic variation (or it) is possible in the length of the N-terminus signal peptide, which in turn can lead to a difference in protein folding, intracellular processing or subcellular localization (Sakamoto et al., 2008). In the allele C of rs2294008, the translation is predicted to start from the next ATG codon, resulting in a nine-amino-acid truncation (Wu et al., 2009). Also, risk allele T of rs2294008, supposedly is associated with lower transcriptional activity of tumor suppressive PSCA gene (Wu et al., 2009). In our study, T allele of rs2294008 was associated with decreased risk for pre-menopausal breast cancer. Therefore, genetic variation of rs2294008 by risk reduction can be analyzed as a risk factor in the breast cancer.

Recent many genome wide association studies confirmed that prostate, endometrium, breast, gastric and bladder cancer susceptibility linked with the 8q24 locus (Garcia-Closas and Chanock, 2008; Li et al., 2010; Thomas et al., 2009). While 8q24 itself is non-coding and non-genic region, it encompasses established cancer related regions (128,101,433~128,828,043 bp from telomere); the prostate cancer region 3, breast/prostate cancer region, prostate cancer region 1, bladder cancer region, and MYC (Chu et al., 2010). Though the PSCA gene is located downstream from 8q24 loci and far from the reported breast cancer region, our results would indicate that PSCA gene SNPs are indeed related with breast cancer risk. Our results show that genetic variation of rs2976395 was associated with the risk for ER (-) tumor. Especially, effect of rs296395 is not clarified in previous PSCA gene research so in future studies, potential of rs2976395 as a prognostic marker in ER (-) breast cancer should be considered in this regard that the sex HR status is a key factor for determining the prognosis and therapy of breast cancer (Rugo, 2008).

HR (-) breast cancers account for around 20~25% of all cases and unfortunately ER (-) breast cancers are more aggressive and unresponsive to anti-estrogens (Gluz et al., 2009; Orlando et al., 2010). A patient with hormone sensitive breast cancer has HR (+) disease which response to treatment that specifically block or interface with the function of estrogen or progesterone (Rugo, 2008), but case of HR (-) disease is difficult to get a solution of treatment. For the ER (-) cancer, other brand-new targeted therapies are urgently needed.

Many researches about ER confirmed that lymph node metastasis status, tumor grade, tumor size and ER status are important and especially ER or PR status rather than combinations of a number of factors is much more useful when treatment of early breast cancer for adjuvant systemic therapy (Williams et al., 2006) so addition ER

and PR indicators to the prognostic index could improve cancer prognostic ability (Buck et al., 2004).

Meanwhile PSCA and Ki-67 is correlated with each other and particularly highly expression of Ki-67 and p53 can be used as prognostic markers in triple negative breast cancer (TNBC) are reported (Han et al., 2011).

Other studies said that systematic treatment for ER (-) breast cancer is required in cases of other molecular targets based on alteration, over expression of oncogene and reexpression of suppressor gene (Rochefort et al., 2003). And oncogene or suppression genes associated with ER (-) breast cancer patient group affect to the survival rate or other prognostic factors so our results also can be studied with these concerns (Cianfrocca and Gradishar, 2009).

As breast cancer is one of the relatively well pharmacogenetic studied cancers, so various type drug was developed. For example, selective estrogen receptor modulators (SERMs; Tamoxifen, raloxifen, or lasofoxifene), selective estrogen receptor down-regulators (SERDs; Fulvestrant), and aromatase inhibitors (AIs; anastrozole, exemestane, and letrozole) are representative of the breast cancer therapy (Uray and Brown, 2011). Because most of breast cancer drug was used to hormone-sensitive breast cancer, existence of hormone receptor is key of anticancer drug treatment (Frasor et al., 2003). Especially, it has previously linked genetic variants in known pathways with treatment response. For example, CYP2D6 variants have been correlated with tamoxifen response (Offit and Robson, 2010). Meanwhile the association of genetic polymorphisms (eg. CYP19A1) with aromatase activity is correlated with sex hormones levels and estrogen-dependent cancer such as breast cancer and prostatic cancer (Chen et al., 2009; Czajka-Oraniec and Simpson, 2010). While antihormone drug (SERMs, SERD and AIs) do prevent the development of many ER (+) breast cancers, these drugs do not prevent ER (-) breast cancer. In mice level study, growth factor pathways activated by epidermal growth factor receptor (EGFR), HER2, and insulin growth factor receptor (IGFR), which are activated in many ER (-) breast cancers, can be a target for ER(-) breast cancer prevention (Uray and Brown, 2011). With our results, we can apply PSCA gene SNP variations for ER (-) breast cancer patients to pharmacogenetic drug design.

While PSCA merit, relatively easy identified in blood level as molecular diagnostic method, PSCA could be considered both potential as cancer marker and possibility as vaccination (Matera, 2010). Several immunotherapeutic strategies targeting PSCA have been explored including monoclonal antibodies, antibodies conjugated to cytotoxins, genetically engineered T cells, PSCA vaccination and peptide-loaded dendritic cells (DC). Due to the restricted pattern of expression, PSCA is an attractive candidate target protein for immunotherapy. Also, in vitro and in vivo studies have shown that PSCA immunotherapy can be not only safe but also quite effective in the treatment of prostate cancer (Raff et al., 2009). Based on that PSCA associated with breast cancer, directly introduction of vaccination know-how developed in prostate cancer to breast cancer must be possible.

As a result, the variations of PSCA gene are accessible candidate of SNP marker, and PSCA could be an important candidate gene that is thought to cancer therapy and vaccines development in breast cancer. Meanwhile other statistically significant SNPs classified at the most intron region are needed further studies.

In this study, the PSCA gene variations according to our result using MALDI-TOF MS make an impact on breast cancer. Especially their association also was revealed in pre-menopausal and ER (-) breast cancer patients Korean women. This paper is the first case analyzing that PSCA gene is concerned with ER (-) breast cancer (rs2976395) and pre-manopausal women (rs2294008). So our result can affect to the new diagnostic approach and drug development (including vaccination) in future breast cancer therapy. The newly excavated marker should be valuable in evaluating the likely impact and cost-effectiveness of new potential prognostic factors and adjuvant therapies.

In further studies, effect of PSCA genes for breast cancer should be performed and also we need to consider other genes in 8q24 loci. Study more in detail related to the breast cancer susceptibility by epidemiological and environmental effect also is needed.

Acknowledgement

This work was supported by the National Cancer Center Grant in Korea (NCC-1110270 and 0610550). We gratefully thank to the 456 patient and 461 normal sample contributors for their valuable contributions.

References

AleAhmad S, Casey G, Sweeney P, et al (2009). Prostate stem cell antigen DNA vaccination breaks tolerance to self-antigen and inhibits prostate cancer growth. *Mol Ther*, **17**, 1101-8.

Ahn SH (2004). Clinical characteristics of breast cancer patients in Korea in 2000. *Arch Surg*, **139**, 27-30, discussion 1.

Benson JR, Jatoi I, Keisch M, et al. (2009). Early breast cancer. *Lancet*, **373**, 1463-79.

Berndt SI, Potter JD, Hazra A, et al. (2008). Pooled analysis of genetic variation at chromosome 8q24 and colorectal neoplasia risk. *Hum Mol Genet*, **17**, 2665-72.

Buck MB, Fritz P, Dippon J, et al. (2004). Prognostic significance of transforming growth factor beta receptor II in estrogen receptor-negative breast cancer patients. *Clin Cancer Res*, **10**, 491-8.

Chen JQ, Brown TR, Russo J (2009). Regulation of energy metabolism pathways by estrogens and estrogenic chemicals and potential implications in obesity associated with increased exposure to endocrine disruptors. *Biochim Biophys Acta*, **1793**, 1128-43.

Chu LW, Meyer TE, Li Q, et al. (2010). Association between genetic variants in the 8q24 cancer risk regions and circulating levels of androgens and sex hormone-binding globulin. *Cancer Epidemiol Biomarkers Prev*, **19**, 1848-54.

Cianfrocca M, Gradishar W (2009). New molecular classifications of breast cancer. *CA Cancer J Clin*, **59**, 303-13.

Coffey DS (2001). Similarities of prostate and breast cancer: Evolution, diet, and estrogens. *Urology*, **57**, 31-8.

Czajka-Oraniec I, Simpson ER (2010). Aromatase research and its clinical significance. *Endokrynol Pol*, **61**, 126-34.

Ferlay J, Shin HR, Bray F, et al. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.

Fletcher O, Johnson N, Gibson L, et al. (2008). Association of genetic variants at 8q24 with breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, **17**, 702-5.

Frasor J, Danes JM, Komm B, et al. (2003). Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. *Endocrinology*, **144**, 4562-74.

Garcia-Closas M, Chanock S (2008). Genetic susceptibility loci for breast cancer by estrogen receptor status. *Clin Cancer Res*, **14**, 8000-9.

Gluz O, Liedtke C, Gottschalk N, et al. (2009). Triple-negative breast cancer--current status and future directions. *Ann Oncol*, **20**, 1913-27.

Habel LA, Capra AM, Oestreicher N, et al. (2007). Mammographic density in a multiethnic cohort. *Menopause*, **14**, 891-9.

Han JS, Cao D, Molberg KH, et al. (2011). Hormone receptor status rather than HER2 status is significantly associated with increased Ki-67 and p53 expression in triple-negative breast carcinomas, and high expression of Ki-67 but not p53 is significantly associated with axillary nodal metastasis in triple-negative and high-grade non-triple-negative breast carcinomas. *Am J Clin Pathol*, **135**, 230-7.

Imyanitov EN (2009). Gene polymorphisms, apoptotic capacity and cancer risk. *Hum Genet*, **125**, 239-46.

Jung KW, Park S, Kong HJ, et al. (2011). Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2008. *Cancer Res Treat*, **43**, 1-11.

Kim BK, Lee JW, Park PJ, et al. (2009). The multiplex bead array approach to identifying serum biomarkers associated with breast cancer. *Breast Cancer Res*, **11**, R22.

Li J, Humphreys K, Heikkinen T, et al. (2010). A combined analysis of genome-wide association studies in breast cancer. Breast Cancer Res Treat.

Lopez-Otin C, Diamandis EP (1998). Breast and prostate cancer: an analysis of common epidemiological, genetic, and biochemical features. *Endocr Rev*, **19**, 365-96.

Matera L (2010). The choice of the antigen in the dendritic cell-based vaccine therapy for prostate cancer. *Cancer Treat Rev*, **36**, 131-41.

Matsuo K, Tajima K, Suzuki T, et al. (2009). Association of prostate stem cell antigen gene polymorphisms with the risk of stomach cancer in Japanese. *Int J Cancer*, **125**, 1961-4.

Offit K, Robson ME (2010). New pharmacogenomic paradigm in breast cancer treatment. *J Clin Oncol*, **28**, 4665-6.

Onay VU, Briollais L, Knight JA, et al. (2006). SNP-SNP interactions in breast cancer susceptibility. *BMC Cancer*, **6**, 114.

Orlando L, Schiavone P, Fedele P, et al. (2010). Molecularly targeted endocrine therapies for breast cancer. *Cancer Treat Rev*, **36** Suppl 3, S67-71.

Ozet A, Yavuz AA, Komurcu S, et al. (2000). Bilateral male breast cancer and prostate cancer: a case report. *Jpn J Clin Oncol*, **30**, 188-90.

Raff AB, Gray A, Kast WM (2009). Prostate stem cell antigen: a prospective therapeutic and diagnostic target. *Cancer Lett*, **277**, 126-32.

Reiter RE, Gu Z, Watabe T, et al. (1998). Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci U S A*, **95**, 1735-40.

Rochefort H, Glondu M, Sahla ME, et al. (2003). How to target estrogen receptor-negative breast cancer? *Endocr Relat Cancer*, 10, 261-6.

Rugo HS (2008). The breast cancer continuum in hormone-receptor-positive breast cancer in postmenopausal women: evolving management options focusing on aromatase inhibitors. *Ann Oncol*, **19**, 16-27.

Sakamoto H, Yoshimura K, Saeki N, et al (2008). Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer. *Nat Genet*, **40**, 730-40.

Smith LF (2007). Palpable cancer of the breast and negative mammography: the ongoing dilemma. *J Surg Oncol*, **96**, 451-2.

Thomas G, Jacobs KB, Kraft P, et al. (2009). A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat Genet*, **41**, 579-84.

Uray IP, Brown PH (2011). Chemoprevention of hormone receptor-negative breast cancer: new approaches needed. *Recent Results Cancer Res*, **188**, 147-62.

Wiechec E, Hansen LL (2009). The effect of genetic variability on drug response in conventional breast cancer treatment. *Eur J Pharmacol*, **625**, 122-30.

Williams C, Brunskill S, Altman D, et al. (2006). Cost-effectiveness of using prognostic information to select women with breast cancer for adjuvant systemic therapy. *Health Technol Assess*, **10**, iii-iv, ix-xi, 1-204.

Wu X, Ye Y, Kiemeney LA, et al. (2009). Genetic variation in the prostate stem cell antigen gene PSCA confers susceptibility to urinary bladder cancer. *Nat Genet*, **41**, 991-5.

Yang X, Lippman ME (1999). BRCA1 and BRCA2 in breast cancer. *Breast Cancer Res Treat*, **54**, 1-10.

Yeager M, Xiao N, Hayes RB, et al. (2008). Comprehensive resequence analysis of a 136 kb region of human chromosome 8q24 associated with prostate and colon cancers. *Hum Genet*, **124**, 161-70.