Multiplex genotyping of 1107 SNPs from 232 candidate genes identified an association between IL1A polymorphism and breast cancer risk

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Abstract. We sought to identify genes and polymorphisms associated with breast cancer risk among Korean women using multiplex genotyping. The SNPs (n=1536) of 264 candidate genes were genotyped using the Illumina Golden Gate™ assay. These genes are involved in the pathways controlling apoptosis/anti-apoptosis, the immune and inflammatory responses, cytokines, DNA repair, cell adhesion, and cell cycle/proliferation. Breast cancer cases (n=209) were recruited from Seoul National University Hospital. Age-matched control subjects (n=209) were selected from a health examinees cohort. Gene-based and SNP-based tests were performed. The final analysis includes 117 cases and 164 controls with 1107 SNPs in 232 genes. Gene-based analyses showed that *IL1A*, *TNFRSF10B*, *TNFRSF1B*, *ICAM*, and *TNFSF9* were significantly associated with breast cancer risk ($p<0.01$). *IL1A* was the most significant gene associated with breast cancer risk [p for likelihood ratio test, 1 degree of freedom (df)=$6x10^{-7}$; FDR-adjusted p-value, 1df=$4x10^{-4}$, 2df=0.0071, respectively]. Individual SNP-based analyses revealed that the rare allele of the *IL1A* SNP rs2856836, Ex7-592T→C, was strongly associated with breast cancer risk (FDR-adjusted p-value, 1df=0.0027, 2df=0.0162). This SNP was found to increase risk for breast cancer [odds ratio (OR)=2.88, 95% confidence interval (CI)=1.58-5.27 for heterozygote and OR=8.17, 95% CI=2.23-29.99 for rare homozygote]. In summary, we identified a common genetic variant in *IL1A* strongly associated with breast cancer risk.

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Introduction

It is estimated that approximately 5% of breast cancer cases are attributable to rare high-penetrance mutations in a small number of specific genes, such as *BRCA1* and *BRCA2* (1). It is hypothesized that the significant unexplained fraction of familial risk is likely to be explained by a polygenic model involving more common variants with weak associations with risk (2). The search for genetic variants that cause disease has focused on the use of study designs that compare cases and controls and have lead to the recent development of highly multiplexed single nucleotide polymorphism (SNP) genotyping assays to screen for candidate genetic variants in an affordable, high-throughput manner (3,4).

The present study used the Illumina GoldenGate assay to evaluate common variants of candidate genes associated with breast cancer risk and targeted 1536 SNPs from 264 genes. The gene set chosen for the present study was originally chosen for the study of non-Hodgkin lymphoma susceptibility. This gene set includes genes involved in the pathways of apoptosis/anti-apoptosis, immune and inflammatory responses, cytokines, DNA repair, cell adhesion, and cell cycle/proliferation. The involvement of these pathways in breast cancer carcinogenesis and progression has led to intensive investigations of the relationship between many of these genes and breast cancer risk (5-14).

We performed an exploratory small scale study to identify possible associations between genetic polymorphisms and breast cancer risk in Korean women.

Materials and methods

Study subjects. In this study, cases were patients diagnosed with histologically confirmed breast cancer from 2002 to 2004 at the Seoul National University Hospital, Seoul, Korea. Control subjects frequency-matched by age were selected from the healthy women who voluntarily receive routine medical check-up at centers in Seoul and Gyeonggi-do (a province adjacent to Seoul), Korea, during the same period. This study was approved by the Institutional Review Board for Human Research of Seoul National University Hospital (IRB No. H-0503-144-004). Informed consent was

obtained from all study subjects before participation in the study. The subjects with previous histories of cancer, hysterectomy, and oophorectomy, and those for whom DNA samples were unavailable, were excluded. Finally a total of 209 cases and 209 controls were selected. Information on demographic characteristics included education, marital status, family history of breast cancer in first-degree relatives, reproductive and menstrual factors, and lifestyle habits including smoking status and alcohol consumption. This information was collected by well-trained interviewers using a structured questionnaire.

Genotyping. DNA samples were sent from Seoul National University, Korea, to the Core Genotyping Facility (CGF) at the National Cancer Institute, USA (http://cgf.nci.nih.gov/). The Illumina oligonucleotide pool assay (OPA) was designed and the GoldenGate™ assay (Illumina Inc., San Diego, USA) was performed at the CGF. The concentrations of genomic DNA were measured in 96-well formats using PicoGreen technology (Molecular Probes, Eugene, USA).

Of the 209 case-control DNA sample pairs, DNA quality of 117 case samples and 164 control samples were adequate for the final assay with overall genotype call rate of 67.2%.

The candidate genes and SNPs were selected from the gene set used for the SNP500Cancer project (http://snp500cancer.nci.nih.gov). SNP selection favored non-synonymous SNPs, those previously evaluated in relation to cancer risk, and those with evidence of functional significance (15). Tag SNPs for each candidate gene were selected from the region 20 kb from the start of transcription (exon 1) and 10 kb from the end of the last exon. Tag SNPs were chosen from the set of SNPs that had been genotyped in the International HapMap Project (http://www.hapmap.org/index.html) and in later iterations using TagZilla (http://tagzilla.nci.nih.gov). The following parameters were used for Tag SNP selection: i) minor allele frequency (MAF) >5% in the HapMap Caucasian (CEU) sample; and ii) Illumina SNP score >0.4.

A gene set represented by 1536 SNPs was selected for the OPA from the SNP500Cancer database. This gene set was originally selected for the study of non-Hodgkin's lymphoma susceptibility. We used this gene set as most of the genes were involved in pathways with a role in breast carcinogenesis and cancer progression. Of the set of 1536 SNPs chosen for this study, 429 SNPs were not analyzed: 166 were not polymorphic, 172 had low MAF values (<3%), 77 failed in the assay, and 14 deviated from the Hardy-Weinberg equilibrium (exact p-value <0.001). The remaining 1107 SNPs of 232 genes were evaluated for association analysis. The overall completion rate for genomic DNA samples was 99.5%.

Statistical analysis. The χ^2 test for genotype distribution was conducted to evaluate the deviation from the Hardy-Weinberg equilibrium in each case and control group. Breast cancer risk was estimated as odds ratios (ORs) and 95% confidence intervals (95% CIs) by unconditional logistic regression analyses, adjusted for age (years), family history of breast cancer in first-degree relatives (yes or no), and alcohol consumption (non-drinker or drinker). The breast cancer risk estimates were calculated using SAS version 9.1 (SAS Institute, Cary, NC, USA).

The association between the case-control status and each individual SNP was tested using the likelihood-ratio test (LRT) with 2-degrees of freedom (df) and the linear trend test according to previously described methods (16). Gene-based p-values for association were also calculated using the LRT. We performed two gene-based tests for association: i) an LRT for each gene comparing models with and without terms for heterozygous and homozygous variant genotypes for each SNP in a given gene (df=2 number of SNPs per gene); ii) an LRT for each gene comparing models with and without terms for each SNP (genotypes coded as 0, 1, and 2) in a given gene (df=number of SNPs per gene). Computing p-values by permutations for multiple comparisons were obtained by running 1000-300000 permutations. False discovery rate (FDR) is the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypothesis among all the genes or SNPs analyzed in this report. The Benjamini and Hochberg method (17) was used to calculate FDR values using 'multtest' package in the R project for statistical analyses.

Haplotype associations, adjusted for the covariates described above, were assessed by unconditional logistic regression using the haplo.stats program (http://mayo-research.mayo.edu/mayo/research/schaid_lab/software.cfm). The statistical analyses were performed using the R project 'genetics' and 'dgc.genetics' packages (http://www.r-project.org).

Results

Breast cancer risk was associated with a family history of breast cancer in first-degree relatives (OR=3.59, 95% CI=1.09-11.77) and alcohol consumption (OR=2.06, 95% CI=1.21-3.51) (Table I). The mean age and the distribution of risk factors were not different between subjects selected for analysis and those deleted from the initial case-control set.

High-quality genotype data were generated from 1107 SNP assays covering 232 candidate genes (range: 1-42 SNPs per gene). Approximately 50% of the SNPs were located in introns, 7% in exons, 26% in promoter region, and 15% in 3'-untranslated regions (UTRs).

Gene-based analysis revealed promising associations with breast cancer risk for 5 of the 232 genes including *IL1A*, *TNFRSF10B*, *TNFRSF1B*, *ICAM1* and *TNFSF*, based on global p-values less than the significance level of 0.01 for the LRT using the trend test (1df) or genotypic associations (2df) (Table II). The most significant association with breast cancer risk was observed for *IL1A* (interleukin 1 α) (global trend $p=6.58\times10^{-7}$, permuted $p=1.64\times10^{-6}$). Among the five genes, only *IL1A* was significantly associated with breast cancer risk, with an FDR-adjusted p-value <0.05. The FDR-adjusted p-value for *IL1A* was 0.0004 and 0.0071 using the trend test and genotypic associations, respectively.

Individual SNP-based analyses showed that the most significant SNP was rs2856836 located in the 3'-UTR of *IL1A* (Table III). The ORs of this SNP for heterozygote and homozygote variants compared with the common homozygote was 2.88 (95% CI: 1.58-5.27) and 8.17 (95% CI: 2.23-29.99), respectively, indicating that the minor allele is

Table I. Characteristics of 117 breast cancer cases and 164 control subjects.

	Cases, N (%)	Controls, N (%)	OR (95% CI)	p-value	Adjusted OR (95% CI)[a]	p-value
Age (mean ± SD), years	49.8±7.8	50.5±7.6	0.98			
Body mass index, kg/m^2	23.4±2.9	23.2±2.7	1.02			
Education						
Under high school	29 (25.9)	49 (31.2)	1.00 (ref.)		1.00 (ref.)	
At or over high school	83 (74.1)	108 (68.8)	1.29 (0.75, 2.23)	0.3440	1.83 (0.92, 3.61)	0.0819
Family history of breast cancer in 1st relatives						
No	107 (91.4)	154 (97.4)	1.00 (ref.)		1.00 (ref.)	
Yes	10 (8.6)	4 (2.6)	3.59 (1.09, 11.77)	0.0343	1.82 (0.38, 8.62)	0.4490
Alcohol consumption						
Non-drinker	75 (64.1)	129 (78.6)	1.00 (ref.)		1.00 (ref.)	
Drinker	42 (35.9)	35 (21.3)	2.06 (1.21, 3.51)	0.0075	2.58 (1.27, 5.21)	0.0081

[a]Odds ratios (OR) were adjusted for other covariates.

Table II. Global, permuted, and FDR-adjusted p-values for the associations between breast cancer and five candidate genes showing most significant results.

Gene symbol	Chromosome	No. of SNPs per gene	Trend test[a]			Genotype associations[b]		
			Global p-value	Permuted p-value[c]	FDR adjusted p-value	Global p-value	Permuted p-value	FDR adjusted p-value
IL1A	2	4	0.000000658	0.00000164[d]	0.0004	00.0000139	0.00003[e]	0.0071
TNFRSF10B	8	10	0.0011	0.0012	0.1416	0.0229	0.052	1.0000
TNFRSF1B	1	8	0.2459	0.34	1.0000	0.0026	0.006	0.7198
ICAM1	19	2	0.2812	0.26	1.0000	0.0153	0.013	0.8732
TNFSF9	19	5	0.1576	0.21	1.0000	0.0086	0.014	0.8732

[a]Likelihood-ratio test with degrees of freedom, number of SNPs per gene included in the model. [b]Likelihood-ratio test with degrees of freedom, number of SNPs per gene included in the model x2. [c]Permutated p-value marked [d]5000000 permutations; [e]500000 permutations; and the rest, 1000-10000 permutations.

associated with an increased risk of breast cancer [p for trend test = 2.46×10^{-6} and p for LRT (2df) = 1.51×10^{-5}].

Table IV presents the FDR-adjusted p-values for the association between breast cancer and 12 common genetic variations listed in Table III. Only *IL1A* rs2856836 gave FDR-adjusted p-values (2df and 1df) <0.05.

Consistent with the results of SNP analyses, haplotype analyses revealed a strong association between *IL1A* and breast cancer risk, with a global p-value of 2.6×10^{-5} (Table V). The A-G-A-G-C haplotype of *IL1A* confers a 6.7-times greater risk for the carrier compared with the most common haplotype ($p=4.6 \times 10^{-5}$). Haplotype analyses also revealed a significant association between *TNFRSF10B* and breast cancer risk with a global p-value of 2.7×10^{-4}. The A-C-A-C haplotype of *TNFRSF10B* was associated with a significantly increased risk (OR=3.4, 95% CI: 1.7-6.7, $p=4.5 \times 10^{-4}$). By comparison, the T-A-G-G haplotype of *FVT1* was associated with a significantly reduced risk for breast cancer.

Discussion

We conducted a large-scale association study using the GoldenGate assay to evaluate 1536 SNPs in 264 candidate genes to identify SNPs associated with breast cancer risk among Korean women. The most significant gene, according to gene-based analyses, was *IL1A* and the most significant individual SNP was found in the 3'-UTR of *IL1A* (rs2856836, Ex7-592T→C). This *IL1A* SNP was identified as strongly associated with breast cancer risk following multiple testing adjustments, and the rare allele of this SNP was found to increase the risk of breast cancer.

This finding is consistent with previous biological studies showing that interleukin (IL)-1 has a role in breast cancer development and progression. The IL-1 family of cytokines (IL-lα, IL-1ß), the IL-1 receptor antagonist (IL-1RA), and IL-1 receptors (IL-1RI and IL-1RII) are frequently expressed in breast cancer cell lines, in human breast cancer tissue,

Table III. Association between individual SNPs and breast cancer risk.

Gene	SNP	Genotype	Cases	Controls	OR (95% CI)	p-value	Adjusted OR (95% CI)[a]	p-value	Global p-value (2df)	Global p-value (1df)
IL1A	rs2856836	TT	66	137	1.00 (ref.)		1.00 (ref.)		0.000015	0.000002
g.Ex7-592T→C		CT	37	24	3.20 (1.77, 5.78)	0.0001	2.88 (1.58, 5.27)	0.0006		
		CC	14	3	9.68 (2.69, 34.87)	0.0005	8.17 (2.23, 29.99)	0.0015		
IL12A	rs583911	GG	51	94	1.00 (ref.)		1.00 (ref.)		0.0004	0.5699
g.IVS2-409G→A		AG	65	56	2.14 (1.31, 3.51)	0.0025	1.95 (1.17, 3.23)	0.0100		
		AA	1	14	0.13 (0.02, 1.03)	0.0534	0.14 (0.02, 1.10)	0.0615		
INSL3	rs12462622	AA	58	85	1.00 (ref.)		1.00 (ref.)		0.0013	0.1813
g.*377C→A		AC	56	61	1.35 (0.82, 2.20)	0.2381	1.28 (0.77, 2.14)	0.3386		
		CC	2	18	0.16 (0.04, 0.73)	0.0176	0.13 (0.03, 0.59)	0.0089		
TNFSF9	rs348373	CC	61	106	1.00 (ref.)		1.00 (ref.)		0.0014	0.2353
g.*8264C→T		CT	55	51	1.87 (1.14, 3.07)	0.0128	1.99 (1.19, 3.34)	0.0091		
		TT	1	7	0.25 (0.03, 2.07)	0.1976	0.12 (0.01, 1.21)	0.0718		
BCL2	rs6567326	CC	43	33	1.00 (ref.)		1.00 (ref.)		0.0025	0.0199
g.IVS2-44616C→A		AC	46	93	0.38 (0.21, 0.68)	0.0010	0.35 (0.20, 0.64)	0.0007		
		AA	24	38	0.49 (0.25, 0.96)	0.0378	0.47 (0.23, 0.96)	0.0368		
TNFRSF10B	rs4460370	CC	78	82	1.00 (ref.)		1.00 (ref.)		0.0043	0.0015
g.IVS3-374C→T		CT	36	65	0.58 (0.35, 0.97)	0.0383	0.59 (0.35, 1.00)	0.0517		
		TT	3	17	0.19 (0.05, 0.66)	0.0091	0.18 (0.05, 0.65)	0.0089		
IL5	rs2706399	TT	91	112	1.00 (ref.)		1.00 (ref.)		0.0055	0.2687
g.*9800T→C		CT	19	51	0.46 (0.25, 0.83)	0.0102	0.46 (0.25, 0.86)	0.0141		
		CC	5	1	6.15 (0.71, 53.61)	0.0999	5.82 (0.65, 52.30)	0.1158		
LOC96597	rs9904659	AA	31	65	1.00 (ref.)		1.00 (ref.)		0.0079	0.2101
g.-1717G→A		AG	67	68	2.07 (1.20, 3.56)	0.0091	2.35 (1.33, 4.15)	0.0033		
		GG	19	31	1.29 (0.63, 2.62)	0.4909	1.24 (0.58, 2.61)	0.5793		
IL1RN	rs315927	CC	63	65	1.00 (ref.)		1.00 (ref.)		0.0089	0.2367
g.-9226C→T		CT	36	81	0.46 (0.27, 0.77)	0.0035	0.45 (0.26, 0.78)	0.0043		
		TT	17	18	0.97 (0.46, 2.06)	0.9459	1.04 (0.48, 2.26)	0.9183		
ICAM1	rs3093030	CC	59	75	1.00 (ref.)		1.00 (ref.)		0.0090	0.1121
g.*1439C→T		CT	54	65	1.06 (0.64, 1.74)	0.8295	1.15 (0.69, 1.93)	0.5968		
		TT	4	24	0.21 (0.07, 0.64)	0.0062	0.24 (0.08, 0.75)	0.0136		
GPX3	rs8177441	CC	46	48	1.00 (ref.)		1.00 (ref.)		0.0091	0.0095
g.IVS2-467G→C		CG	59	77	0.80 (0.47, 1.36)	0.4061	0.88 (0.51, 1.53)	0.6492		
		GG	12	38	0.33 (0.15, 0.71)	0.0044	0.32 (0.14, 0.71)	0.0049		
IL1RN	rs10207930	CC	16	18	1.00 (ref.)		1.00 (ref.)		0.0139	0.2011
g.-14734C→A		CA	36	80	0.46 (0.27, 0.77)	0.0033	0.47 (0.27, 0.80)	0.0058		
		AA	65	66	0.90 (0.42, 1.92)	0.7903	0.99 (0.45, 2.18)	0.9781		

[a]ORs are adjusted for age, family history of breast cancer in first-degree relatives, and alcohol consumption.

and within the tumor microenvironment (18). The local expression of members of the IL-1/IL-1R cytokine family can control, via autocrine and/or paracrine mechanisms, the expression of other protumorigenic cytokines, such as IL-8, and subsequently activate tumorigenesis via angiogenesis, tumor proliferation, and invasion (19-21).

The *IL1A* gene is mapped to chromosome 2q13 and harbors several polymorphisms, including one in the 5'-UTR regulatory region (rs1800587, -889C→T) and one in exon 5 [rs17561, +4845G→T (Ala114Ser)] that are commonly studied. Several previous studies report an association between polymorphisms in IL1A and breast cancer risk. Sigurdson *et al* (5)

Table IV. FDR-adjusted p-values for significant association between breast cancer and common genetic variations.

Gene	SNP	Base-genotype	p-value (2df)	FDR adjusted p-value	p-value (1df)	FDR adjusted p-value
IL1A	rs2856836	TT	0.000015	0.0162	0.000002	0.0027
IL12A	rs583911	GG	0.0004	0.2400	0.5699	0.9847
INSL3	rs12462622	AA	0.0013	0.3682	0.1813	0.9847
TNFSF9	rs348373	CC	0.0014	0.3682	0.2353	0.9847
BCL2	rs6567326	CC	0.0025	0.5283	0.0199	0.9345
TNFRSF10B	rs4460370	CC	0.0043	0.5417	0.0015	0.5528
IL5	rs2706399	TT	0.0055	0.5417	0.2687	0.9847
LOC96597	rs9904659	AA	0.0079	0.5417	0.2101	0.9847
IL1RN	rs315927	CC	0.0089	0.5417	0.2367	0.9847
ICAM1	rs3093030	CC	0.0090	0.5417	0.1121	0.9847
GPX3	rs8177441	CC	0.0091	0.5417	0.0095	0.9345
IL1RN	rs10207930	CC	0.0139	0.6207	0.2011	0.9847

Table V. Significant association between haplotypes and breast cancer risk.

Gene	SNP	Haplotype	Controls (%)	Cases (%)	OR (95% CI)[a]			p-value	Global p-value
		1-2-3-4-5							
IL1A	1: rs17042407	A-G-A-G-T	108 (65.9)	67 (57.3)		1.00 (ref.)			0.000026
	2: rs11687624	G-A-C-A-T	32 (19.4)	14 (12.2)	0.808	0.474	1.377	0.434	
	3: rs12612788	A-A-C-A-T	5 (3.0)	2 (1.7)	0.861	0.237	3.128	0.821	
	4: rs3783516	A-A-C-G-C	8 (5.2)	11 (9.1)	1.256	0.591	2.671	0.555	
	5: rs2856836	A-G-A-G-C	4 (2.4)	14 (12.2)	6.710	2.724	16.530	0.000046	
		G-A-C-A-C	3 (1.6)	7 (5.7)	2.959	0.846	10.355	0.0905	
TNFRSF10B	1: rs11785599	G-C-G-G	60 (36.8)	37 (31.5)		1.00 (ref.)			0.00027
	2: rs4460370	A-C-G-G	15 (9.0)	11 (9.3)	1.199	0.590	2.438	0.617	
	3: rs883429	G-C-G-C	4 (2.4)	5 (4.5)	2.342	0.718	7.641	0.159	
	4: rs1047275	A-C-G-C	23 (14.0)	19 (16.5)	1.562	0.871	2.802	0.136	
		A-T-A-C	46 (28.4)	20 (17.3)	0.752	0.451	1.252	0.274	
		A-C-A-C	12 (7.0)	21 (18.2)	3.410	1.733	6.710	0.00045	
FVT1	1: rs2551408	A-C-A-G	103 (62.5)	75 (63.7)		1.00 (ref.)			0.00061
	2: rs2850767	T-C-A-C	41 (25.0)	33 (28.2)	1.107	0.726	1.687	0.637	
	3: rs11663788	T-A-G-G	17 (10.7)	4 (3.8)	0.275	0.119	0.637	0.0029	
	4: rs2850764	T-A-A-C	3 (1.8)	2 (2.1)	1.351	0.396	4.609	0.631	

[a]ORs are adjusted for age, family history of breast cancer in first-degree relatives, and alcohol consumption.

reported a significant increase in breast cancer risk for the minor alleles of *IL1A* A114S (rs17561) with an OR=1.2 for heterozygotes and an OR = 1.5 for homozygotes in the US Radiologic Technologists cohort. Conversely, Balasubramanian *et al* (22) reported a trend for the rare allele of rs17561 to confer a protective effect against breast cancer (p=0.05). The *IL1A*-889C→T polymorphism has been studied in two different cohorts and has been shown not to be associated with breast cancer risk (7,23).

There is little data on the effect of the SNPs rs2856836 on expression or function of IL-1A. Kristensen *et al* (24) identified two germline SNPs in *IL1A* (rs1800587 and rs2856836) associated with gene expression in the patients' tumor indicating a potential regulatory role of rs1800587 and rs2856836 in breast cancer. This finding suggests that the *IL1A* SNP rs2856836, identified in the present study as significantly associated with breast cancer risk, might be a functional SNP. Another group reported a strong association between this SNP and ankylosing spondylitis (25).

The human genes encoding IL-1α (*IL1A*), IL-1ß (*IL1B*), and IL-1RA (*IL1RN*) are located within a 430-kb region on chromosome 2q14.2 (26). Lee *et al* (6) reported that an 86-bp

variable number of tandem repeats (VNTR) polymorphism (5-6 alleles) in intron 2 of *IL-1RN* was associated with a decrease in breast cancer risk with marginal significance (OR=0.7, 95% 95% CI=0.48-1.05). Consistent with this result, we found that the two SNP on the 5'-UTR of *IL1RN* (rs315927 and rs10207930) were associated with a decrease in breast cancer risk. The ORs for the heterozygote genotypes of the two SNPs were similar [0.45 (95% CI: 0.26-0.78) for rs315927 CT genotype, and 0.47 (95% CI: 0.27-0.80) for rs10207930 CA genotype].

Kammerer *et al* (11) reported an OR of 0.63 for the *ICAM1* K469E GG versus AA in a large-scale breast cancer association study. In a conflicting report, Cox *et al* (27) found a non-significant association for the same SNP and breast cancer risk with an OR of 0.85 (95% CI: 0.67-1.08). In the present study, we revealed that the rare homozygote TT of the SNP within the 3'-UTR of *ICAM1* (rs3093030) had a significant protective effect on breast cancer risk (OR=0.24, 95% CI: 0.08-0.75).

In the present study, an intronic SNP of *TNFRSF10B* (rs4460370) showed significant p-values for 2df and 1df analyses (0.0043 and 0.0015, respectively). The rare allele T of this SNP appeared to confer a protective effect against breast cancer with a significant gene-dosage relationship [OR for heterozygote = 0.59 (95% CI: 0.35-1.00) and OR for rare homozygote = 0.18 (95% CI: 0.05-0.65)]. A previous study investigated the association between another polymorphism (1-5801C/T) of *TNFRSF10B* and breast cancer risk, and found that the association was not significant (28).

Eleven genes in this study have been investigated in the previous Korean SNP association studies (*XRCC1*, *XRCC3*, *XRCC4*, *LIG1*, *LIG4*, *RAD5*, *ERCC1*, *ERCC2*, *LTA*, *IL1B*, *CASP8*) (29-34). However, only one SNP, *CASP8* D302H (rs1045485) was studied both in a previous study and in this study. This SNP was not polymorphic in Korean population in either study (34).

A recent large-scale genome-wide association study has identified SNPs in five loci associated with breast cancer risk: *TNRC9*, *FGFR2*, *MAP3K1*, *H19*, and *LSP1* (35). These results were confirmed for *FGFR2* and *TNRC9* in two subsequent independent studies (4,36). Cancer Genetics Markers of Susceptibility (CGEMS), a three-year initiative of the National Cancer Institute conducts large scale genome-wide association studies with follow-up replication to identify common gene variations affecting breast cancer risk. The raw genotype data are available online to accredited investigators upon request (http://cgems.cancer.gov/). These efforts and results suggest that SNP-chip-based multiplex genotyping technology provides a promising and powerful platform for identifying genes and polymorphisms associated with breast cancer susceptibility, and will replace the candidate gene approach for the discovery of cancer-associated polymorphisms. Due to the high cost and requirement for state-of-the-art technology, the whole genome association study could not be commonly available. The present study supports the validity of multiplex genotyping technology of moderate number of genes as a genetic screening tool for candidate gene discovery.

The most serious limitation of the present study is the small sample size. Subsequent study to validate the results in large scale independent data set should follow. Due to the small sample size, this study also has low statistical power with possible false negative results. The single-stage study design and single hospital-based case collection is another weak point of this study. The tag SNPs selected for this study were primarily identified from Caucasian population, because the gene and SNP set used in this study was originally designed for Caucasians, but not for Korean. The data about the coverage rate of these tag SNPs for the Korea genome and LD blocks is lacking.

In conclusion, we used highly multiplexed SNP genotyping to identify an *IL1A* polymorphism in Korean women that may affect an individual's susceptibility to breast cancer. Further studies using a large validation set are needed to support this finding.

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