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

WONSHIK HAN1,2, SO YOUNG KANG3, DAEHEE KANG1,3, SUE K. PARK1,3, JI-YOUNG LEE3, HO KIM4, AE KYUNG PARK4 and DONG-YOUNG NOH1,2

1Cancer Research Institute, Departments of 2Surgery, and 3Preventive Medicine, 4Graduate School of Public Health, Seoul National University College of Medicine, Seoul, Korea

Received September 25, 2009; Accepted December 21, 2009

DOI: 10.3892/or_00000696

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.

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, hystere-
tomy, and oophorectomy, and those for whom DNA samples were unavai-
able, 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)
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.
ci.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 lymph-
a cancer susceptibility. We used this gene set as most of the
genes were involved in pathways with a role in breast carcino-
genesis 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 χ² 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=1.21-3.51) (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=6.58x10⁻⁷, permuted p=1.64x10⁻⁶). 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 homo-
zygote 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

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

Table IV presents the FDR-adjusted p-values for the association between breast cancer and 12 common genetic variations listed in Table III. Only \textit{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 \textit{IL1A} and breast cancer risk, with a global p-value of 2.6 \times 10^{-5} (Table V). The A-G-A-G-A haplotype of \textit{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 \textit{TNFRSF10B} and breast cancer risk with a global p-value of 2.7 \times 10^{-4}. The A-C-A-C haplotype of \textit{TNFRSF10B} was associated with a significantly increased risk for breast cancer \(p=4.5 \times 10^{-4}\). By comparison, the T-A-G-G haplotype of \textit{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 \textit{IL1A} and the most significant individual SNP was found in the 3'-UTR of \textit{IL1A} (rs2856836, Ex7-592T\(\rightarrow\)C). This \textit{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-1\(\alpha\), IL-1\(\beta\)), 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,
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 poly-
morphisms in IL1A and breast cancer risk. Sigurdson et al (5)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>Adjusted OR (95% CI)</th>
<th>Global p-value (2df)</th>
<th>Global p-value (1df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1A</td>
<td>rs2856836</td>
<td>TT</td>
<td>66</td>
<td>137</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.000015</td>
<td>0.000002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.Ex7-592T- C</td>
<td>CT</td>
<td>37</td>
<td>24</td>
<td>3.20 (1.77, 5.78)</td>
<td>0.0001</td>
<td>2.88 (1.58, 5.27)</td>
<td>0.0006</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>14</td>
<td>3</td>
<td>9.68 (2.69, 34.87)</td>
<td>0.0005</td>
<td>8.17 (2.23, 29.99)</td>
<td>0.0015</td>
<td></td>
</tr>
<tr>
<td>IL12A</td>
<td>rs583911</td>
<td>GG</td>
<td>51</td>
<td>94</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0004</td>
<td>0.5699</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.JVS2-409G-A</td>
<td>AG</td>
<td>65</td>
<td>56</td>
<td>2.14 (1.31, 3.51)</td>
<td>0.0025</td>
<td>1.95 (1.17, 3.23)</td>
<td>0.0100</td>
<td></td>
</tr>
<tr>
<td>INSL3</td>
<td>rs12462622</td>
<td>AA</td>
<td>58</td>
<td>85</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0013</td>
<td>0.1813</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.'377C-A</td>
<td>AC</td>
<td>56</td>
<td>61</td>
<td>1.35 (0.82, 2.20)</td>
<td>0.2381</td>
<td>1.28 (0.77, 2.14)</td>
<td>0.3386</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>2</td>
<td>18</td>
<td>0.16 (0.04, 0.73)</td>
<td>0.0176</td>
<td>0.13 (0.03, 0.59)</td>
<td>0.0899</td>
<td></td>
</tr>
<tr>
<td>TNFSF9</td>
<td>rs348373</td>
<td>CC</td>
<td>61</td>
<td>106</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0014</td>
<td>0.2353</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.'8264C-T</td>
<td>CT</td>
<td>55</td>
<td>51</td>
<td>1.87 (1.14, 3.07)</td>
<td>0.0128</td>
<td>1.99 (1.19, 3.34)</td>
<td>0.0091</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>1</td>
<td>7</td>
<td>0.25 (0.03, 2.07)</td>
<td>0.1976</td>
<td>0.12 (0.01, 1.21)</td>
<td>0.0718</td>
<td></td>
</tr>
<tr>
<td>BCL2</td>
<td>rs6567326</td>
<td>CC</td>
<td>43</td>
<td>33</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0025</td>
<td>0.0199</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.JVS2-44616C-A</td>
<td>AC</td>
<td>46</td>
<td>93</td>
<td>0.38 (0.21, 0.68)</td>
<td>0.0010</td>
<td>0.35 (0.20, 0.64)</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>24</td>
<td>38</td>
<td>0.49 (0.25, 0.96)</td>
<td>0.0378</td>
<td>0.47 (0.23, 0.96)</td>
<td>0.0368</td>
<td></td>
</tr>
<tr>
<td>TNFRSF10B</td>
<td>rs4460370</td>
<td>CC</td>
<td>78</td>
<td>82</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0043</td>
<td>0.0015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.JVS3-374C-T</td>
<td>CT</td>
<td>36</td>
<td>65</td>
<td>0.58 (0.35, 0.97)</td>
<td>0.0383</td>
<td>0.59 (0.35, 1.00)</td>
<td>0.0517</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>3</td>
<td>17</td>
<td>0.19 (0.05, 0.66)</td>
<td>0.0091</td>
<td>0.18 (0.05, 0.65)</td>
<td>0.0089</td>
<td></td>
</tr>
<tr>
<td>IL5</td>
<td>rs2706399</td>
<td>TT</td>
<td>91</td>
<td>112</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0055</td>
<td>0.2687</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.'9800T-C</td>
<td>CT</td>
<td>19</td>
<td>51</td>
<td>0.46 (0.25, 0.83)</td>
<td>0.0102</td>
<td>0.46 (0.25, 0.86)</td>
<td>0.0141</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>5</td>
<td>1</td>
<td>6.15 (0.71, 53.61)</td>
<td>0.0999</td>
<td>5.82 (0.65, 52.30)</td>
<td>0.1158</td>
<td></td>
</tr>
<tr>
<td>LOC96597</td>
<td>rs9904659</td>
<td>AA</td>
<td>31</td>
<td>65</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0079</td>
<td>0.2101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.'171TG-A</td>
<td>AG</td>
<td>67</td>
<td>68</td>
<td>2.07 (1.20, 3.56)</td>
<td>0.0091</td>
<td>2.35 (1.33, 4.15)</td>
<td>0.0033</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>19</td>
<td>31</td>
<td>1.29 (0.63, 2.62)</td>
<td>0.4909</td>
<td>1.24 (0.58, 2.61)</td>
<td>0.5793</td>
<td></td>
</tr>
<tr>
<td>IL1RN</td>
<td>rs315927</td>
<td>CC</td>
<td>63</td>
<td>65</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0089</td>
<td>0.2367</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.'9226C-T</td>
<td>CT</td>
<td>36</td>
<td>81</td>
<td>0.46 (0.27, 0.77)</td>
<td>0.0035</td>
<td>0.45 (0.26, 0.78)</td>
<td>0.0043</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>17</td>
<td>18</td>
<td>0.97 (0.46, 2.06)</td>
<td>0.9459</td>
<td>1.04 (0.48, 2.26)</td>
<td>0.9183</td>
<td></td>
</tr>
<tr>
<td>ICAM1</td>
<td>rs3093030</td>
<td>CC</td>
<td>59</td>
<td>75</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0090</td>
<td>0.1121</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.'1439C-T</td>
<td>CT</td>
<td>54</td>
<td>65</td>
<td>1.06 (0.64, 1.74)</td>
<td>0.8295</td>
<td>1.15 (0.69, 1.93)</td>
<td>0.5968</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>4</td>
<td>24</td>
<td>0.21 (0.07, 0.64)</td>
<td>0.0062</td>
<td>0.24 (0.08, 0.75)</td>
<td>0.0136</td>
<td></td>
</tr>
<tr>
<td>GPX3</td>
<td>rs8177441</td>
<td>CC</td>
<td>46</td>
<td>48</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0091</td>
<td>0.0095</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.JVS2-467G-C</td>
<td>CG</td>
<td>59</td>
<td>77</td>
<td>0.80 (0.47, 1.36)</td>
<td>0.4061</td>
<td>0.88 (0.51, 1.53)</td>
<td>0.6492</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>12</td>
<td>38</td>
<td>0.33 (0.15, 0.71)</td>
<td>0.0044</td>
<td>0.32 (0.14, 0.71)</td>
<td>0.0049</td>
<td></td>
</tr>
<tr>
<td>IL1RN</td>
<td>rs10207930</td>
<td>CC</td>
<td>16</td>
<td>18</td>
<td>1.00 (ref.)</td>
<td>1.00 (ref.)</td>
<td>0.0139</td>
<td>0.2011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>g.'14734C-A</td>
<td>CA</td>
<td>36</td>
<td>80</td>
<td>0.46 (0.27, 0.77)</td>
<td>0.0033</td>
<td>0.47 (0.27, 0.80)</td>
<td>0.0058</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>65</td>
<td>66</td>
<td>0.90 (0.42, 1.92)</td>
<td>0.7903</td>
<td>0.99 (0.45, 2.18)</td>
<td>0.9781</td>
<td></td>
</tr>
</tbody>
</table>

4ORs are adjusted for age, family history of breast cancer in first-degree relatives, and alcohol consumption.

Table III. Association between individual SNPs and breast cancer risk.
reported a significant increase in breast cancer risk for the minor alleles of \textit{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 \textit{et al.} \cite{22} reported a trend for the rare allele of rs17561 to confer a protective effect against breast cancer (p=0.05).

The \textit{IL1A}-889C→T polymorphism has been studied in two different cohorts and has been shown not to be associated with breast cancer risk \cite{7,23}.

There is little data on the effect of the SNPs rs2856836 on expression or function of IL-1A. Kristensen \textit{et al.} \cite{24} identified two germline SNPs in \textit{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 \textit{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 \cite{25}.

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

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Base-genotype</th>
<th>p-value (2df)</th>
<th>FDR adjusted p-value</th>
<th>p-value (1df)</th>
<th>FDR adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{IL1A}</td>
<td>rs2856836</td>
<td>TT</td>
<td>0.000015</td>
<td>0.0162</td>
<td>0.000002</td>
<td>0.0027</td>
</tr>
<tr>
<td>\textit{IL12A}</td>
<td>rs583911</td>
<td>GG</td>
<td>0.0004</td>
<td>0.2400</td>
<td>0.5699</td>
<td>0.9847</td>
</tr>
<tr>
<td>\textit{INSL3}</td>
<td>rs12462622</td>
<td>AA</td>
<td>0.0013</td>
<td>0.3682</td>
<td>0.813</td>
<td>0.9847</td>
</tr>
<tr>
<td>\textit{TNFSF9}</td>
<td>rs348373</td>
<td>CC</td>
<td>0.0014</td>
<td>0.3682</td>
<td>0.2353</td>
<td>0.9847</td>
</tr>
<tr>
<td>\textit{BCL2}</td>
<td>rs6567326</td>
<td>CC</td>
<td>0.0025</td>
<td>0.5283</td>
<td>0.0199</td>
<td>0.9345</td>
</tr>
<tr>
<td>\textit{TNFRSF10B}</td>
<td>rs4460370</td>
<td>CC</td>
<td>0.0043</td>
<td>0.5417</td>
<td>0.0015</td>
<td>0.5528</td>
</tr>
<tr>
<td>\textit{IL5}</td>
<td>rs2706399</td>
<td>TT</td>
<td>0.0055</td>
<td>0.5417</td>
<td>0.2687</td>
<td>0.9847</td>
</tr>
<tr>
<td>\textit{LOC96597}</td>
<td>rs9904659</td>
<td>AA</td>
<td>0.0079</td>
<td>0.5417</td>
<td>0.2101</td>
<td>0.9847</td>
</tr>
<tr>
<td>\textit{IL1RN}</td>
<td>rs315927</td>
<td>CC</td>
<td>0.0089</td>
<td>0.5417</td>
<td>0.2367</td>
<td>0.9847</td>
</tr>
<tr>
<td>\textit{ICAM1}</td>
<td>rs3093030</td>
<td>CC</td>
<td>0.0090</td>
<td>0.5417</td>
<td>0.1121</td>
<td>0.9847</td>
</tr>
<tr>
<td>\textit{GPX3}</td>
<td>rs8177441</td>
<td>CC</td>
<td>0.0091</td>
<td>0.5417</td>
<td>0.0095</td>
<td>0.9345</td>
</tr>
<tr>
<td>\textit{IL1R}</td>
<td>rs10207930</td>
<td>CC</td>
<td>0.0139</td>
<td>0.6207</td>
<td>0.2011</td>
<td>0.9847</td>
</tr>
</tbody>
</table>

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

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

*ORs are adjusted for age, family history of breast cancer in first-degree relatives, and alcohol consumption.*
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% 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 *ICAMI* 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 *ICAMI* (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 (rs13011G/C) 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*, *RADS*, *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 *ESPI* (35). These results were confirmed for *FGFR2* and *TNRC9* in two subsequent independent studies (34,36). 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.

**Acknowledgements**

We would like to thank patients who participated in this project and all hospital and laboratory staff. This study was supported by a grant from the Korea Health 21 R&D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (03-PJ10-PG13-GD01-0002 and A050558).

**References**


