Interleukin-1 and Interleukin-6 Gene Polymorphisms and the Risk of Breast Cancer in Caucasian Women

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Abstract

Purpose: Genetic polymorphisms of cytokine-encoding genes are known to predispose to malignant disease. Interleukin (IL)-1 and IL-6 are crucially involved in breast carcinogenesis. Whether polymorphisms of the genes encoding IL-1 (IL1) and IL-6 (IL6) also influence breast cancer risk is unknown.

Experimental Design: In the present case-control study, we ascertained three polymorphisms of the IL1 gene cluster [−889 C/T polymorphism of the IL1α gene (IL1A), −511 C/T polymorphism of the IL1β promoter (IL1B promoter), a polymorphism of IL1β exon 5 (IL1B exon 5)], an 86-bp repeat in intron 2 of the IL1 receptor antagonist gene (IL1RN), and the −174 G/C polymorphism of the IL6 gene (IL6) in 269 patients with breast cancer and 227 healthy controls using PCR and pyrosequencing.

Results: Polymorphisms within the IL1 gene cluster and the respective haplotypes were not associated with the presence and the phenotype of breast cancer. The IL6 polymorphism was significantly associated with breast cancer. Odds ratios for women with one or two high-risk alleles versus women homozygous for the low-risk allele were 1.5 (95% confidence interval, 1.04–2.3; P = 0.04) and 2.0 (95% confidence interval, 1.1–3.6; P = 0.02), respectively. No association was ascertained between presence of the IL6 polymorphism and various clinicopathologic variables.

Conclusions: Although polymorphisms within the IL1 gene cluster do not seem to influence breast cancer risk or phenotype, presence of the −174 C/IL6 allele increases the risk of breast cancer in Caucasian women in a dose-dependent fashion.

The interleukin-1 (IL-1) family consists of the two proinflammatory cytokines IL-1α and IL-1β (1, 2). IL-1α and IL-1β are produced by monocytes, macrophages, and epithelial cells and exhibit similar biological characteristics including host response to microbial invasion, inflammation, and tissue injury (1). The activity of IL-1α and IL-1β is modulated by an endogenous IL-1 inhibitor, i.e., IL-1 receptor antagonist (IL-1Ra), by binding to the IL-1 receptor without exerting an effector function (2).

With respect to breast cancer, in vitro studies showed that IL-1 inhibits malignant cell growth by reducing the ability of insulin-like growth factor I mediated DNA-synthesis (3). Interestingly, other in vitro studies showed that IL-1 is an autocrine and paracrine inducer of prometastatic genes (4). In human breast cancer, an important role of IL-1 and IL-1Ra expression was noted in various studies (5–7). These studies suggest that the IL-1 system is vital in the local control of tumor growth (6), important in regulating “protumorigenic” activities within the tumor microenvironment (7), and contributes to angiogenesis, tumor proliferation, and tumor invasion (5).

IL-6, a phosphorylated glycoprotein containing 185 amino acids, is a pleiotropic cytokine involved in different physiologic and pathophysiologic processes such as inflammation, bone metabolism, synthesis of C-reactive protein, and carcinogenesis (8, 9). IL-6 has also been shown to inhibit the growth of various breast cancer cell lines (10), shows antiapoptotic effects (11), and modulates the estrogen receptor and progesterone receptor content of these cells (12). IL-6 is thought to increase the activity of the 17-β-hydroxysteroid dehydrogenase, which converts estrone to estradiol, a process that may contribute to the increased concentration of estrogen around breast tumors (13). Furthermore, IL-6 has been shown to be involved in intercellular signaling between mesenchyme and breast cancer epithelium. These findings from in vitro studies extend to human breast cancer as elevated serum IL-6 levels were found to be independent prognosticators (14, 15). Of note, both ILs,
IL-1 and IL-6 Gene Polymorphisms and Breast Cancer

Materials and Methods

Approval for this study was obtained by the Institutional Review Boards at the Martin-Luther-University Medical School, Halle-Wittenberg, Germany and the Medical University of Vienna, Vienna, Austria. Two hundred and sixty-nine consecutive patients with breast cancer treated between 1999 and 2001 at the Departments of Obstetrics and Gynecology, Martin-Luther-University Medical School, Halle-Wittenberg, Germany and at the Medical University of Vienna, Vienna, Austria, were included in this study. Enrollment of cases and controls was started in November 1999 and ended in January 2003. Rejection rates of cases and controls were 1.3% and 4.2%, respectively, mostly due to language difficulties. All patients were of Caucasian origin and consented to participating in our study. Histologic staging of breast cancer was done according to the current classification of the International Union Against Cancer. Healthy Caucasian women from the same geographic area without breast cancer in their personal histories (n = 228) were used as controls. These women were visiting outpatient departments, mostly seeking counseling for various reasons, mainly for postmenopausal disorders or risk assessment for malignancies. No difference in age was ascertained between patients with breast cancer and controls (54.9 (12.5) versus 53.3 (10.2) years, respectively, \( P = 0.1 \)). Signed written consent was obtained from all participating women. To avoid confounding by ethnicity, only women of Austrian and German ethnic background were included.

DNA was extracted from patients' blood. Pyrosequencing and PCR were done according to established protocols as described previously (35, 36). Where appropriate, \( x^2 \) analysis was done. Odds ratios and 95% confidence intervals (CI) are given accordingly. For statistical analyses with respect to the IL6 polymorphism, we used a genotype model (IL6 – 174 G/C versus IL6 – 174 C/C versus IL6 – 174G/C). Within the IL1 gene locus, we did haplotype analysis and repeated the association analysis on the haplotype level using permutation analysis. \( P \) values <0.05 were considered statistically significant. For statistical analysis, we used the SPSS statistical software system (SPSS 11.0, SPSS Inc., Chicago, IL) and SAS/Genetics V9.1 (2003 SAS Institute Inc., Cary, NC).

Results

pT1, pT2, pT3, and pT4 tumors were found in 140, 99, 12, 18, respectively. G1, G2, and G3 tumors were found in 20, 150, and 99 cases, respectively. Histologically, 185 tumors were graded as ductal, 33 as lobular, 5 as tubular, 4 as cribriform, 9 as medullary, 5 as mucinous carcinoma, 6 as undifferentiated, 3 as scirrhous, 5 as papillotubular, and 14 other histologic types of breast cancer. One hundred and forty-six patients were lymph node–negative whereas 110 patients were lymph node–positive; lymph node status was unknown in 13 patients. Estrogen and progesterone receptor positivity was noted in 196 and 173 tumors, respectively.

Hardy-Weinberg equilibrium was tested for the –889 C/T IL1A \( (P = 0.2, P = 0.01) \), the –511 C/T IL1B promoter \( (P = 0.5, P = 0.5) \), the IL1B exon 5 \( (P = 0.7, P = 0.04) \), the IL1RN \( (P = 0.05, P = 0.2) \), and the –174 G/C IL6 \( (P = 0.5, P = 0.9) \) genotypes in patients with breast cancer and controls. Unadjusted odds ratios (95% CI) and the respective \( P \) values are shown in Table 1. No significant associations were ascertained between –889 C/T IL1A, –511 C/T IL1B promoter,

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Table 1. Genotype frequencies of the IL1A, IL1B promoter, IL1B exon 5, IL1RN, and IL6 polymorphisms

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Breast cancer</th>
<th>Controls</th>
<th>( P )</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL1A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–889 C/C</td>
<td>123</td>
<td>102</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>–889 C/T</td>
<td>124</td>
<td>112</td>
<td>0.7*</td>
<td>0.9 (0.6-1.3)*</td>
</tr>
<tr>
<td>–889 T/T</td>
<td>22</td>
<td>13</td>
<td>0.5†</td>
<td>1.4 (0.7-2.9)†</td>
</tr>
<tr>
<td><strong>IL1B promoter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–511 C/C</td>
<td>124</td>
<td>88</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>–511 C/T</td>
<td>114</td>
<td>111</td>
<td>0.1*</td>
<td>0.7 (0.5-1.1)*</td>
</tr>
<tr>
<td>–511 T/T</td>
<td>31</td>
<td>28</td>
<td>0.5†</td>
<td>0.8 (0.4-1.4)†</td>
</tr>
<tr>
<td><strong>IL1B exon 5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1/E1</td>
<td>159</td>
<td>119</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>E1/E2</td>
<td>97</td>
<td>99</td>
<td>0.1*</td>
<td>0.7 (0.5-1.1)*</td>
</tr>
<tr>
<td>E2/E2</td>
<td>13</td>
<td>9</td>
<td>1.0†</td>
<td>1.1 (0.5-2.6)†</td>
</tr>
<tr>
<td><strong>IL1RN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/I</td>
<td>145</td>
<td>111</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>96</td>
<td>89</td>
<td>0.3*</td>
<td>0.8 (0.6-1.2)*</td>
</tr>
<tr>
<td>II/II</td>
<td>18</td>
<td>15</td>
<td>0.9†</td>
<td>0.9 (0.4-1.9)†</td>
</tr>
<tr>
<td>Others</td>
<td>10</td>
<td>12</td>
<td>0.2†</td>
<td>0.5 (0.2-1.3)†</td>
</tr>
<tr>
<td><strong>IL6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–174 G/G</td>
<td>78</td>
<td>91</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>–174 G/C</td>
<td>139</td>
<td>105</td>
<td>0.04*</td>
<td>1.5 (1.04-2.3)*</td>
</tr>
<tr>
<td>–174 C/C</td>
<td>52</td>
<td>31</td>
<td>0.02†</td>
<td>2.0 (1.1-3.6)†</td>
</tr>
</tbody>
</table>

* \( x^2 \) test, a heterozygous versus wild-type calculation was done.
† \( x^2 \) test, a homozygous versus wild-type calculation was done.
\( ^* \) \( x^2 \) test, other genotypes versus wild-type calculations was done.

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IL1B exon 5, and IL1RN, and the presence of breast cancer. A test for marker-trait association showed no significant association between the 16 haplotypes within the IL1 gene family and the presence of breast cancer (P = 0.7). The −174 G/C polymorphism was associated with the presence of breast cancer in a dose-dependent fashion. Odds ratios for women with one and two polymorphic alleles versus women with a wild-type genotype were 1.5 (95% CI 1.04-2.3, P = 0.04) and 2.0 (95% CI 1.1-3.6, P = 0.02), respectively.

No associations between the investigated polymorphisms −889 C/T IL1A (P = 0.3, P = 0.6, P = 0.9, P = 0.9, P = 0.5), −511 C/T IL1B promoter (P = 0.06, P = 0.06, P = 0.6, P = 0.4, P = 0.2), IL1B exon 5 (P = 0.3, P = 0.07, P = 0.2, P = 0.9, P = 0.2), the IL1RN (P = 0.5, P = 0.6, P = 0.8, P = 0.2, P = 0.3), and −174 G/C IL6 (P = 0.7, P = 0.9, P = 0.3, P = 0.3, P = 0.3) and the clinicopathologic variables, tumor stage, lymph node involvement, tumor grade, presence of estrogen, and progesterone receptor were noted in our series.

Discussion

As prophylactic interventions in order to reduce breast cancer risk have gained rising popularity, the clinical importance of breast cancer risk assessment has increased dramatically in recent years (37, 38). Testing of mutations and polymorphisms in candidate genes of breast cancer has been proposed as a powerful tool in assessing an unaffected woman’s personal risk for disease (39–41).

Although numerous genetic association studies dealing with breast cancer have been published, few candidate genes for this disease have been established. Cytokines in general are thought to be involved in numerous physiologic and pathologic conditions. Among cytokines, IL-1 and IL-6 probably seem to play the most important role in breast carcinogenesis (12, 13). IL-1 and IL-6 strongly interact with each other and act synergistically, subsequently increasing their effect. A number of well-defined gene polymorphisms within the IL1 gene cluster and the IL6 gene have already been linked to various malignant diseases (26–31). Although data with respect to the −174 G/C IL6 polymorphism in breast cancer have been published, no data are available whether IL1 and IL6 gene polymorphisms can serve as “candidate genes” for breast cancer.

Our relatively large case-control trial in Caucasian women falls short of showing any significant effect of IL1 gene polymorphisms on breast cancer risk. Only a statistical trend was ascertained when the −511 C/T IL1B promoter was associated with the phenotype of breast cancer, as this polymorphism was related to tumor stage and lymph node involvement. With respect to genotype frequencies of the controls, our results compare favorably with those previously published for Caucasians (42–44). Of note, the investigated polymorphisms were not all in Hardy-Weinberg equilibrium. This finding is surprising. Of note, the genotyping procedures used in the present study have been rigorously validated and were previously used in various studies. Furthermore, we selected women as controls, only if they were healthy Caucasian women from the same geographic area without breast cancer in their personal histories after assessing a detailed medical history. Therefore, we can exclude any bias with respect to selection of controls and the genotyping procedure.

Our data seem promising with respect to the −174 G/C IL6 polymorphism. As previously published for other malignancies (29), the −174 G/C IL6 polymorphism likely is important for carcinogenesis. Previously published data are conflicting (34, 35). We cannot confirm data on the association between the IL6 polymorphism and clinicopathologic variables. Furthermore, our study cannot comment on the prognostic impact of the IL6 polymorphism, as patient follow-up is incomplete. Of note, this was not the aim of the present study.

We provide new data with respect to a significant association between the IL6 polymorphism and breast cancer. To imply causality, findings from association studies should be discussed along the Bradford Hill criteria (45): (a) temporality, (b) strength, (c) consistency, (d) biological gradient, (e) specificity, (f) biological plausibility, and (g) analogy. Our results are in accordance with most of these criteria. The IL6 polymorphisms are inherited (a), therefore present before eventual breast cancer occurs; a doubling of risk was ascertained with two mutant alleles present (b); the results are in accordance with previously published studies in other malignancies (c and g; ref. 29); a dose-dependent effect was noted (d). Our results are also biologically plausible (f). The −174 G/C IL6 polymorphism is known to reduce gene transcription rate subsequently reducing protein levels. IL-6 is known to inhibit tumor cell growth. Therefore, it is not surprising that women with decreased protein expression are at an increased risk for developing breast cancer. It is not surprising that a gene polymorphism cannot meet the ‘specificity’ criterion, as the IL6 gene and the encoded protein IL-6 are involved in a wide variety of conditions and diseases.

We present data on a novel, biologically plausible “candidate gene” for breast cancer. The presence of the −174 G/C IL6 polymorphism increases the risk of breast cancer in a dose-dependent fashion.


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