The Cyclooxygenase-2 (PTGS2) 8473T>C Polymorphism is Associated with Breast Cancer Risk

Uwe Langsenlehner,1 Babak Yazdani-Biuki,2 Tanja Eder,3 Wilfried Renner,4 Thomas C. Wascher,2 Bernhard Paulweber,5 Werner Weitzer,1 Hellmut Samonigg,1 and Peter Kripl1

Abstract  Cyclooxygenase-2 (COX-2) is involved in carcinogenesis, immune response suppression, apoptosis inhibition, angiogenesis, and tumor cell invasion and metastasis. The gene for COX-2, designated as PTGS2, carries a common polymorphism at position 8473 in the 3′-untranslated region (PTGS2 8473T>C), which has been associated with susceptibility to malignant disease. To investigate the role of this polymorphism for breast cancer, we determined the prevalence of PTGS2 genotypes in 500 women with breast cancer and 500 sex- and age-matched healthy control subjects. Homozygous carriers of the 8473-CC genotype were more frequent among patients (12.4%) than among controls (6.6%; \( P = 0.002 \)). The odds ratio for carriers of this genotype for breast cancer was 2.1 (95% confidence interval, 1.3–3.3). Among patients, estrogen receptor positivity was less frequent among carriers of a CC genotype (63.9%) than among carriers of a TT or TC genotype (76.9%; \( P = 0.028 \)). Tumor size, histologic grade, presence of primary lymph node metastases, progesterone receptor positivity, or age at diagnosis were not associated with PTGS2 genotypes. We conclude that the homozygous PTGS2 8473-CC genotype may be associated with breast cancer risk.

Cyclooxygenase (COX), the rate-limiting enzyme in the production of prostaglandins from arachidonic acid, exists in at least two isoforms, COX-1 and COX-2. Whereas COX-1 is constitutively expressed in almost all tissues mediating physiologic processes, COX-2, undetectable in most tissues, can be up-regulated by cytokines, growth factors, and tumor promoters (1, 2). Cellular expression of COX-2 is increased in the earliest stages of carcinogenesis and through tumor development and invasive tumor growth (3). COX-2-derived prostaglandins participate in carcinogenesis, immune response suppression, apoptosis inhibition, angiogenesis, and tumor cell invasion and metastasis. COX-2 overexpression is observed in breast cancer and has been associated with indicators of poor prognosis, such as lymph node metastasis, poor differentiation, and large tumor size (4). Furthermore, inhibition of COX-2 by nonsteroidal anti-inflammatory drugs has been associated with a protective effect against a variety of cancers (5) and may be effective in the prevention and treatment of breast cancer (6, 7).

The gene for COX-2, designated as PTGS2, carries a common T>C polymorphism at position 8473 in the 3′-untranslated region (8). The 8473-C variant has been associated with an increased risk for non–small cell lung cancer as well as colorectal cancer (8, 9). In contrast to these results, two other studies reported a protective effect of the same genetic polymorphism against lung cancer (10, 11). In the present study, we investigated the role of this PTGS2 8473T>C polymorphism for breast cancer.

Materials and Methods

The study included 500 consecutive female patients with histologically confirmed prevalent breast cancers without any other cancer diagnosis besides breast cancer, and a population-based and age-matched control group of 500 healthy women. Characteristics of the study population have been described previously (12). All patients were recruited between January 2002 and July 2002 from patients attending the Division of Oncology, Department of Internal Medicine, Medical University of Graz, Graz, Austria. For each patient, one healthy female age-matched (±1 year) control subject was included. Controls were selected from two Austrian population-based screening studies, the Salzburger Atherosklerose Präventionsprogramm bei Personen mit hohem Infarkt Risiko (13) and the Grazer Diabetes Screening Programme.

The study was done according to the Austrian Gene Technology Act and has been approved by the local ethical committee. Written informed consent was obtained from all participating subjects.

Genotypes were determined by a 5′-nuclease assay (TaqMan) with primers and probes designed and manufactured using Applied’s “Assay-by-Design” custom service (Applied Biosystems, Vienna, Austria). PCR reaction and evaluation of fluorescence data were done as described previously (14). For each set of reactions, one negative control containing water instead of DNA was added to check for contamination. Finally, 95 samples were reanalyzed and results were identical for all samples.

Statistical analysis was done using SPSS 11.0 for Windows. Numerical values were analyzed by Student’s \( t \) test, proportions of
groups were compared by χ² test. Odds ratios and 95% confidence intervals were calculated by logistic regression to estimate the risk for breast cancer. Threshold for significance was \( P < 0.05 \).

### Results

**PTGS2** genotypes were determined successfully in all patients and all but one control. Genetic data are summarized in Table 1. The homozygous **PTGS2** 8473-CC genotype was found more often in patients than in controls (χ² test, \( P = 0.002 \)). The homozygous CC genotype, but not the heterozygous TC genotype, was associated with breast cancer, suggesting a recessive effect of the C variant on breast cancer risk.

Among patients with breast cancer, the **PTGS2** 8473T>C genotypes showed no association with tumor size, histologic grade, presence of primary lymph node metastases, progesterone receptor positivity, or age at diagnosis (Table 2). Estrogen receptor positivity was less frequent among patients carrying a CC genotype (63.9%) than among those with a TT or TC genotype (76.9%; \( P = 0.028 \)).

Analysis of the **PTGS2** sequence using the MatInspector Online Tool (15) showed that the **PTGS2** 8473T>C polymorphism did not create or disrupt any potential binding sites for transcription factors.

The potential role of the **PTGS2** 8473T>C polymorphism for mRNA structure was analyzed using the Vienna RNA secondary structure server (16). The input sequence contained the polymorphic site and 250 bp of the 3' as well as the 5' flanking regions. The T>C exchange interrupted a predicted 25 bp stem, potentially altering mRNA stability and expression.

### Discussion

The main finding of our study is an overrepresentation of the homozygous **PTGS2** 8473-CC genotype among patients with breast cancer, suggesting an association of this genetic variant with the disease. To the best of our knowledge, the present study is the first one in which the role of the cyclooxygenase-2 (**PTGS2**) 8473T>C polymorphism for breast cancer risk was investigated.

**PTGS2** encodes the COX-2 enzyme, which has been recognized to have an important role for breast cancer development (3, 4). Our data are in line with the results of two previous studies, which found a similar association between the **PTGS2** 8473-CC genotype with non–small cell lung cancer (8) and colorectal cancer (9). Two other studies described a protective effect of the same genetic variant against lung cancer, which seems to be in clear contrast with our results (10, 11). The reason for this discrepancy is currently unclear, but may be due to the different etiologies of breast and lung cancer. Furthermore, the effects of **PTGS2** gene polymorphisms on cancer risk are likely to be influenced by gene-environment interactions, such as smoking, which is the most important risk factor for lung cancer and strongly induces the expression of COX-2 (17). Unfortunately, in the present study, no data is available on environmental factors such as nonsteroidal anti-inflammatory drug use and diet that could potentially interact with **PTGS2** genotype.

Another explanation for the discrepant associations between the **PTGS2** 8473T>C polymorphism and different types of cancer could be ethnic differences between studies. The **PTGS2** 8473T>C polymorphism could be in linkage disequilibrium with other causal genetic variants, and this linkage disequilibrium would likely differ across different ethnic populations.

Little is known about the functionality of the **PTGS2** 8473T>C polymorphism. The 3' untranslated region of the murine gene for COX-2 contains several regulatory elements altering mRNA stability and translational efficiency (18). This suggests that polymorphisms in the corresponding region of the human gene for COX-2 could similarly influence COX-2 expression. Nevertheless, analysis of the **PTGS2** 8473T>C polymorphism using the MatInspector Online Tool showed no creation or disruption of a potential transcription factor binding site. Analysis of the predicted mRNA secondary structure indicated that the 8473T>C exchange interrupts a 25 bp stem and creates an additional loop. This suggests a potential effect on the mRNA stability and expression, but the results of this in silico analysis remain to be proven by in vitro data. Finally, as has been stated above, the **PTGS2** 8473T>C polymorphism may be nonfunctional, but could be in linkage disequilibrium with a true causal variant.

Higher COX-2 activity has been associated with an increased tumor risk and poor prognosis, and inhibition of COX-2 reduces the incidence of a variety of cancers. We therefore hypothesize that expression of the **PTGS2** 8473-C allele, which was associated with an increased breast cancer risk in the present study, may be higher than that of the "wild-type" 8473-T allele. Further in vitro analyses of the genetic regulation of COX-2 expression will be necessary before a conclusion on the functionality of the **PTGS2** 8473T>C polymorphism can be drawn.

The **PTGS2** 8473T>C polymorphism was not associated with tumor size, histologic grade, presence of primary lymph node metastases, progesterone receptor positivity, or age at diagnosis. Interestingly, estrogen receptor positivity was less frequent among carriers of the CC genotype than among carriers of a T allele. This may be in line with data from Wulfg and coworkers, who reported an inverse relationship between COX-2 activity and estrogen receptor content of breast cancer tissue (19). Nevertheless, our analysis of the relation between **PTGS2** genotype and estrogen receptor positivity was not based on an a priori hypothesis and should be regarded as hypothesis-generating rather than hypothesis-testing.

<table>
<thead>
<tr>
<th><strong>PTGS2</strong> 8473T&gt;C genotype</th>
<th>Controls</th>
<th>Patients with breast cancer</th>
<th>Odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT, ( n ) (%)</td>
<td>234 (46.9)</td>
<td>214 (42.8)</td>
<td>0.85 (0.66-1.09); TT vs. TC + CC</td>
</tr>
<tr>
<td>TC, ( n ) (%)</td>
<td>232 (46.5)</td>
<td>224 (44.8)</td>
<td>1.06 (0.81-1.35); TC vs. TT</td>
</tr>
<tr>
<td>CC, ( n ) (%)</td>
<td>33 (6.6)</td>
<td>62 (12.4)</td>
<td>2.05 (1.30-3.25); CC vs. TT</td>
</tr>
<tr>
<td>C allele frequency</td>
<td>0.299</td>
<td>0.348</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The cyclooxygenase-2 (**PTGS2**) 8473T>C polymorphism and breast cancer risk
The present study included incident as well as prevalent cases, therefore, a survival bias cannot be excluded. Nevertheless, due to the rather modest effect and the low frequency of the CC risk-genotype, it is unlikely that the results of the present study have been strongly distorted due to survival bias.

One of the major problems of genetic association studies is the increasing risk of type 1 error (false positive) findings after multiple testing. The present study is the 11th investigation of genetic risk factor in the same study population. Applying statistical corrections for multiple testing (e.g., Bonferroni correction) can be used to reduce this type 1 error risk, but at the same time, increases type 2 error risk (false negative). Applying Bonferroni correction to the present study lowers the threshold for significance to 0.0045 (0.05/11), leaving our results still statistically significant.

The polymorphism we analyzed in the present study reflects only a part of the variability of the PTGS2 gene (20). In September 2005, the National Center for Biotechnology Information single nucleotide polymorphism database (http://www.ncbi.nlm.nih.gov) contained 32 common (heterozygosity >10%) human polymorphisms for the PTGS2 gene encoding COX-2. Further studies including other PTGS2 variants will very likely provide additional insight into the role of COX-2 genetics for breast cancer risk.

Recent studies have indicated that inhibition of COX-2 by nonsteroidal anti-inflammatory drugs may be useful in the prevention and treatment of breast cancer (5–7, 21). In view of the potential role of PTGS2 polymorphisms for COX-2 activity and cancer risk, it might be interesting to explore their role for the pharmacogenetics of COX-2 inhibitors in future studies.

**References**

**Table 2. The cyclooxygenase-2 (PTGS2) 8473T/C polymorphism and characteristics of patients with breast cancer**

<table>
<thead>
<tr>
<th>Tumor size (cm)</th>
<th>TT</th>
<th>TC</th>
<th>CC</th>
<th>p (CC vs. TC + TT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>114 (54.5)</td>
<td>104 (47.7)</td>
<td>35 (57.4)</td>
<td>0.36</td>
</tr>
<tr>
<td>&gt;2</td>
<td>95 (45.5)</td>
<td>114 (52.3)</td>
<td>26 (42.6)</td>
<td></td>
</tr>
<tr>
<td>Histologic grade</td>
<td>1 and 2</td>
<td>107 (51.4)</td>
<td>102 (47.5)</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>3 and 4</td>
<td>101 (48.6)</td>
<td>111 (52.1)</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td>Negative</td>
<td>106 (51.2)</td>
<td>91 (41.9)</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>101 (48.8)</td>
<td>126 (58.1)</td>
<td></td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>Negative</td>
<td>49 (23.6)</td>
<td>49 (22.6)</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>159 (76.4)</td>
<td>168 (77.4)</td>
<td></td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>Negative</td>
<td>74 (35.7)</td>
<td>69 (32.1)</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>133 (64.3)</td>
<td>146 (67.9)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Numbers are n (%).

**Tumor Size**
- TT: 114 (54.5)
- TC: 104 (47.7)
- CC: 35 (57.4)

**Histologic Grade**
- 1 and 2: 107 (51.4)
- 3 and 4: 101 (48.6)

**Lymph Node Metastases**
- Negative: 106 (51.2)
- Positive: 101 (48.8)

**Estrogen Receptor**
- Negative: 49 (23.6)
- Positive: 159 (76.4)

**Progesterone Receptor**
- Negative: 74 (35.7)
- Positive: 133 (64.3)

**Age at diagnosis, y (mean ± SD)**
- TT: 57 ± 11
- TC: 56 ± 11
- CC: 56 ± 11

**p (CC vs. TC + TT)**
- TT: 0.36
- TC: 0.75
- CC: 0.88

**NOTE:** Numbers are n (%).
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