Associations between GPX1 Pro198Leu polymorphism, erythrocyte GPX activity, alcohol consumption and breast cancer risk in a prospective cohort study

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Breast cancer may be related to oxidative stress. Breast cancer patients have been reported to have lower antioxidant enzyme activity than healthy controls and the polymorphism GPX1 Pro198Leu has been associated with risk of lung and breast cancer. The purpose of the present nested case-control study was to determine whether GPX1 Pro198Leu and glutathione peroxidase (GPX) activity in prospectively collected blood samples are associated with breast cancer risk among postmenopausal women and whether GPX activity levels are associated with other known breast cancer risk factors. We matched 377 female breast cancer cases with 377 controls all nested within the prospective ‘Diet, Cancer and Health’ study of 57 000 Danes. Carriers of the variant T-allele of GPX1 Pro198Leu were at 1.43-fold higher risk of breast cancer compared with non-carriers (95% CI = 1.07–1.92). Pre-diagnostic GPX activity tended to be lower in cases compared with controls. GPX activity was positively correlated with intake of alcohol (P < 0.0001) and the catalytic activity was lowered 5% for each additional copy of the variant T-allele (P = 0.0003). Alcohol intake was correlated with increased GPX activity for the C-allele but not for the T-allele. Results from this prospective study suggest that the GPX1 Pro198Leu-associated lowered GPX activity is associated with higher breast cancer risk among Danish women.

Introduction

In the Western world the incidence rate of breast cancer is high especially among postmenopausal women. In Denmark, one in ten women will develop breast cancer before the age of 75. Although the risk factors identified for breast cancer are mainly endocrine and reproductive in character, it has also been suggested that breast cancer in part may be related to oxidative stress (1–6) and specifically lipid hydroperoxides have been implicated (7,8). The antioxidant enzyme glutathione peroxidase 1 (GPX1, EC 1.11.1.9) is part of the enzymatic antioxidant defence preventing oxidative damage to DNA, proteins and lipids, by detoxifying hydrogen- and lipid peroxides. A polymorphism in the GPX1 gene (GPX1 Pro198Leu, rs1050450) encoding the isoenzyme GPX1 expressed in erythrocytes (9) as well as in several epithelial tissues including breast, has been associated with risk of lung (10) and breast cancer (11). The latter study reported a 1.9-fold [95% confidence interval (CI) = 1.0–3.6] higher risk of breast cancer among homozgyous carriers of the variant allele. They found that the GPX1 198Leu enzyme had lower selenium-activation of GPX activity when transfected into MCF-7 cells. This indicates that the amino acid substitution may have a biological phenotype. No correlation between genotype and GPX activity in erythrocytes was reported in a study where genotype and activity were correlated in 66 persons (12). GPX activity has been measured in the blood compartment and shown to be lower in women with breast cancer compared with healthy controls (13–16).

It is believed that environmental as well as genetic factors are implicated in the development of breast cancer and consequently it is important to assess both genetic and non-genetic variability in the activities of defence enzymes in relation to cancer. Several factors have been observed to affect the activity of GPX. In a recent human intervention study it was shown that the intake of fruit and vegetables significantly increased the activity of GPX in human erythrocytes (17) and selenium supplementation is well known to increase GPX activity in populations with a low intake of this trace element (< 40 µg/day) (18,19). Alcohol induces lipid peroxidation and has been reported also to decrease erythrocyte GPX activity in some human studies but not in others (20–22). It is thus plausible that different dietary and lifestyle factors may influence GPX levels, but whether this plays a role in breast cancer risk is not known.

We wanted to clarify whether erythrocyte GPX activity levels were correlated with GPX1 Pro198Leu genotype and whether GPX1 Pro198Leu and GPX activity were associated with subsequent risk of developing breast cancer in postmenopausal women. We performed a nested case-control study within the ‘Diet, Cancer and Health’ prospective cohort study.

Materials and methods

Subjects

The subjects were selected from the Danish ‘Diet, Cancer and Health’ study, an ongoing prospective cohort study (23). Between December 1993 and May 1997, 79729 women aged 50–64 years, born in Denmark, living in the Copenhagen and Aarhus areas and having no previous cancers at the time of invitation, were invited to participate in the study. A total of 29 875 women accepted the invitation.

They were asked to fill in a 192-item food frequency questionnaire of which 44 of the items exclusively concerned intake of fruit, vegetables, or fruit/vegetable juice (24,25) and a lifestyle questionnaire, including questions about reproductive factors, health status, social factors and lifestyle habits.

Of the initial 29 875 women, a total of 326 women were excluded from the study because they were diagnosed with a cancer prior to enrolment. Further 4844 women who were not postmenopausal at study entry and 8 women who

Abbreviations: CI, confidence intervals; GPX1, glutathione peroxidase 1; Hb, haemoglobin; HRT, hormone replacement therapy; OR, odds ratio; RR, rate ratio.
Oxidative stress and breast cancer

Results
Baseline characteristics of women diagnosed with breast cancer and their matched controls are presented in Table I as

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (N = 377)</th>
<th>Controls (N = 377)</th>
<th>IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fruit and vegetable intake g/day(^a)</td>
<td>363 (126, 781)</td>
<td>322 (103, 814)</td>
<td>1.07 (1.00–1.14)</td>
</tr>
<tr>
<td>Alcohol intake g/day(^a)</td>
<td>11 (0, 44)</td>
<td>10 (1, 43)</td>
<td>1.09 (1.00–1.20)</td>
</tr>
<tr>
<td>Present smoking (%)</td>
<td>33</td>
<td>37</td>
<td>0.84 (0.62–1.15)</td>
</tr>
<tr>
<td>Selenium intake μg/day(^a)</td>
<td>62 (29, 126)</td>
<td>59 (28, 131)</td>
<td>1.01 (0.97–1.06)</td>
</tr>
<tr>
<td>Duration of HRT use(^a)</td>
<td>6 (1, 20)</td>
<td>5 (1, 21)</td>
<td>1.00 (0.97–1.03)</td>
</tr>
<tr>
<td>Benign breast disease (%)</td>
<td>20</td>
<td>14</td>
<td>1.49 (1.00–2.23)</td>
</tr>
<tr>
<td>School education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (%)</td>
<td>29</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>Medium (%)</td>
<td>47</td>
<td>49</td>
<td>1.10 (0.77–1.57)</td>
</tr>
<tr>
<td>High (%)</td>
<td>24</td>
<td>17</td>
<td>1.58 (1.01–2.60)</td>
</tr>
<tr>
<td>Parous (%)</td>
<td>86</td>
<td>87</td>
<td>1.59 (0.84–3.03)</td>
</tr>
<tr>
<td>Number of births(^a)</td>
<td>2 (0, 3)</td>
<td>2 (0, 4)</td>
<td>0.94 (0.78–1.13)</td>
</tr>
<tr>
<td>Age at first birth, years(^a)</td>
<td>23 (18, 32)</td>
<td>23 (18, 30)</td>
<td>1.03 (0.99–1.08)</td>
</tr>
<tr>
<td>Body mass index(^a)</td>
<td>25 (20, 34)</td>
<td>25 (20, 33)</td>
<td>1.02 (0.99–1.06)</td>
</tr>
</tbody>
</table>

Observed median values (5 and 95%) or fractions of the distribution of intake of fruit and vegetables, alcohol, smoking and selenium and potential breast cancer confounders among breast cancer cases and controls.

\(^a\)Risk estimate/100 g increment/day for fruits and vegetables; per 10 g increment/day for alcohol; and per 10 μg increment/day for selenium.

\(^b\)Among HRT users, risk estimate per additional year.

\(^c\)Risk estimate per additional birth/additional year of age/1 kg/m\(^2\).

\(^d\)IRR's are mutually adjusted.

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**Table I.** Baseline characteristics of study participants selected from the Danish ‘Diet, Cancer and Health’ prospective cohort study
The table gives the increase in GPX activity per dose.

**Table III.** Associations between erythrocyte GPX activity, dietary and lifestyle factors and GPX1 Pro198Leu genotype among postmenopausal women in the ‘Diet, Cancer and Health’ study

<table>
<thead>
<tr>
<th>Dietary and lifestyle factors</th>
<th>GPX activity (U/g Hb)</th>
<th>Percent explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit and vegetables, per 100 g/day</td>
<td>+0.3</td>
<td>0.42</td>
</tr>
<tr>
<td>Alcohol, per 10 g/day</td>
<td>+2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Present smokers</td>
<td>-2.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Selenium, per 10 µg/day</td>
<td>+0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>GPX1 Pro198Leu genotype, per allele</td>
<td>-4.2</td>
<td>0.0003</td>
</tr>
<tr>
<td>Variation explained totally</td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

The findings regarding fruit and vegetable intake, alcohol and HRT use have been reported elsewhere (31–33). The genotype distribution of GPX1 Pro198Leu was in Hardy-Weinberg equilibrium among the controls. In Table II, both unadjusted risk estimates and risk estimates adjusted for duration of HRT, previous benign breast disease, length of school education, parity (parous yes/no, number of births, age at first birth) and BMI are presented. Adjustment did not change the risk estimates. Heterozygous carriers of the variant T-allele had a 1.48-fold higher risk of breast cancer (95% CI = 1.09–2.01) and homozygous carriers of the T-allele had a 1.22-fold higher risk of breast cancer (95% CI = 0.70–2.12) than homozygous carriers of the C-allele (Table II). Thus, carriers of the variant allele had a 1.43-fold higher risk of breast cancer (95% CI = 1.07–1.92) than homozygous carriers of the wild-type C-allele had.

GPX activity was measured in erythrocytes that were collected at the time of entry into the ‘Diet, Cancer and Health’ cohort. There was little difference in GPX activity between cases and controls (Table II). Low GPX activity was associated with a lowered risk of breast cancer that was not statistically significant (IRR = 0.70, 95% CI = 0.45–1.10, Table II). When subdivided into quartiles on the basis of GPX activity, higher GPX activity was associated with lower risk of breast cancer, although only the risk estimate for the fourth quartile was significantly lowered (IRR = 0.65, 95% CI = 0.43–0.99) (Table II). Sample storage time (3–8 years) was not correlated with activity of GPX (r² = 0.0028, P = 0.12) (results not shown). In univariate analyses (Table III), the correlation between intake of fruit and vegetables, alcohol, smoking, selenium intake and GPX1 Pro198Leu genotype was investigated. Intake of fruit and vegetables was not found to be correlated with GPX activity (P = 0.42), but a positive correlation between alcohol intake and GPX activity was found (P < 0.0001). In smokers, GPX activity tended to be lower compared with non-smokers (P = 0.08). Dietary selenium tended to be correlated with higher levels of GPX activity (P = 0.05). GPX1 Pro198Leu genotype and GPX activity were strongly correlated. GPX activity was lowered 4.2 U/g Hb corresponding to 5% for each additional copy of the variant T-allele (P = 0.0003). This correlation was found both in cases and in controls (Table IV). Thus, the two strongest predictors found for GPX activity were alcohol consumption and GPX1 Pro198Leu genotype. The investigated variables explained 6% of the total variation in GPX activity (Table III).

In the present study group, the variant allele of GPX1 Pro198Leu polymorphism was correlated with lower GPX activity and associated with increased risk of breast cancer. This indicates that low GPX activity may be a risk factor for breast cancer. Alcohol intake is, on the other hand, both a risk factor for breast cancer and positively correlated with GPX activity. We investigated whether the GPX1 Pro198Leu polymorphism modified the positive correlation between GPX activity and alcohol intake. Among homozygous C-allele carriers, GPX activity increased 2.8 U/10 g alcohol/day (P < 0.0001) (Table IV). Among heterozygous carriers, GPX activity only increased 1.8 U/10 g increased alcohol intake (P = 0.01). Among homozygous T-allele carriers, GPX activity did not increase with increased alcohol intake. Thus, it seems that alcohol increases GPX activity in the wild-type C-allele, but not in the variant T-allele. Mutual adjustment of GPX activity and alcohol did not change their risk estimates.
in relation to breast cancer (Table II). Among homozygous carriers of the T-allele, GPX activity was lower among present smokers compared with non-smokers (Table IV). This decrease was much smaller and not statistically significant, among homozygous and heterozygous carriers of the wild-type C-allele.

The correlation between GPX activity and intake of fruit and vegetables, and selenium intake were not modified by GPX1 Pro198Leu genotype (results not shown).

Table V shows risk estimates for the combinations of GPX1 genotypes and alcohol consumption. Since only 3% of the present study group were non-drinkers, low alcohol consumption was defined as ≤3 g/day (~<2 drinks/week). This subgroup constitutes 25% of the study group. Homozygous C-allele carriers who drank >3 g alcohol/day had a 1.52-fold (95% CI = 0.94–2.45) higher risk of breast cancer than similar non-drinkers. Non-drinking heterozygous T-allele carriers had a 1.47-fold (95% CI = 0.78–2.78) higher risk of breast cancer compared with non-drinking homozygous C-allele carriers. Non-drinking homozygous T-allele carriers had a 2.19-fold (95% CI = 0.82–5.89) higher risk of breast cancer compared with non-drinking homozygous C-allele carriers. Heterozygous and homozygous T-allele carriers who drank >3 g alcohol/day had 2.09 (95% CI = 1.31–3.35) and 1.33-fold (95% CI = 0.63–2.80) higher risk of breast cancer than non-drinking homozygous C-allele carriers.

Discussion

In this prospective study we found relationships between a well-known GPX1 polymorphism, erythrocyte GPX activity and breast cancer risk among postmenopausal women. We also found that alcohol intake and GPX1 Pro198Leu genotype were the strongest predictors for GPX activity and that GPX1 genotype modified the correlation between alcohol intake and GPX activity.

We found evidence of erythrocyte GPX activity induction by alcohol. Others have reported either a decrease in GPX activity in alcoholics or found no effect of alcohol consumption on erythrocyte GPX activity among middle-aged individuals (21,22,34). These studies included much fewer persons than the present study. We found that the correlation between alcohol consumption and GPX activity was modified by the GPX1 Pro198Leu genotype. Thus, differences in genotype distribution between the study groups may contribute to the contradictory findings.

We found that GPX activity tended to be lower in present smokers. It has previously been reported that smoking results in lower GPX activity (35). When subdivided by GPX1 Pro198Leu genotype, lowered GPX activity was only associated with the variant T-allele.

We found that carriers of the variant T-allele of the GPX1 Pro198Leu polymorphism had a slightly higher risk of breast cancer compared with homozygous wild-type individuals. It was reported previously that carriers of the variant allele were at higher risk of lung cancer compared with homozygous carriers of the wild-type allele (10), whereas no association was found with regard to basal cell carcinoma (36) or colorectal cancer (37). Few studies have explored GPX1 Pro198Leu polymorphism in relation to breast cancer. A case-control study by Hu and Diamond (11) found a higher frequency of homozygosity for the variant allele in breast cancer tissue when compared with lymphocyte DNA from controls. Whether this reflects higher risk for developing breast cancer by carrying this genotype or loss of heterozygosity during tumour development could not be determined in that study. A larger case-control study of 399 pre- and postmenopausal women with breast cancer compared with 372 healthy women by Knight et al. (38) could not confirm these results. They reported a slightly lower, but not statistically significant, risk among carriers of the variant allele (OR = 0.89, 95% CI = 0.67–1.18). No association between the polymorphism and breast cancer risk was observed in the prospective Nurses’ Health Study where 1323 women with breast cancer were compared with 1910 controls (39). Thus, heterozygous carriers had a slightly lower risk of breast cancer (OR = 0.91, 95% CI = 0.77–1.07) and homozygous carriers had 1.07-fold increased risk of breast cancer (95% CI = 0.82–1.40). The allele frequency of the variant allele in this study group was 0.28 and thus very similar to a previous study of Danes (36). The allele frequency was slightly higher, 0.325 and 0.394 in the two

### Table IV. Associations between GPX1 Pro198Leu genotypes and erythrocyte GPX activity among postmenopausal women in the ‘Diet, Cancer and Health’ study and genotype specific effects of alcohol intake and current smoking

<table>
<thead>
<tr>
<th>GPX1 Genotype</th>
<th>GPX activity (U/g Hb) median (5, 95%)</th>
<th>Change in GPX activitya</th>
<th>Change in GPX activityb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Cases Controls</td>
<td>All P-value</td>
<td>All P-value</td>
</tr>
<tr>
<td>CC</td>
<td>90 (63, 132) 88 (58, 139) 92 (64, 125)</td>
<td>+2.8 &lt;0.0001</td>
<td>–0.83 0.72</td>
</tr>
<tr>
<td>CT</td>
<td>87 (61, 124) 88 (63, 121) 84 (56, 127)</td>
<td>+1.8 0.01</td>
<td>–2.87 0.25</td>
</tr>
<tr>
<td>TT</td>
<td>83 (54, 117) 80 (56, 110) 84 (53, 125)</td>
<td>–1.7 0.35</td>
<td>–12.55 0.008</td>
</tr>
<tr>
<td>P_trend</td>
<td>0.0003 0.02 0.008</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GPX activity is presented as medians (5, 95%), as change in GPX activity per 10 g alcohol intake per day and as change in GPX activity depending on smoking status (current smoker/present non-smoker).

*Activity for present smokers compared with non-smokers, measured in U/g Hb.

### Table V. Rate ratios for breast cancer in relation to GPX1 genotype and alcohol intake among postmenopausal women in the ‘Diet, Cancer and Health’ study

<table>
<thead>
<tr>
<th>GPX1 Pro198Leu</th>
<th>Alcohol intake</th>
<th>N</th>
<th>RR (95% CI)</th>
<th>N</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤3 g/day</td>
<td>&gt;3 g/day</td>
<td>All Cases</td>
<td>Controls</td>
<td>All Cases</td>
</tr>
<tr>
<td>CC</td>
<td>104</td>
<td>104</td>
<td>777</td>
<td>777</td>
<td>1.52 (0.94–2.45)</td>
</tr>
<tr>
<td>CT</td>
<td>65</td>
<td>65</td>
<td>239</td>
<td>239</td>
<td>2.09 (1.31–3.35)</td>
</tr>
<tr>
<td>TT</td>
<td>21</td>
<td>21</td>
<td>48</td>
<td>48</td>
<td>1.33 (0.63–2.80)</td>
</tr>
</tbody>
</table>

P-value for interaction = 0.30.
previously mentioned studies (11, 38). Danish women in the ‘Diet Cancer and Health’ cohort have high alcohol consumption (40), which is associated with an increased breast cancer risk. The different associations between GPX1 Pro198Leu and breast cancer risk may be a consequence of different alcohol consumptions in the different populations.

Only 3% of the women in the present study group are abstainers and this subgroup is in others respects very different from the rest of the study group. We therefore chose to increase the non-drinker group to include women with a low alcohol intake of ≤3 g/day. Our results indicate that the GPX1-198Leu variant has 10% lower GPX activity than the wild-type enzyme and that the activity of the variant enzyme is not increased in response to alcohol consumption. In the rather larger group of heterozygous carriers, who presumably have equal amounts of GPX1-198Leu and GPX1-198Pro, alcohol intake was associated with the same ca. 50% increased risk as was observed for homozygous wild-type carriers. This would indicate that the association between alcohol intake and breast cancer is independent of the GPX1 genotype. On the other hand, the risk estimates for the small group of homozygous T-allele carriers indicated that alcohol consumption was not associated with increased breast cancer risk for the variant T-allele. Therefore, larger studies preferably with a larger group of abstainers are required to resolve the biological interplay between alcohol consumption and GPX1 genotypes.

We found a highly significant correlation between the GPX1 polymorphism and erythrocyte GPX activity. GPX activity was lowered gene-dose dependently. This is in contrast with a much smaller study by Forsberg (12) who could not detect a difference in GPX activity between the genotypes. Increased alcohol consumption was correlated with increased GPX in homozygous C-allele carriers and to a lesser extent among heterozygous carriers. However, the lack of alcohol induction of the GPX1-198Leu enzyme could not explain the correlation between genotype and GPX activity (results not shown), indicating that the Pro to Leu substitution is correlated with lowered GPX activity. It is, however, entirely possible that the change in activity is caused by another polymorphism that co-segregates with the studied polymorphism. There are several such candidate polymorphisms in GPX (http://egp.gs.washington.edu/directory.html). Lower activity associated with carrying the variant allele has been reported for another polymorphic antioxidant enzyme, (Cu-Zn)-superoxide dismutase (41) and was explained by increased instability of the enzyme. A non-conservative substitution such as the Pro to Leu substitution could affect the stability or catalytic activity of the enzyme. Recently, it was shown that exposure of cells to adenosine led to a two-fold induction of GPX activity by increasing the stability of the GPX1 mRNA (42).

GPX activity was slightly lower in breast cancer cases than in controls, but this did not reach statistical significance. A number of case-control studies have measured erythrocyte antioxidant enzyme activities in breast cancer. Significantly lower activities of GPX in breast cancer patients compared with healthy controls was found by Kumar et al. (14), and confirmed by Kumaraguruparan et al. (15) and Abiaka et al. (13). To our knowledge, this is the first study where GPX measurements are based on pre-diagnostic blood samples.

Results from this prospective study suggest that the GPX1 Pro198Leu-associated lowered GPX activity is associated with higher breast cancer risk among Danish women.

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Conflict of Interest Statement

None declared.

References


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