Functional effects of a common single-nucleotide polymorphism (GPX4c718t) in the glutathione peroxidase 4 gene: interaction with sex$^{1-3}$

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ABSTRACT

Background: Selenium is essential for health in humans. Selenium is present as selenocysteine in selenoproteins such as the glutathione peroxidases (GPx). Selenocysteine incorporation requires specific structures in the 3′ untranslated region (3′ UTR) of selenoprotein mRNAs.

Objective: This study investigated the functional significance of the single-nucleotide polymorphism (SNP) GPX4c718t within the 3′ UTR of the GPX4 gene.

Design: A selenium supplementation trial was carried out with prospectively genotyped individuals of both homozygote genotypes for this SNP. Blood samples were analyzed at baseline, after a 6-wk supplementation with 100 μg Se as sodium selenite/d, and during a 6-wk washout period. RNA-protein binding studies were carried out in vitro.

Results: Both lymphocyte GPX1 protein concentrations and plasma GPX3 activity increased significantly after selenium supplementation in CC but not TT participants. After selenium withdrawal, there was a significant fall in both lymphocyte GPX4 protein concentrations and GPX4 activity in TT but not in CC participants; this effect was modulated by sex. RNA-protein binding assays showed that both T and C variants of transcripts corresponding to the GPX4 3′ UTR formed complexes in vitro and that the C variant bound more strongly than did either the T variant or the GPX1 3′ UTR.

Conclusions: The GPX4c718t SNP both alters protein binding to the 3′ UTR in vitro and influences the concentration of lymphocyte GPX4 and other selenoproteins in vivo. The latter is consistent with competition for selenium in selenoprotein synthesis, and, at low selenium intake, the SNP thus may influence susceptibility to disease. Am J Clin Nutr 2008;87:1019–27.

INTRODUCTION

The micronutrient selenium is essential for human health. Relatively high intakes can be associated with lower risks of cancer, cardiovascular disease (1–3), and viral infection (4). Conversely, lower selenium status is associated with a greater risk of some diseases (1–3) and greater mortality (5). The biological functions of selenium are exerted predominantly by means of the amino acid selenocysteine that is present in selenoproteins involved in many aspects of metabolism (6).

The incorporation of selenocysteine into selenoproteins involves the recoding of a UGA codon and the formation of a complex involving an RNA stem-loop structure, the selenocysteine-insertion sequence (SECIS), which is located in the 3′ untranslated region (3′ UTR) of selenoprotein mRNA (7) and a number of RNA-binding proteins including SBP2 protein (SECIS-binding protein 2) and ribosomal protein L30, a specific elongation factor (EFsec) and the tRNA(ser)(t) (8–14).

The phospholipid hydroperoxide glutathione peroxidase (GPx4) is an intracellular antioxidant selenoprotein that was identified first by its ability to reduce lipid hydroperoxides, as reviewed by Brigelius-Flohe (15). GPx4 is expressed in most tissues but is found at particularly high concentrations in the testes, where it also functions in sperm maturation. The protein has also been proposed to have roles in leukotriene biosynthesis and the regulation of cytokine signaling pathways (15). GPX4-null mice die in utero between stages E7.5 and E8.5, which confirms its physiologic importance (16, 17). The lethal phenotype can be rescued by overexpression of a human GPX4 transgene that protects the animals from oxidative damage (18, 19).

A limited number of studies have investigated genetic variations within the GPX4 gene that could potentially alter its function (20–24). Although the level of GPx4 expression is correlated with sperm function, no link has been found between a series of genetic variations and fertility (20–22). In whites, there is a C→T single-nucleotide polymorphism (SNP) located within the GPX4 gene region corresponding to the 3′ UTR, near the SECIS element at position 718 (23). The C variant of this SNP has a higher frequency in patients with colorectal cancer (24), which is suggestive of a link between the SNP and disease. This GPX4c718t polymorphism (rs713041) could modulate the synthesis of GPx4 by altering the affinity of the selenocysteine insertion machinery for its SECIS element. However, to date there is no direct evidence of its functional effects on GPx4.

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synthesis or selenoprotein metabolism. The aim of the present work was to test the hypothesis that the GPX4c718t SNP has functional consequences. To do this, we assessed lymphocyte GPX4 and other selenoprotein biomarkers in prospectively genotyped healthy volunteers before and after selenium supplementation and assessed the effects of the single-nucleotide change in vitro in RNA-protein binding assays.

SUBJECTS AND METHODS
Selenium supplementation trial

Forty unrelated volunteers (male and female) aged 20–60 y who were nonsmokers and generally healthy were recruited from the general population in and around Newcastle (United Kingdom). Exclusion criteria included cardiovascular, hepatic, gastrointestinal and thyroid disorders; cancer; excessive alcohol consumption (>30 units/wk); and chronic intake of antiinflammatory drugs (25). Persons already taking selenium or multivitamin or vitamin E supplements also were excluded. For all participants, weight and height were measured, and basal metabolic index (BMI) was calculated. Peripheral blood samples were drawn from the antecubital vein into 10-mL EDTA-tubes (BD Vacutainer; Becton-Dickinson), processed on the same day, typically within 6 h of sample collection, and stored at −80 °C until assays were performed.

An initial 10-mL blood sample was collected for GPX4 genotype assessment. Volunteers were asked to give an additional 30-mL blood sample (baseline) and then take a daily supplement type assessment. Volunteers were asked to give an additional extension cycle at 72 °C for 7 min. An aliquot of each PCR denaturation (94 °C for 30 s), annealing (53.5 °C for 30 s), and extension (72 °C for 1 min) was followed by 30 cycles of extension cycle at 94 °C for 4 min, which was followed by 30 cycles of denaturation (94 °C for 30 s), annealing (53.5 °C for 30 s), and extension (72 °C for 1 min). The PCR was completed by a final extension cycle at 72 °C for 7 min. An aliquot of each PCR product was subjected to electrophoresis in a 1 X Tris-acetate-EDTA-2%-agarose gel and visualized with ethidium bromide. Genotype was determined by restriction fragment analysis, digesting the PCR products with SstI (Promega) overnight at 37 °C to generate either 2 fragments of 159 and 62 bp (C allele) or 3 fragments of 97, 62, and 62 bp (T allele). Subsequently digested products were resolved on a 4%-Tris-borate-EDTA polyacrylamide gel and visualized with ethidium bromide. Genotype was confirmed by direct sequencing of PCR product for 10% of samples. For this, PCR products were purified by using a polyethylene glycol (PEG) mix (26.2% wt:vol PEG 8000, 6.6 mmol MgCl2/L, 0.6 mol sodium acetate/L; pH 5.2), washed with 70% (by vol) ethanol, air-dried, and sent for sequencing (MWG- Biotech).

Plasma and erythrocyte preparation, lymphocyte isolation, and measurement of selenoprotein markers

After fractionation of the blood by centrifugation (950 × g, 4 °C, 15 min), plasma was further centrifuged at 730 × g for 12 min at 4 °C to obtain platelet-free plasma. Erythrocytes were frozen immediately. Lymphocytes were reconstituted in an equal volume of RPMI 1640/Hepes medium (GibcoBRL, Invitrogen) and isolated onto a Histopaque H1077 gradient (Sigma-Aldrich) as described elsewhere (25). After isolation, lymphocyte pellets were stored at −80 °C. Lymphocyte GPX1 activity and plasma GPX3 activity were calculated by the method of Paglia and Valentine (26), as modified by Brown et al (27), using hydrogen peroxide as a substrate. One unit of GPX1 or GPX3 activity is defined as that which oxidizes 1 μmol NADPH/min. Lymphocyte GPX4 activity was measured by the method of Weitzel et al (28) using phosphatidyl choline hydroperoxide as a substrate. One unit of GPX4 activity is defined as that which oxidizes 1 μmol NADPH/min. Lymphocyte GPX1 and GPX4 protein concentrations were measured by competitive enzyme-linked immunosorbent assay (ELISA (29)) using polyclonal antibodies and recombinant proteins GPX1 and GPX4 obtained from Labfrontier.

Cell culture and protein extraction from colon adenocarcinoma cells

Human colon adenocarcinoma cells (Caco-2) were grown in Dulbecco’s Modified Essential Medium (Invitrogen) supplemented with 10% fetal calf serum (Sigma), 1% penicillin and streptomycin (Invitrogen), and 1% nonessential amino acid (Invitrogen). Cells were grown to ~90% confluence, and cytoplasmic protein extracts were prepared by a modification of the method of Mahtani et al (30). Cells were cooled on ice for 5 min, rinsed once in ice-cold phosphate-buffered saline (PBS), and harvested by scraping. Cells were pelleted by centrifugation at 600 × g for 10 min at 4 °C and washed once in ice-cold PBS. The pellet was then lysed by the addition of lysis buffer [10 mmol HEPELS/L, pH 7.6; 3 mmol MgCl2/L, 40 mmol KCl/L, 2 mmol dithiothreitol/L, 5% glycerol, 0.5% NP-40, and mini-EDTA–free protease inhibitor cocktail (Roche)] using 100 μL buffer20 × 106 cells. Cells were lysed on ice for 10 min and disrupted by being passed 10 times through a 21-gauge needle. Nuclei were removed by centrifugation at 600 × g for 10 min at 4 °C. The supernatant fluid containing cytoplasmic proteins was aliquoted and snap-frozen. Protein content was measured by the Bradford method (Sigma).

In vitro transcription

Templates for transcription of human GPX4 T and C variants and GPX4 sequences corresponding to the regions of the 3’UTRs containing the SECIS structure were generated by PCR from genomic DNA from participants who were either TT or CC for GPX4c718t. PCR was performed with 100 ng template genomic DNA by using forward primers that contained the T7 promoter sequence (underlined) followed by a sequence specific for GPX4

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**Note:** The text appears to be part of a larger scientific article discussing the effects of selenium supplementation on selenoprotein levels and the genetic basis of those effects. The focus is on methodological details related to sample collection, DNA extraction, and the assessment of lymphocyte GPX4 activity and genotype. The text includes technical details such as PCR conditions, DNA extraction methods, and protein isolation techniques, which are common in molecular biology research. The discussion on GPX4c718t SNP and its functional consequences is also a critical part of the study.
(TAATACGACTCATAAGGACCTGCCCCTATTTTCTA) or GPX1 (TAATACGACTCATAAGGCGAAGCTCTGCTGCTCAAG), and reverse primers specific for GPX4 (GTCTGTTTATTCCCACAAGG) or GPX1 (CTGACACCCGGCACTTTATTAGTG). Annealing temperatures were 53.5 °C for GPX4 and 64 °C for GPX1. PCR products were purified by using a QIAquick PCR purification kit (Qiagen) and were of 239 (T7-GPX4) and 253 (T7-GPX1) base pairs, respectively. In vitro transcription was performed, according to the manufacturer’s protocol, by using either a digoxigenin (DIG)-RNA-labeling kit (Roche) for the synthesis of labeled GPX4 probes corresponding to C or T variants or a MEGASHortscript kit (Ambion) to generate competitor unlabeled probes for GPX1 and the 2 GPX4 variants. Probes were subsequently purified by a phenol and chloroform extraction and ethanol precipitation. The precipitated RNA probes were finally resuspended in diethylpyrocarbonate-treated water in the presence of RNasin (Promega).

Electromobility-shift assay

Electromobility-shift assay (EMSA) reactions were carried out on ice for 25 min. Protein extract was incubated with 100 pmol digoxigenin-labeled RNA probe (previously heated to 70 °C and cooled to 40 °C at a rate of 1.3 °C/min to refold slowly in the correct conformation) in binding buffer (20 mmol HEPES/L, pH 7.6; 3 mmol MgCl2/L; 40 mmol KCl/L; 2 mmol dithiothreitol/L; protease inhibitor cocktail; and 5% glycerol) in a final 20 µL-reaction volume. For competition experiments, unlabeled transcripts were added simultaneously with the labeled transcripts. After the binding reaction, 40 units of RNase T1 were added and incubation was continued for 20 min on ice. Loading buffer (3 µL; 90% glycerol and 0.025% bromophenol blue) was then added, and complexes were separated by electrophoresis at 4 °C for 2.5 h at 20 V/cm by using 4% (wt:vol) non-denaturing PEGs in 0.5× Tris-borate EDTA. RNA-protein interactions were detected by hybridizing the gels with a digoxigenin-labeled RNA probe.
complexes were subsequently transferred onto a Hybond-N+ nylon membrane (Amersham Biosciences) for 1 h at 10V and UV-crosslinked to the membrane for 2 min. The membrane was then incubated in a blocking solution (2% wt:vol bovine serum albumin and 0.5 ng salmon sperm DNA in PBS) for 1 h, which was next incubated for 1 h with anti-DIG-alkaline phosphatase antibody (1:5000 dilution, Roche). After 3 washes in PBS, the membrane was rinsed in milliQ water and incubated with BM purple reagent (Roche) for 10 min to show the digoxigenin-labeled RNA-protein complexes. Band intensity was quantified by using UVIDEC software (version 11.01; Uvitec).

Statistical analysis

Biochemical variables were analyzed by using factorial analysis of variance (ANOVA). In the case of time differences, the responses to selenium supplementation and withdrawal were analyzed by using a paired t test of the values at different pairs of timepoints, and the interactions between time and other factors were examined by ANOVA of these differences. Results of the paired t test are reported only when the interaction between time and other factors was significant at $P < 0.05$. When a significant interaction between 2 factors was observed by ANOVA, secondary analysis was carried out to determine the main effects of each factor within subgroups. One-factor ANOVA was used to compare the binding capacity of competitor probes in the EMSA experiment.

RESULTS

Selenium supplementation study

To investigate the functionality of $GPX4c718t$, a selenium supplementation trial (the SELGEN study) was carried out in 40 prospectively genotyped healthy human volunteers, of whom 22 were $CC$ and 18 were $TT$ for the $GPX4c718t$ SNP. As part of the SELGEN study, volunteers also were genotyped for 2 SNPs in the selenoprotein P ($SEPP$) gene. Because statistical analysis of the genotypes frequencies showed that $GPX4c718t$ was independent of the 2 $SEPP$ SNPs, for clarity, the data are presented here only in terms of $GPX4c718t$. The study design is shown in Figure 1. Volunteer characteristics and compliance are summarized in Table 1. Volunteers were asked to take a single daily selenium supplement as sodium selenite (100 $\mu$g Se/d) for 6 wk. Blood samples were drawn before supplementation and on the last day of supplementation and at 2-wk intervals during a 6-wk washout period. Baseline (presupplementation) mean plasma selenium concentrations were 1.15 ± 0.03 $\mu$mol/L, and the concentration was significantly ($P < 0.001$) increased, by 20%, to 1.36 ± 0.02 $\mu$mol/L by selenium supplementation (Table 1). In addition, supplementation resulted in a significant increase in $SEPP$ concentrations (25) and plasma GPx3 activity (see below). The increases in plasma selenium and $SEPP$ concentrations and in GPx3 activity indicate both the subjects’ compliance and the effectiveness of the supplementation in raising selenium status. Selenium withdrawal led to a rapid decrease in plasma selenium by 2 wk and a further fall at 4 wk, after which time the concentration was similar to baseline (25). Most of the decline in plasma selenium occurred by 2 wk.

TABLE 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM</th>
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<tbody>
<tr>
<td>Plasma selenium ($\mu$mol/L)</td>
<td></td>
</tr>
<tr>
<td>Before supplementation</td>
<td>1.15 ± 0.03</td>
</tr>
<tr>
<td>After supplementation</td>
<td>1.36 ± 0.02</td>
</tr>
<tr>
<td>Compliance (%)</td>
<td>98 ± 0.6</td>
</tr>
</tbody>
</table>

$^1 n = 40$. SELGEN study, selenium supplementation trial. $^2 \pm$ SEM (all such values). $^3 A 20\%$ increase in plasma selenium was seen after supplementation, $P < 0.001$. $^4$ Percentage ± SEM.

Effects of sex and $GPX4c718t$ on GPx4 activity and protein concentrations

Lymphocyte GPx4 activity and protein concentrations were measured in samples taken before and after supplementation and during the washout period. Sex had a significant effect ($P = 0.028$) on GPx4 protein concentration at baseline: females had higher concentrations than did males (Figure 1B), but no effect of sex was observed after supplementation or during the washout period. In contrast, males had a higher mean GPx4 activity than did females at baseline, but this difference was not significant (Figure 1C). As a result, a sex effect was observed on the ratio of GPx4 activity to GPx4 protein at baseline ($P = 0.027$): males had a higher ratio than did females (Figure 1D). This difference may reflect differences between the sexes in the regulation of enzyme activity.

Presupplementation GPx4 protein concentrations and activity levels were similar in participants who were $TT$ or $CC$ for $GPX4c718t$ (Figure 2A and B). Supplementation had little effect on either activity or protein, but, after the withdrawal of selenium, there was a fall in GPx4 protein concentrations and activity in $TT$ but not in $CC$ persons. Significant genotype × time, sex × time, and genotype × sex × time interactions were observed for GPx4 protein concentrations, particularly between before supplementation and after supplementation ($P = 0.047$) and 2 wk after withdrawal of supplementation ($P = 0.017$). ANOVA confirmed that the difference in GPx4 activity between genotypes was significant at 2 ($P = 0.022$) and 4 ($P = 0.034$) wk after selenium withdrawal, with lower activity in $TT$ participants. A similar effect of the $GPX4c718t$ genotype was observed on GPx4 protein after a 2-wk withdrawal ($P = 0.022$). Moreover a significant sex × $GPX4c718t$ interaction was observed for GPx4 protein 4 wk after withdrawal ($P = 0.044$). Most of this genotype effect was attributable to females—lower GPx4 protein concentrations were seen in $TT$ than in $CC$ females after supplementation and at 2 ($P = 0.018$) and 4 ($P = 0.031$) wk of the washout—and the difference was less evident in males. Overall, the data
indicate that, during washout of the selenium supplement, GPx4 activity and protein concentration are influenced by genotype for GPX4c718t and, in addition, GPx4 protein concentrations are influenced by sex.

Effect of polymorphism on other selenoprotein biomarkers

There is a hierarchy in selenoprotein synthesis that results from a competition for available selenium and for components of the selenoprotein synthetic machinery (31–33). Therefore, in addition to GPx4 protein and activity, plasma GPx3 activity, lymphocyte GPx1 activity, and protein and plasma TR1 concentrations were measured. GPX4c718t genotype affected the response to supplementation of the GPx1 protein concentration and its decrease 2 and 6 wk after selenium withdrawal (Figure 3A): persons of CC genotype had significant differences in GPx1 protein (P = 0.019, 0.019, and 0.023, respectively; paired t test) (Figure 3A). Further statistical analysis showed that the increase in GPx1 protein concentrations after supplementation was significant (P = 0.017, paired t test) in CC females (Figure 3B) but not in males (Figure 3C). Both the difference between sex and the sex × time interaction were significant (post- to presupplementation differences—genotype × sex interaction, P = 0.033; sex main effect, P = 0.006; week 6 washout-postsupplementation differences—genotype × sex interaction, P = 0.053; sex main effect, P = 0.007). In addition, sex × time × genotype interactions were significant. Finally, TT females showed a rapid drop in GPx1 activity by 2 wk after selenium withdrawal (P = 0.016), but TT males showed no significant decrease in GPx1 activity during the washout period (data not shown).

In addition, the GPX4c718t genotype influenced plasma concentrations of the selenoproteins GPx3 and TR1. GPX4c718t genotype affected GPx3 activity after supplementation (P = 0.029): CC participants had a higher GPx3 activity than did TT participants (Figure 4A). Moreover a significant response to selenium supplementation occurred only in CC volunteers (P = 0.005, paired t test), and this difference in response was significant between genotypes (P = 0.045, ANOVA). Similarly, there was a significant sex × GPX4c718t genotype interaction that determines plasma TR1 concentrations at all time-points (P ≤ 0.045 for all; Figure 4B). TT males and CC females showed higher TR1 concentrations than did CC males and TT females after supplementation and throughout the washout period (Figure 4B).

Effect of GPx4 polymorphism on in vitro RNA-protein binding

EMSA experiments were carried out to determine whether GPX4c718t SNP modulated the ability of RNA transcripts containing the GPX4 3’UTR (including the SECIS) to form specific

![Figure 2](https://academic.oup.com/ajcn/article-abstract/87/4/1019/4633397)
RNA-protein complexes and compete with GPx1 mRNA for protein binding. Both T and C variants of digoxigenin-labeled GPx4 transcripts formed complexes with proteins extracted from Caco-2 cells, as judged by lower transcript mobility (Figure 5A, B). Quantification of band intensities showed that unlabeled C transcripts competed strongly with labeled T transcripts at 5× molar excess so that complex formation was disrupted by 45%, whereas competition of unlabeled T transcripts was significantly weaker, and complex formation was affected by only 35% at 10–15× molar excess (P = 0.0013, one-factor ANOVA; Figure 5C). In the complementary experiments, the C variant competed more strongly with itself than with an unlabeled T competitor (Figure 5D). These competition experiments suggest that the C variant transcripts bind protein more strongly than do the T variant transcripts.

Unlabeled GPX1 transcripts competed with labeled T GPx4 transcripts for complex formation (Figure 5B), and quantification of band intensities showed that this competition required a higher molar excess (10–15×) of GPX1 competitor compared with the C competitor, which indicated that the C variant has stronger protein binding than does either the T variant or the GPX1 SECIS element (Figure 5C, D). GPX1 competitor transcripts lowered RNA–protein complex formation with the T variant by 60% and that with the C variant by only 30% (Figure 5C, D). Competition experiments with unlabeled rat metallothionein-I 3′UTR transcripts (a negative control lacking a SECIS element) showed no competition for binding to GPX4 and therefore confirmed the specificity of protein binding to GPX4 and GPX1 probes (data not shown). Overall, the data indicate that the C variant GPX4 transcripts bind proteins more strongly than do the T variant and GPX1 transcripts, which is consistent with the in vivo observation that GPX4c718t influences both GPX4 and GPX1 protein expression (Figure 3).

**FIGURE 3.** Mean (±SEM) effects of GPX4c718t genotype on lymphocyte GPx1 protein concentrations, measured by using an enzyme-linked immunosorbent assay. Presupp, before supplementation (baseline); postsupp, after supplementation. Data are shown from 22 CC (11 female, 11 male) and 18 TT (13 female, 5 male) participants on the basis of GPX4c718t genotype (A) and of genotype for females (B) and males (C). *Significant responses in GPx1 activity to selenium supplementation and withdrawal were assessed by paired t test. ** Significant differences were indicated by 2 symbols joined by a line: *P < 0.05, **P < 0.01.

**FIGURE 4.** Mean (±SEM) effects of GPX4c718t genotype on plasma GPx3 activity and plasma thioredoxin reductase 1 (TR1) protein concentrations. Presupp, before supplementation (baseline); postsupp, after supplementation. GPx3 activity (A) was measured by spectrophotometric assay, and TR1 concentrations (B) were measured by radioimmunoassay. A: Data are presented for persons of CC (n = 22) and TT (n = 18) genotype. *Significant difference between CC and TT participants after supplementation, P < 0.05 (ANOVA). ** Significant responses to supplementation and wash-out at 2 wk (paired t test): *P < 0.05, **P < 0.01. B: Data are shown for groups of males (5 TT, 11 CC) and females (13 TT, 11 CC) as a function of genotype for GPX4c718t. *** Significant differences between CC and TT participants at all time-points (ANOVA): *P < 0.05, **P < 0.01, ***P < 0.001.
Effect of GPx4 polymorphism on relative activity of GPx1 and GPx4

To assess the relative effects of the SNP on GPx1 and GPx4 in vivo, the ratio of GPx1 activity to GPx4 activity was calculated from the lymphocyte enzyme activities determined in the supplementation trial (Figure 6). An effect of GPX4 c718t was evident 2 wk after selenium withdrawal (P = 0.047). For CC participants, the ratio of the 2 enzyme activities returned to presupplementation values after 2 wk of selenium withdrawal, whereas the ratio stayed significantly higher in TT participants during the washout period at 2 and 4 wk. This observation suggests that CC persons maintain GPx4 synthesis preferentially over GPx1 when selenium intake falls, which is consistent with the stronger competition of the C variant transcripts in the RNA-binding assays.

DISCUSSION

SNPs in selenoprotein genes have been described, but detailed data on their functionality are less comprehensive (25, 34–37). A T/C variation has been described in the GPX4 gene at position 718 with the 3'UTR (23) close to the predicted SECIS structure, which is essential for selenoprotein synthesis (38), and this SNP has recently been confirmed (36). All 3 genotypic variants at this locus have been reported to occur at relatively high frequency in whites, Chinese, and South Asian ethnic groups (results not}

![Image](https://academic.oup.com/ajcn/article-abstract/87/4/1019/4633397)
shown), which indicates that, if functional, this SNP could affect a significant number of people. However, to date, there is only indirect evidence—namely, measurement of 5′lipoxigenase metabolite concentrations (23)—that the SNP may be functional. The present work provides direct evidence, both in vivo and in vitro, that this SNP has functional consequences related to selenium metabolism.

First, the fall in GPx4 activity and protein concentrations in lymphocytes after withdrawal of a daily selenium supplement is affected by GPX4c718t genotype (Figure 2). The data suggest that CC persons maintain GPX4 concentrations better than do TT persons when selenium intake falls. The effects of the SNP were observed after withdrawal of the selenium supplement but not at baseline. This may be because initial interindividual variations in selenium intake mask the influence of the polymorphism at baseline. Alternatively, revelation of the genotype effect after selenium supplementation is stopped may be due to the sudden decrease in selenium intake that leads to a transient negative selenium balance and the need to prioritize the available selenium.

Second, in vivo, GPX4c718t influences the concentrations of other selenoprotein biomarkers. After the supplementation, plasma GPx3 increased in CC but not in TT participants, and plasma TR1 was greater in CC than in TT females. In addition, presupplementation concentrations of GPx1 were higher in TT than in CC participants. At first, it may be surprising that an SNP in one selenoprotein gene influences the expression of other selenoproteins. However, there is a strong body of evidence of competition among selenoproteins for available selenium, in the form of selenocysteine that is required for synthesis of the selenoproteins, and for the selenium incorporation machinery (31–33, 39, 40). Furthermore, the mechanism for this competition may involve, at least in part, differences in 3′ UTR sequences (32, 33, 41–43). GPX4 variants may influence the position of GPX4 in the hierarchy of demands on the components of the selenium synthesis apparatus, with consequential effects on the synthesis of other selenoproteins.

Third, in vitro binding experiments showed that GPX4 transcripts with the C variant compete more strongly with either the T variant transcripts or GPX1 3′ UTR transcripts for protein binding, which suggests that the C and T variants differ in their ability to form RNA-protein complexes. Our hypothesis is that the T and C allelic variants differ in their capacity to promote selenocysteine incorporation into GPx4; this possibility is supported by recent data indicating that the C variant of the GPX4 3′ UTR is stronger than the T variant at driving synthesis of a selenoprotein reporter (24). In addition, the hierarchy in selenoprotein synthesis (31–33, 44) would predict that GPX4c718t CC participants could maintain their GPX4 concentrations as selenium intake falls, whereas the abundances of some other selenoproteins would be more sensitive to selenium supply; this prediction was confirmed in the present experiments for GPx1, TR1, and GPx3. Because the selenoproteins and their mRNAs are present in different abundances [e.g., there is 4 times as much GPx1 as GPx4 in many tissues (44)]; the effects of the TIC variation in the 3′ UTR most likely reflect not only a competition for available selenocysteine but also the ability of different transcripts to compete for the selenoprotein synthetic machinery. Thus, the stronger in vitro protein binding of C variant than of the GPX1 3′ UTR is paralleled by a greater response of GPx1 protein to supplementation in persons of CC genotype than in those of TT genotype.

The effect of GPX4c718t on GPx4 expression was dependent on sex, and it was evident in females but not males. This could result from differing, perhaps less stringent, demands for GPx4 in females that would make synthesis of the protein susceptible to the SNP. Additional regulation of GPx4 expression in males may apply because of the role of GPx4 in male fertility or of sex differences in immune function. It it interesting that, although the effects of the SNP on GPx4 activity and protein concentrations after withdrawal of the selenium supplement occurred in parallel, sex effects on activity and protein concentrations at baseline did not. As a result, GPx4 activity:GPx4 protein was lower in females than in males, which indicates that regulatory processes may be affecting GPx4 expression in males, who maintain lymphocyte GPx4 at a higher concentration than do females. The mechanism underlying this effect is not known, but it may well involve differences in the stability of GPx4 protein. To our knowledge, this report is the first time an effect of sex on human lymphocyte GPx4 expression has been described. However, in rats, there is a similar sexual dimorphism in the synthesis of the selenoprotein Dio1 in the liver and kidney (45). This sex difference was abolished by ovariectomy or orchidectomy, which suggests a role of sex steroids in posttranscriptional regulation of selenoproteins.

In conclusion, the present data provide evidence that an SNP in the GPX4 gene, a TIC variation at position 718 close to the SECIS, has functional consequences. Pilot studies suggest that this SNP may affect susceptibility to colon cancer (24). Such observations, taken with the present data on functionality, indicate the potential importance of this SNP in the GPX4 gene and could be especially relevant when selenium intake is suboptimal. We propose that the SNP alters protein binding to the 3′ UTR, which in turn modulates the ability of the GPX4 3′ UTR both to promote GPx4 synthesis and to compete for components of the selenoprotein synthetic machinery—and thus influence the synthesis of a range of selenoproteins.

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FIGURE 6. Mean (±SEM) effects of GPX4c718t genotype on the ratio of lymphocyte GPx1 activity to lymphocyte GPx4 activity, measured by spectrophotometric assay. Values shown are for groups of participants of (n = 22) and TT (n = 18) genotype. Presupp, before supplementation (baseline); postsupp, after supplementation. *Significant differences between CC and TT participants at 2 and 4 wk after withdrawal of the selenium supplement, P < 0.05 (ANOVA).
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The authors’ responsibilities were as follows—CM: the supplementation trial, genotyping, RNA protein binding; and data analysis; LKC and FN: measurements of selenoprotein biomarkers; GHW: helped with the statistics; JEH, JCM, and JRA: designed and supervised the project; and JEH and CM: wrote the manuscript with contributions from JCM and JRA. None of the authors had a personal or financial conflict of interest.

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