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ORIGINAL RESEARCH COMMUNICATION

Relative Abundance of Selenoprotein P Isoforms in Human Plasma Depends on Genotype, Se Intake, and Cancer Status

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Abstract

Selenium (Se), a dietary trace metal essential for human health, is incorporated into selenoproteins as selenocysteine. Selenoprotein P (SePP), the major plasma selenoprotein, has both transport and antioxidant functions. In humans, it exists in plasma as two isoforms of ~50 and 60 kDa. This study investigated the effect of polymorphisms in the *SEPP-1* gene, Se supplementation, and disease status on the proportions of SePP plasma isoforms. SePP was isolated from plasma from healthy volunteers, before and after a 6-week supplementation with 100 μg sodium selenite, and from colon cancer patients and controls. SePP isoform distribution was analysed by Western blot. In healthy volunteers, the relative abundance of each isoform depended on two *SEPP-1* polymorphisms: rs3877899, predicted to cause an Ala-to-Thr amino acid change at position 234, and rs7579, located in the 3′-untranslated region of *SEPP-1* mRNA. The difference between genotypes disappeared after Se supplementation. A genotype-dependent reduction was seen in the proportion of the 60-kDa isoform in patients with colorectal cancer compared with controls. We conclude that functional polymorphisms in the *SEPP-1* gene influence the proportion of SePP isoforms in plasma. An elevated proportion of the 60-kDa isoform of SePP may increase selenoprotein synthesis and reduce colorectal cancer risk. *Antioxid. Redox Signal.* 11, 2631–2640.

Introduction

SELENIUM (Se) is an essential micronutrient, and intakes of Se above or below the optimal range have profound effects on human health (23). Although severe Se deficiency is rare, suboptimal Se intake is observed in many areas of the world, such as Europe and New Zealand (24, 32), and may increase susceptibility to various cancers (4, 13, 33). In contrast, in several but not all studies, Se supplementation is associated with lower cancer incidence (8–10, 15, 24). However, the link between Se intake and cancer susceptibility remains equivocal. This diversity in experimental findings probably reflects factors such as the form of dietary Se used in studies, the specific cancer studied, and genetic variations within and between the study populations, which may modulate the response to Se intake or status. Se is present as the amino-acid selenocysteine (Sec) in selenoproteins, of which ~25 are found in humans. Many of these selenoproteins, for example, glutathione peroxidases and thioredoxin reductases, have antioxidant functions in protecting cells from oxidative stress or in regulation of the redox state (6, 16). As Sec is essential for the antioxidant activity of selenoenzymes, Se is often referred to as a dietary antioxidant.

Selenoprotein P (SePP) is atypical among selenoproteins because it is the only selenoprotein containing more than one Sec residue in its sequence. Ten Sec forms exist in the human SePP, nine of which are in a Sec-rich C-terminal domain, and one, in the N-terminal region (27). Plasma SePP accounts for ~65% of plasma Se content (1) and, at present, plasma SePP concentration is used widely as a biomarker of Se status because it is sensitive to Se intakes over a wide range (34). In plasma, SePP is heavily glycosylated and transports Se from the liver to other tissues. SePP has been proposed to have both transport and antioxidant functions (7, 14, 27, 30).

Several isoforms of SePP have been detected. In the rat, four plasma isoforms correspond to alternative premature terminations at UGA codons normally encoding for Sec in the

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full-length protein (17). In humans, Western blot analysis of plasma SePP reveals two bands corresponding to proteins of approximate molecular mass 50 and 60 kDa, respectively (19). The role and nature of these isoforms remain unclear. Enzymatic cleavage of N-linked glycans induces a shift in molecular weight of both isoforms, suggesting that they are not alternative *N*-glycosylated SePP forms (19, 31). Additionally, the Se/protein ratio of the 60-kDa isoform is higher than that of the 50-kDa form, suggesting that the proteins differ in length (19).

Two functional polymorphisms (SNPs), rs3877899 and rs7579, in the human *SEPP-1* gene lead to alterations in Se metabolism (18). Rs387789 is predicted to cause an Ala-to-Thr change at codon 234 in SePP and rs7579 to cause a G-to-A base change in the 3′ untranslated region of the mRNA (Fig. 1). The present study investigated how these SNPs affect SePP function by analyzing the profile of plasma SePP isoforms in healthy volunteers in relation to genotype, Se supplementation, and gender. In addition, we compared SePP isoforms in plasma from patients with colon cancer and from control subjects.

Materials and Methods

Plasma samples from healthy volunteers

Forty-five healthy volunteers (male and female, nonsmokers), aged 20–60 years, were recruited from the general population in and around Newcastle-upon-Tyne (England) as part of the SelGen Study (18). Recruitment was prospective, depending on genotype for rs3877899 and rs7579 in the *SEPP-1* gene. Informed and written consent was obtained from all volunteers, and ethical permission was granted by the Sunderland Local Research Ethical Committee. Persons already taking Se or multivitamin or vitamin E supplements, having cardiovascular, hepatic, gastrointestinal, or thyroid disorders, cancer, excessive alcohol consumption (>30 units/week), or long-term intake of antiinflammatory drugs were excluded. Volunteers were supplemented with 100 μg sodium selenite for 6 weeks, followed by a 6-week washout period. This level of supplementation is known to increase Se status and is considered safe (8, 34). Peripheral blood samples (30 ml) were drawn from the antecubital vein into EDTA-tubes (BD Vacutainer; Becton-Dickinson, Oxford, U.K.), processed on the same day, typically within 6 h of sample collection, and stored at −80°C until assays were performed. These samples were collected before supplementation, at the end of the supplementation, and every 2 weeks during the washout period. After fractionation of the blood by centrifugation (4°C, 15 min, 950 *g*), plasma was further centrifuged for 12 min at 4°C, 730 *g*, to obtain platelet-free plasma, frozen, and stored at −80°C.

Plasma samples from colorectal cancer patients and healthy controls

Plasma samples were obtained from 21 healthy volunteers and 20 colon cancer patients participating in the FAB2 Study (22). In brief, ethical approval was granted by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority, University of Newcastle upon Tyne, and University of Northumbria. All volunteers gave informed written consent. Exclusion criteria included (a) current supplemental vitamin use or had taken vitamin supplements within the preceding 3 months; (b) pregnant or breast-feeding women; (c) those with hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, or inflammatory bowel disease; (d) consumption of any medicines known to interact with folate; and (e) aged younger than 40 years.

Genotyping

Both polymorphisms are present in the same 722bp-PCR product. PCR was performed with 100-ng template genomic DNA by using forward (cacgcattattcctatctctataagcttg) and reverse (ggaaatgaaattgtgtctagactaaattgg) primers and the Expand High Fidelity PCR system (Roche, Burgess Hill, U.K.) in a ThermoHybaid Px2 thermocycler with the following conditions: an initial denaturating step at 94°C for 4 min,

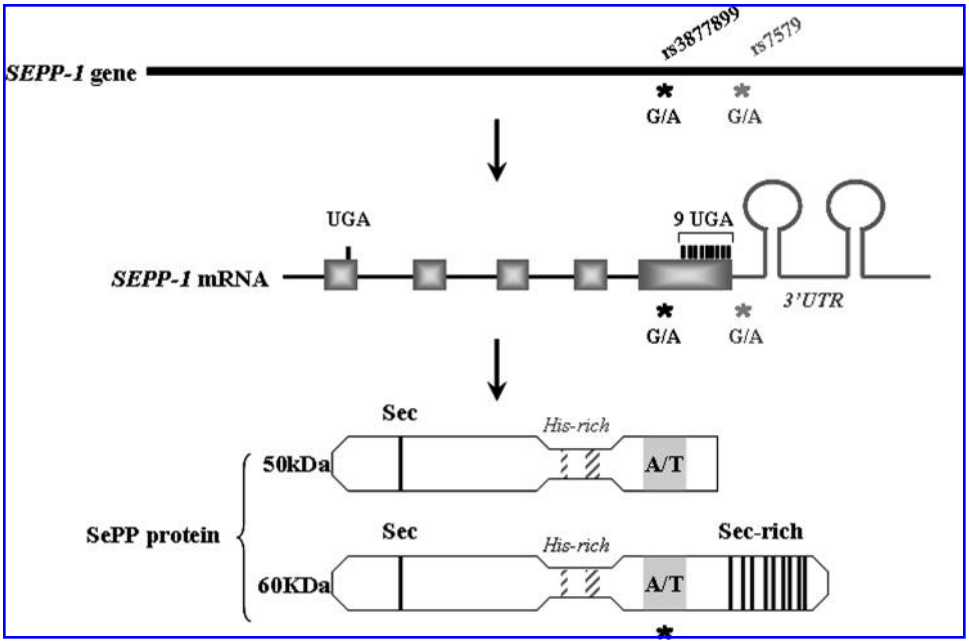


FIG. 1. Scheme indicating the position of polymorphisms rs3877899 and rs7579 in the *SEPP-1* gene in relation to the coding sequence, 3′UTR, and SECIS elements. Positions of both SNPs in the gene and corresponding mRNA and protein are shown by a *star*. The change in base (G to A) or amino acid (A to T) also are indicated. In the mRNA structure, the exons are indicated as *rectangles*, and the stem loops of the two SECIS elements in the 3′untranslated region (3′UTR) and the 10 Sec-encoding UGA codons are displayed. In the SePP protein structure, the position of the amino acid change is indicated for both isoforms in relation to the position of Sec and the histidine-rich domains.

followed by 30 cycles of denaturation (94°C for 30 s), annealing (61.2°C for 30 s), and extension (72°C for 1 min). PCR products were subjected to electrophoresis in a 1x Tris-Acetate-EDTA, 2% agarose gel, and visualized with ethidium bromide. PCR products were subsequently PEG purified and sent for sequencing (MWG-Biotech, London, U.K.).

Protein extraction from plasma

SePP was semipurified from plasma by using a His-spin column (Sigma, Gillingham, U.K.) and following manufacturer's instructions. Columns were equilibrated in 600-μl equilibration buffer, pH 8 (50 m*M* Na_2HPO_4, 300 m*M* NaCl) in the presence of complete mini EDTA-free protease-inhibitor cocktail (Roche) by centrifugation at 3,000 rpm for 1 min, at room temperature. Plasma, 3×500 μl, was then applied to the column and centrifuged at 3,000 rpm for 1 min, at room temperature. The column was washed 3 times in equilibration buffer. SePP was then eluted off the column by 500-μl elution buffer, pH 8 (50 m*M* Na_2HPO_4, 300 m*M* NaCl, 100 m*M* imidazole) in the presence of complete mini EDTA-free protease-inhibitor cocktail. Samples were then divided into two aliquots: one was denatured in sample buffer (45 m*M* Tris, pH 6.8, 10% glycerol, 1% wt/vol SDS, 0.01% wt/vol bromophenol blue, 50 m*M* DTT, 1% vol/vol β-mercaptoethanol) for 4 min at 95°C for Western blot analysis, and the other aliquot was snap-frozen and stored at −80°C.

For SePP purification on heparin resin, plasma samples were first incubated for 1 h, at 4°C, rotating in the presence of 5% wt/vol polyethylene glycol. Samples were then centrifuged to remove the polyethylene glycol for 15 min at 2,500 *g* at 4°C. The heparin Sepharose 6 fast-flow resin (GE Healthcare, Chalfont, St. Giles, U.K.) was poured into a 1.5-ml column and washed twice by two-column volume of PBS (20 m*M* Na_2HPO_4, 0.15 *M* NaCl, 0.5 m*M* EDTA, pH 7.4, containing EDTA-free protease inhibitors). Plasma samples were then applied to the column. The heparin column was washed twice in two-column volume of PBS (20 m*M* Na_2HPO_4, 0.15 *M* NaCl, 0.5 m*M* EDTA, pH 7.4, EDTA-free protease inhibitors). Elution was performed in ½-column volume of high-salt PBS (20 m*M* Na_2HPO_4, 0.6 *M* NaCl, 0.5 m*M* EDTA, pH 7.4, EDTA-free protease inhibitors). Samples were then denatured as described earlier.

SDS-PAGE and immunoblotting

Proteins were separated by electrophoresis through an SDS-7%-polyacrylamide gel and electrotransferred onto a

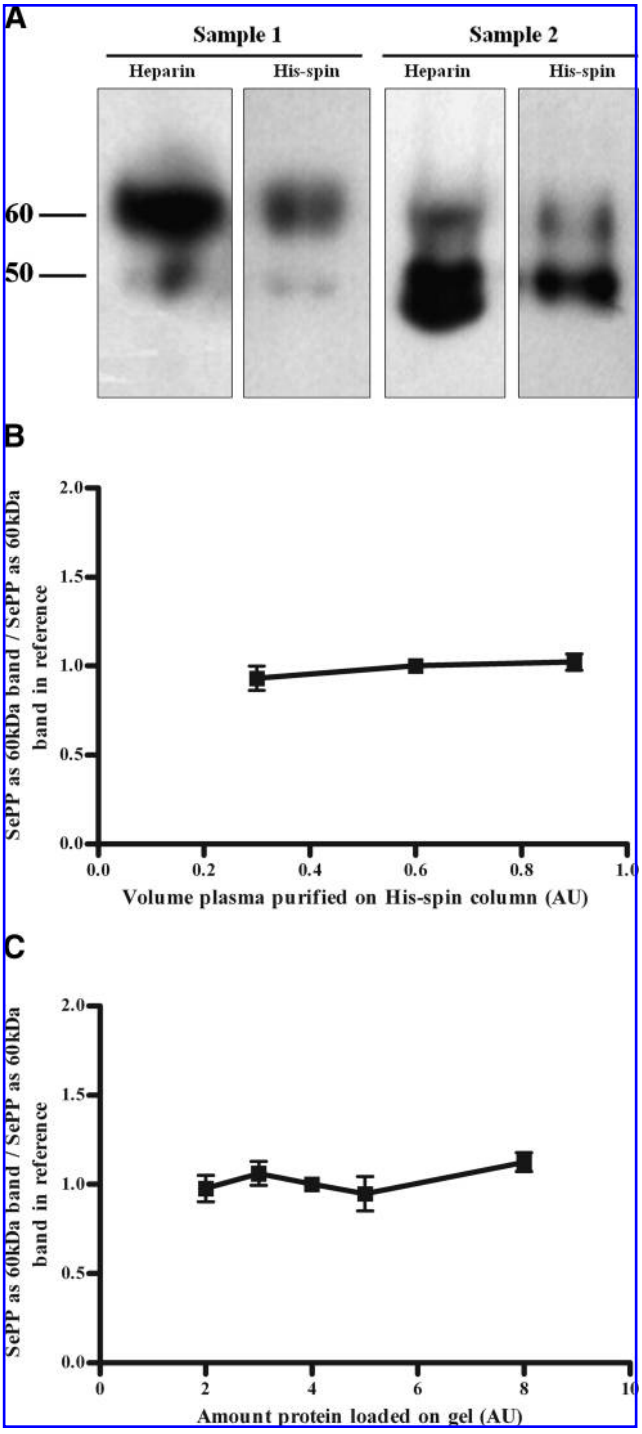
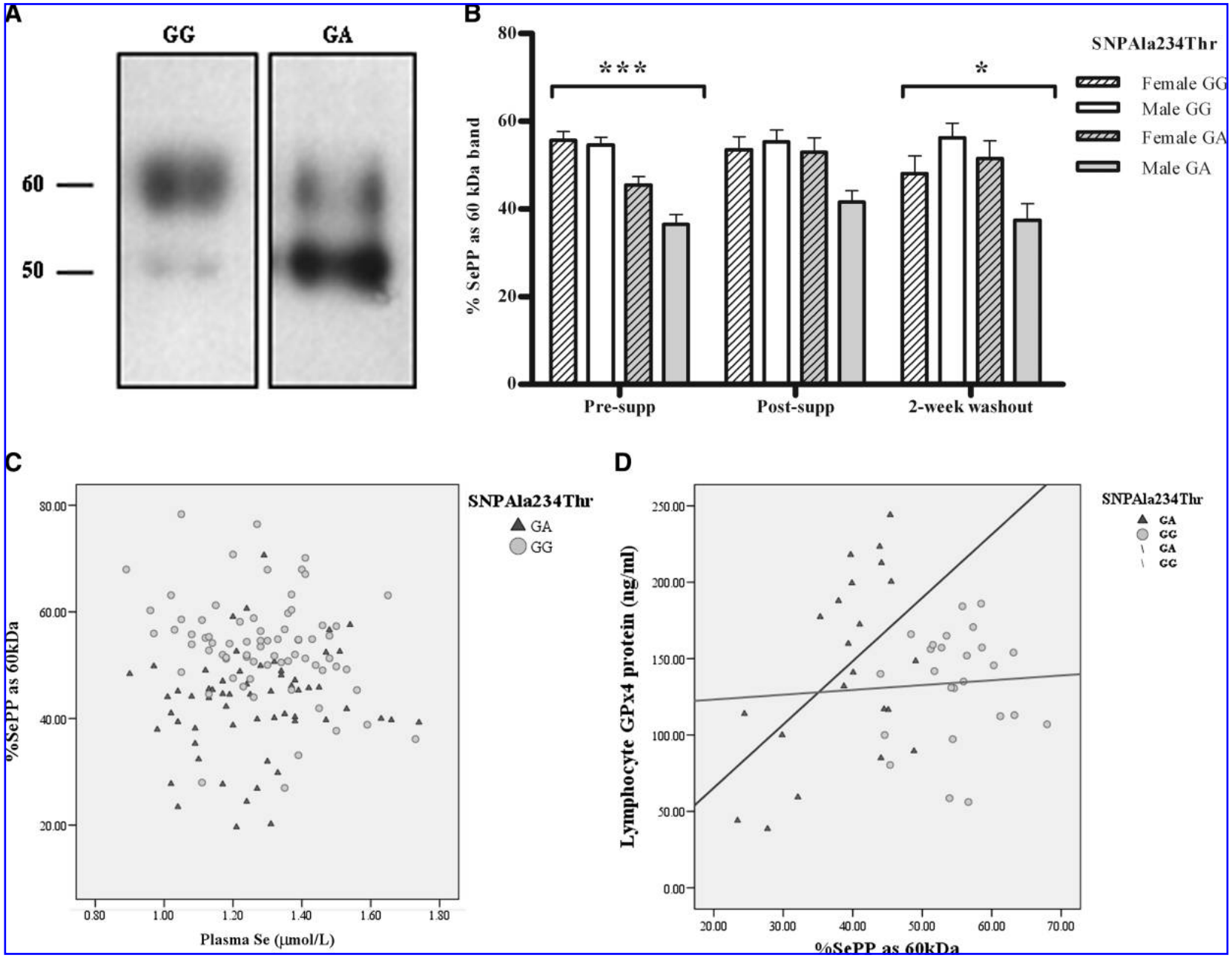


FIG. 2. Semipurification of human SePP from plasma samples. Human SePP was purified from plasma samples obtained from healthy volunteers either on heparin sepharose 6 fast flow resin or His-spin column (Sigma). (**A**) Western blot analysis of two samples is shown as an example and reveals two bands of 60 and 50 kDa, respectively. The band pattern is dependent on the sample but not on the semipurification method. Band intensity was quantified and expressed as the proportion of total SePP present as the 60-Da isoform (in arbitrary units, AUs). (**B**) Three different volumes of plasma from three different samples were purified on His-spin columns. Quantification of the bands revealed that the proportion of the 60-kDa isoform present is independent of the volume of plasma sample purified on the His-spin column. For each sample, one value was taken as an internal reference to normalize the data against this reference. Data are presented as the ratio of the proportion of total SePP present as the 60-kDa isoform to the proportion of total SePP present as the 60-kDa isoform in this reference. (**C**) The isoform pattern is independent of the amount of protein loaded on the gel. Different protein amounts from 10 independent samples were loaded on a gel, and for each sample, 1 point was taken as an internal reference against which the data were normalized. Data are presented as the ratio of the proportion of total SePP present as the 60-kDa isoform to the proportion of total SePP present as the 60-kDa isoform in the reference.


A
GG
GA
60
50
B
SNPAla234Thr
Female GG
Male GG
Female GA
Male GA
% SePP as 60 kDa band
80
60
40
20
0
***
*
Pre-supp
Post-supp
2-week washout
C
SNPAla234Thr
GA
GG
%SePP as 60kDa
80.00
60.00
40.00
20.00
0.80
1.00
1.20
1.40
1.60
1.80
Plasma Se (μmol/L)
D
SNPAla234Thr
GA
GG
GA
GG
Lymphocyte GPx4 protein (ng/ml)
250.00
200.00
150.00
100.00
50.00
0.00
20.00
30.00
40.00
50.00
60.00
70.00
%SePP as 60kDa

PVDF membrane (Roche). Membranes were blocked in PBS containing 0.05% Tween 20 and 5% nonfat dry milk, and then incubated for 1 h with polyclonal anti-human SePP antibody diluted 1:2,000 in PBS containing 0.05% Tween 20 and 5% nonfat dry milk. The antibody was raised in rabbits to a peptide from the published protein sequence (KQPPAW SIRDQDPML) (11) by Eurogentec (Seraing, Belgium) and affinity purified by using this peptide (Eurogentec). Membranes were then washed 5 times in PBS-0.05% Tween 20, and incubated for 1 h with the secondary anti-rabbit antibody (Sigma) diluted 1:5,000 in blocking solution. After a further five washes of the membranes, immunodetection was carried out by using a chemiluminescence kit (GE Healthcare, Chalfont, St. Giles, U.K.) on BioMax autoradiogram film (Kodak, Hermel Hempstead, U.K.). Band intensity was quantified by using UVIband software (Uvitec, Cambridge, U.K.).

Measurement of lymphocyte GPx4 and plasma selenium concentration

Lymphocyte GPx4 protein levels were measured with competitive ELISA assay as described in (18). Total plasma Se was measured by inductively coupled plasma mass spectrometry, as described previously (18).

Statistical analyses

Statistical analyses were carried out by using the SPSS 15.0 software. Ratios of the two isoforms were analyzed by using analysis of variance with the factors gender, genotypes, and supplementation, and their interactions. When a significant interaction between two factors was observed, secondary analysis was carried out to determine the main effects of each factor within subgroups. Data analysis was carried out on postsupplementation values adjusted for presupplementation values and washout values adjusted for postsupplementa tion values. Subsequently, the responses to Se supplementation and discontinuation were analyzed by using a paired *t* test. The Mann–Whitney *U* test was used to examine differences between healthy controls and cancer patients.

Results

Purification of SePP from plasma samples and detection of two isoforms by Western blotting

SePP possesses two histidine-rich domains, and this property has been used to purify SePP through an Ni-NTA-agarose column (19). Therefore, by using His-spin columns originally developed to purify His-Tag proteins, we performed a single-step semipurification of SePP from small amounts of plasma (0.3–1.5 ml). In initial experiments, for comparative purposes, we also purified SePP by using a heparin column used previously to purify SePP in bulk (1, 19). Eluates from both His-spin and heparin columns were found to contain SePP, as identified by Western blotting with anti-SePP antibodies (Fig. 2A). Two bands, with molecular masses of $\sim$60 and 50 kDa, respectively, were detected, consistent with previous observations (19, 31). Importantly, the relative intensity of the two bands varied from sample to sample (Fig. 2A), suggesting important interindividual differences in the distribution of the two isoforms.

Comparison of SePP purification from the same plasma sample, by using either a heparin column or a His-spin column, showed a similar band pattern (for example, compare lanes 1 and 2, or 3 and 4, in Fig. 2A), indicating that the distribution of SePP isoforms was not influenced by the purification method. To determine whether the distribution of SePP isoforms was affected by either the amount of plasma processed or the amount of SePP present in the sample, two experiments were carried out. First, three different volumes of plasma from three different samples were purified on His-spin columns, and the samples were subjected to Western blotting. Second, different amounts of protein from 10 independent plasma samples were loaded on a gel and quantified by Western blotting (Fig. 2C). To determine the proportion of total SePP present in the form of the 60-kDa isoform, the amounts of each of the two isoforms were quantified, and data are presented as the percentage of total SePP represented by the 60-kDa band. The proportion of the 60-kDa isoform present was independent of both the volume of plasma sample (Fig. 2B) and of the amount of loaded protein (Fig. 2C). These data indicate that the intensities of the two bands do not reflect variation in sample preparation but reflect the proportion of the two isoforms of SePP present in the plasma sample. In addition, comparable proportions of the two isoforms were obtained when a second anti-SePP antibody (Lab Frontier, Seoul, Korea) was used (data not shown).

Effect of SNP Ala 234Thr (rs3877899) on SePP isoform pattern

SePP was semipurified from plasma samples from healthy volunteers taking part in the SelGen Study, a human Se-intervention trial in which volunteers were prospectively genotyped for the two SNPs in the *SEPP-1* gene, a G/A polymorphism (rs3877899) resulting in an amino acid change at Ala-to-Thr at position 234 and a G/A polymorphism (rs7579) located in the 3′ untranslated region of the *SEPP-1* gene (18). In both cases, the AA genotype was relatively rare; therefore, we recruited people of GG or GA genotype and supplemented them with 100 μg sodium selenite/day for 6 weeks followed by a 6-week washout period. Blood samples were collected before and after supplementation and during the washout period (for details, see ref. 18). The supplementation increased plasma Se concentration and the

FIG. 3. Effect of SNP Ala234Thr (rs3877899) polymorphism in the *SEPP-1* gene on the SePP isoform pattern. Semipurified plasma SePPs from healthy human volunteers of either GG or GA genotype for SNP Ala 234 Thr were analyzed by Western blotting. Band intensity was quantified and expressed as the proportion of total SePP present as the 60-kDa isoform (in arbitrary units, AUs). (**A**) Intensity of the 60-kDa isoform was greater in GG volunteers than in GA volunteers. (**B**) Quantification of Western blot from 45 volunteers (24 GG + 21 GA) at baseline, before (pre-supp), after (post-supp) supplementation, and 2 weeks after Se discontinuation (2-week washout), depending on gender. (**C**) Scatterplot graph of %SePP as 60 kDa as a function of plasma Se concentration (micromolar). Data from all time points are shown. (**D**) Scatterplot graph of lymphocyte GPx4 protein (nanograms per milliliter) before supplementation as a function of %SePP as 60 kDa.

concentration of total plasma SePP in a genotype- and gender-specific manner (18). These effects were also seen in the subset of SelGen Study volunteers studied in this investigation. At baseline, mean plasma Se was $1.15 \pm 0.02\ \mu M$, and this increased significantly ($p < 0.001$) to 1.41 ± 0.03 after supplementation.

Similarly, mean baseline plasma SePP concentration was $4.6 \pm 0.17\ \mu g/ml$, and this increased significantly ($p < 0.001$) to 5.97 ± 0.15 after Se supplementation. To determine whether the effect of SNP Ala234Thr was related to the SePP isoform pattern and to assess the effects of supplementation, we analyzed plasma samples from a randomly selected subset of volunteers with equal numbers of GG and GA genotype before and after supplementation, and during the washout period. At baseline (before supplementation), the top band (60 kDa) was stronger than the lower band (50 kDa) in GG volunteers, whereas in GA volunteers, the lower band was stronger (Fig. 3A). This difference was confirmed as statistically significant ($p < 0.001$; $F = 58$) when the bands were quantified (Fig. 3B).

In addition to the main effect of SNP Ala234Thr on the proportion of the 60-kDa isoform of SePP before supplementation (Fig. 3B), a significant interaction was noted between genotype and gender ($p = 0.043$), but there was no main effect of gender ($p = 0.189$). Overall, GA volunteers had less 60-kDa isoform compared with GG for both genders, and male subjects had less 60-kDa isoform compared with female subjects for both genotypes.

After supplementation, no difference was seen between genotypes in the proportion of the 60-kDa isoform ($p = 0.309$). A significant ($p = 0.007$) SNP-supplementation interaction was found in determining the relative abundance of the 60-kDa isoform (Fig. 3B). Additionally, a significant interaction occurred between gender and genotype after supplementation ($p = 0.024$). This interaction persisted for 2 weeks after completing the Se supplementation ($p = 0.006$), with GA male subjects having less of the 60-kDa isoform compared with GG male subjects and both GA and GG female subjects. GA females responded to supplementation with a significant increase in the proportion of SePP as the 60-kDa isoform (paired t test, $p = 0.009$), whereas GA male subjects did not respond (paired t test, $p = 0.109$). The proportion of the 60-kDa isoform increased after Se supplementation in GA female subjects to a level comparable with that found in GG individuals, and 2 weeks after stopping Se supplementation, the proportion of the 60-kDa isoform in GG and GA female subjects remained similar ($p = 0.2$).

In view of the known contribution of SePP to blood Se concentrations and Se transport and delivery (7, 14, 27, 30), the proportion of SePP in the 60-kDa band was analyzed in relation to both plasma Se concentration and lymphocyte glutathione peroxidase levels. Within the plasma Se range in our study population (0.8–1.7 μM), no correlation was found between the proportion of SePP in the 60-kDa band and plasma Se concentration (Fig. 3C), whereas total plasma SePP concentration was highly correlated with plasma Se for both genotypes ($p < 0.001$; data not shown). As illustrated in Fig. 3C, the proportion of SePP in the 60-kDa band was independent of plasma Se concentration but was significantly affected by genotype. Additionally, a positive correlation appeared between the concentration of lymphocyte GPx4 protein and the percentage of the 60-kDa isoform before supplementation (Pearson correlation, 0.512; $p = 0.015$) in individuals who were GA for the SNP Ala234Thr, but not in GG individuals (Fig. 3D). This observation is consistent with previous data showing that this SNP influences the expression of lymphocyte selenoproteins (18).

Effect of SNP r25191g/a (rs 7579) on SePP isoform pattern

For the SNP r25191g/a, GG volunteers had a lower proportion of the 60-kDa isoform compared with GA or AA before supplementation ($p = 0.011$; Fig. 4A), and this genotype effect was abolished by Se supplementation ($p = 0.113$). However, 2 weeks after Se discontinuation, the effect of the genotype for SNP r25191g/a reappeared ($p = 0.032$), with the proportion of the 60-kDa isoform being markedly lower in AA volunteers and a decrease in this isoform compared with that after supplementation (paired t test, $p = 0.02$). No main effect of gender and no evidence that gender affected the genotype–Se supplementation interaction was noted on the proportion of the 60-kDa SePP isoform.

We also analyzed the effect of combinations of the genotypes for both SNPs (SNP Ala234Thr, SNP r25191g/a) in the *SEPP-1* gene on the SePP isoform pattern. Before supplementation, a highly significant main effect of the combination ($p < 0.001$; $F = 18$) was observed with (GA, GG for SNP Ala234Thr, SNP r25191g/a, respectively) and (GA, GA) showing a lower proportion of the 60-kDa isoform (Fig. 4B). The proportion of the 60-kDa isoform was greater in the presence of the A allele for SNP r25191g/a, with a higher proportion of the 60-kDa isoform in individuals of (GG, AA) genotype than in those with other combinations. This effect of the combination of the two SNP genotypes disappeared after Se supplementation ($p = 0.12$) but was restored 2 weeks after Se discontinuation ($p = 0.023$). This is consistent with previous observations that these two SNPs interact in determining plasma Se concentration and lymphocyte GPx1 activity (18). When analyzing the combination of the two SNPs, no main effect of gender and no interaction between gender and the genotype combination was noted.

SePP isoform pattern in colon cancer patients

To determine whether the profile of the isoform changed with disease, plasma samples from 21 controls and 20 colon cancer patients from the FAB2 Study (22) were purified on His-spin columns and analyzed by Western blotting. As shown in Fig. 5A, for those with the GG genotype of the Ala234Thr SNP, the proportion of the 60-kDa isoform was significantly ($p = 0.001$) lower in colon cancer patients than in healthy controls (the 60-kDa isoform provided $37 \pm 3.6\%$ and $56 \pm 2.5\%$ of the SePP in cancer patients and healthy controls, respectively). In contrast; the proportions of the isoforms were similar between colon cancer patients and healthy controls for the GA genotype of the Ala234Thr SNP. For the SNP r25191g/a polymorphism, the proportion of the 60-kDa isoform was similar for all genotypes in colon cancer patients and lower (significantly so for the GA genotype; Mann–Whitney U test, $p = 0.004$) than for the healthy controls (Fig. 5B). For both polymorphisms, the proportion of 60-kDa isoform of SePP in the healthy controls was very similar to that found for the group of healthy volunteers who took part in the SelGen trial (Fig. 5A).

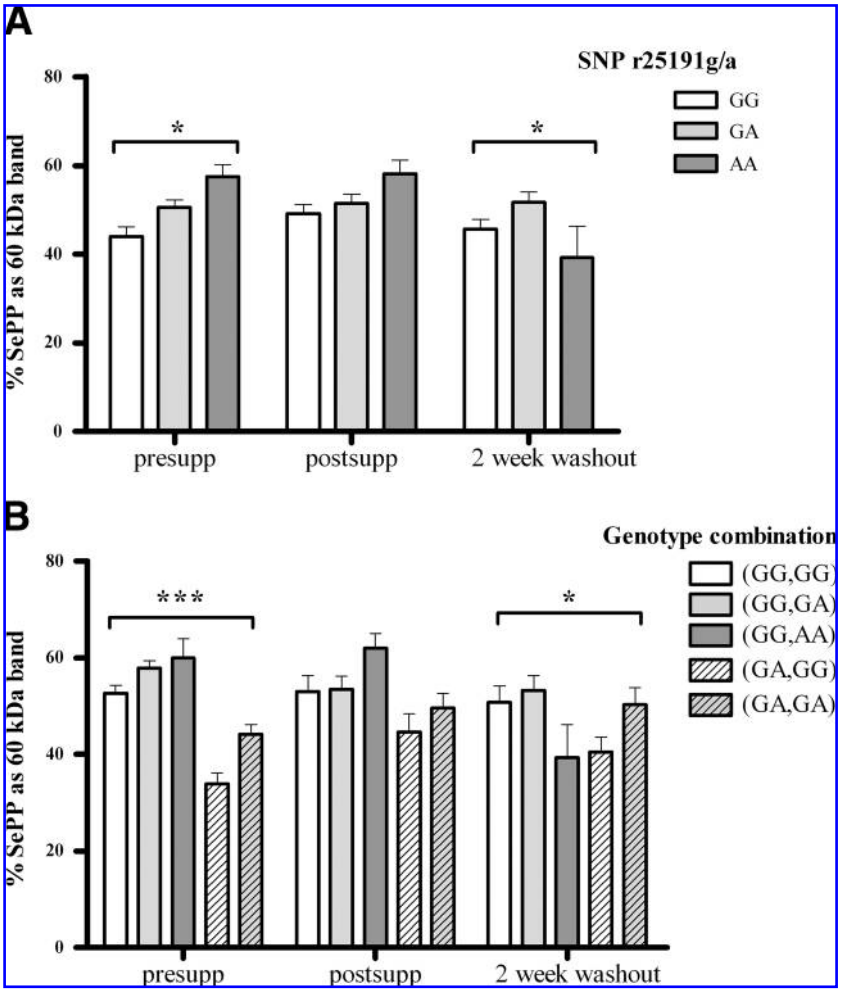


FIG. 4. Effect of SNP r25191g/a polymorphism and of the combination of the genotype for two SNPs in the *SEPP-1* gene on the SePP isoform pattern. Quantification of Western blot depending on (**A**) the SNP r25191g/a polymorphism and (**B**) the combination of genotypes for both SNPs. Genotype combination is defined as (genotype for SNP Ala 234 Thr, genotype for SNP r25191g/a). Band intensity was quantified and expressed as the proportion of total SePP present as the 60-kDa isoform (in arbitrary units, AUs).

Discussion

In addition to its antioxidant function, SePP plays a key role in Se supply to tissues for the synthesis of other selenoproteins, including antioxidant enzymes (12, 27, 29). On this basis, we predicted that genetic variations in the *SEPP-1* gene may affect not only the antioxidant functions of SePP but also its capacity to deliver Se for synthesis of downstream selenoproteins that play a determining role in many cell functions, thus influencing the cellular response to oxidative stress. In humans, two SePP isoforms have been detected by Western blotting (19, 31).The present data show that the relative abundance of these two isoforms is influenced by genotype, Se supplementation, and cancer status.

Two SNPs in the *SEPP-1* gene, rs3877899 (Ala234Thr) and rs7579 (r25191g/a), modulate the response to Se supplementation in humans (18). Both 60-kDa and 50-kDa SePP bands were detected in plasma from all study subjects, but the relative abundance of the two isoforms was influenced by both the SNPAla234Thr and SNP r25191g/a, with a higher proportion of the 60-kDa band in volunteers of the GG genotype for the SNP Ala234Thr and GA for SNP r25191g/a compared with the other genotypes (Figs. 2A and B and 4A). Additionally, a strong effect of the combination of the two SNPs was observed on the distribution pattern of the isoforms (Fig. 4B). Additionally, a positive correlation was found between the concentration of lymphocyte GPx4 protein and the percentage of the 60-kDa isoform before supplementation in individuals who were GA for the SNP Ala234Thr, but not in GG individuals (Fig. 3D). This observation is consistent with previous data showing that these polymorphisms in the SePP gene affected the expression of other selenoproteins (18) and suggests that the difference in isoform pattern could modulate the availability of Se for selenoprotein synthesis. We hypothesize that the SNP r25191g/a, located in the 3'UTR of the mRNA, affects SePP synthesis, because it is located close to one SECIS structure of *SEPP-1* mRNA (Fig. 1). Although it is predicted not to alter the SECIS structure itself, the SNP may alter the efficiency of selenocysteine incorporation by affecting the RNA–protein interactions involved in SePP synthesis. In contrast, the SNP rs3877899 that alters Ala 234 to Thr in the SePP amino-acid sequence may regulate the stability or uptake of the protein by cells. Both mechanisms could alter the availability of plasma SePP for other tissues. Consistent with this hypothesis are the observations that supplementation of healthy volunteers with 100-μg sodium selenite for 6 weeks resulted in a significant increase in the percentage of the 60-kDa isoform in GA volunteers for SNP Ala234Thr (Fig. 3A) and GG for the r25191g/a SNP (Fig. 4A).

Although no overall difference was found between female and male subjects in the distribution of the two isoforms, significant interactions occurred between gender and the

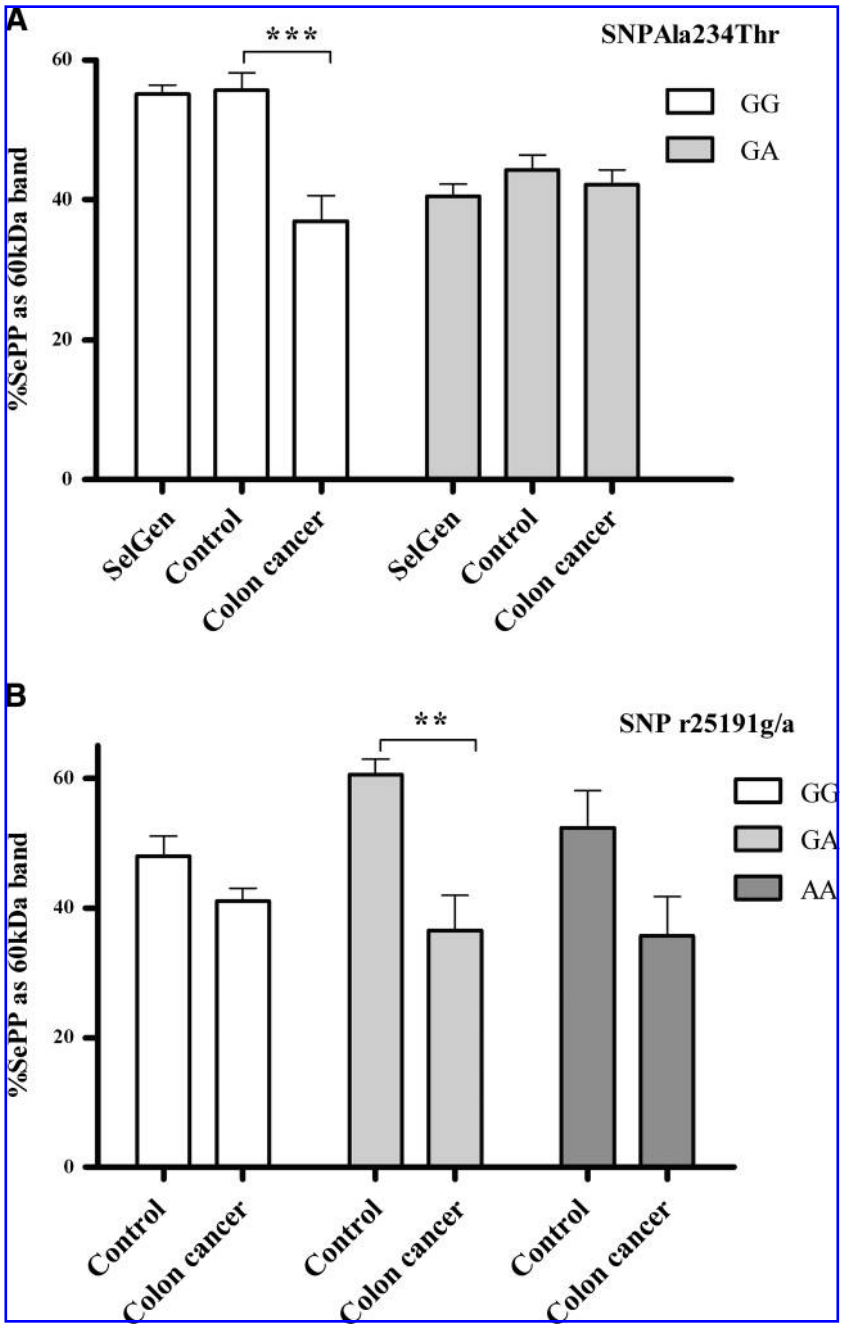


FIG. 5. SePP isoform pattern in colon cancer patients and healthy controls. SePP from volunteers from the FAB2 study (21 healthy controls and 20 patients with colorectal cancer) was semipurified on His-spin columns. Western blot analysis revealed differences in SePP isoform pattern between controls and colon cancer patients depending on (**A**) Ala 234Thr polymorphism, or (**B**) SNP r25191g/a SNP. Band intensity was quantified and expressed as the proportion of total SePP present as the 60-kDa isoform (in arbitrary units, AUs). For comparison, in (**A**), the %SePP as 60 kDa from the SelGen volunteers before supplementation is presented.

genotype for SNP Ala234Thr. This could explain the previous observation that responses of blood cell and plasma selenoprotein activities to supplementation were affected by gender and genotype for SNP Ala234Thr and SNP r25191g/a (18). Although the function and nature of the two SePP isoforms remain unresolved, this difference in isoform distribution could potentially influence Se supply to extrahepatic tissues. For example, the observation of more positive effects of Se supplementation in male volunteers compared with females volunteers in other studies may reflect differences in genotype for these polymorphisms or gender–genotype interactions or both (28). Since livers from male mice incorporates Se into selenoproteins more efficiently than do livers from females (25), we speculate that the gender–genotype interaction effects on the proportion of SePP in plasma as the 60-kDa band reflects greater incorporation of Se into other tissue selenoproteins in males.

It is possible that the two SePP isoforms described in the present study may differ in their C-terminal region, which is rich in Sec, with the 50-kDa form lacking the C-terminal region and being poorer in Sec content. Supplementing volunteers with Se increases the proportion of the 60-kDa isoform (Fig. 3B), and this effect is influenced by genotype; both these observations are consistent with such a hypothesis. Moreover, C-terminal peptides of SePP resulting from a proteolytic cleavage of the full-length protein are capable of supplying Se to Jurkat cells (27).

Se supplementation may affect mortality from colon cancer (26, 33), and recently two studies (5, 21) linked SNPs in selenoprotein genes with susceptibility to colon cancer. In addition,

expression of SePP, GPx1, and GPx3 is altered in the colonic epithelium of cancer patients (2, 20), whereas expression of GPx2 is maintained or even increased (3, 20). Interestingly, we observed that, in colon cancer patients, the proportion of the 60-kDa isoform of SePP in plasma was lower in patients of GG genotype for SNP Ala234Thr (Fig. 5A) compared with the levels observed in either GA patients or healthy volunteers. This difference suggests that colon cancer patients of GG genotype have less of the isoform previously reported to have the greater Se content (17). This difference could arise because (a) individuals with a higher proportion of the 60-kDa isoform and the GG genotype are less susceptible to colon cancer; (b) in cancer patients, a higher demand for Se occurs, and in people of the GG genotype, this is reflected in changes in the proportion of SePP that is present as the 60-kDa isoform; (c) colorectal cancer patients have a lower plasma Se concentration, which may reflect decreased absorption of Se and subsequent synthesis of SePP; or (d) a combination of these factors.

The lower proportion of the 60-kDa isoform in GA individuals for SNP r25191g/a among cancer patients compared with healthy volunteers (Fig. 5B) may have implications for understanding of the Se–genotype interactions in influencing the risk of colon cancer. One possibility suggested by the present data is that maintenance of a high proportion of the 60-kDa isoform may enhance the antioxidant and anticarcinogenic properties of Se by increasing Se availability for the synthesis of selenoproteins with such antioxidant properties. Alternatively, the putative greater efficiency of incorporation of Se into selenoproteins promoted by the 60-kDa isoform may allow more Se to be metabolized into nonselenoprotein metabolites that may themselves be anticarcinogenic (31).

In summary, the present data show that SePP has two isoforms in human plasma that can be detected and quantified by Western blotting. The relative proportions of these two isoforms is influenced by genotype for two SNPs within the *SEPP-1* gene, by gender, by Se supplementation, and by cancer status. In addition to providing evidence that these two SNPs in *SEPP-1* are functional, the findings indicate the importance of carrying out future, more extensive genotyping studies to investigate relations between Se supply, these SNPs in *SEPP-1*, and SePP isoform distribution and potential consequences for susceptibility to colon cancer.

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Abbreviations Used

Ala = alanine
DTT = dithiothreitol
FAB2 = folate and vitamin B_2
GPx = glutathione peroxidase
kDa = kilodalton
Ni-NTA agarose column = nickel-nitrilotriacetic acid agarose column
PAGE = polyacrylamide gel electrophoresis
PBS = phosphate-buffered saline
PCR = polymerase chain reaction
PEG = polyethylene glycol
PVDF = polyvinylidene fluoride
SDS = sodium dodecyl sulfate
Sec = selenocysteine
SECIS = selenocysteine insertion sequence
SePP = selenoprotein P
SEPP-1 = human selenoprotein P gene
SNP = single-nucleotide polymorphism
Thr = threonine
UTR = untranslated region

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