Variation in the Selenoenzyme Genes and Risk of Advanced Distal Colorectal Adenoma

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Abstract

Background: **Epidemiologic and animal studies provide evidence for a chemopreventive effect of selenium on colorectal cancer, which may be mediated by the antioxidative and anti-inflammatory properties of selenoenzymes. We therefore investigated whether genetic variants in selenoenzymes abundantly expressed in the colon are associated with advanced colorectal adenoma, a cancer precursor.**

Methods: **Cases with a left-sided advanced adenoma (n = 772) and matched controls (n = 777) screen negative for polyps based on sigmoidoscopy examination were randomly selected from participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. The underlying genetic variation was determined by resequencing. We genotyped 44 tagging single nucleotide polymorphisms (SNP) in six genes [glutathione peroxidase 1-4 (*GPX1, GPX2, GPX3*, and *GPX4*), selenoprotein P (*SEPP1*), and thioredoxin reductase 1 (*TXNRD1*)] to efficiently predict common variation across these genes.**

Results: **Four variants in *SEPP1* were significantly associated with advanced adenoma risk. A rare variant in the 5′ region of *SEPP1* (*-4166C>G*) was present in nine cases but in none of the controls (exact P = 0.002). Three SNPs located in the 3′ region of *SEPP1*, which is overlapping with the promoter region of an antisense transcript, were significantly associated with adenoma risk: homozygotes at two *SEPP1* loci (31,174 bp 3′ of *STP A>G* and 43,881 bp 3′ of *STP G>A*) were associated with increased adenoma risk [odds ratio (OR), 1.48; 95% confidence interval (95% CI), 1.00-2.19 and OR, 1.53; 95% CI, 1.05-2.22, respectively] and the variant *SEPP1* 44,321 bp 3′ of *STP C>T* was associated with a reduced adenoma risk (*CT* versus *CC* OR, 0.85; 95% CI, 0.63-1.15). Furthermore, we observed a significant 80% reduction for advanced colorectal adenoma risk for carriers of the variant allele at *TXNRD1 IVS1-181C>G* (OR, 0.20; 95% CI, 0.07-0.55; P_{trend} = 0.004). Consistent with the individual SNP results, we observed a significant overall association with adenoma risk for *SEPP1* and *TXNRD1* (global P = 0.02 and 0.008, respectively) but not for the four *GPX* genes.**

Conclusion: **Our study suggests that genetic variants at or near the *SEPP1* and *TXNRD1* loci may be associated with advanced colorectal adenoma. As this is the first study to comprehensively investigate this hypothesis, confirmation in independent study populations is needed.** (Cancer Epidemiol Biomarkers Prev 2008;17(5):1144–54)

Introduction

An increasing number of studies suggests that the essential trace element selenium is a preventive agent for colorectal carcinogenesis. Evidence arises from the Nutritional Prevention of Cancer Trial, a randomized study to evaluate selenium supplementation and skin cancer prevention, which observed, as secondary endpoint, a 61% reduction in colorectal cancer (1). This association was further confirmed, although slightly attenuated, when based on 2 additional years of follow-up (2). Furthermore, several blood-based observational studies, including ours (3), support a beneficial effect of selenium on colorectal carcinogenesis (3-10).

Important biological activities of the essential trace element selenium are mediated through the function of selenoenzymes. Selenium is incorporated into the active center of selenoenzymes as selenocysteine, which was only recently discovered as the 21st naturally occurring amino acid (11). Compared with other amino acids, selenocysteine occurs infrequently in a small number of proteins (12); however, it is located in the active center of the selenoenzymes. The unique redox characteristics of selenocysteine confer important antioxidant properties to these selenoenzymes, which can reduce reactive oxygen species and thereby prevent damage of important biomolecules, including DNA, RNA, lipids, proteins, and membranes; reactive oxygen species–induced DNA damage is known to promote tumor progression (13-18). Because of the direct contact of the colonic epithelial cells with microbial- and food-derived reactive oxygen species, the gastrointestinal tract may be particularly susceptible to oxidative damage (19-23). In addition, selenium and selenoenzyme activity may

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reduce inflammatory processes, known triggers for colorectal carcinogenesis (24, 25).

In our study, we focus on the six antioxidative selenoenzymes, glutathione peroxidase 1-4 (*GPX1, GPX2, GPX3*, and *GPX4*; refs. 1-4), selenoprotein P (*SEPP1*), and thioredoxin reductase 1 (*TXNRD1*), which are expressed in the gastrointestinal tract (26-30). Interestingly, the gastrointestinal tract expresses all four common *GPX* (*GPX1-GPX4*), which may suggest a significant role in colonic function (e.g., as a barrier for reactive oxygen species; refs. 26, 31, 32). Support for the importance of these selenoenzymes comes from an animal study showing that targeted disruption of both cytosolic and gastrointestinal *GPX* (*GPX1* and *GPX2*, respectively) genes results in accumulation of lipid hydroperoxides (33) and high susceptibility to inflammation and colon cancer in mice (25, 34). Preliminary data suggest that genetic variants in some of these selenoenzymes may affect their function and cancer risk; however, only a limited number of the genetic variants in selenoenzymes has been identified or studied so far.

To investigate the association between polymorphisms in six selenoenzymes genes abundantly expressed in the colon (*GPX1-GPX4, SEPP1*, and *TXNRD1*) and advanced colorectal adenoma risk, we conducted a case-control study nested within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. In this large and well-characterized population, case and control status were identified following a standard protocol. To capture the underlying genetic variation across each of the six genes, we resequenced all functionally important regions and genotyped selected tagging single nucleotide polymorphisms (tagSNP). Gene-environmental interactions were explored for important factors, such as serum selenium concentration and smoking.

Materials and Methods

Study Population. This case-control study was nested within the Prostate, Lung, Colorectal and Ovarian Trial, which was designed to evaluate selected methods for the early detection of these cancers and to investigate etiologic factors and early markers of cancer (35, 36). In brief, the Prostate, Lung, Colorectal and Ovarian Trial recruited 154,952 men and women ages 55 to 74 years at 10 centers in the United States (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St. Louis, MO; and Washington, DC) between 1993 and 2001. Participants were randomized to routine care or screening for prostate cancer (prostate-specific antigen testing and digital rectal examination), lung cancer (chest X-ray), colorectal cancer (sigmoidoscopy), and ovarian cancer (CA125 testing and transvaginal ultrasound). If the sigmoidoscopy identified polyps or other suspect lesions, participants were advised to get further follow-up examination through their own medical care providers, which usually resulted in a full colonoscopy with polypectomy or surgical procedures, if indicated. All medical and pathologic reports of the follow-up examinations were obtained and coded by trained medical record abstractors. Written informed consent was obtained from participants and the trial received approval from the institutional review boards of the U.S. National Cancer Institute and the 10 study centers.

Identification of Cases and Controls. Cases and controls for this study were selected from participants randomized to the screening arm between September 1993 and September 1999, who had undergone a successful sigmoidoscopic examination at baseline (insertion to at least 50 cm with >90% of mucosa visible or a suspect lesion identified), completed a baseline risk factor questionnaire, donated a blood sample, and consented to participate in etiologic studies (n = 42,037). Of these participants, we excluded 4,834 with a self-reported history of cancer (except basal-cell skin cancer), ulcerative colitis, Crohn's disease, familial polyposis, colorectal polyps, or Gardner's syndrome. We randomly selected 772 cases for study from among 1,234 cases with advanced distal adenoma (≥10 mm in size, containing high-grade dysplasia, or villous characteristics) in the distal colon (descending colon and sigmoid or rectum) and 777 controls with a negative screening sigmoidoscopy (that is, no polyp or other suspect lesion; n = 26,651) frequency matched to cases by gender and self-reported ethnicity. Five cases and four controls either had no DNA or had discrepancies on repeated fingerprint analyses and were excluded from all analysis, leaving 767 cases and 773 controls for the study. Approximately 63% of the cases had at least one distal adenoma considered to be histologically aggressive (high-grade dysplasia or villous characteristics).

Genotype Analysis. To determine the underlying genetic variation in each gene (*GPX1-GPX4, SEPP1*, and *TXNRD1*), we resequenced the promoter regions, 5′ and 3′ untranslated regions, including the selenocysteine insertion sequence, as well as all exons and intron-exon boundaries in a multiethnic panel of 102 subjects, including 31 Caucasians and 24 African Americans (details are described elsewhere; ref. 37). To efficiently predict the common variation across each gene, we selected tagSNPs based on the resequencing data using the method developed by Clayton.[7] The selection criteria were minimum haplotype r^2 = 0.9 and minor allele frequency > 5% in Caucasians or African Americans, the predominant ethnic groups in this study population. We preselected any nonsynonymous SNPs and those located in the selenocysteine insertion sequence as tagSNPs. TagSNPs were determined separately within the subgroup of Caucasians and African Americans, with the goal to maximize the overlap between tagSNPs to minimize the total number of tagSNPs.

In total, 44 tagSNPs (*GPX1*, n = 6; *GPX2*, n = 6; *GPX3*, n = 11; *GPX4*, n = 7; *SEPP1*, n = 6; and *TXNRD1*; n = 8) were successfully genotyped. These 44 tagSNPs resulted in substantial coverage of the common SNPs (minor allele frequency > 5%). The average r^2 and minimal r^2 are as follows: *GPX1*, 0.73 and 0.33; *GPX2*, 0.81 and 0.22; *GPX3*, 0.83 and 0.62; *GPX4*, 0.92 and 0.53; *SEPP1*, 0.73 and 0.38; and *TXNRD1*, 0.72 and 0.20, respectively. The fraction of common SNPs captured by the tagSNPs with $r^2 \geq 0.8$ and $r^2 \geq 0.5$ is 0.75 and 0.75 for *GPX1*, 0.59 and 0.73 for *GPX2*, 0.67 and 1.0 for *GPX3*, 0.88 and

[7] http://www-gene.cimr.cam.ac.uk/clayton/software/stata/

1.0 for *GPX4*, 0.40 and 0.90 for *SEPP1*, and 0.43 and 0.71 for *TXNRD1*, respectively.

DNA was extracted from whole-blood or buffy-coat samples using QIAamp DNA Blood Midi or Maxi kits (Qiagen). Genotyping was done using SNPlex or TaqMan assays at the National Cancer Institute Core Genotyping Facility (38). Assays were validated and optimized as described in the SNP500 Cancer Web page.[8] Laboratory personnel were blinded to case-control status, and replicate quality-control samples (46 individuals assayed two to six times per polymorphism) were interspersed in the plates. Replicate samples displayed >99% concordance for all SNPs, except for *GPX1* 1,553 bp 3′ of *STP* (97%), *GPX2* 2,680 bp 3′ of *STP* (97%), *GPX2 IVS1-604* (98%), *GPX3 IVS1+2268* (96%), *GPX3 IVS4-14* (95%), *GPX3 Ex5+314* (96%), and *TXNRD1 IVS1-181* (98%). Given the high concordance rates, all SNPs were included in the analysis. Depending on the batch, 0.5% to 1.7% of the subjects had insufficient DNA for genotyping and a small percentage of participants (<1%) was found to have discrepancies on repeated DNA fingerprint analysis and hence were excluded from the analysis. Of those with sufficient DNA, genotyping was successfully completed for 96.5% to 99.5% of the participants depending on the genotype. Genotype frequency among Caucasian controls were consistent with Hardy-Weinberg proportions ($P > 0.05$), except for *SEPP1* 44,321 bp 3′ of *STP* ($P = 0.03$), *TXNRD1 Ex15+410* ($P = 0.0008$), and *TXNRD1 -27129* ($P = 0.04$).

Assessment of Questionnaire and Serum-Based Covariates. At initial screening, all participants were asked to complete a questionnaire about sociodemographic factors, height, weight, medical history (including current and former use of aspirin and other nonsteroidal anti-inflammatory drugs), and risk factors for cancer (including smoking and physical activity). Usual dietary intake over the 12 months before enrollment was assessed with a 137-item food frequency questionnaire, including an additional 14 questions about intake of vitamin and mineral supplements and 10 questions about meat cooking practice (39). Daily dietary nutrient intake was calculated by multiplying the daily frequency of each consumed food item by the nutrient value of the sex-specific portion size (40) using the nutrient database from the U.S. Department of Agriculture (41). Serum selenium concentrations were determined using inductively coupled plasma mass spectrometry (for details, see refs. 3, 42).

Statistical Analysis. To estimate the association between individual SNPs and colorectal adenoma, we calculated odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression analysis. Genotypes were evaluated using indicator variables with the common homozygote as reference. Test for a linear trend was assessed by including a single variable for each SNP, coded as the number of variant alleles, in the regression model. For genotypes with small cell numbers (<5), we used exact test statistics.

All ORs were adjusted for the matching factors, sex, and race as well as for age at randomization, study center, and ethnicity. Adjusting for additional potential risk factors of colorectal tumors, including physical activity, body mass index, smoking, alcohol intake, aspirin use, ibuprofen use, educational attainment, energy intake, red meat intake, folate intake, fiber intake, and calcium intake, did not materially affect the results (data not shown); hence, these variables were not included in the final analysis.

To adjust for multiple comparisons, we conducted global tests of association by simultaneously including all of the SNPs in a given gene in the logistic regression model and then comparing it with a null model that includes none of the SNPs (43). In this multivariate analysis, each SNP was coded by two dummy variables corresponding to homozygous and heterozygous variant genotypes. The resulting likelihood ratio χ^2 value had $2k$ df, with k denoting the number of SNPs in a given gene. This multiloci global test automatically adjusts for multiple testing based on the df of the corresponding χ^2 test. Moreover, the multiloci test can efficiently capture the multivariate linkage disequilibrium pattern within a gene and hence can be more efficient than tests based on SNPs for detecting associations when the true causal variant in the region may not have been genotyped.

We explored interaction of any significant SNPs with serum selenium, age, and smoking. We coded selenium and age as continuous variables. For smoking, we combined current smokers and those that quit smoking <10 years ago as recent smokers to avoid small cell numbers and to account for the relevant period of advanced adenoma development. Former smokers were defined as those that quit smoking ≥10 years ago. To test for statistical significance of multiplicative interaction, we compared models with and without the cross-product terms using likelihood ratio test.

We used polytomous logistic regression analysis to examine the association between individual SNPs and adenoma subtypes, defined by histologic characteristics (tubular, tubular/villous, and villous) and multiplicity (single adenoma, more than one adenoma), and to test for heterogeneity to assess differences between these subtypes.

We used Haploview to assess pairwise linkage disequilibrium measurements (D and r^2) and define haplotype blocks based on Gabriel's algorithm (44). As the linkage disequilibrium pattern may differ between ethnic groups, we explore linkage disequilibrium and conducted haplotype analysis within Caucasian only (sample size of any other ethnic group was too small). Within each haplotype block, haplotype frequency and ORs and 95% CI were estimated using HaploStats (version 3.32), assuming an additive model based on the generalized linear model (45, 46). Haplotypes were estimated using expectation-maximization algorithm and overall difference between cases and controls was evaluated using the global score test statistic adjusted for age, sex, and screening center (46). We used the most common haplotype as reference.

Results

Advanced adenoma cases were slightly older at the time of colorectal cancer screening than controls (average,

[8] http://snp500cancer.nci.nih.gov

Table 1. Characteristics of the study population

Characteristics	Cases	Controls
n	772	777
Age at screening (y), mean	63.1	61.8
Female, %	30.8	30.9
Ethnic origin, %		
African American	2.9	3.0
Caucasian	93.9	93.8
Others*	3.2	3.2
Education		
<12 y	9.1	6.4
High school graduate	24.9	22.7
Some college or college graduate, %	66.0	70.9
Smoking status, %		
Never	34.0	40.9
Former smokers†	35.6	39.2
Recent smokers‡	25.7	14.4
Cigar or pipe (never cigarettes)	4.7	5.6
BMI		
<25	26.2	28.0
25 to <30	45.2	46.6
≥30	28.6	25.4
First-degree relative with colorectal cancer, %	12.3	9.0
Serum selenium (ng/mL), mean§	135.2	137.2

*Includes Hispanic (0.9%), Asian (1.7%), Pacific Islander (0.4%), and native American/Alaskan Native (0.3%) for both cases and controls.
†Former smokers = quite smoking ≥10 y ago.
‡Recent smokers = current smokers or quit smoking <10 y ago.
§Limited to 757 cases and 768 controls with available serum selenium measurements.

63.1 and 61.8 years, respectively; Table 1). The distributions by gender (~31% were female) and ethnic origin (~94% were Caucasian) were similar for cases and controls because we matched on these two variables. Cases were less likely to have graduated from college and were more likely to be smokers, to be obese, and to have a family history of colorectal cancer. Serum selenium concentrations were lower in cases than controls.

Individual analysis of polymorphisms in the *GPX* genes revealed a borderline nonsignificant association between *T* variant located in the 3 region (273 bp 3′ of *STP*) of *GPX4* and advanced colorectal adenoma (P_{trend} = 0.07; Table 2), which was significant when restricted to Caucasians (P_{trend} = 0.04). Haplotype analysis provided similar results: those carrying the haplotype with the rare allele at 273 bp 3′ of *STP* were associated with a borderline significant inverse adenoma risk (OR, 0.81; 95% CI, 0.67-0.99; P = 0.04; Table 3). Adjusting for all other polymorphisms in *GPX4* had little effect on 273 bp 3′ of *STP*. No associations with adenoma risk were observed for any of the other polymorphisms in the four *GPX* genes and the global test for association was not significant for any of the four *GPX* genes (Tables 2 and 3).

Within *SEPP1*, four SNPs were significantly associated with risk of advanced colorectal adenoma. In the promoter region, the variant *SEPP1 -4166G* was present in nine cases (of which eight were Caucasian) but none of the control subjects (Fisher's exact test P = 0.002; Table 2). Furthermore, significant associations were observed between colorectal adenoma risk and variants at two loci in the 3 region of *SEPP1* (31,174 bp 3′ of *STP* and 43,881 bp 3′ of *STP*) with an OR of 1.48 (95% CI, 1.00-2.19) for *GG* versus *AA* and OR of 1.53 (95% CI, 1.05-2.22) for *AA* versus *GG*, respectively. Results were very similar for Caucasians only (data not shown). In addition, the variant at a third loci in the 3 region of *SEPP1* (44,321 bp 3′ of *STP*) was inversely associated with risk of colorectal adenoma (OR for *CT* versus *CC*, 0.73; 95% CI, 0.57-0.92), which remained the same when restricted to Caucasian (data not shown). *SEPP1* 31,174 bp 3′ of *STP* and 43,881 bp 3′ of *STP* were strongly correlated (r^2 = 0.90) among Caucasians, so that it was not possible to differentiate the effects of one polymorphism from the other statistically. The correlation of these two SNPs with the third significant SNP in the 3 region (44,321 bp 3′ of *STP*) was moderate ($r^2 \leq 0.37$). Haplotype analysis containing the three significant polymorphisms in the 3 region did not reveal significant results, which might be expected because we observed association for two SNPs (31,174 bp 3′ of *STP* and 43,881 bp 3′ of *STP*) under a recessive model, whereas the haplotype analysis assumes an additive genetic model. Adjustment for the other *SEPP1* polymorphisms did not significantly alter the ORs observed for any of the four *SEPP1* SNPs. The global P value testing for an overall association within the gene was significant (global P = 0.02).

We also found a significant global test for *TXNRD1* (global P = 0.008) due to a strong association for *IVS1-181C>G*: carriers of the variant allele had a statistically significant 80% reduction in advanced colorectal adenoma risk (OR for *GG+CG* versus *CC*, 0.20; 95% CI, 0.07-0.55), with a strong linear trend (P = 0.004; Table 2). Results for Caucasian only were very similar (data not shown). This polymorphism was not correlated ($r^2 < 0.01$) with any other genotyped SNPs in this gene. Adjustment for the other *TXNRD1* polymorphisms did not significantly alter the ORs observed for *TXNRD1 IVS1-181*. No other genotype or haplotype in *TXNRD1* was significantly associated with adenoma risk.

As adenoma subtypes differ in their tendency to undergo malignant transformation, we assessed differences by histologic characteristics (villous, tubular villous, and tubular) and number of tumors (single and multiple adenoma); however, we did not observe any meaningful differences. Furthermore, associations between tagSNPs in any of the selenoenzymes and advanced adenoma risk did not vary by serum selenium concentrations, age, or smoking: the number of observed interactions significant at $P < 0.05$ (n = 7) did not exceed the expected number of interactions significant at $P < 0.05$ by chance alone given the total number of tests (n = 132; expected number: 0.05 × 132 = 6.6). Of these seven interactions with $P < 0.05$, we observed two with serum selenium (*GPX1* 2,616 bp 3′ of *STP*, P = 0.04; *RAB15* 3,306 bp 3′ of *STP*, P = 0.01), three with age (*GPX1* 2,616 bp 3′ of *STP*, P = 0.04; *GPX3 IVS1+1494*, P = 0.04; *GPX4* 273 bp 3′ of *STP*, P = 0.02), and two with smoking (*GPX1* 2,616 bp 3′ of *STP*, P = 0.03; *GPX3 IVS1-1961*, P = 0.02).

Discussion

Selenoenzymes have antioxidative properties important to prevent oxidative stress, which can lead to DNA damage and neoplastic progression. Several selenoenzymes are expressed in the large intestine, which is exposed to microbial- and food-borne oxidative stress

Table 2. OR (95% CI) for association between polymorphisms in selenoenzyme genes and advanced distal colorectal adenoma

Gene/polymorphism	Cases	Controls	OR (95% CI)	P_{trend}
GPX1				
GPX1 -1039 (rs3448)				
GG	384	407	1.00 (—)	
AG	309	291	1.14 (0.91-1.42)	
AA	46	55	0.89 (0.58-1.37)	0.70
GPX1 -648 (rs3811699)				
AA	352	359	1.00 (—)	
AG	310	337	0.94 (0.76-1.18)	
GG	81	60	1.31 (0.90-1.91)	0.46
GPX1 Ex1+35 (rs1800668)				
CC	347	355	1.00 (—)	
CT	298	333	0.92 (0.74-1.16)	
TT	80	60	1.31 (0.90-1.91)	0.51
GPX1 Ex2-224 (*P200L*; rs1050450)				
CC	351	355	1.00 (—)	
TC	288	331	0.89 (0.71-1.11)	
TT	77	57	1.32 (0.90-1.94)	0.63
GPX1 1,553 bp 3′ of *STP* (rs8179172)				
TT	737	758	1.00 (—)	
AT	10	5	1.94 (0.63-6.02)	0.25
GPX1 2,616 bp 3′ of *STP*				
GG	679	669	1.00 (—)	
GC	64	80	0.80 (0.56-1.14)	
CC	3	1	2.33 (0.23-23.76)	0.34
Global *P*				0.14
GPX2				
RAB15 3,306 bp 3′ of *STP* (rs3742599)				
GG	515	517	1.00 (—)	
GT	210	223	0.97 (0.77-1.22)	
TT	22	18	1.23 (0.64-2.36)	0.92
GPX2 -1102 (rs2296327)				
GG	475	462	1.00 (—)	
GA	244	262	0.97 (0.78-1.21)	
AA	32	37	0.92 (0.55-1.52)	0.70
GPX2 IVS1-604 (rs4902346)				
TT	464	460	1.00 (—)	
CT	252	268	0.97 (0.77-1.21)	
CC	31	34	0.86 (0.51-1.44)	0.57
GPX2 IVS1-444 (rs2071566)				
GG	369	368	1.00 (—)	
AG	297	298	1.01 (0.81-1.27)	
AA	75	88	0.81 (0.57-1.17)	0.46
GPX2 823 bp 3′ of *STP* (rs12172810)				
CC	469	465	1.00 (—)	
CT	245	262	0.95 (0.76-1.19)	
TT	31	34	0.85 (0.51-1.44)	0.51
GPX2 2,680 bp 3′ of *STP*				
AA	656	669	1.00 (—)	
AT	88	90	0.95 (0.69-1.31)	
TT	4	4	1.06 (0.25-4.43)	0.81
Global *P*				0.84
GPX3				
GPX3 -2301 (rs2042235)				
CC	493	496	1.00 (—)	
TC	232	238	0.98 (0.78-1.23)	
TT	24	29	0.82 (0.47-1.46)	0.62
GPX3 -1005 (rs1946234)				
AA	554	565	1.00 (—)	
CA	179	174	1.00 (0.78-1.29)	
CC	10	10	1.00 (0.40-2.49)	0.97
GPX3 -581 (rs8177406)				
TT	560	572	1.00 (—)	
TC	179	176	0.99 (0.77-1.27)	
CC	10	13	0.79 (0.33-1.85)	0.73
GPX3 IVS1+1494 (rs3828599)				
GG	419	419	1.00 (—)	
GA	294	301	0.97 (0.78-1.20)	
AA	35	41	0.79 (0.49-1.29)	0.45

(*Continued on the following page*)

Table 2. OR (95% CI) for association between polymorphisms in selenoenzyme genes and advanced distal colorectal adenoma (Cont'd)

Gene/polymorphism	Cases	Controls	OR (95% CI)	P_{trend}
GPX3 IVS1+2268 (rs4958434)				
GG	533	539	1.00 (—)	
AG	204	211	0.97 (0.77-1.23)	
AA	13	12	1.15 (0.51-2.58)	0.94
GPX3 IVS1-1961 (rs8177426)				
GG	514	519	1.00 (—)	
GA	218	228	0.95 (0.76-1.20)	
AA	14	14	1.00 (0.46-2.17	0.73
GPX3 IVS2-89 (rs869975)				
GG	634	644	1.00 (—)	
GA	102	114	0.86 (0.64-1.17)	
AA	5	3	1.48 (0.33-6.57)	0.47
GPX3 IVS4-14 (rs8177447)				
CC	529	541	1.00 (—)	
CT	210	209	1.02 (0.81-1.29)	
TT	11	13	0.93 (0.41-2.13)	0.94
GPX3 Ex5+314 (rs11548)				
CC	638	632	1.00 (—)	
CT	101	127	0.76 (0.57-1.03)	
TT	6	4	1.19 (0.31-4.55)	0.12
GPX3 947 bp 3′ of *STP* (rs8177454)				
GG	731	747	1.00 (—)	
CG	17	14	1.30 (0.62-2.70)	0.49
GPX3 1,785 bp 3′ of *STP* (*TNIP1* 796 bp 3′ of *STP*) (rs2277940)				
AA	638	643	1.00 (—)	
AG	104	116	0.85 (0.63-1.15)	
GG	6	3	1.84 (0.43-7.93)	0.51
Global *P*				0.94
GPX4				
GPX4 -1928 (rs757229)				
GG	203	189	1.00 (—)	
GC	378	373	0.95 (0.74-1.23)	
CC	166	199	0.80 (0.59-1.07)	0.14
GPX4 -1831 (rs3746165)				
AA	203	191	1.00 (—)	
GA	380	373	0.97 (0.76-1.25)	
GG	168	199	0.82 (0.61-1.10)	0.25
GPX4 IVS4-134 (rs4807543)				
GG	660	675	1.00 (—)	
TG	86	86	0.97 (0.70-1.35)	0.88
GPX4 Ex7+77 (rs713041)				
CC	231	266	1.00 (—)	
TC	368	359	1.13 (0.90-1.43)	
TT	146	133	1.24 (0.91-1.67)	0.15
GPX4 273 bp 3′ of *STP* (rs2075710)				
CC	425	396	1.00 (—)	
TC	242	268	0.84 (0.67-1.05)	
TT	46	56	0.76 (0.49-1.16)	0.07
GPX4 463 bp 3′ of *STP* (rs2074451)				
GG	208	226	1.00 (—)	
TG	373	377	1.07 (0.84-1.36)	
TT	160	149	1.16 (0.86-1.56)	0.34
GPX4 588 bp 3′ of *STP* (rs2074452)				
CC	400	409	1.00 (—)	
TC	292	292	1.03 (0.83-1.28)	
TT	55	61	0.96 (0.64-1.44)	0.97
Global *P*				0.72
SEPP1				
SEPP1 -4166				
CC	740	763	1.00 (—)	
CG	9	0	(—)	0.002*
SEPP1 Ex5+166 (rs3877899)				
GG	445	459	1.00 (—)	
AG	264	268	0.99 (0.79-1.24)	
AA	43	39	0.98 (0.61-1.56)	0.90
SEPP1 Ex5+710 (rs6413428)				
TT	441	458	1.00 (—)	
CT	264	263	1.02 (0.82-1.27)	
CC	47	42	1.00 (0.64-1.57)	0.91

(*Continued on the following page*)

Table 2. OR (95% CI) for association between polymorphisms in selenoenzyme genes and advanced distal colorectal adenoma (Cont'd)

Gene/polymorphism	Cases	Controls	OR (95% CI)	P_{trend}
SEPP1 31,174 bp 3′ of *STP* (rs12055266)				
AA	397	399	1.00 (—)	
AG	282	304	0.95 (0.76-1.18)	
GG	71	54	1.48 (1.00-2.19)	0.26
SEPP1 43,881 bp 3′ of *STP* (rs3797310)				
GG	370	376	1.00 (—)	
GA	296	326	0.95 (0.76-1.19)	
AA	80	60	1.53 (1.05-2.22)	0.17
SEPP1 44,321 bp 3′ of *STP* (rs2972994)				
CC	241	202	1.00 (—)	
CT	352	408	0.73 (0.57-0.92)	
TT	157	154	0.85 (0.63-1.15)	0.18
Global *P*				0.02
TXNRD1				
TXNRD1 -27129 (rs34195484)				
GG	404	423	1.00 (—)	
TG	281	268	1.13 (0.91-1.42)	
TT	63	68	1.03 (0.71-1.51)	0.47
TXNRD1 -24735 (rs4077561)				
CC	240	247	1.00 (—)	
TC	378	380	1.00 (0.79-1.27)	
TT	137	137	1.00 (0.74-1.35)	0.99
TXNRD1 Ex1+116 (rs1128446)				
CC	433	469	1.00 (—)	
GC	278	251	1.17 (0.94-1.46)	
GG	41	43	0.95 (0.60-1.51)	0.43
TXNRD1 IVS1-181 (rs35009941)				
CC	742	741	1.00 (—)	
CG+GG †	5	22	0.20 (0.07-0.55)	0.004
TXNRD1 IVS4+56 (rs5018287)				
GG	215	227	1.00 (—)	
GA	370	375	1.02 (0.80-1.29)	
AA	159	158	1.04 (0.77-1.39)	0.81
TXNRD1 IVS4+85 (rs6539137)				
TT	612	608	1.00 (—)	
AT	126	139	0.92 (0.70-1.20)	
AA	8	10	0.95 (0.37-2.47)	0.55
TXNRD1 IVS12+158 (rs10778322)				
CC	335	366	1.00 (—)	
TC	337	321	1.14 (0.92-1.42)	
TT	79	75	1.10 (0.77-1.58)	0.32
TXNRD1 Ex15+410 (rs35776976)				
CC	744	761	1.00 (—)	
CT	5	0	(—)	
TT	0	1	(—)	0.69*
Global *P*				0.008

NOTE: OR (95% CI) adjusted for age, gender, screening center, and ethnicity.
*P value corresponds for the polymorphism *SEPP1 -4166* and *TXNRD1 Ex15+410* to the Fisher's exact and not to the P_{trend}.
†Only one case and two controls were homozygote rare allele (GG); therefore, the dominate model was presented.

(19-23). Furthermore, selenoenzymes may reduce inflammatory processes. Despite the biological rational for an effect of these genes on colorectal cancer, this hypothesis was previously only investigated to a very limited extent. In our comprehensive analysis of the common variation in six selenoenzymes, genetic variants in *SEPP1* and *TXNRD1* were significantly associated with advanced colorectal adenoma risk after adjusting for multiple comparisons. Furthermore, our findings may suggest an association with advanced adenomas and a variant in the 3 region of *GPX4*. However, this SNP did not result in an overall significant association of the *GPX4* gene as observed for *SEPP1* and *TXNRD1*.

SEPP1 contains 10 selenocysteines and is the only selenoprotein with more than one selenocysteine. *SEPP1* binds ~40% to 60% of the selenium in plasma and is found in several tissues, including the colon, where it becomes down-regulated during tumor development (26, 47-49). *SEPP1* has three important functions: transport of selenium to target tissues, intracellular selenium binding and supply, and protection against oxidative stress (47, 50-54). Our finding of an association with a SNP in the promoter region may point toward an effect on gene expression. Interestingly, the promoter is cytokine responsive, suggesting a role of SEPP1 on inflammation, a risk factor for colorectal cancer (55, 56). Another group (48, 57) examined *SEPP1*, suggesting a potential effect of a complex repeat structure in the promoter region; however, the results are difficult to interpret due to limited information on the study population and controls were selected from different sources than cases. As our study was limited

Table 3. OR (95% CI) for association between haplotypes of selenoenzyme genes and advanced distal colorectal adenoma in Caucasians

Haplotype	Cases (%)	Controls (%)	OR (95% CI)	*P*
GPX1 (2,616 bp 3′ of *STP*, *Ex2-224*, *Ex1+35*, *-648*)				
CCCA	4.7	5.5	0.87 (0.61-1.25)	0.32
GCCA	62.8	63.8	1.00 (—)	
GTTG	32.0	30.6	1.06 (0.89-1.25	0.73
Other	0.5	0.1	5.20 (—)	<0.01
Global *P*				0.68
GPX2 (823 bp 3′ of *STP*, *IVS1-444*, *IVS1-604*)				
CAT	7.9	8.3	0.92 (0.7-1.2)	0.53
CGT	71.8	69.9	1.00 (—)	
TAC	20.1	21.2	0.93 (0.77-1.12)	0.42
Other	0.2	0.6	0.41 (0.38-0.46)	
Global *P*				0.79
GPX3 (*-2301*, *-1005*, *-581*)				
CAT	68.2	68.4	1.00 (—)	
CCC	13.0	12.4	1.00 (0.79-1.26)	0.99
TAT	18.6	19.0	0.98 (0.8-1.19)	0.84
Other	0.2	0.2	1.35 (1.3-1.4)	<0.01
Global *P*				0.97
GPX4 (*-1928*, *-1831*)				
CG	47.3	50.1	0.91 (0.78-1.05)	0.20
GA	52.7	49.8	1.00 (—)	
Other	0.0	0.1	0.71 (0.71-0.71)	<0.01
Global *P*				0.26
GPX4 (273 bp 3′ of *STP*, 463 bp 3′ of *STP*)				
CG	30.0	28.9	1.00 (0.83-1.2)	1.00
CT	47.2	44.9	1.00 (—)	
TG	22.3	26.0	0.81 (0.67-0.99)	0.04
Other	0.5	0.2	2.27 (2.15-2.41)	<0.01
Global *P*				0.16
SEPP1 (44,321 bp 3′ of *STP*, 43,881 bp 3′ of *STP*, 31,174 bp 3′ of *STP*, *Ex5+710*, *Ex5+166*)				
CAGTG	28.3	27.6	1.10 (0.92-1.32)	0.28
CGACA	23.7	22.5	1.06 (0.88-1.29)	0.53
TGATG	44.3	46.4	1.00 (—)	
Other	3.7	3.5	1.23 (0.8-1.89)	0.34
Global *P*				0.75
TXNRD1 (*-27129*, *-24735*)				
GC	28.4	30.0	0.93 (0.77-1.11)	0.40
GT	43.5	42.4	1.00 (—)	
TC	28.1	27.7	1.02 (0.85-1.23)	0.80
Global *P*				0.68
TXNRD1 (*Ex1+116*, *IVS4+56*, *IVS4+85*, *IVS12+158*)				
CAAC	10.0	10.7	1.00 (0.77-1.28)	0.97
CATT	9.0	9.1	1.02 (0.78-1.34)	0.87
CGTC	52.8	54.3	1.00 (—)	
GATT	23.9	21.8	1.10 (0.91-1.33)	0.32
Other	4.3	4.1	1.01 (0.68-1.52)	0.95
Global *P*				0.94

NOTE: Global *P* test for association between all haplotypes and colorectal adenoma. Haplotype and global test adjusted for age, sex, and center Haplotype blocks were determined by Haploview 3.32.

to SNPs, we have no data on this complex repeat polymorphism.

We further found significant associations for three polymorphisms in the 3 region of *SEPP1*. We note that one of these three SNPs (44,321 bp 3′ of *STP*) was out of Hardy-Weinberg equilibrium ($P = 0.03$), which may explain the significant association for this one SNP. All three SNPs are located in the promoter region of an uncharacterized antisense transcript overlapping *SEPP1* (BC039102, we are calling it asSEPP137). The function of *asSEPP1* is unknown, but this unique genomic organization may be clinically relevant because some antisense transcripts regulate the expression of the overlapping gene post-transcriptionally; furthermore, the expression of *SEPP1* is impaired in some prostate tumors (58). Interestingly, the SNP 44,321 bp 3′ of *STP* is predicted to disrupt a putative STAF transcription factor binding site of *asSEPP1* based on MatInspector (59). STAF is a zinc finger protein originally identified as activating the RNA polymerase III promoter of the selenocysteine tRNA gene (60). Altogether, these data suggest that STAF and *asSEPP1* might be part of a complex regulatory mechanism regulating the production of *SEPP1*. Additional studies will be necessary to define the functional importance of the identified variants.

TXNRD1 is involved in antioxidant defense as part of the thioredoxin system, which exists in nearly all cells (61). Its high reactivity is attributed to selenocysteine (62). *TXNRD1* is also involved in the regulation of transcription factors, protein-DNA interaction, and growth control and it reduces nucleotides for DNA synthesis (61, 63-65) *TXNRD1* is highly expressed in various tumor tissues likely in direct response to stress (64-66). We observed a highly significant association for

advanced adenoma with *TXNRD1 IVS1-181*, which remained significant after adjusting for multiple comparisons within the gene. Only one other group investigated *TXNRD1* in relation to breast cancer risk and survival and reported no association for any of the four genotyped tagSNPs, not including *IVS1-181* (67-69). *TXNRD1 IVS1-181* is located -30 bp from the ATG of E1A-like inhibitor of differentiation 3 (*EID3*), a small gene nested within the intron of *TXNRD1*. Little is know about this gene, indicating that *EID3* inhibits transcription and blocks cellular differentiation of cultured muscle cells. Importantly, the C-to-G polymorphism at *IVS1-181* is predicted to change a SRY to NkX-2 transcription factor binding site identified via MatInspector (59). Accordingly, this SNP may not indicate an association with *TXNRD1* but instead with *EID3*. Replication and functional studies are needed to provide additional evidence.

We observed a borderline significantly reduced risk for one polymorphism in *GPX4* (273 bp 3′ of *STP*) among Caucasians. This SNP is only ~150 bp 3 of the selenocysteine insertion sequence, which is required for selenocysteine insertion into the active center of the protein, a highly complex mechanism (11). *GPX4*, a ubiquitously expressed enzyme, and compared with other *GPX*, has the unique ability to directly reduce destructive phospholipid hydroperoxide within the cell and membrane, thus promoting membrane integrity (70). Besides antioxidative properties, GPX4 is involved in regulating inflammatory processes (71); *GPX4* reduces lipid hydroperoxides, which activate lipoxygenase and cyclooxygenase (72). Compared with the other isozymes of the GPX family, the resistance of *GPX4* to selenium deprivation may suggest its vital role, which is further suggested by the finding that mice with disrupted *GPX4* genes die *in utero* (73, 74). One group investigated the association between tagSNPs in *GPX4* and breast cancer and reported a significant association for two SNP (*Ex7+77* and *GPX4 -1928*) with survival but not risk of breast cancer (67-69). These two SNPs were not significantly associated with adenoma risk in our study; however, the nonsignificant associations were in the same direction seen for breast cancer survival. Based on these limited data, further follow-up studies may be warranted given the biological relevance of GPX4.

Besides the suggested association for one *GPX4* polymorphism, we observed no significant association for any other SNP in the four *GPX* genes. The redundant expression of the *GPX* genes in the gastrointestinal tract may limit the effect of any single genetic variant on cancer risk and may explain the limited evidence for an association between the four *GPX* genes and colorectal adenoma risk. Similar *GPX* knockout studies showed that colon cancer risk only increased when both *GPX1* and *GPX2* were knocked out simultaneously but not if only one gene was (25, 34, 75-77).

The study design enabled us to randomly select cases and controls derived from the same source population and screened following a standardized procedure; in particular, cases were not screened based on symptoms. The large study population allowed us to confine the analysis to cases with advanced adenoma, which have a higher potential for malignant transformation and are a particularly meaningful intermediate outcome for studying factors related to colorectal cancer. Cases were identified from the initial baseline screening, so we cannot distinguish the effect of genetic variants on the temporal component of adenoma formation. We investigated potentially important interaction with selenium based on serum measurements, which are preferred over dietary assessment due to a large variation of selenium concentrations in the same foods. However, only a few participants had low selenium concentrations. It may be possible that specific variants in selenoenzymes only affect the function among individuals with selenium deficiency, which would result in limited power to investigate interactions with selenium. TagSNPs selection was based on resequencing data to allow a comprehensive analysis of the common genetic variation in all six genes. However, except for *GPX1*, we did not have the resources to resequence the entire gene, making it possible that we missed some of the genetic variation. Nonetheless, our resequencing was focused on the most functionally relevant regions of the genes (promoter, coding regions, intron, exon boundaries, and selenocysteine insertion sequence). Due to the use of sigmoidoscopy as screening tool, we focused our analysis on left-sided adenoma. Because differences in the associations with right-sided and left-sided colorectal neoplasia have been reported, the extrapolation of our findings with all adenomas (right-sided and left-sided) or right-sided adenoma should be done with caution.

In summary, we observed significant associations for advanced colorectal adenoma and genetic variants in *SEPP1* and *TXNRD1*, which remained significant after adjusting for multiple comparisons. However, as this is the first study to comprehensively analyze the genetic variation in selenoenzymes and risk of colorectal adenoma, further studies are needed to replicate these interesting findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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