Carcinogenesis vol.31 no.6 pp.1074–1079, 2010
doi:10.1093/carcin/bgq076
Advance Access publication April 8, 2010

Genetic variants in selenoprotein genes increase risk of colorectal cancer

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Low selenium (Se) status correlates with increased risk of colorectal cancer (CRC). Since Se exerts its biological roles through the selenoproteins, genetic variations in selenoprotein genes may influence susceptibility to CRC. This study analysed 12 single-nucleotide polymorphisms (SNPs) in selenoprotein genes [glutathione peroxidase 1 (*GPX1*), *GPX4*, 15 kDa selenoprotein (*SEP15*), selenoprotein S (*SELS*), selenoprotein P *(SEPP1)* and thioredoxin reductase 2 (*TXNRD2*)] and in genes that code for a key protein in Se incorporation [SECIS-binding protein 2 (*SBP2*)] and in antioxidant defence [superoxide dismutase 2 (*SOD2*)] in relation to sporadic CRC incidence. CRC patients (832) and controls (705) from the Czech Republic were genotyped using allele specific PCR. Logistic regression analysis showed that three SNPs were significantly associated with an altered risk of CRC: rs7579 (*SEPP1*), rs713041 (*GPX4*) and rs34713741 (*SELS*). The association of these SNPs with disease risk remained after data stratification for diagnosis and adjustments for lifestyle factors and sex. Significant two-loci interactions were observed between rs4880 (*SOD2*), rs713041 (*GPX4*) and rs960531 (*TXNRD2*) and between *SEPP1* and either *SEP15* or *GPX4*. The results indicate that SNPs in *SEPP1*, *GPX4* and *SELS* influence risk of CRC. We hypothesize that the two-loci interactions reflect functional interactions between the gene products. We propose that these variants play a role in cancer development and represent potential biomarkers of CRC risk.

Introduction

Selenium (Se) is a micronutrient that is essential for human health (1,2). There is evidence that low Se status is associated with increased risk of colorectal cancer (CRC), whereas a higher Se intake may lower CRC mortality (3,4) and higher Se status is usually associated with reduced risk of colonic adenoma recurrence (3,5). Daily supplementation with 200 μg Se has been found to result in lower CRC mortality, especially in those individuals with low Se status prior to supplementation (6). Such effects may be particularly relevant to populations where Se intake is relatively low, for example, in European countries such as Czech Republic (7). Additionally, the Czech Republic has one of the highest reported incidences of CRC worldwide, especially for the male population (8). The potential anticarcinogenic properties of Se may be due to its presence as the amino acid selenocysteine in ∼25 human selenoproteins (9,10). They include the glutathione peroxidases (GPxs) 1 and 4 (GPx1 and GPx4) that protect cells from damaging oxidative radicals, selenoprotein P (SePP) that transports Se to tissues (11), the thioredoxin reductases that function in redox control (12), the 15 kDa selenoprotein (Sep15) and members of a novel family of thioredoxin-like proteins (1,2,10) and selenoprotein S (SelS) that is involved in inflammation (13). Therefore, it is possible that genetic variations in the genes encoding the selenoproteins may influence cell protection mechanisms and susceptibility to cancer.

Several single-nucleotide polymorphisms (SNPs) in selenoprotein genes have been shown to have functional consequences. These include: rs1050450 that causes a Pro–Leu amino acid change in GPx1 that affects protein activity and conformation (14–16); rs713041 that causes a C–T substitution in a region of the *GPX4* gene corresponding to the 3′-untranslated region (3′-UTR) of the messenger RNA and alters the protein binding to the 3′-UTR and reporter gene activity (17,18); rs5859 in the 3′-UTR of the *SEP15* mRNA that affects reporter gene activity (19); rs34713741 in the *SELS* promoter (20) and two SNPs in *SEPP1*, rs7579 and rs3877899, which both affect blood selenoprotein levels *in vivo* (21,22). However, the data on the association of allelic SNPs in selenoprotein genes with risk of CRC are very limited. Although an earlier study reported no association between variants in the promoter of the human *SEPP1* gene and altered CRC risk (23), in a more recent study, three variants in *SEPP1* (rs3797310, rs2972994 and rs12055266) and one in the thioredoxin reductase 1 gene *TXNRD1* showed an association with risk of advanced colorectal adenoma (24). In addition, increased risk of CRC has been found to be associated with the C variant allele of rs713041 in *GPX4* (17).

The aim of the present work was to assess the association of functional SNPs in selenoprotein genes with sporadic CRC risk. We focused on functional SNPs from selenoprotein genes that play key roles in cell protection mechanisms, redox control, endoplasmic reticulum (ER) function, inflammation and Se transport. In addition, since risk of breast cancer has been found to be affected by an interaction between rs1050450 in *GPX1* and rs4880 in the manganese superoxide dismutase *SOD2* gene (25), the participants were also genotyped for rs4880. A large population from the Czech Republic was genotyped for these SNPs and the results showed that rs7579 (*SEPP1*), rs34713741 (*SELS*) and rs713041 (*GPX4*) influence risk of CRC.

Subjects and methods

Subjects

The study population comprised 832 patients with CRC and 705 controls with no evidence of colorectal malignancy. Cases and controls were aged ≥29 years, were of Czech origin and consented to provide biological samples for genetic analysis. Cases with histologically confirmed positively diagnosed CRC were recruited (between September 2004 and February 2006) from patients attending nine oncology departments in the Czech Republic (two in Prague and the others in the towns of Benesov, Brno, Liberec, Ples, Pribram, Usti nad Labem and Zlin). During the study period, a total of 968 cases with CRC provided blood samples; of these, 16 individuals were excluded because they met the Amsterdam criteria I and II (26,27) for hereditary CRC. Controls were selected from individuals who attended the same hospitals for exploratory colonoscopy during the period when cases were being recruited and who, having undergone colonoscopy after either evidence of macroscopic bleeding, a positive occult faecal blood test, or abdominal pain of unknown origin, showed no evidence of malignancy or idiopathic bowel diseases (28). Controls had no diagnosis of chronic disease necessitating repeated admittance to hospital (28). Among 739 recruited controls, 705 controls were included in the study. In all, 136 cases and 34 controls were excluded because there was incomplete lifestyle and potential risk factor information or biological material

Abbreviations: 95% CI, 95% confidence intervals; CRC, colorectal cancer; ER, endoplasmic reticulum; GPX, glutathione peroxidase; OR, odds ratio; SBP2, SECIS-binding protein 2; SELS, selenoprotein S; SEP15, 15 kDa selenoprotein; SEPP, selenoprotein P; SNP, single-nucleotide polymorphism; SOD, superoxide dismutase; TXNRD2, thioredoxin reductase 2; UTR, untranslated region.

was lacking. The participating subjects were properly informed and signed a written consent and approval form for genetic analysis in accord with the Helsinki declaration. The design of the study was approved by the Ethical Committee of the Institute of Experimental Medicine, Prague, Czech Republic. All subjects were interviewed using a structured questionnaire to determine demographic characteristics and potential risk factors for CRC. Study subjects provided information on their education, living area, lifestyle habits, body mass index, diabetes, family/personal history of cancer and long-term (at least six consecutive months) drug use.

SNP genotyping

All participants were genotyped for 12 SNPs in selenoprotein genes: rs1050450 in *GPX1*, rs713041 in *GPX4*, rs5859 and rs5845 in the *SEP15* gene, rs34713741 in *SELS*, rs7579, rs3877899, rs12055266, rs3797310 and rs2972994 in *SEPP1* and rs9605031and rs3211684 in thioredoxin reductase 2 (*TXNRD2*) and SECIS-binding protein 2 (*SBP2*) genes, respectively, and also rs4880 in manganese *SOD2*. The genotyping was performed by KBiosciences (Hoddesdon, Hertfordshire, EN11 OEX, UK) using a competitive allele specific PCR system (KASPar). The genotyping assay was validated using a random 10% of samples as duplicate quality controls with complete concordance. Samples with unclear or failed genotype calls were excluded from the analysis. Details of allele probe sequences are available on request. The genotyping success rate was 92%.

Statistical analysis

To estimate the association between individual SNPs and CRC, we calculated odds ratios (ORs) and 95% confidence intervals (95% CI) using logistic regression analysis and STATA 7.0 statistical software. For each SNP, ORs are presented with reference to the most frequent homozygous genotype. Genotypes were evaluated using indicator variables with the common homozygote as reference. Two models were tested: a recessive model in which each genotype was compared with the homozygote for the frequent allele to assess the effect of each genotype on the risk of CRC and a dominant model in which heterozygotes and homozygotes for the rare allele were pooled together and compared with the homozygote for the frequent allele to assess the effect of the presence of at least one rare allele on the risk of developing CRC. Data were analysed with and without adjustment for sex, body mass index, diabetes, smoking and alcohol consumption. Subgroup analysis was carried out for both sexes. Logistic regression of two-loci interaction was performed for selected SNPs when either (i) the SNPs showed a main effect or (ii) multivariate logistic regression suggested an interaction and when in addition, the likelihood ratio test showed significant interactions ($P \leq 0.05$); ORs are presented with reference to the double homozygous genotype for the most frequent allele. Haplotype analysis, using Haploview software, was used to determine pairwise linkage disequilibrium measurements (D and r^2) for SNPs in *SEPP1* gene.

A study power calculation, using the 'Quanto' software (29), showed that the study had 80% power to detect OR risk increases in the dominant (1.3) and recessive (95% CI 1.7–1.4) models and reductions in the dominant (0.7) and recessive (95% CI 0.4–0.6) models when minor allele frequency was 24 and 49%, respectively.

Results

Minor alleles for SNPs in SEPP1, GPX4 and SELS genes increase risk of CRC

The overall study population included 832 CRC cases and 705 controls. Details of cases and controls are shown in Table I. For all SNPs studied, except for rs3877899 and rs7579 in *SEPP1*, the allelic variants were in Hardy–Weinberg equilibrium. Deviation from Hardy–Weinberg equilibrium can be evident when the SNP in question is related to disease risk (30,31) and therefore the risk association observed for rs7579 and the borderline non-significant association of genotype for rs3877899 with disease risk (see below) may explain the deviation of these allelic variants from Hardy–Weinberg equilibrium, although a previous study of a male UK population found rs3877899 (*SEPP1*) to be in Hardy–Weinberg equilibrium (32).

Association of individual SNPs with CRC risk was assessed by logistic regression. Two models of logistic regression were tested, as described in Subjects and Methods: a recessive model and a dominant model. In the absence of adjustment for other variables (body mass index, sex, smoking and alcohol consumption), three SNPs were found to be significantly associated with an altered risk of CRC (see Table II): rs7579 in *SEPP1* gene (located in region corresponding to the 3′-UTR, essential for selenoprotein synthesis) with AA having an OR of 1.67 (95% CI 1.07–2.60), $P = 0.023$ with reference to GG individuals; rs713041 in *GPX4* (region corresponding to the 3′-UTR) with CT having an OR of 1.33 (95% CI 1.05–1.68), $P = 0.018$ with reference to CC individuals and rs34713741 (*SELS* gene, in the promoter) with TT having an OR of 1.68 (95% CI 1.16–2.43), $P = 0.006$ with reference to CC individuals. In all cases, the presence of one or two copies of the rare allele was associated with an increased risk of CRC. Overall, when the data were stratified according to diagnosis, the association of these three SNPs with disease risk remains similar for colon (including sigmoid) and rectal cancers. After adjustment for the above-mentioned variables, the association of genotype with disease risk for rs7579 in *SEPP1*, rs713041 in *GPX4* and rs3471374 in *SELS* remained statistically significant (data not shown), and carriage of the leucine allele in *GPX1* rs1050450 was associated with decreased risk of CRC [OR = 0.26 (95% CI 0.095–0.71), $P = 0.009$].

Table I. Description of CRC cases and controls

	Cases ($n = 832$)	Controls ($n = 705$)
Males/females (%)	58.4/41.6	52.6/47.4
Average age (years) ± SD	61.3 ± 11.6	54.2 ± 15.4
Rectal cancer (%)	31.9	—
Colon cancer (%)	68.1	—
Smoking habit (%)		
Non-smokers	53.2	54.9
Ex-smokers	32.6	24.0
Smokers	14.2	21.1
Positive family history of cancer (%)		
No	45.5	39.6
Yes	54.5	60.4
Percentage of strictly vegetarian (%)	12.7	6.2
Alcohol consumption (%)		
No	48.0	43.1
Yes	52.0	56.9
Mean grams of alcohol/day	24.2 ± 24.8	22.2 ± 21.9
Living place (%)		
City	54.4	56.2
Suburbs	14.4	18.6
Country	31.2	25.2
Education level (%)		
Basic	34.6	25.8
High school	51.0	55.6
University	14.4	18.6
Mean BMI (kg/m^2) ± SD	26.7 ± 4.4	26.4 ± 4.6

Cases were diagnosed as colon or rectal cancer. BMI, body mass index.

Further analysis was carried out to determine the influence of sex (Table II). Women homozygous for the variant TT genotype of rs34713741 in *SELS* showed a significantly increased risk of CRC [OR = 1.83 (95% CI 1.05–3.18), $P = 0.032$ with reference to CC individuals]. For rs7579 in *SEPP1*, the increased risk was far stronger in women of GA + AA genotypes [OR = 1.43 (95% CI 1–2.05), $P = 0.048$, with reference to GG individuals] as compared with men [OR = 1.11 (95% CI 0.81–1.52), $P = 0.534$]. Surprisingly, the presence of one or two rare T alleles for rs2972994 (*SEPP1* promoter) was significantly associated with CRC risk for both sexes but ORs observed in men carrying one [OR = 1.41 (95% CI 1–1.99), $P = 0.05$] or two [OR = 1.4 (95% CI 1.01–1.93), $P = 0.043$] T allele with reference to CC individuals were the opposite of those for women carrying one [OR = 0.66 (95% CI 0.45–0.97), $P = 0.035$] or two [OR = 0.69 (95% CI 0.48–0.99), $P = 0.045$] T alleles (Table II).

SNP–SNP interactions modulate the CRC risk

Since selenoproteins are linked metabolically through the presence of selenocysteine and functionally through their roles in cell protection

Table II. OR for association between SNPs in selenoprotein and Se metabolism genes and CRC risk

Gene (polymorphism)	Cases/controls (both sexes)	OR (95% CI)	*P* value	Cases/controls (males)	OR (95% CI)	*P* value	Cases/controls (females)	OR (95% CI)	*P* value
SOD2 (rs4880)									
Total	719/657			368/304			264/273		
CC	189/174	1.00 (—)		109/75	1.00 (—)		59/76	1.00 (—)	
CT	358/318	1.03 (0.80–1.33)	0.831	177/157	0.76 (0.53–1.10)	0.148	139/125	1.43 (0.94–2.17)	0.091
TT	172/165	0.95 (0.71–1.28)	0.759	82/72	0.78 (0.50–1.20)	0.251	66/72	1.18 (0.73–1.90)	0.494
CT + TT	530/483	1.00 (0.79–1.27)	0.980	259/229	0.77 (0.54–1.08)	0.132	205/197	1.34 (0.91–1.98)	0.143
SELS (rs34713741)									
Total	735/663			379/304			269/278		
CC	323/324	1.00 (—)		167/153	1.00 (—)		115/137	1.00 (—)	
CT	320/284	1.14 (0.91–1.42)	0.256	173/130	1.23 (0.90–1.69)	0.193	114/115	1.18 (0.83–1.69)	0.363
TT	92/55	**1.68 (1.16–2.43)**	**0.006**	39/21	1.71 (0.96–3.04)	0.067	40/26	**1.83 (1.05–3.18)**	**0.032**
CT + TT	412/339	1.23 (0.99–1.51)	0.058	212/151	1.30 (0.96–1.76)	0.089	154/141	1.30 (0.93–1.82)	0.126
GPX4 (rs713041)									
Total	729/664			375/305			266/276		
CC	229/249	1.00 (—)		122/111	1.00 (—)		85/106	1.00 (—)	
CT	364/301	**1.33 (1.05–1.68)**	**0.018**	189/142	1.23 (0.88–1.73)	0.221	131/116	1.41 (0.96–2.06)	0.077
TT	136/114	1.29 (0.95–1.76)	0.101	64/52	1.11 (0.71–1.74)	0.644	50/54	1.15 (0.72–1.86)	0.556
CT + TT	500/415	**1.32 (1.06–1.65)**	**0.015**	253/194	1.20 (0.87–1.65)	0.259	181/170	1.33 (0.93–1.89)	0.116
SEPP1 (rs7579)									
Total	690/629			356/289			253/261		
GG	260/269	1.00 (—)		138/119	1.00 (—)		86/112	1.00 (—)	
GA	369/323	1.18 (0.94–1.48)	0.158	192/150	1.10 (0.80–1.53)	0.552	143/137	1.34 (0.93–1.94)	0.113
AA	61/37	**1.67 (1.07–2.60)**	**0.023**	26/20	1.12 (0.60–2.11)	0.723	24/12	**2.47 (1.16–5.23)**	**0.019**
GA + AA	430/360	1.23 (0.99–1.53)	0.068	218/170	1.11 (0.81–1.52)	0.534	167/149	**1.43 (1.00–2.05)**	**0.048**
SEPP1 (rs3877899)									
Total	732/657			376/302			269/273		
GG	427/409	1.00 (—)		237/193	1.00 (—)		144/168	1.00 (—)	
GA	258/204	1.21 (0.96–1.52)	0.100	117/91	1.05 (0.75–1.46)	0.787	105/87	1.41 (0.98–2.02)	0.063
AA	47/44	1.02 (0.66–1.58)	0.917	22/18	1.00 (0.52–1.91)	0.989	20/18	1.30 (0.66–2.54)	0.451
GA + AA	305/248	1.18 (0.95–1.46)	0.136	139/109	1.04 (0.76–1.42)	0.814	125/105	1.39 (0.99–1.95)	0.060
SEPP1 promoter (rs12055266)									
Total	826/680			442/321			301/294		
AA	474/377	1.00 (—)		247/177	1.00 (—)		171/164	1.00 (—)	
AG	296/261	0.90 (0.73–1.12)	0.346	168/121	0.99 (0.73–1.35)	0.974	105/115	0.88 (0.62–1.23)	0.445
GG	56/42	1.06 (0.70–1.62)	0.785	27/23	0.84 (0.47–1.52)	0.565	25/15	1.60 (0.81–3.14)	0.173
AG + GG	352/303	0.92 (0.75–1.13)	0.449	195/144	0.97 (0.73–1.30)	0.839	130/130	0.96 (0.69–1.33)	0.800
SEPP1 promoter (rs3797310)									
Total	827/688			442/323			299/296		
GG	442/351	1.00 (—)		228/160	1.00 (—)		161/155	1.00 (—)	
GA	320/289	0.88 (0.71–1.09)	0.234	184/137	0.94 (0.70–1.27)	0.698	110/123	0.86 (0.61–1.21)	0.387
AA	65/48	1.08 (0.72–1.60)	0.721	30/26	0.81 (0.46–1.42)	0.462	28/18	1.50 (0.80–2.82)	0.210
GA + AA	385/337	0.91 (0.74–1.11)	0.346	214/163	0.92 (0.69–1.23)	0.576	138/141	0.94 (0.68–1.30)	0.717
SEPP1 promoter (rs2972994)									
Total	826/682			442/320			300/295		
CC	218/188	1.00 (—)		105/97	1.00 (—)		91/68	1.00 (—)	
CT	413/344	1.04 (0.81–1.32)	0.778	232/152	**1.41 (1.00–1.99)**	**0.050**	144/163	**0.66 (0.45–0.97)**	**0.035**
TT	195/150	1.12 (0.84–1.50)	0.438	105/71	1.37 (0.91–2.06)	0.134	65/64	0.76 (0.48–1.21)	0.247
CT + TT	608/494	1.06 (0.84–1.33)	0.609	337/223	**1.40 (1.01–1.93)**	**0.043**	209/227	**0.69 (0.48–0.99)**	**0.045**
SEP15 (rs5859)									
Total	682/626			348/286			249/261		
CC	423/388	1.00 (—)		226/181	1.00 (—)		151/163	1.00 (—)	
CT	229/214	0.99 (0.78–1.25)	0.920	108/97	0.92 (0.65–1.28)	0.610	90/88	1.08 (0.75–1.57)	0.666
TT	30/24	1.15 (0.66–2.00)	0.623	14/8	1.41 (0.58–3.45)	0.446	8/10	0.86 (0.33–2.23)	0.753
CT + TT	259/238	1.00 (0.80–1.26)	0.969	122/105	0.95 (0.69–1.32)	0.778	98/98	1.06 (0.74–1.52)	0.744
GPX1 (rs1050450)									
Total	681/637			354/293			247/264		
CC	354/355	1.00 (—)		186/157	1.00 (—)		129/152	1.00 (—)	
CT	306/259	1.18 (0.95–1.48)	0.133	153/116	1.11 (0.81–1.54)	0.513	116/109	1.25 (0.88–1.78)	0.207
TT	21/23	0.92 (0.50–1.68)	0.777	15/20	0.63 (0.31–1.28)	0.202	2/3	0.79 (0.13–4.77)	0.793
CT + TT	327/282	1.16 (0.94–1.44)	0.173	168/136	1.04 (0.76–1.42)	0.792	118/112	1.24 (0.88–1.76)	0.225
TXNRD2 (rs9605031)									
Total	724/653			377/301			262/274		
CC	389/328	1.00 (—)		204/148	1.00 (—)		137/139	1.00 (—)	
CT	287/273	0.89 (0.71–1.11)	0.286	150/127	0.86 (0.62–1.18)	0.340	107/116	0.94 (0.66–1.33)	0.713
TT	48/52	0.78 (0.51–1.18)	0.241	23/26	0.64 (0.35–1.17)	0.147	18/19	0.96 (0.48–1.91)	0.910
CT + TT	335/325	0.87 (0.70–1.07)	0.194	173/153	0.82 (0.61–1.11)	0.201	125/135	0.94 (0.67–1.32)	0.718
SBP2 (rs3211684)									
Total	734/658			379/301			268/276		
TT	661/577	1.00 (—)		349/266	1.00 (—)		240/242	1.00 (—)	
TG	73/81	0.79 (0.56–1.10)	0.161	30/35	0.65 (0.39–1.09)	0.104	28/34	0.83 (0.49–1.41)	0.493
GG	0/0			0/0			0/0		
TG + GG	73/81	0.79 (0.56–1.10)	0.161	30/35	0.65 (0.39–1.09)	0.104	28/34	0.83 (0.49–1.41)	0.493

OR, 95% confidence interval (CI) and *P* values were calculated for each SNP analysed using logistic regression. ORs were calculated in the total study population and for males and females separately. Case and control groups were compared and significant associations are indicated in bold. For each SNP, ORs are presented with reference to the most frequent homozygous genotype.

from oxidative stress and redox control, we carried out an analysis to assess if CRC risk is influenced by two-loci genotype combinations of variants in different selenoprotein genes. Multivariate logistic regression including all SNPs revealed changes in ORs and statistical significance of disease risk associations for several SNPs when interactions between selenoprotein gene variants were considered (data not shown). Therefore, further analysis was carried out to examine two-loci interactions when the likelihood ratio test was significant for SNPs with a main effect or when suggested by multivariate logistic regression analysis and only statistically significant interactions (as assessed by logistic regression) are presented in Table III.

Different two-loci interactions were observed between rs4880 (*SOD2*), rs713041 (*GPX4*) and rs9605031 (*TXNRD2*). There was a markedly increased risk for CRC in individuals who were TT for rs713041 (*GPX4*) and either CT [OR = 3.28 (95% CI 1.45–7.43), $P = 0.004$] or TT [OR = 3.03 (95% CI 1.22–7.48), $P = 0.016$] for rs4880 (*SOD2*) with reference to individuals homozygous CC for both SNPs. Additionally, the risk of CRC was increased by the interaction of rs4880 (*SOD2*) with rs9605031 (*TXNRD2*) and rs713041 (*GPX4*) with rs9605031 (*TXNRD2*) (Table III).

SNPs in *SEPP1* gene (rs3877899, rs7579, rs3797310 and rs12055266) were shown to interact significantly with rs5859 in *SEP15* and rs713041 in *GPX4*. A decrease in risk [OR = 0.26 (95% CI 0.1–0.69), $P = 0.007$] was observed in individuals having an AA genotype for rs3877899 in *SEPP1* and a CT genotype for rs713041 in *GPX4* with reference to homozygous GG/CC individuals. The interaction of rs5859 (*SEP15*) with rs3877899 (*SEPP1*) was associated with an increased risk of CRC for individuals carrying at least one rare A allele for rs3877899, whereas a decreased risk of CRC was observed for the three other SNPs. This may reflect to a certain extent the fact that rs7579, rs3797310 and rs12055266 are in linkage disequilibrium ($r^2 > 0.95$), whereas rs3877899 is not in linkage disequilibrium with the other studied SNPs in the *SEPP1* gene. rs32116884 (*SBP2*) interacts with rs34713741 (*SELS)* and with rs713041 (*GPX4)* to decrease risk for CRC. Additionally, an interaction between rs34713741 in *SELS* and rs1050450 in *GPX1* was observed for double heterozygotes for the two SNPs (Table III); the absence of significant effect for the homozygotes probably reflects the small numbers of carriers who are homozygous for the rare alleles.

Discussion

Low Se status has been associated with increased incidence of CRC (5). In this study, we report novel associations of sporadic CRC risk with several polymorphisms in selenoprotein genes. Logistic regression analysis showed that three SNPs were associated with CRC risk: rs7579 in *SEPP1*, rs713041 in *GPX4* and rs34713741 in *SELS*. Two of the variants (rs7579 in *SEPP1* and rs713041 in *GPX4*) are present in gene regions corresponding to the 3′-UTR and known to be functional (17,18,22), whereas the third (rs34713741 in *SELS*) is in the promoter region.

SePP is the major selenoprotein in plasma and is crucial for Se supply to the different organs for the synthesis of other selenoproteins (33). The present data show that individuals having at least one A allele for rs7579 (a polymorphism resulting in a G/A base change in the 3′-UTR of the *SEPP1* messenger RNA) exhibited an increased risk for CRC. Genetic variations in *SEPP1* would be expected to lead to altered Se metabolism in various tissues and data from an earlier Se supplementation trial have indicated that rs7579 and rs3877899 affect selenoprotein activities in lymphocytes, compatible with an effect of the SNPs on Se supply for selenoprotein synthesis (21). rs7579 also affects the proportion of SePP isoforms found in plasma (22). The increased risk of CRC associated with the A allele of rs7579 is consistent with our recent finding in a different cohort that CRC disease status modulates the influence of rs7579 genotype on SePP isoform distribution in plasma (22).

A previous study of a series of SNPs in *SEPP1* in colorectal adenoma patients (24) showed that the ORs for four SNPs (rs3877899, rs12055266, rs3797310 and rs2972994) were similar to the values observed in the Czech population studied here. However, for the four SNPs together, they observed a global P value = 0.02, whereas in this study, grouping and multivariate analysis did not reveal a change in CRC risk when the SNPs were considered together (data not shown). The difference in results probably lies in different characteristics of the studied groups, clinical outcomes (adenoma is a precancerous lesion, not necessarily resulting in CRC) or environmental factors. However, both studies indicate the importance of variants in *SEPP1* in conferring an increased risk of adenoma or CRC.

rs713041 in the *GPX4* gene results in a C/T base change in the 3′-UTR GPx4 messenger RNA, in a region close to the base of the SECIS element that is involved in Se incorporation during GPx4 synthesis. The present data show a statistically significant effect of the CT genotype for rs713041 on CRC risk and a similar, but not statistically significant, effect of the TT genotype. This lack of statistically significant effect of TT genotype is probably due to the low numbers of individuals homozygous for this allele and overall, the data suggest that genotype for rs713041 influences CRC risk. However, the observation is not consistent with the decreased risk of CRC associated with the TT genotype found in a previous study of a smaller population from a different European background (17). Further study of another large population is required to clarify the effect of genotype for rs713041 on CRC risk.

Table III. Two-loci interactions between selenoprotein genes, SBP2 and SOD2 genes in relation to CRC risk

SNP–SNP interaction	Genotype combination	Cases/controls	OR (95% CI)	P value
rs4880 (*SOD2*) × rs713041 (*GPX4*)	CT × TT	65/47	3.28 (1.45–7.43)	0.004
	TT × TT	48/34	3.03 (1.22–7.48)	0.016
rs713041 (*GPX4*) × rs9605031 (*TXNRD2*)	CT × TT	26/19	3.58 (1.22–10.52)	0.020
	TT × TT	13/7	5.85 (1.56–21.95)	0.009
rs4880 (*SOD2*) × rs9605031 (*TXNRD2*)	CT × CT	150/130	1.93 (1.12–3.32)	0.018
	CT × TT	25/15	3.99 (1.28–12.41)	0.017
	(CT + TT) × (CT + TT)	261/236	1.91 (1.17–3.12)	0.010
rs5859 (*SEP15*) × rs3877899 (*SEPP1*)	CT × GA	98/58	2.32 (1.40–3.85)	0.001
	TT × GA	17/6	4.72 (1.32–16.85)	0.017
	(CT + TT) × (GA + AA)	131/86	2.00 (1.26–3.17)	0.003
rs5859 (*SEP15*) × rs7579 (*SEPP1*)	CT × AA	13/18	0.22 (0.08–0.6)	0.003
rs5859 (*SEP15*) × rs3797310 (*SEPP1*)	CT × AA	13/21	0.28 (0.13–0.73)	0.009
rs5859 (*SEP15*) × rs12055266 (*SEPP1*)	CT × GG	10/19	0.23 (0.08–0.63)	0.004
rs3877899 (*SEPP1*) × rs713041 (*GPX4*)	AA × CT	25/29	0.26 (0.10–0.69)	0.007
	(GA + AA) × (CT + TT)	187/155	0.59 (0.37–0.92)	0.022
rs34713741 (*SELS*) × rs1050450 (*GPX1*)	CT × CT	142/94	1.78 (1.11–2.87)	0.016
rs34713741 (*SELS*) × rs3211684 (*SBP2*)	CT × TG	25/46	0.38 (0.18–0.79)	0.010
rs713041 (*GPX4*) × rs3211684 (*SBP2*)	CT × TG	28/40	0.40 (0.19–0.85)	0.017

Only significant interactions between two loci, as identified by logistic regression, are presented. Loci are identified by rs number and OR values with 95% CI are shown. ORs are presented with reference to the double homozygous genotype for the most frequent allele. P values for comparison of case and control groups are shown.

The association of rs34713741, an SNP in the *SELS* gene promoter, with CRC suggests that SelS plays an important role in normal colorectal function. SelS is present in the ER where it contributes to the processing and removal of misfolded proteins to target them for proteosomal degradation. The protein is induced by ER stress and has been reported to play a role in inflammatory signalling (20). SNPs in *SELS* promoter have been reported to influence inflammatory pathways (20) and since increased CRC risk has been associated with gut inflammation (34), it is possible that the association of rs34713741 with CRC risk observed here reflects an effect of SelS on inflammation. However, a previous study on a small number of patients did not reveal any association between six polymorphisms in *SELS* gene and autoimmune inflammatory diseases (35).

Additionally, a sex-specific association with CRC for genotype for rs2972994 (*SEPP1* promoter) was revealed when the population was analysed for men or women only, showing a significant and opposite effects of the association in men or women. Interestingly, a similar observation, despite not being significant, was reported for an SNP in another selenoprotein gene, *SEP15* (1125 G/A transition within 3′-UTR) (36). These observations are consistent with previous reports showing differences in Se metabolism and selenoprotein expression between the sexes (21,22,37,38). We speculate that the influence of sex could reflect differences in Se requirements to maintain selenoprotein expression, hormonal or lifestyle differences.

Furthermore, we observed that the OR for risk of CRC due to presence of the SNPs in *GPX4*, *SEPP1* and *SELS* genes could be altered by combinations of particular genotypes. Notable interactions were observed between rs713041 in *GPX4* and rs4880 in *SOD2* and rs965031 in *TXNRD2*, between rs7579 in *SEPP1* and rs5859 in *SEP15* and between rs34713741 in *SELS* and rs1050450 in *GPX1* and rs3211684 in *SBP2*. These interactions suggest that the disease risk associated with the variants in *SEPP1*, *GPX4* and *SELS* is modified by occurrence of SNPs in other selenoprotein or related genes. Interestingly, a similar interaction between rs4880 in *SOD2* and rs3877899 in *SEPP1* has also been found to modify risk of prostate and bladder cancer (32,39).

We hypothesize that these genetic interactions between SNPs in selenoprotein genes or between SNPs in selenoprotein genes and SNPs in either the gene encoding a key protein in selenoprotein synthesis (*SBP2*) or the antioxidant protein manganese *SOD2* could reflect functional interactions, as illustrated in Figure 1. Thus, observed genetic interactions (Table III) may reflect biological interactions between SePP, manganese SOD, GPx1 and 4 and thioredoxin reductases in terms of delivery of Se for redox and radical scavenging reactions in both cytoplasmic and mitochondrial compartments, maintenance of redox state and production of controlled redox signals. Functional effects of SNPs in *GPX4*, *TXNRD2* and *SOD2* could lead to altered ability of cells in preventing oxidative damage and overall changes in that metabolic pathway may result in an increased susceptibility to cancer. In the rat colon, high Se diet was shown to protect against chemically induced mitochondrial dysfunction, acute inflammation and secondary necrosis (40).

Selenoproteins SeP15 and SelS are present in the ER and respectively act to allow proper folding of protein via the synthesis of disulphide bonds and to remove misfolded proteins from the ER lumen to target them for proteosomal degradation (13,20). SelS plays as well a role in inflammatory signalling (20). Thus, it is possible that observed genetic interactions between variants in these genes and SNPs *SEPP1* gene or in genes involved in the control of the redox status affect the ability of cells to deal with abnormal proteins, thus affecting functional changes in apoptotic and inflammatory signalling.

In conclusion, the present data show that common gene variants in *SEPP1*, *GPX4* and *SELS* influence risk of CRC. In turn, the effect of these SNPs is further modified by interactions with SNPs in other selenoprotein genes and modulated by sex. Since the study was hypothesis driven and focused on a small number of biochemically linked functional variants in selenoprotein-related genes, we did not correct our data for multiple testing as we did not believe the loss of

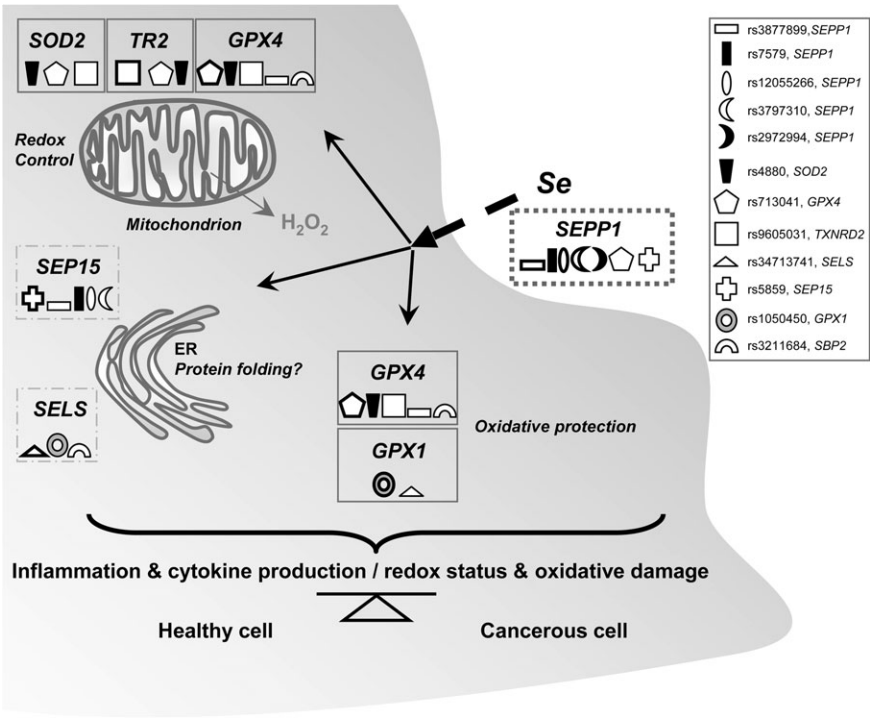

Fig. 1 Genetic and biological interactions between selenoproteins in relation to colorectal function. Scheme shows the overlap between identified genetic interactions in the context of selenoprotein subcellular localization and biological function. Genetic interactions are illustrated with pictograms illustrating SNPs analysed in this study. rs numbers are presented as pictograms as indicated in the frame on the right of the figure. Each of the other boxes corresponds to a specific gene and contains pictograms for this gene and for each interaction with another locus. Black arrows indicate the delivery of selenium to the different selenoproteins. Mitochondrion and ER are indicated.

statistical power to be justified. Despite both this and the low statistical power to assess the reported SNP–SNP interactions, the results highlight the potentially important role played by selenoproteins and selenium in colorectal function and in protecting cells from carcinogenesis. However, further studies are needed to clarify the interactions between Se intake and genetic background in modulating susceptibility to CRC, particularly in relation to whether progression from adenoma to cancer is influenced by altered Se intake. The effects in the present study were observed in a population from Czech Republic, a region of Europe known to have a low Se status (7) and it is probable that functional consequences of selenoprotein gene variants are influenced by the Se status of the population. We hypothesize that the variant alleles lowering functional efficiency of particular selenoproteins may contribute to an increased CRC risk but that such effects can be mitigated by an increased Se intake resulting in an enhancement of selenocysteine supply for selenoprotein synthesis. Interestingly, in the case of one selenoprotein variant and breast cancer risk, it is known that the effect of rs1050450 in *GPX1* is influenced by Se status (41). The effects of these variants on CRC risk are relatively small (in line with most common cancer susceptibility variants) but the concordance in results from the earlier study on adenoma risk (24) and the present one on CRC risk strongly suggests that selenoprotein variants, possibly in combination with low Se status, play a role in both adenoma and cancer development and could represent potential valuable biomarkers of CRC risk.

Funding

Czech Science Foundation (GACR 310/07/1430 and GACR 305/09/P194); Internal Grant Agency of the Czech Ministry of Health (IGA 8563-5/2005). Work in JEH's laboratory is supported by NuGO, the European Union-funded Network of Excellence in Nutrigenomics (FP6-505360).

Acknowledgements

We thank Dr Caroline Relton for advice on statistical analysis.

Conflict of Interest Statement: None declared.

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Received February 16, 2010; revised March 29, 2010; accepted April 2, 2010