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Glutathione Peroxidase (GPX) tagSNPs: Associations with Rectal Cancer but not with Colon Cancer

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Abstract

Glutathione peroxidases (GPXs) are selenium-dependent enzymes that reduce and, thus, detoxify hydrogen peroxide and a wide variety of lipid hydroperoxides. We investigated tagSNPs in *GPX1-4* in relation to colorectal neoplasia in three independent study populations capturing the range of colorectal carcinogenesis from adenoma to cancer. A linkage-disequilibrium (LD)-based tagSNP selection algorithm ($r^2 \geq 0.90$, MAF $\geq 4\%$) identified 21 tagSNPs. We used an identical Illumina platform to genotype *GPX* SNPs in three population-based case-control studies of colon cancer (1424 cases/1780 controls), rectal cancer (583 cases/775 controls), and colorectal adenomas (485 cases/578 controls). For gene-level associations, we conducted principal components analysis (PCA); multiple logistic regression was used for single SNPs. Analyses were adjusted for age, sex, and study center and restricted to non-Hispanic white participants. Analyses of cancer endpoints were stratified by molecular subtypes. Without correction for multiple testing, one polymorphism in *GPX2* and three polymorphisms in *GPX3* were associated with a significant risk reduction for rectal cancer at $\alpha=0.05$, specifically for rectal cancers with *TP53* mutations. The associations regarding the three polymorphisms in *GPX3* remained statistically significant after adjustment for multiple comparisons. The PCA confirmed an overall association of *GPX3* with rectal cancer ($p=0.03$). No other statistically significant associations were observed. Our data provide preliminary evidence that genetic variability in *GPX3* contributes to risk of rectal cancer but not of colon cancer and thus provide additional support for differences in underlying pathogenetic mechanisms for colon and rectal cancer.

INTRODUCTION

Glutathione peroxidases (GPXs) are selenium-dependent enzymes that reduce and, thus, detoxify hydrogen peroxide and a wide variety of lipid hydroperoxides (Topppo et al., 2009). Decreased activity of these antioxidant enzymes may increase oxidative stress and damage to several biomolecules, including DNA, which may initiate or promote neoplastic transformation in affected tissues (Brigelius-Flohé and Kipp 2009). Furthermore, a persistent increase in reactive oxygen species may trigger chronic inflammation, which is considered a risk factor for colorectal cancers (Moore et al., 2010).

There are four major GPX isoenzymes (GPX1-4) encoded by distinct genes, and the isoenzymes vary in tissue distribution and substrate specificity (Topppo et al., 2009). All four GPX isoenzymes are expressed in the colorectal tissue (Mörk et al., 1998), suggesting that they have an important biological role at this site and raising the possibility that genetic variability in the *GPXs* influences the risk of colorectal neoplasia. This hypothesis has been supported by animal models (Chu et al., 2004a, b) and has partly been tested in epidemiological studies (Méplan et al., 2008; Peters et al., 2008; Hansen et al., 2009). However, the available epidemiological evidence is scarce and mainly limited to the candidate SNPs in *GPX1* and *GPX4* (Méplan et al., 2008; Hansen et al., 2009).

For a more comprehensive approach, we evaluated tagSNPs in *GPX1-4* in relation to colorectal neoplasia in three independent study populations (Potter et al., 1996; Slattery et al., 1997, 2003) that capture the range of colorectal carcinogenesis.

MATERIALS AND METHODS

Study Design and Data Collection

The analyses are based on three US population-based case-control studies of colorectal adenomas (Potter et al., 1996), colon cancer (Slattery et al., 1997), and rectal cancer (Slattery et al., 2003). Methods have been described in detail elsewhere (Potter et al., 1996; Slattery et al., 1997; Slattery et al., 2003); a brief description is provided here.

Adenoma study (Potter et al., 1996)—Colorectal adenoma cases (n=485) and polyp-free controls (n=578) were recruited through a large multiclinic gastroenterological practice in the Twin Cities area of Minnesota (numbers refer to non-Hispanic whites with DNA). In brief, eligible participants were aged 30–74 years, with a first diagnosis of colorectal adenoma between 1991 and 1994, no known genetic syndrome associated with increased risk of colon neoplasia, and no individual history of cancer (except non-melanoma skin cancer), prior colorectal polyps, or inflammatory bowel disease. All participants underwent colonoscopy. The participation rate among all patients who underwent colonoscopy was 68%.

Colon and rectal cancer studies (Slattery et al., 1997, 2003)—Colon cancer cases (n=1424) and controls (n=1780) and rectal cancer cases (n=583) and controls (n=775) were recruited from Utah, the Northern California Kaiser Permanente Medical Care Program (KPMCP), and the Twin Cities area of Minnesota (colon only) (numbers refer to non-Hispanic whites with DNA). Eligible participants were aged 30–79 years with no previous diagnosis of colorectal cancer and no diagnosis of familial adenomatous polyposis, Crohn's disease, or ulcerative colitis. Colon cancer cases were first diagnosed between 1991 and 1994 (Slattery et al., 1997). Rectal cancer cases - including cancer of the rectosigmoid junction or rectum only - were first diagnosed between 1997 and 2001 (Slattery et al., 2003). Participation among contacted colon cancer cases was 76% (69% among controls);

participation among contacted rectal cancer cases was 73% (69% among controls), but not all participants provided blood for DNA extraction.

Questionnaire data—Information on diet, physical activity, smoking, anthropometry, medical history, including family history of cancer, demographics, NSAIDs use and reproductive history were obtained by questionnaire as described previously (Potter et al., 1996; Slattery et al., 1997; Slattery et al., 2003). The referent period for colon and rectal cancer studies was the calendar year two years prior to the date of diagnosis or selection.

Tumor markers—Tumor DNA was obtained from paraffin-embedded tissue as described (Slattery et al., 2000a) and categorized according to their genetic profile into tumors with *TP53* or *KRAS2* mutations, with microsatellite instability (MSI) or with the CpG-island methylator phenotype (CIMP+) as previously described (Samowitz et al., 2000; Slattery et al., 2000b; Samowitz et al., 2002; Samowitz et al., 2005).

TagSNP Selection and Genotyping

We applied a linkage-disequilibrium (LD)-based tagSNP selection algorithm ($r^2 \geq 0.90$, MAF $\geq 4\%$), which identified 21 tagSNPs, including the candidate SNPs (*GPX1* P200L and *GPX4* 2573 C>T), representing common genetic variation in Europeans (Supplementary Table 1).

We used the same genotyping platform (Illumina™ GoldenGate bead-based genotyping technology) in all three studies. Intraplate and interplate replicates and blinded duplicates were included (at 5%) as quality control measures. Data from 30 CEPH trios (Coriell Cell Repository, Camden, NJ) genotyped by HapMap were used to confirm reliability and reproducibility. Genotypes were excluded from analyses if any of the following was true: GenTrain Score < 0.4, 10% GC Score < 0.25, AB T Dev > 0.1239, Call Frequency < 0.85, Replicate Errors > 2, P-P-C Errors > 2, < 85% concordance with blinded or non-blinded duplicates and Hardy-Weinberg p-values < 0.0001 (Poole et al., 2010).

Candidate *GPX1* SNP P200L (rs1050450) failed to pass the QC criteria set for the GoldenGate platform. Given the potential importance of this non-synonymous SNP, we genotyped this SNP separately in colon and rectal cancer studies using a pre-developed Taqman allelic discrimination assay. This assay was validated using the HapMap 30 CEPH trios and intra- and inter-plate duplicates, with a success rate of 98% in the colon study and 97% in the rectal study. The polyp study was not genotyped.

Statistical Analysis

Single SNP analyses—Unconditional logistic regression was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for the associations between genotypes and outcomes. Genotypes were modeled using indicator variables for the heterozygous and homozygous variant genotypes (unrestricted or co-dominant model); the dominant model (combining heterozygous and homozygous variants) was used if < 10 cases or controls were observed. Models were adjusted for age, sex and study site as applicable. For trend tests genotypes were treated as a continuous variable. Because of racial differences in genotype frequencies, analyses were restricted to non-Hispanic whites (representing 97% in the adenoma study and 91% and 82% in the colon and rectal cancer study, respectively). A two-side p-value < 0.05 was considered statistically significant. We calculated p-values for correlated tests (p_{ACT}) to adjust for multiple comparisons at the gene level using the method by Conneely and Boehnke (2007).

Principal component analyses (PCA) (Gauderman et al., 2007)—We determined the number of principal components that explained at least 80% of the variance in a gene and performed logistic regression using those components. Gene-level significance was determined using a likelihood-ratio test, comparing a model that contained the principal components and one that did not. The PCA were also adjusted for age, sex, and study site as applicable.

Tumor marker analyses—Tumors were defined by specific molecular alterations: any *TP53* mutation, any *KRAS2* mutation, and MSI+ or CIMP+, defined as at least 2 of 5 markers methylated (Samowitz et al., 2000, 2002, 2005; Slattery et al., 2000b). The proportion of MSI+ tumors in the rectal cases was <3% and thus not investigated. In order to compare cancer patients with specific molecular types of tumors to population-based controls, a generalized estimating equation (GEE) with a multinomial outcome was used, because tumors can have multiple mutations and the case subjects could thus contribute to multiple outcomes (Burton et al., 1998). A co-dominant model with three genotype categories was used when sample sizes were sufficient (≥ 10 subjects); otherwise, a dominant model was used. A recessive model also was analyzed, when indicated by co-dominant ORs.

RESULTS

Characteristics of the study populations included in the analyses are shown in Table 1. Compared to controls, adenoma cases tended to be older and were more likely to be male; the cancer case-controls studies were frequency-matched for age and sex. The 2007 colorectal cancer cases overall were distributed approximately equally in rectum, distal colon and proximal colon.

An overview of the 21 tagSNPs in *GPX1-4* together with information regarding their exclusion or inclusion is provided in the Supplementary Table 1 and the pairwise LDs are shown in the Supplementary Figure 1.

Without correction for multiple testing, four polymorphisms in *GPX2* and *GPX3* were associated with a significant risk reduction for rectal cancer (Table 2). Carrying one or more variant alleles of rs4902347 in *GPX2* were associated with a risk reduction for rectal cancer (OR=0.78, 0.60–1.00), but this association did not remain statistically significant after adjustment for multiple comparisons ($p_{ACT}=0.14$). The variant genotypes of rs3828599, rs736775 and rs8177447 in *GPX3* were associated with a risk reduction for rectal cancer of approximately 40–50% for the homozygous variant genotype compared to the wildtype genotype ($p\text{-trend}=0.01$). The association between the SNPs on *GPX3* and rectal cancer remained statistically significant after adjustment for multiple comparisons (with p_{ACT} -values of 0.04, 0.03, and 0.04, respectively, for rs3828599, rs736775, and rs8177447). No other SNPs in *GPX1-4* showed significant associations for rectal cancer and no statistically significant association was observed for colon cancer or adenomas (data not shown). In PCA, genetic variation in *GPX3* was significantly associated with decreased rectal cancer risk ($p=0.03$; data not shown).

Table 3 shows associations stratified by rectal cancer molecular subtypes for SNPs in *GPX3* that were associated with overall risk of rectal cancer. For the three associations in *GPX3* mentioned above, the observed risk reduction of the variant genotypes appears to be attributable to rectal cancers with *TP53* mutations (31–44% risk reduction with homozygous variant genotype); in this subgroup analysis, the association was marginally significant for rs3828599 ($p=0.06$) and statistically significant for rs736775 ($p=0.04$) and rs8177447 ($p=0.03$) at $\alpha=0.05$. No association was observed for rectal cancers with CIMP+ or *KRAS2* mutations.

DISCUSSION

Our data suggest that genetic variability in *GPX3* contributes to risk of rectal cancer, but not of colon cancer. The homozygous variant genotypes of rs3828599, rs736775 and rs8177447 in *GPX3* were associated with a 40–50% risk reduction for rectal cancer, specifically for rectal cancers with *TP53* mutations. These associations remained statistically significant after adjustment for multiple comparisons and the PCA for *GPX3* confirmed an overall association with rectal cancer. This finding may indicate that oxidative stress and inflammation play a strong role in rectal carcinogenesis.

More than one decade ago it was proposed that colorectal cancers occurring proximal versus distal to the splenic flexure involve distinct genetic abnormalities (Bufill 1990). Our data support the increasing evidence that rectal cancer has a unique pathogenetic mechanism and should be considered a different entity from colon cancer (Kapiteijn et al., 2001; Frattini et al., 2004). A recent study examined the 16 genetic loci identified by genome-wide association studies to be associated with CRC risk according to tumor site and found a difference in genotype frequencies between patients with colon versus rectal cancer for five of these SNPs (Lubbe et al., 2011). Given that pooling the results for biologically different groups could hide meaningful differences, it seems important to consider the stratification by colon and rectal cancer in future studies. To the best of our knowledge, variants in *GPX3* have not been identified in genome-wide association studies on CRC risk so far, but according to the aforementioned, it cannot be excluded that the association with rectal cancer was hidden in studies that considered colon and rectal cancer only as combined endpoint. We observed an association between SNPs in *GPX3* with rectal cancer risk but not with its precursor lesions, i.e., rectal adenomas. This finding raises the possibility that genetic variability in these SNPs affects the progression from adenoma to cancer rather than the initiation of neoplasia. However, the number of rectal adenomas in our study was small (n=77), limiting the statistical power.

Few epidemiological studies have investigated the association between genetic variability in *GPXs* and risk for colorectal neoplasia. A nested case-control study by Hansen et al. (2009) including 375 colorectal cancer cases and 779 controls matched on gender showed an increased risk of colorectal cancer associated with alcohol consumption and smoking among subjects carrying the homozygous variant genotype of the candidate SNP in *GPX1* (P200L). This association was not observed in our study. Hansen et al. (2009) did not investigate other SNPs on *GPX1-4*. A lack of association between polymorphisms in *GPX1-4* and colorectal adenomas was indicated by a study of 772 cases with left-sided advanced adenomas and 777 matched controls within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (Peters et al., 2008). Another study investigating candidate SNPs in *GPX1* (rs1050450) and in *GPX4* (rs713041) in 729 CRC cases and 664 controls reported an increased risk of CRC for the variant genotypes of rs713041 (2573 C>T) (Méplan et al., 2008); this is at variance with results in the present study, but we could only assess the association with colon cancer because the SNP failed quality control criteria in the rectal cancer study.

Our study has several strengths. By investigating several tagSNPs we achieved extensive coverage of genetic variability in *GPX1-4*. The study design, comprising an adenoma study as well as a colon and rectal cancer study, captured the range of colorectal carcinogenesis; the comparability of the results was ensured by using standardized methods in all three studies. Furthermore, the data on molecular subtyping in the colon and rectal cancer studies allowed exploration of subtype-specific associations. Due to the number of statistical tests performed in this study, some of the associations observed could be due to chance. We therefore used stringent methods to account for multiple comparisons.

Nevertheless, replication studies will be needed to confirm our findings. Incorporation of genotyping data for other selenoproteins and parallel measurements of blood selenium levels would allow assessment of potential interaction effects and would help to further elucidate the role of *GPX1-4* in colorectal carcinogenesis. Furthermore, a study that is designed and powered to further stratify according to ethnic subgroups would be of interest to explore potential differences within non-Hispanic whites.

In conclusion, our data provide the first evidence that genetic variability in *GPX3* contributes to risk of rectal cancer but not of colon cancer and thus provide additional support for distinct etiological mechanisms for colon and rectal cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Brigelius-Flohé R, Kipp A. Glutathione peroxidases in different stages of carcinogenesis. *Biochim Biophys Acta*. 2009; 1790:1555–1568. [PubMed: 19289149]
- Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. *Ann Intern Med*. 1990; 113:779–788. [PubMed: 2240880]
- Burton P, Gurrin L, Sly P. Extending the simple linear regression model to account for correlated responses: an introduction to generalized estimating equations and multi-level mixed modelling. *Stat Med*. 1998; 17:1261–1291. [PubMed: 9670414]
- Chu FF, Esworthy RS, Doroshow JH. Role of Se-dependent glutathione peroxidases in gastrointestinal inflammation and cancer. *Free Radic Biol Med*. 2004a; 36:1481–1495. [PubMed: 15182851]
- Chu FF, Esworthy RS, Chu PG, Longmate JA, Huycke MM, Wilczynski S, Doroshow JH. Bacteria-induced intestinal cancer in mice with disrupted Gpx1 and Gpx2 genes. *Cancer Res*. 2004b; 64:962–968. [PubMed: 14871826]
- Conneely KN, Boehnke M. So many correlated tests, so little time! Rapid adjustment of P values for multiple correlated tests. *Am J Hum Genet*. 2007; 81:1158–1168. [PubMed: 17966093]
- Frattini M, Balestra D, Suardi S, Oggionni M, Alberici P, Radice P, Costa A, Daidone MG, Leo E, Pilotti S, Bertario L, Pierotti MA. Different genetic features associated with colon and rectal carcinogenesis. *Clin Cancer Res*. 2004; 10:4015–4021. [PubMed: 15217933]
- Gauderman WJ, Murcray C, Gilliland F, Conti DV. Testing association between disease and multiple SNPs in a candidate gene. *Genet Epidemiol*. 2007; 31:383–395. [PubMed: 17410554]
- Hansen RD, Krath BN, Frederiksen K, Tjønneland A, Overvad K, Roswall N, Loft S, Dragsted LO, Vogel U, Raaschou-Nielsen O. GPX1 Pro(198)Leu polymorphism, erythrocyte GPX activity, interaction with alcohol consumption and smoking, and risk of colorectal cancer. *Mutat Res*. 2009; 664:13–19. [PubMed: 19428376]
- Kapiteijn E, Liefers GJ, Los LC, Kranenbarg EK, Hermans J, Tollenaar RA, Moriya Y, van de Velde CJ, van Krieken JH. Mechanisms of oncogenesis in colon versus rectal cancer. *J Pathol*. 2001; 195:171–178. [PubMed: 11592095]
- Lubbe SJ, Whiffin N, Chandler I, Broderick P, Houlston RS. Relationship between 16 susceptibility loci and colorectal cancer phenotype in 3,146 patients. *Carcinogenesis*. 2011 in press.

- Méplan C, Crosley LK, Nicol F, Horgan GW, Mathers JC, Arthur JR, Hesketh JE. Functional effects of a common single-nucleotide polymorphism (GPX4c718t) in the glutathione peroxidase 4 gene: interaction with sex. *Am J Clin Nutr*. 2008; 87:1019–1027. [PubMed: 18400727]
- Moore MM, Chua W, Charles KA, Clarke SJ. Inflammation and cancer: causes and consequences. *Clin Pharmacol Ther*. 2010; 87:504–508. [PubMed: 20147899]
- Mörk H, Lex B, Scheurlen M, Dreher I, Schütze N, Köhrle J, Jakob F. Expression pattern of gastrointestinal selenoproteins--targets for selenium supplementation. *Nutr Cancer*. 1998; 32:64–70. [PubMed: 9919613]
- Peters U, Chatterjee N, Hayes RB, Schoen RE, Wang Y, Chanock SJ, Foster CB. Variation in the selenoenzyme genes and risk of advanced distal colorectal adenoma. *Cancer Epidemiol Biomarkers Prev*. 2008; 17:1144–1154. [PubMed: 18483336]
- Poole EM, Hsu L, Xiao L, Kulmacz RJ, Carlson CS, Rabinovitch PS, Makar KW, Potter JD, Ulrich CM. Genetic variation in prostaglandin E2 synthesis and signaling, prostaglandin dehydrogenase, and the risk of colorectal adenoma. *Cancer Epidemiol Biomarkers Prev*. 2010; 19:547–557. [PubMed: 20086108]
- Potter JD, Bostick RM, Grandits GA, Fosdick L, Elmer P, Wood J, Grambsch P, Louis TA. Hormone replacement therapy is associated with lower risk of adenomatous polyps of the large bowel: the Minnesota Cancer Prevention Research Unit Case-Control Study. *Cancer Epidemiol Biomarkers Prev*. 1996; 5:779–784. [PubMed: 8896888]
- Samowitz WS, Curtin K, Schaffer D, Robertson M, Leppert M, Slattery ML. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. *Cancer Epidemiol Biomarkers Prev*. 2000; 9:1193–1197. [PubMed: 11097226]
- Samowitz WS, Curtin K, Ma KN, Edwards S, Schaffer D, Leppert MF, Slattery ML. Prognostic significance of p53 mutations in colon cancer at the population level. *Int J Cancer*. 2002; 99:597–602. [PubMed: 11992552]
- Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, Wolff RK, Slattery ML. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroenterology*. 2005; 129:837–845. [PubMed: 16143123]
- Slattery ML, Potter J, Caan B, Edwards S, Coates A, Ma KN, Berry TD. Energy balance and colon cancer--beyond physical activity. *Cancer Res*. 1997; 57:75–80. [PubMed: 8988044]
- Slattery ML, Curtin K, Anderson K, Ma KN, Edwards S, Leppert M, Potter J, Schaffer D, Samowitz WS. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. *J Natl Cancer Inst*. 2000a; 92:1831–1836. [PubMed: 11078760]
- Slattery ML, Edwards SL, Palmer L, Curtin K, Morse J, Anderson K, Samowitz W. Use of archival tissue in epidemiologic studies: collection procedures and assessment of potential sources of bias. *Mutation Research*. 2000b; 432:7–14. [PubMed: 10729707]
- Slattery ML, Edwards S, Curtin K, Ma K, Edwards R, Holubkov R, Schaffer D. Physical activity and colorectal cancer. *Am J Epidemiol*. 2003; 158:214–224. [PubMed: 12882943]
- Toppo S, Flohé L, Ursini F, Vanin S, Maiorino M. Catalytic mechanisms and specificities of glutathione peroxidases: variations of a basic scheme. *Biochim Biophys Acta*. 2009; 1790:1486–1500. [PubMed: 19376195]

Table 1

Characteristics of the three study populations^a

	Adenoma study			Colon cancer study			Rectal cancer study		
	Cases (N=485)	Controls (N=578)	p-value	Cases (N=1424)	Controls (N=1780)	p-value	Cases (N=583)	Controls (N=775)	p-value
Age									
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
	58.0 (9.6)	52.9 (11.0)	<0.01	65.2 (9.7)	65.1 (10)	NA ^b	62.3 (10.8)	62.6 (10.5)	NA ^b
Location									
	N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
Proximal	104 (22)	NA	NA	688 (50)	NA	NA	NA	NA	NA
Distal	300 (62)	NA		700 (50)	NA		NA	NA	
Rectal	77 (16)	NA		NA	NA		583	775	
Sex									
Male	304 (63)	227 (39.3)	<0.01	797 (56)	946 (53)	NA ^b	346 (59)	428 (55)	NA ^b
Female	181 (37)	351 (60.7)		627 (44)	834 (47)		237 (41)	347 (45)	
Study site									
Kaiser Northern California	NA	NA	NA	617 (43)	647 (36)	<0.01	349 (60)	449 (58)	0.48
Minnesota	485 (100)	578 (100)		565 (40)	791 (44)		NA	NA	
Utah	NA	NA		242 (17)	342 (19)		234 (40)	326 (40)	
Regular use of Aspirin or NSAIDs									
Yes	180 (37.1)	257 (44.5)	0.02	562 (39.5)	865 (48.6)	<0.01	263 (45.1)	417 (53.8)	<0.01
No	305 (62.9)	321 (55.6)		862 (60.5)	915 (51.4)		320 (54.9)	358 (46.2)	
Smoking in pack years (amt per day)^c									
0	163 (34.4)	276 (49.0)	<0.01	840 (47.3)	581 (41.0)	<0.01	271 (46.5)	401 (51.7)	0.05
1-25 (20)	152 (32.1)	171 (30.4)		336 (18.9)	250 (17.6)		106 (18.2)	133 (17.2)	
>25 (21+)	159 (33.5)	116 (20.6)		599 (33.8)	586 (41.4)		206 (35.3)	241 (31.1)	
Body mass index									
Normal/Underweight	159 (33.5)	225 (39.8)	0.10	475 (33.5)	708 (39.8)	<0.01	184 (31.7)	258 (33.5)	0.31
Overweight (25-29.9)	204 (43.0)	213 (37.7)		578 (40.7)	726 (40.9)		242 (41.7)	325 (42.2)	
Obese (30+)	111 (23.4)	127 (22.5)		366 (25.8)	343 (19.3)		155 (26.7)	187 (24.3)	

^aNumbers may not total to 100% due to rounding and missing values

^bNA – these were matching factors

^cFor adenoma and rectal cancers, pack-years are reported. For colon cancer, number of cigarettes per day is reported.

Table 2

Selected tagSNPs in *GPX2* and *GPX3* and risk of colorectal neoplasia, adjusted for age, sex, and study center

Colorectal adenoma ^d																Colorectal cancer ^b					
dbSNP ID	genotype	Cases, N (%)	Controls, N (%)	OR	95%CI	p (2df)	p-trend	Cases, N (%)	Controls, N (%)	OR	95%CI	p (2df)	p-trend	Cases, N (%)	Controls, N (%)	p-trend [p _{ACT}] ^d					
GPX 2																					
rs4902347 ^c	GG	372 (77)	452 (78)	1.00				1061 (76)	1338 (77)	1.00				456 (80)	574 (75)						
1756 G>A	GA or AA	110 (23)	129 (22)	1.02	0.75–1.39	0.89	NA	338 (24)	409 (23)	1.05	0.89–1.24	0.84	0.70	114 (20)	188 (25)	0.05 [0.14] ^d					
GPX 3																					
rs3828599	CC	249 (52)	309 (53)	1.00				821 (58)	1015 (57)	1.00				340 (58)	409 (53)						
1580 C->T	CT	195 (41)	233 (40)	1.06	0.81–1.38			528 (37)	670 (38)	0.97	0.83–1.12			214 (37)	298 (39)	0.85 0.68–1.07					
	TT	37 (8)	39 (7)	1.33	0.80–2.20	0.55	0.33	71 (5)	91 (5)	0.97	0.70–1.35	0.90	0.68	29 (5)	66 (9)	0.01 [0.06] ^d 0.01 [0.04] ^d					
rs736775	CC	178 (37)	221 (38)	1.00				584 (45)	711 (44)	1.00				237 (41)	282 (36)						
9133 C->T	CT	237 (49)	277 (48)	1.12	0.85–1.48			514 (40)	668 (42)	0.92	0.79–1.08			275 (47)	354 (46)	0.91 0.72–1.15					
	TT	67 (14)	84 (14)	0.99	0.67–1.48	0.67	0.79	193 (15)	227 (14)	1.02	0.82–1.27	0.54	0.83	70 (12)	138 (18)	0.01 [0.04] ^d 0.01 [0.03] ^d					
rs8177447	CC	324 (67)	391 (67)	1.00				994 (70)	1238 (70)	1.00				413 (71)	500 (65)						
7241 C->T	CT	137 (28)	174 (30)	0.93	0.70–1.24			393 (28)	489 (27)	1.00	0.85–1.17			155 (27)	235 (31)	0.79 0.62–1.01					
	TT	22 (5)	15 (3)	1.88	0.93–3.82	0.16	0.51	33 (2)	47 (3)	0.89	0.56–1.39	0.87	0.77	14 (2)	35 (5)	0.02 [0.08] ^d 0.01 [0.04] ^d					

^a Adjusted for age and sex

^b Adjusted for age, sex, and study center

^c Dominant model is shown because there were 10 or fewer subjects with the homozygous variant model

^d P-values adjusted for correlated tests (PACT), which takes into account the multiple comparison issue

Table 3

Associations between selected tagSNPs in *GPX3* and rectal cancer subtypes^{a,b}

dbSNP ID	genotype	TP53 mutation				KRAS2 mutation				CIMP+							
		Cases, N (%)	Controls, N (%)	OR	95%CI	p	Cases, N (%)	Controls, N (%)	OR	95%CI	p	Cases, N (%)	Controls, N (%)	OR	95%CI	p	
GPX 3																	
rs3828599	CC	125 (60)	409 (53)	1.00			74 (55)	409 (53)	1.00			29 (62)	409 (53)	1.00			
1580 C->T	CT or TT	84 (40)	364 (47)	0.77	0.58–1.03	0.06	60 (45)	364 (47)	0.97	0.69–1.37	0.57	18 (38)	364 (47)	0.75	0.41–1.34	0.24	
rs736775	CC	92 (44)	282 (36)	1.00			54 (40)	282 (36)	1.00			18 (38)	282 (36)	1.00			
9133 C->T	CT/TT	117 (56)	492 (64)	0.74	0.66–1.14	0.04	80 (60)	492 (64)	0.83	0.59–1.17	0.35	29 (62)	492 (64)	1.00	0.56–1.80	0.77	
rs8177447	CC	151 (73)	500 (65)	1.00			93 (70)	500 (65)	1.00			32 (68)	500 (65)	1.00			
7241 C->T	CT/TT	57 (27)	270 (35)	0.72	0.53–0.99	0.03	40 (30)	270 (35)	0.85	0.59–1.24	0.24	15 (32)	270 (35)	0.95	0.52–1.75	0.67	

^aAdjusted for age, sex, and study center

^bAs the proportion of MSI+ tumors in the rectal cancer cases was <3%, there was insufficient power to examine MSI with genotype data of rectal cancer patients