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Polymorphisms in estrogen- and androgen-metabolizing genes and the risk of gastric cancer

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Androgens and estrogens may play a role in gastric cancer etiology. To investigate the association of gastric cancer with single-nucleotide polymorphisms (SNPs) in six genes (*COMT*, *CYP1B1*, *CYP17A1*, *CYP19A1*, *HSD17B1* and *SHBG*) involved in estrogen and androgen synthesis and metabolism, 58 haplotype-tagging SNPs were genotyped in 295 gastric cancer cases and 415 controls from a population-based study in Poland. We assessed differences in haplotype frequency between cases and controls using a global score test and calculated multivariate odds ratios (ORs) and 95% confidence intervals (CIs) for individual haplotypes using logistic regression. We found associations in one linkage disequilibrium (LD) block containing the 3′ untranslated region of *COMT* (rs9332377, rs165728, rs165849 and rs1110478), global score test (df = 4, *P* = 0.033). Relative to the most frequent GATA haplotype, the GATG haplotype was associated with statistically significant increased gastric cancer risk (OR = 1.50, 95% CI: 1.06–2.12; false discovery rate (FDR) value = 0.459) and the AACA haplotype with borderline increased risk (OR = 1.36, 95% CI = 1.00–1.85; FDR = 0.50). We also found associations for the LD block containing part of the *SHBG* coding region (rs6258, rs6259, rs2955617, rs1641544 and rs1641537). The CACCC haplotype was associated with statistically significant lower gastric cancer risk relative to the referent CGACC haplotype (OR = 0.55, 95% CI = 0.34–0.90; FDR = 0.459), but the overall score test was statistically non-significant. No other statistically significant associations were observed. In summary, we found possible associations between gastric cancer and polymorphisms in *COMT*, involved in estrogen inactivation, and *SHBG*, a modulator of hormone bioavailability. These findings should be interpreted cautiously until replicated in other studies.

Introduction

Gastric cancer is the second leading cause of cancer death worldwide (1). Incidence rates vary widely by geographic area. Men are two times more likely to develop gastric cancer than women in both high- and low-incidence areas (2). The ratio of male to female cases is greatest during the reproductive years and become more similar after the age of 60, corresponding with menopause (2). As normal and cancerous gastric tissue expresses steroid hormone receptors that bind to estrogens and androgens (3,4), one hypothesis suggests that sex hormones may play an etiological role in gastric cancer (2). The overall results from nearly 10 epidemiologic studies that investigated associations between menstrual and reproductive factors and gastric cancer risk (5–12) suggest an association between age of menopause, years of fertility or hormone replacement therapy and gastric cancer risk, although the associations did not reach statistical significance and case numbers were small in most studies. Two types of clinical data also support the hypothesis. Prostrate cancer patients treated with estrogen had reduced risk of developing gastric cancer (13) and women treated with the estrogen antagonist tamoxifen had a statistically non-significant increased risk of gastric cancer (14,15). Finally, male rats are more likely to develop gastric cancer than female rats; but tumor numbers become similar after male rats are treated with estrogen. Castrating male rats also lowers gastric cancer risk (16). Similar results have been found in a mouse model of gastric cancer (17).

A number of genes encode enzymes that synthesize and metabolize androgens and estrogens. *CYP17A1* converts pregnenolone to dehydroepiandrosterone and progesterone to 17 alpha-hydroxyprogesterone (18). *CYP19A1* converts the androstenedione to estrone and testosterone to estradiol (19). *HSD17B1* converts androstenedione to testosterone and estrone to estradiol (19). *SHBG* encodes a plasma glycoprotein that transports androgens and estrogens in the circulation and that regulates the availability of estrogens and androgens (20). Among other functions, *CYP1B1* and *COMT* convert estrogens to compounds that lack estrogenic activity. *CYP1B1* catalyzes the C4 hydroxylation of estradiol to 4-hydroxy catechol estrogens, which can form adducts with DNA. *COMT* further metabolizes 4-hydroxy catechol estrogens into stable methylated catechol estrogens that do not form DNA adducts (21,22).

As androgens and estrogens may be related to gastric cancer risk, we hypothesized that genetic polymorphisms in these genes might be associated with gastric cancer.

Abbreviations: BPC3, Breast and Prostate Cohort Consortium; CI, confidence interval; FDR, false discovery rate; HW, Hardy–Weinberg; LD, linkage disequilibrium; NCI, National Cancer Institute; OR, odds ratio; SNP, single-nucleotide polymorphism; UTR, untranslated region.

Materials and methods

Study design

The methods of this population-based case–control study of gastric cancer have been described in detail previously (23). Cases are Caucasian residents of Warsaw, Poland, aged 21–79 years, who were newly diagnosed with gastric cancer (ICD-0 151) between 1994 and 1996 and identified by physicians in each of the 22 hospitals serving the study area. All the cases were confirmed to be gastric adenocarcinoma by study pathologists. Controls were randomly selected from a computerized registry of Warsaw residents and frequency matched to cases by sex and 5 year age groups. Informed consent was obtained from all participants and Institutional Review Boards of the USA National Cancer Institute (NCI) and the M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland, approved the study.

Trained interviewers collected demographic, lifestyle and medical information, including tobacco use, alcohol consumption, family history of gastric cancer, childhood living conditions, history of selected medical conditions, medication use and occupational history, and typical diet before 1990. Among 515 eligible cases and 549 eligible controls, in-person interviews were conducted with 324 cases (62.9%) and 480 controls (87.4%). Proxy interviews were conducted with the next of kin for 140 cases (27.2%) who were too ill to be interviewed or deceased. Of 464 gastric cancer cases and 480 controls with successful interviews, genomic DNA was obtained from peripheral blood lymphocytes of 305 cases (65.7%) and 427 controls (90.0%) as described previously (24).

Selection of haplotype tagging single-nucleotide polymorphisms

Data from the Breast and Prostate Cohort Consortium (BPC3) (25) were used to identify haplotype tagging single-nucleotide polymorphisms (SNPs) for common haplotypes in *COMT*, *CYP1B1*, *CYP17A1*, *CYP19A1*, *HSD17B1* and *SHBG*. A two-stage approach was used by the Consortium to comprehensively measure genetic variation in these genes. First, a genomic region beginning 20 kb upstream of the transcription start site and ending 10 kb downstream

Table I. Polymorphisms examined in this study

Gene	Chromosomal region	LD block[a]	RS #	Nearest gene to SNP, nucleotide change[b]	Genotyping platform	MAF in controls
COMT	22q11.21	Block 1	rs4485648	TXNRD2, IVS3+475A>G	TaqMan	0.168
			rs7290221	*COMT*, IVS1−6042C>G	SNPlex	0.435
			rs4646312	*COMT*, IVS1−385T>C	SNPlex	0.341
			rs4633	*COMT*, Ex3−104C>T	TaqMan	0.453
		Block 2	rs9332377	ARVCF, 3058 bp 3′ of STP G>A	SNPlex	0.159
			rs165728	ARVCF, 1727 bp 3′ of STP G>A	TaqMan	0.092
			rs165849	ARVCF, IVS19+70C>T	SNPlex	0.312
			rs1110478	ARVCF, IVS11+440G>A	SNPlex	0.195
CYP1B1	2p21	Block 1	rs10175368	CYP1B1, −5329G>A	SNPlex	0.321
			rs162556	CYP1B1, −3922C>T	SNPlex	0.432
			rs162557	CYP1B1, −2919C>T	SNPlex	0.275
		Block 2	rs1056836	CYP1B1, Ex3+251G>C	TaqMan	0.449
			rs1800440	CYP1B1, Ex3+315A>G	TaqMan	0.14
			rs162562	CYP1B1, Ex3+939C>A	TaqMan	0.25
			rs9341266	CYP1B1, Ex3−1249C>T	TaqMan	0.029
			rs163086	FAM82A, IVS10−1363C>T	SNPlex	0.199
			rs163077	FAM82A, IVS10−8520T>C	SNPlex	0.262
CYP17A1	10q24.3	Block 1	rs7097872	CYP17A1, −9517G>A	SNPlex	0.444
			rs2486758	CYP17A1, −361G>A	SNPlex	0.2
			rs743572	CYP17A1, Ex1+27T>C	SNPlex	0.416
			rs4919687	CYP17A1, IVS1−99C>T	TaqMan	0.298
		Block 2	rs4919682	CYP17A1, 6128 bp 3′ of STP G>A	TaqMan	0.302
			rs10883782	CYP17A1, 6526 bp 3′ of STP C>T	TaqMan	0.115
			rs619824	CYP17A1, 9170 bp 3′ of STP G>T	SNPlex	0.465
			rs6892	CYP17A1, 14588 bp 3′ of STP C>T	SNPlex	0.179
CYP19A1	15q21.1	Block 1	rs2446405	CYP19A1, −111683A>T	SNPlex	0.149
			rs2470144	CYP19A1, IVS1−5604A>G	SNPlex	0.386
			rs2445762	CYP19A1, IVS1−1587A>G	SNPlex	0.228
			rs1004984	CYP19A1, IVS2+2484C>T	SNPlex	0.278
			rs1902584	CYP19A12, IVS2+4359T>A	SNPlex	0.039
		Block 2	rs3751591	CYP19A1, IVS2+9303T>C	SNPlex	0.145
			rs2445759	CYP19A1, IVS2+15172C>A	TaqMan	0.059
			rs936306	CYP19A1, IVS2+36415G>A	SNPlex	0.139
			rs1902586	CYP19A1, IVS2−35706C>T	SNPlex	0.058
		Block 3	rs749292	CYP19A1, IVS2−23584C>T	SNPlex	0.494
			rs1008805	CYP19A1, IVS2−14452C>T	SNPlex	0.376
		Block 4	rs727479	CYP19A1, IVS3+418G>T	TaqMan	0.325
			rs28757184	CYP19A1, Ex6−27C>T	TaqMan	0.017
			rs700519	CYP19A1, Ex8+47C>T	TaqMan	0.048
			rs17601241	CYP19A1, IVS8+26C>T	SNPlex	0.071
			rs10046	CYP19A1, Ex11+268C>T	SNPlex	0.423
			rs4646	CYP19A1, Ex11+410T>G	SNPlex	0.221
HSD17B1	17q11–q21	Block 1	rs676387	HSD17B1, IVS4−150C>A	SNPlex	0.205
			rs598126	COASY, Ex3+57A>G	SNPlex	0.475
			rs2010750	MLX, IVS7−174C>T	TaqMan	0.437
SHBG	17p13–p12	Block 1	rs12150660	FXR2, −4064C>A	TaqMan	0.248
			rs13894	SAT2, Ex6+31C>T	SNPlex	0.085
			rs858521	SAT2, IVS4−36C>G	SNPlex	0.373
		Block 2	rs6258	SHBG, Ex4−2C>T	TaqMan	0.01
			rs6259	SHBG, Ex8+6G>A	TaqMan	0.074
			rs2955617	SHBG, 2114 bp 3′ of STP C>A	TaqMan	0.324
			rs1641544	SHBG, 3195 bp 3′ of STP T>C	SNPlex	0.052
			rs1641537	ATP1B2, −9115T>C	TaqMan	0.127

MAF, minor allele frequency; RS, refSNP.
[a]SNPs are listed 5′–3′ across each gene. LD blocks were defined using data from the BPC3 (25).
[b]SNPs in the regulatory regions 20 kb upstream and 10 kb downstream of each gene of interest may include neighboring gene sequences. Further information on the nearest gene and nucleotide change of each SNP can be found at http://snp500cancer.nci.nih.gov.

of the stop codon was chosen. In addition to SNPs in the dbSNP database, SNPs in these regions were identified by sequencing a multiethnic panel of 190 breast and prostate cancer cases. Allele frequencies in Caucasians were calculated, and haplotypes were imputed by the expectation–maximization algorithm (26). We genotyped previously published haplotype-tagging SNPs identified by the BPC3 to predict common haplotypes in Caucasians for *CYP17A1* [two linkage disequilibrium (LD) blocks and nine SNPs, $R_h^2 \geq 0.85$] (27), *CYP19A1* (four LD blocks and 20 SNPs, $R_h^2 \geq 0.75$) (28) and *HSD17B1* (one LD block and four SNPs, $R_h^2 \geq 0.8$) (29,30). Haplotype-tagging SNPs for *CYP1B1* (two haplotype blocks and nine SNPs, $R_h^2 \geq 0.7$) and *COMT* (four LD blocks and 10 SNPs, $R_h^2 \geq 0.9$) have been selected by the BPC3, but have not yet been published. Details can be found on the website http://cgf1.nci.nih.gov/cohort.cfm. Two of three SNPs in LD block #1 of *COMT* failed validation and were not included. We did not analyze haplotypes in this block separately; to improve statistical efficiency without losing predictive power, we combined this block with LD blocks 2 and 3 into a single block. A previous report also analyzed LD blocks 1–3 as a single entity (31).

As BPC3 had not yet chosen tagging SNPs for *SHBG* at the time of genotyping, nine SNPs were chosen by the NCI Core Genotyping Facility from BPC3 LD data using a pair-wise R^2 of ≥0.8 and >5% minor allele frequency in Caucasians with Tagzilla (http://tagzilla.nci.nih.gov/). The BPC3 identified three LD blocks in *SHBG*. To improve statistical efficiency,

we combined *SHBG* LD blocks #1 and #2 into a single block for analysis. One assay in *SHBG* failed validation (rs2908809). Of successfully genotyped SNPs in *SHBG*, rs12150660 had the highest pair-wise LD with rs2908809 ($R^2 = 0.43$), coverage of the remaining SNPs in *SHBG* did not change.

Genotyping

In total, 61 SNPs were genotyped in these six genes. All assays were performed at the NCI's Core Genotyping Facility; description, methods and validation data for the assays can be found at http://snp500cancer.nci.nih.gov. Each assay was sequence validated in the 102 samples of the NCI SNP500Cancer project (32) and additionally validated against 210 HapMap control subjects. Complete concordance was observed for all assays, except for rs174674 and rs5748489 in LD block #1 of *COMT* and rs2908809 in LD block #1 of *SHBG*. These assays, therefore, were not included in this study.

Of the 58 validated assays, 38 polymorphisms were genotyped by SNPlex assay (Applied Biosystems, Foster City, CA) and the remaining 20 SNPs were genotyped by TaqMan (Applied Biosystems). At the laboratory, case–control status was masked and we included quality control samples from the NCI SNP500 project and blinded duplicate samples from our study. Concordance for the blinded quality control samples (~10% of samples) was >97%. Genotypes were successfully obtained from >94% of participants for all SNPs with a median completion rate of 98%.

Table I presents details on genotyped SNPs, their chromosomal region and their refSNP number. Although we also genotyped rs605059 (*HSD17B1*), rs284849 (*CYP17A1*), rs2445765 (*CYP19A1*), rs28566535 (*CYP19A1*) and rs6493494 (*CYP19A1*), these SNPs were in strong LD ($R^2 > 0.9$) with rs598126, rs2486758, rs2446405, rs1902586 and rs749292, respectively, and were not further analyzed. We had sufficient DNA to genotype polymorphisms by TaqMan assays in 710 participants (415 controls and 295 cases), but SNPlex assays required more DNA and therefore these assays were restricted to 676 participants (391 controls and 285 cases). Associations between other covariates and gastric cancer did not change when analyses were restricted to those with a DNA sample (data not shown).

Statistical analysis

We tested for Hardy–Weinberg (HW) equilibrium in controls using an exact test (33). All SNPs passed except for rs1110478 (P-value < 0.001) in *COMT* that was also out of HW equilibrium in Caucasians of the NCI SNP500 reference population (32).

We examined pair-wise LD in controls using Haploview (34). We used Haplo.Stats (version 1.3.1) (35) to compute common haplotype frequencies (those with 5% or greater frequency), odds ratios (ORs) and 95% confidence intervals (CIs) for haplotypes, assuming an additive model. Uncommon haplotypes (<5% prevalence) were included in the models as a single 'rare haplotypes category'. A global score test, adjusted for age, gender, education and smoking (never, former, current and pack-years among smokers) was used to evaluate the overall difference in haplotypes between cases and controls (36). Alternative LD blocks defined within controls of our study (using a solid spline definition with $D' < 0.80$) gave similar results to those presented in this manuscript using BPC3-defined LD blocks.

For individual SNPs, we used unconditional logistic regression in SAS version 9.1 to estimate ORs and 95% CIs for the gastric cancer risk associated with a single copy or two copies of the minor allele relative to carrying no copies. We tested the null hypothesis of no association using a likelihood ratio test that compared models with and without the SNP in question.

All ORs were adjusted for age, gender, education (less than high school, high school or technical training and some college/college graduate) and smoking (never, former, current and pack-years among smokers). Further adjustment for other potential confounding variables including alcohol, caloric intake, intake of fruits, vegetables, sausages, red meats or preserved vegetables, family history of gastric cancer or other cancers, gastroesophageal reflux disease or *Helicobacter pylori* serology did not alter risk estimates. To examine the possible effect modification by sex, we inspected data for men and women separately and formally tested for differences using a likelihood ratio test. All tests were two sided and an alpha level of <0.05 was considered statistically significant.

We evaluated the robustness of our findings by calculating the false discovery rate (FDR) (37) for both P-values from LD blocks and the P-value for trend of individual SNPs. Among all SNPs analyzed, FDR is the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypotheses.

Results

By design, controls matched cases in their age and sex distribution. Cases smoked more and had less education than controls (Table II).

Table II. Distribution of selected covariates in cases and controls from Warsaw, Poland

	Gastric cancer cases ($n = 295$)		Controls ($n = 415$)		P-value[a]
	No.	%	No.	%	
Age (years)[b]					0.97
<50	37	12.5	51	12.3	
50–59	54	18.3	72	17.4	
60–69	117	39.7	163	39.3	
≥70	87	29.5	129	31.1	
Gender[b]					0.69
Male	196	62.4	269	64.8	
Female	99	33.6	146	35.2	
Education					0.002
Less than high school	140	47.5	157	37.8	
High school or technical training	94	31.9	123	29.6	
Some college/college graduate	61	20.7	135	32.5	
Smoking status[c]					<0.001
Never	85	29.0	168	40.5	
Former	85	29.0	132	31.8	
Current	123	42.0	115	27.7	
Pack-years among ever smokers					0.014
≤16.5	47	22.6	62	25.1	
>16.5–28	38	18.3	64	25.9	
>28–40	48	23.1	65	26.3	
>40	75	36.1	56	22.7	

[a]Fisher's exact test for distribution of differences between gastric cancer cases and control participants.
[b]The study design frequency-matched controls by age and gender to the cases.
[c]Totals do not add up to 295 cases or 415 controls due to missing data.

We found no association with LD blocks in *CYP1B1*, *CYP17A1*, *CYP19A1* or *HSD17B1* (Table III). In contrast, we found associations in one of two LD blocks in *COMT* and one of two LD blocks in *SHBG*. In *COMT*, a LD block (block #2) containing the 3′ untranslated region (UTR) (rs9332377, rs165728, rs165849 and rs1110478) had a statistically significant global score test ($P = 0.033$). This block had four common haplotypes with >5% prevalence. Relative to the most common haplotype (GATA), the GATG haplotype was associated with statistically significant increased risk of gastric cancer (OR: 1.50, 95% CI: 1.06–2.12), whereas the AACA haplotype had borderline increased risk (OR: 1.36, 95% CI: 1.00–1.85). For *SHBG*, LD block #2 (rs6258, rs6259, rs2955617, rs1641544 and rs1641537), which contains part of the *SHBG* coding region, had a statistically non-significant global score test ($P = 0.14$), but the CACCC haplotype had statistically significant lower risk than the referent CGACC haplotype (OR: 0.55, 95% CI: 0.34–0.90).

We next examined the individual SNPs within the two LD blocks that showed evidence for an association with gastric cancer risk (block #2 in *COMT* and block #2 in *SHBG*) (Table IV). For *COMT*, we found evidence for an association between gastric cancer risk and rs1110478 (OR per variant allele of 1.31, 95% CI: 1.02–1.67) and statistically non-significant increased risk with rs9332377 (OR per variant allele of 1.24, 95% CI: 0.93–1.67) and rs165849 (OR per variant allele of 1.23, 95% CI: 0.97–1.56). For *SHBG*, we found an association between rs6259 and gastric cancer risk (OR for GA/AA: 0.59, 95% CI: 0.36–0.96) and statistically non-significant reduced risk with rs2955617 (OR per variant allele of 0.81, 95% CI: 0.64–1.03). Individual data for all SNPs genotyped are presented in supplementary Table 1 (available at *Carcinogenesis* Online). We observed a statistically significant association between rs162557 (−2919C>T of *CYP1B1*) and gastric cancer risk (OR per variant allele of 0.74, 95% CI: 0.56–0.96). But, we did not observe evidence for an association of the LD block containing this SNP and gastric cancer risk ($P = 0.27$).

We evaluated the robustness of our findings using the FDR method. The FDR value for LD block #2 in *COMT* was 0.429. For haplotypes, the FDR value was 0.459 for the GATG haplotype of *COMT* and the

Table III. Haplotype analysis of *COMT*, CYP1B1, CYP17A1, CYP19A1, HSD17B1 and SHBG and gastric cancer risk

Gene	LD block #	Haplotype[a]	% of controls	% of cases	Global P[b]	OR-1[c]	95% CI	OR-2[d]	95% CI
COMT	Block #1	ACTT	35.8	29.3	0.22	1.00	Reference	1.00	Reference
		AGCC	24.0	23.1		1.16	0.87–1.56	1.11	0.82–1.51
		GGTT	9.6	9.7		1.17	0.76–1.79	1.16	0.75–1.79
		ACTC	9.0	9.4		1.25	0.80–1.96	1.25	0.79–1.98
		ACCC	7.8	9.9		1.48	0.96–2.30	1.45	0.92–2.29
	Block #2	GATA	63.2	55.7	0.033	1.00	Reference	1.00	Reference
		AACA	14.9	17.5		1.33	0.99–1.80	1.36	1.00–1.85
		GGCG	9.6	8.2		0.94	0.63–1.42	0.97	0.64–1.47
		GATG	5.2	8.9		1.43	1.02–2.01	1.50	1.06–2.12
CYP1B1	Block #1	TAG	32.1	32.5	0.27	1.00	Reference	1.00	Reference
		CGG	23.0	26.1		1.12	0.83–1.51	1.07	0.78–1.46
		CGA	20.3	17.3		0.75	0.44–1.28	0.80	0.57–1.12
		CAG	17.4	18.5		1.06	0.76–1.47	1.08	0.77–1.51
		CAA	7.3	5.6		0.75	0.44–1.28	0.71	0.41–1.23
	Block #2	GTTGGG	30.2	30.8	0.52	1.00	Reference	1.00	Reference
		CTTGAG	20.1	18.6		0.91	0.66–1.24	0.90	0.65–1.24
		CTGGGA	18.1	17.6		0.95	0.69–1.31	0.88	0.63–1.23
		GCTGGG	14.0	14.9		1.05	0.74–1.49	0.97	0.68–1.39
		GTTGGA	7.4	9.8		1.33	0.86–2.07	1.32	0.84–2.08
		CTGGGG	6.2	4.5		0.71	0.40–1.26	0.69	0.39–1.24
CYP17A1	Block #1	GATC	35.0	33.9	0.63	1.00	Reference	1.00	Reference
		AACT	30.4	32.5		1.11	0.86–1.45	1.04	0.80–1.36
		GGTC	19.4	20.6		1.12	0.82–1.53	1.05	0.76–1.45
		AACC	12.1	11.2		0.98	0.68–1.42	0.93	0.63–1.37
	Block #2	ATTT	30.0	30.0	0.92	1.00	Reference	1.00	Reference
		GTGT	23.4	21.1		0.90	0.67–1.22	0.97	0.72–1.32
		GTTT	17.2	16.8		0.98	0.70–1.37	1.00	0.71–1.41
		GTGC	17.0	18.4		1.09	0.79–1.50	1.11	0.80–1.54
		GCGT	10.9	11.9		1.10	0.77–1.59	1.13	0.78–1.65
CYP19A1	Block #1	TAACT	61.3	61.7	0.48	1.00	Reference	1.00	Reference
		TGGTT	16.2	14.7		0.89	0.66–1.22	0.87	0.64–1.20
		AGGCT	6.9	5.5		0.80	0.50–1.28	0.78	0.48–1.27
	Block #2	TCGC	78.7	76.9	0.85	1.00	Reference	1.00	Reference
		CCGC	6.9	7.5		1.16	0.75–1.81	1.13	0.72–1.77
	Block #3	TT	49.3	46.7	0.88	1.00	Reference	1.00	Reference
		CC	37.8	38.6		1.08	0.85–1.37	1.07	0.84–1.37
		CT	12.8	14.4		1.19	0.85–1.68	1.10	0.77–1.56
	Block #4	TCCCTG	53.9	51.5	0.41	1.00	Reference	1.00	Reference
		GCCCCG	15.1	15.2		1.04	0.77–1.42	1.09	0.79–1.49
		GCCCCT	14.3	15.8		1.15	0.84–1.59	1.14	0.83–1.58
		TCCTCT	6.6	4.7		0.73	0.43–1.22	0.70	0.41–1.20
HSD17B1	Block #1	CAT	42.8	41.2	0.37	1.00	Reference	1.00	Reference
		CGC	31.7	30.4		1.00	0.78–1.30	0.93	0.71–1.21
		AGC	20.3	22.1		1.12	0.83–1.51	1.06	0.78–1.44
		CAC	4.6	6.2		1.40	0.86–2.28	1.30	0.79–2.14
SHBG	Block #1	CCG	37.5	39.7	0.46	1.00	Reference	1.00	Reference
		CCC	29.0	27.2		0.88	0.67–1.16	0.82	0.62–1.09
		ACC	24.9	25.4		0.98	0.74–1.29	0.96	0.72–1.27
		CTC	8.6	7.4		0.79	0.51–1.22	0.76	0.49–1.18
	Block #2	CGACC	67.4	70.3	0.14	1.00	Reference	1.00	Reference
		CGCCC	11.0	9.2		0.79	0.55–1.15	0.78	0.53–1.14
		CGCCT	7.8	9.2		1.15	0.78–1.70	1.01	0.68–1.51
		CACCC	7.5	5.1		0.63	0.39–1.01	0.55	0.34–0.90

[a]Haplotypes include SNPs listed in Table I and are presented 5′–3′. Haplotypes with <5% frequency were grouped together and included in the models as one category, but are not presented here.
[b]A global score test was used to evaluate the overall difference in haplotype frequency in cases and controls.
[c]Model-1: estimates are adjusted for the matching variables (age and gender).
[d]Model-2: estimates are adjusted for age, gender, education and smoking (never, former, current and pack-years among smokers).

CACCC haplotype of *SHBG*; the AACA haplotype of *COMT* had a FDR of 0.50. Whereas for individual SNPs, the FDR value was 0.583 for rs1110478 (*COMT*), rs6259 (*SHBG*) and rs162557 (*CYP1B1*).

Risk estimates did not differ by sex (P for interaction between haplotypes or individual genetic variants and sex was all >0.279), but we had low power for these tests. For example, the OR per variant allele of rs1110478 and gastric cancer risk was 1.12 (0.74–1.69) in women and 1.43 (1.04–1.95) in men; the OR for rs6259 (GA/AA versus GG) was 0.51 (0.17–1.50) in women and 0.62 (0.36–1.08) in men.

Discussion

We systematically investigated genetic variation in six genes (*COMT*, *CYP1B1*, *CYP17A1*, *CYP19A1*, *HSD17B1* and *SHBG*) related to the synthesis and metabolism of estrogen and androgen using

Table IV. Association of selected SNPs and gastric cancer risk

Gene	LD block #	RS #	Genotype	No. of controls (%)	No. of cases (%)	Global P^a	OR[b]	95% CI
COMT	Block #2[c]	rs9332377	GG	272 (71)	185 (67)	0.18	1.00	Reference
			GA	102 (27)	76 (28)		1.10	0.76–1.58
			AA	10 (3)	14 (5)		2.23	0.94–5.28
			Per allele risk				1.24	0.93–1.67
		rs165728	AA	328 (81)	240 (83)	0.44	1.00	Reference
			AG	77 (19)	47 (16)	AG/GG	0.85	0.57–1.28
			GG	2 (0.5)	2 (1)			
		rs165849	TT	183 (48)	114 (41)	0.23	1.00	Reference
			TC	162 (42)	126 (46)		1.27	0.90–1.78
			CC	39 (10)	36 (13)		1.47	0.87–2.50
			Per allele risk				1.23	0.97–1.56
		rs1110478	AA	255 (68)	165 (61)	0.08	1.00	Reference
			AG	94 (25)	74 (28)		1.18	0.81–1.71
			GG	26 (7)	30 (11)		1.91	1.08–3.41
			Per allele risk				1.31	1.02–1.67
SHBG	Block #2[d]	rs6258	CC	405 (98)	288 (98)	0.80	1.00	Reference
			CT	8 (2)	5 (2)		0.87	0.27–2.74
		rs6259	GG	348 (85)	261 (90)	0.029	1.00	Reference
			GA	59 (15)	29 (10)	GA/AA	0.59	0.36–0.96
			AA	1 (0)	1 (0)			
		rs2955617	AA	187 (45)	147 (50)	0.17	1.00	Reference
			AC	188 (45)	123 (42)		0.75	0.54–1.03
			CC	40 (10)	25 (8)		0.74	0.42–1.30
			Per allele risk				0.81	0.64–1.03
		rs1641544	CC	345 (90)	249 (90)	0.86	1.00	Reference
			CT	39 (10)	27 (10)	CT/TT	0.96	0.56–1.63
			TT	1 (0)	0 (0)			
		rs1641537	CC	314 (76)	215 (74)	0.88	1.00	Reference
			CT	92 (22)	70 (24)		1.04	0.72–1.50
			TT	6 (2)	6 (2)		1.34	0.41–4.40
			Per allele risk				1.07	0.78–1.48

RS, refSNP.
[a]Calculated from a likelihood ratio test comparing models with indicator variables for one or two copies of the minor allele to models without.
[b]Estimates are based on an additive effects model and adjusted for age, gender, education and smoking (never, former, current and pack-years among smokers).
[c]Pair-wise R^2: rs9332377/rs165728 (0.01); rs9332377/rs165849 (0.41); rs9332377/rs1110478 (0.01); rs165728/rs165849 (0.22); rs165728/rs1110478 (0.42); rs165849/rs1110478 (0.15).
[d]Pair-wise R^2: rs6258/rs6259 (0); rs6258/rs2955617 (0.02); rs6258/rs1641544 (0); rs6258/rs1641537 (0); rs6259/rs2955617 (0.16); rs6259/rs1641544 (0); rs6259/rs1641537 (0.01); rs2955617/rs1641544 (0.10); rs2955617/rs1641537 (0.28); rs1641544/rs1641537 (0.31).

haplotype-tagging SNPs (25). We examined associations with gastric cancer risk using haplotype blocks and individual SNPs were merited. We found evidence for an association of gastric cancer risk with genetic polymorphisms in *COMT* and *SHBG*, but not with LD blocks in the other genes we examined.

CYP1B1 and *COMT* sequentially metabolize estrogens into methylated catechol estradiols that lack estrogenic activity. We found a statistically significant global *P*-test for a haplotype block in *COMT* (block #2 in our analysis) and gastric cancer risk. These findings were consistent with the results of individual SNPs in this block, with one SNP (rs1110478) having a statistically significant positive association and two other SNPs (rs9332377 and rs165849) showing some evidence for an association. rs1110478 was out of HW equilibrium in both controls and cases of this study and also in the SNP500 Caucasian reference panel, with more individuals homozygous for the minor allele than expected (32). This deviation from HW equilibrium could indicate a genotyping error and may increase the possibility that this association is due to chance (38). But, the genotyping assay for rs1110478 was validated against sequencing in DNA samples from >300 individuals and found to be 100% concordant, genotyping completion rates were >95% and there were no discordant pairs for the blinded quality control samples (~10% of total samples), suggesting that these results are unlikely to be due to a genotyping error. By chance, we would expect that 5% of genotyped SNPs (two of 58 in this study) would be out of HW equilibrium. Two other SNPs (rs9332377 and rs165849) in this block showed increased but not statistically significant association with gastric cancer risk, providing some evidence that the association observed with rs1110478 is not due to a HW error.

No previous studies have examined the association of SNPs in LD block #2 of *COMT* and gastric cancer risk. Specific haplotypes in block #2 were previously shown to be associated with increased risk of breast cancer (31,39) as well as other chronic diseases (40). This LD block contains the 3′ UTR of *COMT*. Though no *in vitro* data are available for these SNPs, 3′ UTRs are important for the stability of many messenger RNA transcripts and thus could affect *COMT* expression. This genomic region also contains the 3′ UTR of the neighboring armadillo repeat deleted in the velocardiofacial syndrome (*ARVCF*) gene that helps maintain adherens junctions (41), another process that may be relevant to gastric cancer (42).

We did not find associations between gastric cancer risk and LD block #1 of *COMT*, which overlaps the gene's coding region. SNPs within this region have been associated with increased risk of breast cancer in some studies, though the summary estimate from a recent meta-analysis does not support an association (43). Recent data from a case–control study in Japan also did not observe an association between SNPs in this block and gastric cancer risk (44).

SHBG is a plasma glycoprotein that transports androgens and estrogens while in circulation and regulates the availability of free estrogens and androgens (20). Although the global *P* test was not statistically significant, one haplotype in LD block #2 in *SHBG* (CACCC) was associated with reduced risk of gastric cancer. This result was consistent with risk estimates for individual SNPs in this block, with those carrying the minor allele of rs6259 showing

a statistically significant association. Though no previous studies have examined this SNP for gastric cancer, carrying the minor allele of rs6259 has been linked to decreased endometrial (45) and breast cancer risk (46) and increased risk of prostate cancer (47) in some studies, but not in others (48–50). One study examined the association with ovarian cancer and found no association (51).

rs6259 is a putatively functional SNP (D356N) that inserts an additional N-glycosylation site (52) and has been associated with modestly increased circulating levels of *SHBG* in those homozygous for the minor allele (50,53,54). But, the association observed in this study was among heterozygotes, where circulating SHBG levels appear similar to those with the major allele (50,53,54). Only one case and one control in this study were homozygous for the minor allele. rs6259 does not affect hormone binding (55), but may affect other aspects including the transport of hormones into the cell (56). *SHBG* may also regulate steroid signaling via alternative pathways, including regulating cyclic adenosine monophosphate levels by binding to its plasma membrane bound receptor (SHBG-R) (57,58). Alternatively, rs6259 could be in high LD with another functional polymorphism in SHBG or a neighboring gene.

We found no association between LD blocks in *CYP1B1*, *CYP17A1*, *CYP19A1* and *HSD17B1* and gastric cancer risk. Previous studies with hormonally associated cancers, such as breast or prostate, have not demonstrated consistent associations (27–30,49,50). One recent Japanese study observed statistically significant associations between several SNPs in *CYP17A1* (rs619824 and rs743572) and *CYP19A1* (rs4646 and rs1902586) and gastric cancer risk (44) that were also genotyped in our study. Our results did not replicate these findings.

Strengths of this study include its population-based design and comprehensive assessment of selected LD blocks, as well as use of haplotypes to limit the number of comparisons. Limitations include the exclusion of a larger percentage of cases (36%) than controls (14%) due to lack of DNA availability. Cases with a blood sample for DNA extraction had a lower tumor stage and grade than cases without a blood sample. If the association between genotypes or other exposures differed between more and less advanced cases, then the risk estimates observed in our study may not reflect the entire distribution of gastric cancer cases. But, the distribution of genotypes did not vary by tumor stage or grade in our study. In addition, demographic and lifestyle factors did not differ between cases and controls with and without a DNA sample, arguing against selection bias as a major determinant of our results.

An additional limitation is that our results are from a single modestly sized study. As we examined genetic variation in 13 LD blocks, statistically significant findings could be due to multiple comparisons and chance. Indeed, the most statistically significant LD block had a FDR of 0.429, the most statistically significant haplotype had a FDR of 0.459 and the most statistically significant individual SNPs had a FDR of 0.583. Therefore, our findings should be interpreted with caution and confirmed in additional studies. We also had limited power to examine associations in men and women separately.

In conclusion, in the first study to comprehensively examine the association of genetic variation in six genes of the steroid hormone metabolism pathway and gastric cancer risk, we found possible associations with genetic variation in *COMT*, involved in estrogen inactivation, and *SHBG*, a modulator of sex hormone bioavailability. We found no association with haplotypes in *CYP1B1*, *CYP17A1*, *CYP19A1* and *HSD17B1*.

Supplementary material

Supplementary Table 1 can be found at http://carcin.oxfordjournals.org/

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