

## Vitamin C transporter gene (*SLC23A1* and *SLC23A2*) polymorphisms, plasma vitamin C levels, and gastric cancer risk in the EPIC cohort

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**Abstract** Vitamin C is known to protect mucosal tissues from oxidative stress and inhibit nitrosamine formation in the stomach. High consumption of fruits, particularly citrus, and higher circulating vitamin C concentrations may

be inversely associated with gastric cancer (GC) risk. We investigated 20 polymorphisms in vitamin C transporter genes *SCL23A1* and *SCL23A2* and GC risk in 365 cases and 1,284 controls nested within the European Prospective Investigation into Cancer and Nutrition cohort. We also evaluated the association between these polymorphisms and baseline plasma vitamin C levels in a subset of participants. Four SNPs were predictors of plasma vitamin C

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levels (*SLC23A1* rs11950646 and rs33972313; *SLC23A2* rs6053005 and rs6133175) in multivariable linear regression models. One SNP (*SLC23A2* rs6116569) was associated with GC risk, in particular non-cardia GC (OR = 1.63, 95 % CI = 1.11–2.39, based on 178 non-cardia cases), but this association was attenuated when plasma vitamin C was included in the logistic regression model. Haplotype analysis of *SLC23A1* yielded no associations with GC. In *SLC23A2*, one haplotype was associated with both overall and non-cardia GC, another haplotype was associated with GC overall, and a third was associated with intestinal-type GC. Common variants in *SLC23A1* and *SLC23A2* may influence plasma vitamin C concentration independent of dietary intake, and variation in *SLC23A2* may influence GC risk. Additional prospective studies in large populations and consortia are recommended. Investigation of variation in vitamin C transporter genes may shed light on the preventative properties of vitamin C in gastric carcinogenesis.

**Keywords** Gastric cancer · Vitamin C · Antioxidants · Genetic susceptibility · *SLC23A1* · *SLC23A2*

## Introduction

Although incidence has been declining, gastric cancer (GC) is still the second leading cause of cancer death worldwide

(Jemal et al. 2010). High consumption of fruits, particularly citrus fruits, has been inversely associated with GC risk (Gonzalez et al. 2006, 2012; Tsugane and Sasazuki 2007). Studies that have specifically investigated dietary intake or plasma levels of L-ascorbic acid (vitamin C) and GC risk also have reported inverse associations, supporting the hypothesis that antioxidants (such as vitamin C) from fruit may be protective against the development of GC (Jenab et al. 2006a, b). Indeed, vitamin C, an enzymatic cofactor and scavenger of reactive oxygen species, has been shown to inhibit nitrosamine formation in the stomach (Tannenbaum et al. 1991) and protect mucosal tissues from the damaging effects of oxidative stress (Drake et al. 1996; Sasazuki et al. 2008).

Infection of the gastric epithelium with *Helicobacter pylori* (*H. pylori cagA+* strains) is the strongest known risk factor for non-cardia GC, but *H. pylori* infection itself is not a sufficient cause for GC development; thus, additional lifestyle, environmental, and host genetic factors also play a role (Polk and Peek 2010; Peek and Blaser 2002). *H. pylori cagA+* infection in the gastric mucosa has been associated with pro-inflammatory responses and mucosal proliferation, as well as lower vitamin C levels in blood and gastric juice, possibly due to malabsorption or decreased stability and bioavailability (Lahner et al. 2012). In addition, very high doses of vitamin C may inhibit *H. pylori* infection, growth, and reinfection (Pal et al. 2011; Zhang et al. 1997). As a consequence, the multistep process

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of gastric cancer carcinogenesis involving chronic *H. pylori* infection may be slowed or partially reversed in some individuals with high intake of vitamin C.

Vitamin C absorption and reabsorption by the gastrointestinal tract and renal system, respectively, is accomplished by active transport across plasma membranes involving two transporter proteins encoded by the sodium-dependent vitamin C transporter genes, *SLC23A1* and *SLC23A2*. *SLC23A1* encodes SVCT1 which is the ascorbate transporter believed to be primarily involved in whole-body homeostasis and circulating vitamin C levels, while SVCT2 encoded by *SLC23A2* is believed to be involved in vitamin C regulation within specific metabolically active tissue (Savini et al. 2008). Both transporter genes have known genetic variants, but little is known about the influence of such variants on the risk for GC.

Only one case–control study has investigated variants in these genes and GC risk, and this study (based on 279 cases and 414 controls) identified an association with a variant in *SLC23A2* (Wright et al. 2009). We conducted a case–control study (based on 365 cases and 1,284 controls) nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort of 20 vitamin C transporter gene polymorphisms and haplotypes and GC risk, with additional consideration of these SNPs as predictors of baseline plasma levels of vitamin C in a subset of participants.

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## Materials and methods

### EPIC cohort study population and dietary and lifestyle assessment

European Prospective Investigation into Cancer and Nutrition is a multicenter prospective cohort of over 500,000 participants recruited through 23 research center from 10 European countries including France, Italy (Florence, Turin, Varese, Naples, Ragusa), Spain (Asturias, Granada, Murcia, Navarra, San Sebastian), the United Kingdom (Cambridge, Oxford), The Netherlands (Bilthoven, Utrecht), Greece, Germany (Heidelberg, Potsdam), Sweden (Malmö, Umeå), Denmark (Aarhus, Copenhagen), and Norway (Riboli et al. 2002). The majority of cohort participants were recruited between 1992 and 1998 at ages between 35 and 70 years. Cohort participants were recruited from the general population residing in geographic areas including towns and provinces (in Greece, Norway and France, participants were recruited from throughout these countries). The French cohort comprised women who were members of a teachers' organization health insurance program, cohorts from Utrecht and Florence comprised women attending population-based breast cancer screening programs, parts of the Italian and Spanish cohorts comprised blood donors, and most of the Oxford cohort comprised vegetarian volunteers. Written informed consent was obtained from all EPIC participants. The EPIC

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cohort and the EurGast study (nested case–control study described below) were approved by ethical committees from the International Agency for Research on Cancer (IARC) and each of the 23 EPIC study centers. Validated, country-specific food intake questionnaires were used to assess regular diet in cohort participants at baseline (Riboli et al. 2002). Vitamin C intake from food sources was estimated using standardized country-specific food composition tables (Slimani et al. 2007). Separate lifestyle questionnaires were used to collect baseline information on non-dietary factors such as tobacco smoking, physical activity, and history of medical conditions.

#### Identification of gastric cancer cases

Follow-up for these analyses was through 2003–2006, depending upon the study center. In general, case identification was based upon information available from population cancer registries except in France, Greece, Germany, and Naples (Italy) where a combination of methods including health insurance records, hospital-based cancer and pathology registries, and active participant tracking was used. GC for these analyses included diagnoses coded as C16 according to the 10th revision of the International Classification of Disease (ICD-10). A total of 665 GC cases were observed (487 adenocarcinomas, 145 non-adenocarcinomas, 7 stump tumors and 26 not specified) of which a total of 444 were first, incident, primary gastric adenocarcinomas (hereafter referred to as gastric cancer or GC). Cases were validated for histologic (diffuse, intestinal, mixed, or not specified using Lauren's classification) and anatomic subtypes (cardia C16.0, non-cardia C16.1-9, mixed, or not specified) by a panel of pathologists who reviewed original pathology reports, tumor slides, and paraffin blocks obtained on GC cases from EPIC centers (Carneiro et al. 2007).

#### Nested case–control study of gastric adenocarcinoma

A nested case–control study of primary incident gastric adenocarcinoma (Eurgast) was designed to efficiently study biomarkers and genetic susceptibility in relation to GC risk in the EPIC cohort (Sala et al. 2012). Each incident primary GC case with an available blood sample was matched by study center, sex, age group ( $\pm 2.5$  years), and date of blood collection ( $\pm 45$  days) to four control participants randomly selected from the cohort at risk at the time of diagnosis of the index case. There were 365 GC cases (181 non-cardias, 107 cardias, and 77 with mixed or unspecified anatomic subsite; and 126 intestinal, 128 diffuse, and 111 with mixed or unspecified histologic subtype) and 1,284 controls with complete genetic information. There were 172 GC cases and 311 controls with both genetic and plasma vitamin C information. *Helicobacter*

*pylori* (*H. pylori*) infection was determined by quantification of *H. pylori*-specific IgG antibodies in the plasma of cases and controls (originally collected at cohort enrollment) using enzyme-linked immunosorbent assay (ELISA) (Pyloriset EIA-GIII<sup>®</sup>) and Western blot (HELICO-BLOT2.1) (Gonzalez et al. 2006, 2011; Palli et al. 2007). Participants were categorized as positive for *H. pylori* infection if they were positive for anti-*H. pylori* antibodies or *cagA* antibodies or both (Gonzalez et al. 2011). Pepsinogen I levels were quantified in nested cases and controls by ELISA using kits from Biohit (Helsinki, Finland) in order to classify the presence or absence of severe chronic atrophic gastritis (SCAG) at the time of cohort enrollment (Palli et al. 2007). Nested participants were classified as having evidence of SCAG if their pepsinogen I level was  $< 22$   $\mu\text{g/l}$ , and no evidence of SCAG if their pepsinogen I level was  $\geq 22$   $\mu\text{g/l}$  (Broutet et al. 2003).

#### Single-nucleotide polymorphism selection and genotyping

This study was part of a candidate gene study of GC susceptibility which genotyped 1,285 single-nucleotide polymorphisms (SNPs) within 249 genetic loci on a customized array using the Illumina Beadstation Platform and GoldenGate technology (Illumina, San Diego, CA) (Duell et al. 2012; Sala et al. 2012). Genomic DNA was extracted from frozen buffy coats and genotyping performed at the Spanish National Genotyping Center (CEGEN) in Barcelona. Selection strategies for this analysis involved choosing common SNPs with haplotype-tagging potential ( $\text{MAF} \geq 5\%$ ) or with known functionality (rs33972313) (Timpson et al. 2010). Four SNPs were selected and successfully genotyped in the 21-kb region containing *SLC23A1*, and 19 SNPs were selected in the 205-kb region containing *SLC23A2*, but three of these were eliminated due to inadequate cluster separation (rs3787455, rs1776964, and rs2064842). Five percent of the samples were genotyped in duplicate to assess genotyping error (proportion concordant—99.2 %).

#### Plasma vitamin C assay

Vitamin C levels ( $\mu\text{mol/L}$ ) were previously determined in thawed plasma samples using a fluorometric method described in detail in our previous study of vitamin C levels and GC risk (Vuilleumier and Keck 1989; Jenab et al. 2006b). The effect of long-term cryopreservation on the stability of plasma vitamin C in the EPIC cohort also has been previously described, and in brief, while samples with higher baseline concentrations tended to lose more vitamin C than samples with lower concentrations, the methods and assays for plasma vitamin C concentration were

determined to be reliable without the addition of stabilizing agents (Jenab et al. 2005).

### Statistical analysis

A Kruskal–Wallis one-way analysis of variance by ranks test was used to compare median plasma vitamin C level for each SNP genotype. Multiple linear regression models of  $\log_e$ -transformed plasma vitamin C levels as the outcome and SNPs as predictors were also evaluated in 311 controls participants. All linear regression models of plasma vitamin C level were adjusted for age (in 5-year categories), sex, country, baseline smoking intensity (never smoker, former smoker quit 20+ years, former quit 11–20 years, former quit  $\leq 10$  years, occasional smoker or pipe or cigar user, current smoker 1–15 cigs./day, current 16–25 cigs./day, current 26+ cigs./day), *H. pylori* infection, and season of blood draw. To improve precision, unconditional logistic regression modeling was used to estimate the odds ratio (OR) for vitamin C SNPs and GC risk adjusting for age (in 5-year categories), sex, country, smoking status (never, former, current), *H. pylori* infection, and season of blood draw. Unconditional regression models included the variables used in the matching process (age at recruitment  $\pm 2.5$  years, sex, center/country, date of blood draw  $\pm 45$  days) and were compared with conditional models where appropriate. Analyses of vitamin C transporter SNPs and GC risk evaluated log-additive (per allele), codominant, dominant, and recessive genetic models. The possibility of population stratification was considered, but since the observed distribution of *P* values for the final 1,285 SNPs that passed QC in Eurgast was well fitted by a uniform distribution, genomic control was not needed (Sala et al. 2012). Vitamin C–SNP interactions were explored for those SNPs found to be significantly associated with GC risk or with plasma vitamin C levels. Cutpoints for vitamin C and dietary intake variables were based on medians or tertiles of the distributions for these variables in controls. Tests for statistical significance of interactions were based on a likelihood ratio test (LRT) and represent departures from a multiplicative model. All statistical tests were two-sided, and all analyses were performed using the R package (v. 2.12.2) and the SNPassoc library (Gonzalez et al. 2007).

### Haplotype analysis

Linkage disequilibrium (LD) between SNPs in each gene region (*SLC23A1* and *SLC23A2*) was assessed and haplotype frequencies inferred using the EM algorithm as implemented in the haplo.stats R package. SNPs with  $>5\%$  missing data were not included in haplotype analyses (rs3914810). ORs for haplotypes with minimum

frequency  $\geq 5\%$  and GC risk were estimated using the most common haplotype as the referent group and were adjusted for age, sex, country, smoking status, *H. pylori* infection, and season of blood draw. Since haploview analysis of downloaded genotype data from the CEU population in Hapmap revealed a single LD block in *SLC23A1* (21-kb), the four SNP markers in this gene were analyzed as one haplotype block. *SLC23A2* is a much larger gene (205 kb), and haplotypes were analyzed in four LD blocks according to haploview analysis of CEU genotype data from Hapmap.

### Results

The median time interval between blood collection and diagnosis for the 365 GC cases was 4.7 years. GC cases and nested controls were similar with regard to age, country, and sex as these were variables used in the nested case–control matching procedure (Table 1). With regard to smoking status, cases were about 80 % more likely to be current smokers at baseline than controls, about 3.2 times more likely to be positive for *H. pylori* infection, and 30 % less likely to have plasma vitamin C concentrations at or above the upper tertile in controls ( $\geq 45 \mu\text{mol/L}$ ), although differences in vitamin C concentration were not statistically significant (Table 1). Cases were 31 % less likely to have questionnaire-based vitamin C intake from food sources at or above the upper tertile in controls ( $\geq 135.8 \text{ mg/day}$ ) and were 60 % more likely to be at or above the upper tertile of the processed meat intake ( $\geq 39.6 \text{ g/day}$ ) (Table 1). All *SLC23A1* and *SLC23A2* SNPs passed Hardy–Weinberg equilibrium in controls (all *P* values  $>0.05$ ). Genotype information for rs3914810 and rs6596473 in *SLC23A2* was missing in 14.9 and 4.2 % of participants, respectively; the remaining SNPs had less than 1.7 % of participants with missing genotype data (data not shown).

Multivariable linear regression models of  $\log_e$ -transformed plasma vitamin C level as the dependent variable and  $\log_e$ -transformed questionnaire-based vitamin C intake as the independent variable indicated that food intake explained only 5 % of the variability in plasma vitamin C levels (*P* value 0.001, data not shown). Median plasma vitamin C levels in controls were evaluated according to the genotype of each SNP in *SLC23A1* and *SLC23A2*. None of the Kruskal–Wallis test statistics evaluating the unadjusted association between genotype and median plasma vitamin C level were statistically significant (all *P* values  $>0.06$ ) (data not shown). In multiple linear regression analyses ( $n = 311$  controls) of the natural log of plasma vitamin C as the dependent variable and *SLC23A1* and *SLC23A2* SNPs as independent variables, regression

**Table 1** Covariates by case and control status and odds ratios (OR) for gastric cancer risk, EPIC-Eurgast study

	Cases		Controls		OR <sup>a</sup> (95 % CI)
	<i>n</i> = 365	%	<i>n</i> = 1,284	%	
Age at recruitment					
Mean (SD)	58.4 (7.9)		58.4 (7.7)		–
Country					–
France	2	0.5	3	0.2	–
Italy	56	15.3	206	16	–
Spain	41	11.2	134	10.4	–
UK	41	11.2	135	10.5	–
The Netherlands	26	7.1	99	7.7	–
Greece	24	6.6	88	6.9	–
Germany	48	13.2	186	14.5	–
Sweden	64	17.5	220	17.1	–
Denmark	61	16.7	205	16	–
Norway	2	0.5	8	0.6	–
Sex					
Men	214	58.6	759	59.1	–
Women	151	41.4	525	40.9	–
Smoking status					
Never	128	35.1	542	42.2	1.00 (Referent)
Former	115	31.5	427	33.3	1.19 (0.88–1.61)
Current	118	32.3	292	22.7	1.84 (1.36–2.50)
Unknown	4	1.1	23	1.8	
<i>H. pylori</i> infection at baseline					
Negative	68	18.8	492	38.7	1.00 (Referent)
Positive <sup>b</sup>	294	81.2	779	61.3	3.19 (2.35–4.33)
Plasma vitamin C <sup>c</sup> (μmol/L, tertiles)					
<31	63	36.63	95	30.55	1.00 (Referent)
31–44.9	55	31.98	107	34.41	0.74 (0.46–1.18)
≥45	54	31.4	109	35.05	0.70 (0.43–1.13)
Vitamin C intake from food sources (mg/d, tertiles)					
<86.76	148	40.55	435	33.88	1.00 (Referent)
86.76–135.82	112	30.68	425	33.1	0.75 (0.57–1.00)
≥135.83	105	28.77	424	33.02	0.69 (0.51–0.93)
Processed meat intake (g/d, tertiles)					
<16.89	111	30.41	435	33.88	1.00 (Referent)
16.89–39.57	108	29.59	424	33.02	1.05 (0.76–1.45)
≥39.58	146	40	425	33.1	1.58 (1.12–2.23)

<sup>a</sup> Adjusted for age at recruitment (5-year categories), sex, and country

<sup>b</sup> *cagA*-positive or anti-*H. pylori* positive, or both

<sup>c</sup> Plasma vitamin C concentration assessed in a subset (172 cases and 311 controls)

coefficients were statistically significant for the following four SNPs: *SLC23A1* rs11950646 (dominant model *P* value: 0.03; log-additive model *P* value 0.02), *SLC23A1* rs33972313 (codominant model *P* value 0.04), *SLC23A2* rs6053005 (recessive model *P* value 0.007), and *SLC23A2* rs6133175 (recessive model *P* value 0.02) (Table 2). In *SLC23A1*, rs11950646 GG or AG genotypes (compared with AA) were associated with a 13 % lower plasma vitamin C concentration (10 % lower per G allele in a log-additive model), and rs33972313 GA heterozygotes were associated with a 24 % lower concentration. In *SLC23A2*,

rs6053005 TT homozygotes and rs6133175 GG homozygotes were associated with 24 % higher plasma vitamin C concentrations (Table 2). For all remaining SNPs and genetic models, there were no statistically significant predictors of plasma vitamin C levels (all  $\beta$  parameter *P* values >0.10, data not shown). We conducted regression analyses of questionnaire-based vitamin C intake (mg/day) from food sources as a predictor of plasma vitamin C levels, stratified by the genotypes of these four SNPs (one at a time) under a recessive or dominant model and found no evidence for effect modification (all *P* values for interaction

**Table 2** Multiple linear regression of log<sub>e</sub>-transformed plasma vitamin C level and vitamin C transporter gene variants, in controls from the EPIC-Eurgast study

Gene, SNP <sup>a</sup>	<i>n</i> = 311	Mean plasma vitamin C (μmol/L)	Model <sup>b</sup>	β parameter	95 % CL	% Change in plasma vitamin C level (95 % CL)	Variant allele/genotype
<i>SLC23A1</i>							
rs11950646							
AA	125	42.01					
AG	128	39.1	A	−0.11	−0.20, −0.017	−10 % (−2, −18 %)	<i>G</i> allele
GG	32	34	D	−0.14	−0.26, −0.011	−13 % (−1, −23 %)	<i>AG</i> + <i>GG</i>
rs33972313							
GG	293	40.4					
GA	17	33.65	C	−0.28	−0.54, −0.016	−24 % (−2, −42 %)	<i>GA</i>
AA	0	–					
<i>SLC23A2</i>							
rs6053005							
CC	79	38.6					
CT	151	39.39					
TT	58	43.69	R	0.21	0.058, 0.37	+24 % (6, 44 %)	<i>TT</i>
rs6133175							
AA	115	39.13					
AG	139	39.42					
GG	37	45.22	R	0.22	0.029, 0.40	+24 % (3, 50 %)	<i>GG</i>

Adjusted for age at recruitment (5-year categories), sex, country, smoking intensity, baseline *H. pylori* infection status, and season of blood draw  
*SNP* single-nucleotide polymorphism, *CL* confidence limit

<sup>a</sup> *SLC23A1* and *SLC23A2* variants not listed were not predictors of plasma vitamin C levels in multiple linear regression models (all β parameter *P* values > 0.10)

<sup>b</sup> Genetic models: *A* allelic or log-additive, *D* dominant, *R* recessive, *C* codominant

>0.30, data not shown), but data were sparse. We also evaluated potential interactions in relation to GC risk between plasma vitamin C or questionnaire-based vitamin C intake from food sources and intakes of processed and red meat (with cutpoints based on both the median and tertiles in controls), but found no evidence for effect measure modification (all *P* values for interaction >0.1, data not shown).

Multivariable logistic regression analysis of the association between *SLC23A1* and *SLC23A2* genotypes and GC risk gave evidence of a positive association for one SNP in *SLC23A2* (rs6116569) (Table 3). The heterozygous genotype was statistically significantly associated with GC risk, while the homozygous variant was also associated with risk, but was not statistically significant (Table 3). In a dominant model (rs6116569 CT + TT vs. CC), the OR for overall GC was 1.36 and 95 % CI 1.04–1.79. The known functional variant in *SLC23A1* (rs33972313) was not associated with GC risk in our study (Table 3).

Supplemental Table S1 gives dominant model ORs and 95 % CIs for each SNP according to GC anatomic location (non-cardia, cardia) and histologic subtype (intestinal,

diffuse). Analysis of rs6116569 (CT + TT vs. CC) and GC risk by anatomic location suggested that the association observed for this SNP with overall GC (Table 3) was for non-cardia GC (OR 1.63, 95 % CI 1.11–2.39, based on 178 non-cardia cases) and not cardia GC (OR 1.01, 95 % CI 0.65–1.57, based on 107 cardia cases); however, the convergence of the logistic regression model for rs6116569 and cardia GC was questionable due to the limited number of cardia cases in some EPIC countries (the corresponding OR without considering country was 1.05, 95 % CI = 0.68–1.63). Analyses of the association between rs6116569 and GC by histology indicated no major differences (for intestinal, OR 1.50, 95 % CI 0.97–2.32, based on 121 cases; and for diffuse, OR 1.20, 95 % CI 0.79–1.83, based on 123 cases). No other SNPs were associated with GC risk by anatomic location or histologic subtype (with the exception of rs1279685 and the intestinal subtype, but the OR was of borderline statistical significance) (Supplemental Table S1). Additional analyses of the association between rs6116569 and GC risk stratified by plasma vitamin C level and by the SNPs found to be associated with plasma vitamin C yielded no evidence for effect measure

**Table 3** Genetic variants in *SLC23A1* and *SLC23A2* and gastric cancer risk, EPIC-Eurgast study

Gene, SNP	Cases <i>n</i> = 365	Controls <i>n</i> = 1,284	OR <sup>a</sup>	95 % CL
<i>SLC23A1</i>				
rs6596471				
AA	195	665	1	Referent
AG	137	519	0.9	0.70, 1.17
GG	31	99	1.09	0.69, 1.71
rs6596473				
GG	163	542	1	Referent
GC	158	565	0.78	0.50, 1.24
CC	29	123	0.93	0.72, 1.21
rs11950646				
AA	163	539	1	Referent
AG	163	581	0.95	0.73, 1.23
GG	36	145	0.82	0.54, 1.25
rs33972313 <sup>b</sup>				
GG	346	1,212	1	Referent
GA + AA	19	68	0.99	0.58, 1.71
<i>SLC23A2</i>				
rs6052937 <sup>b</sup>				
CC	260	926	1	Referent
CA + AA	104	355	1.07	0.81, 1.39
rs3787456 <sup>b</sup>				
GG	342	1,219	1	Referent
GA + AA	22	64	1.27	0.76, 2.11
rs6116569				
CC	92	404	1	Referent
CT	196	616	1.38	1.03, 1.83
TT	76	262	1.32	0.93, 1.88
rs17339746 <sup>b</sup>				
CC	291	1,024	1	Referent
CG + GG	72	255	0.95	0.70, 1.28
rs6052961				
CC	156	493	1	Referent
CT	162	606	0.83	0.64, 1.07
TT	47	181	0.79	0.54, 1.16
rs17339942 <sup>b</sup>				
CC	308	1,096	1	Referent
CT + TT	50	169	1.09	0.77, 1.55
rs1776963 <sup>b</sup>				
CC	239	860	1	Referent
CT + TT	124	414	1.09	0.84, 1.40
rs1715380				
GG	221	788	1	Referent
GC	128	438	1.03	0.80, 1.33
CC	16	58	0.97	0.54, 1.74
rs6133175				
AA	156	554	1	Referent
AG	156	578	0.95	0.73, 1.23
GG	53	152	1.28	0.88, 1.86

**Table 3** continued

Gene, SNP	Cases <i>n</i> = 365	Controls <i>n</i> = 1,284	OR <sup>a</sup>	95 % CL
rs3914810 <sup>c</sup>				
CC	120	404	1	Referent
CT	133	537	0.83	0.62, 1.11
TT	59	150	1.38	0.94, 2.02
rs6139587				
AA	116	385	1	Referent
AT	180	654	0.89	0.67, 1.17
TT	65	226	0.97	0.68, 1.38
rs6053005				
CC	115	388	1	Referent
CT	177	658	0.88	0.67, 1.17
TT	72	231	0.98	0.69, 1.39
rs2326576				
TT	110	407	1	Referent
TC	176	633	1.04	0.79, 1.37
CC	79	243	1.28	0.91, 1.80
rs6053034 <sup>b</sup>				
GG	247	897	1	Referent
GT + TT	118	387	1.14	0.88, 1.48
rs8126456 <sup>b</sup>				
CC	332	1,169	1	Referent
CT + TT	31	110	1.03	0.67, 1.59
rs1279685				
GG	204	746	1	Referent
GA	137	458	1.18	0.91, 1.52
AA	24	79	1.31	0.79, 2.16

SNP single-nucleotide polymorphism

<sup>a</sup> Odds ratios (OR) adjusted for age at recruitment (5-year categories), sex, country, smoking status, baseline *H. pylori* infection status, season of blood draw

<sup>b</sup> Dominant model evaluated when there were fewer than 10 cases or controls with variant genotypes

<sup>c</sup> Genotype data missing for 15 % of participants

modification by these covariates (all *P* values for interaction >0.2, data not shown). We also ran a logistic regression model of the association between rs6116569 and GC risk including a variable for plasma vitamin C level (in addition to age, sex, country, smoking status, *H. pylori* infection, and season of blood draw): the dominant model OR was 1.15, 95 % CI 0.74–1.80, based on 172 GC cases and 311 controls. The OR for rs6116569 and GC risk in the same 172 cases and 311 controls without the variable for plasma vitamin C was 1.16, 95 % CI 0.74–1.81. We then estimated ORs for rs6116569 and GC risk stratified by SCAG status and observed slightly different adjusted ORs (SCAG ‘+’/pepsinogen I < 22 µg/l, OR 1.11, 95 % CI 0.37–3.38; SCAG ‘-’/pepsinogen I ≥ 22 µg/l, OR 1.55,

95 % CI 1.11–2.16) but the difference was not statistically significant (interaction *P* value: 0.66).

We investigated whether plasma vitamin C level modified associations between each *SLC23A1* or *SLC23A2* genotype and GC risk and found no evidence for effect measure modification (all interaction *P* values >0.1, except for rs6052961 which had the lowest *P* value of 0.07) (data not shown).

Finally, we conducted an adjusted analysis of *SLC23A1* haplotype and GC risk and found no evidence for haplotype–disease associations (Table 4). An adjusted analysis of *SLC23A2* haplotypes showed a statistically significant positive association between haplotype CGTC in block 1 and GC (Table 4), and further analysis revealed that the association was observed primarily in non-cardia GC (OR 1.52; 95 % CI 1.14–2.03) (subsite data not in table). When we included a variable for log plasma vitamin C in the model, CGTC haplotype-specific ORs were attenuated and no longer statistically significant (data not shown). A statistically significant positive association between haplotype ATC in block 3 and GC was also observed (Table 4). No overall association was observed between haplotypes in block 4 and GC, but an association between haplotype GCA and intestinal-type GC was identified (OR 1.43; 95 % CI 1.04–1.97) (subtype data not in table). This haplotype contains variant allele A (SNP rs1279685), which by itself was associated with intestinal-type GC (supplemental Table S1).

## Discussion

In this nested case–control study of vitamin C transporter genes and GC risk within the EPIC cohort, we identified one *SLC23A2* marker (rs6116569, intronic) associated with GC risk, and two markers in *SLC23A2* (rs6053005, rs6133175) and two in *SLC23A1* (rs33972313, rs11950646) as predictors of plasma vitamin C concentration. The magnitude of the inverse association between plasma vitamin C concentration and GC risk previously reported in EPIC was moderate (highest vs. lowest quartile, OR 0.55, 95 % CI 0.31–0.97) representing a 45 % lower risk (Jenab et al. 2006b). In the previous analysis which included fewer GC cases (*n* = 215), questionnaire-based vitamin C intake from foods was not associated with GC risk (Jenab et al. 2006b); however, with additional GC cases in the present analysis (*n* = 365), higher intakes of vitamin C from foods were statistically significantly associated with reduced GC risk. In contrast, questionnaire-based vitamin C intake was not a strong predictor of plasma vitamin C concentration in our study, and none of the SNP markers we tested improved this prediction.

Since the four markers (two in *SLC23A2* and two in *SLC23A1*) were only moderate predictors of plasma vitamin

**Table 4** *SLC23A1* and *SLC23A2* haplotype frequencies and gastric cancer risk, EPIC-Eurgast study

Gene–haplotype	Cases	Controls	OR <sup>a</sup>	95 % CI
<i>SLC23A1</i> <sup>b</sup>				
A G A G	0.61	0.6	1	Referent
G C G G	0.23	0.23	0.95	0.77–1.18
A C G G	0.081	0.084	0.81	0.58–1.13
Rare	0.08	0.08	–	–
<i>SLC23A2</i> <sup>c</sup>				
Block 1				
C G C C	0.38	0.43	1	Referent
C G T C	0.33	0.29	1.29	1.04–1.60
A G C C	0.13	0.12	1.29	0.95–1.74
C G T G	0.1	0.1	1.07	0.78–1.45
Rare	0.058	0.052	–	–
Block 2				
C C C G G	0.33	0.31	1	Referent
T C C G A	0.29	0.31	0.85	0.68–1.06
C C C G A	0.13	0.13	0.93	0.70–1.24
C C T C A	0.11	0.1	1.01	0.74–1.38
C T T C A	0.06	0.07	0.94	0.65–1.37
Rare	0.078	0.091	–	–
Block 3				
A T T	0.32	0.34	1	Referent
T C C	0.3	0.32	1.05	0.84–1.30
T C T	0.12	0.11	1.18	0.86–1.63
A T C	0.11	0.093	1.44	1.01–2.05
A C T	0.094	0.11	1.07	0.77–1.49
Rare	0.044	0.028	–	–
Block 4				
G C G	0.58	0.59	1	Referent
G C A	0.24	0.24	1.17	0.95–1.44
T C G	0.12	0.12	1.06	0.81–1.39
Rare	0.05	0.049	–	–

<sup>a</sup> Haplotype odds ratios (OR) adjusted for age at recruitment (5-year categories), sex, country, smoking status, baseline *H. pylori* infection status, and season of blood draw

<sup>b</sup> Inferred using rs659671, rs6596473, rs17339746 and rs33972313

<sup>c</sup> Inferred using rs6052937, rs3787456, rs6116569, rs17339746 (block 1); rs6052961, rs17339942, rs1776963, rs1715380, rs6133175 (block 2); rs6139587, rs6053005, rs2326576 (block 3); rs6053034, rs8126456, rs1279685 (block 4)

C concentration in controls (*n* = 311), it is perhaps not surprising that we did not identify them as predictors of GC risk. None of the identified haplotypes in *SLC23A1* (based on four markers) were associated with GC risk, but three haplotypes in *SLC23A2* (based on 15 markers in 4 LD blocks) were associated with GC risk: one with both non-cardia and overall GC, one with overall GC, and one with the intestinal subtype.

The association between *SLC23A2* rs6116569 and GC risk, in particular non-cardia GC, suggests that this variant

may influence cancer risk in the distal stomach; however, this association (and the haplotype-specific OR) was attenuated and no longer statistically significant when we included a variable for plasma vitamin C concentration in the regression models, suggesting that this variant may not be an independent susceptibility factor for GC risk. *SLC23A2* rs6116569 is a tagSNP with unknown function, and in our data, it showed no detectable effect on plasma vitamin C levels. Our results showing associations between the SNP markers rs11950646, rs6053005, and rs6133175 and plasma vitamin C concentration have not been directly observed in other study populations; however, previous studies have evaluated *SLC23A1* and *SLC23A2* SNPs and blood vitamin C concentrations in large sample sizes (Cahill and El-Sohemy 2009; Timpson et al. 2010).

Cahill and El-Sohemy reported an association between *SLC23A1* rs4257763 and mean serum vitamin C levels from 12-h fasting blood in a cross-sectional study of 1,277 volunteers (39 % Caucasian, and 29 % East Asian and the remainder of mixed race/ethnicity) (Cahill and El-Sohemy 2009). *SLC23A1* rs4257763 is not in HapMap, but has been reported to be in partial LD with rs6596473 (for which we observed no associations) (Cahill and El-Sohemy 2009). Timpson and colleagues performed a large-scale analysis of genetic variants at the *SLC23A1* locus and 6-h fasting blood vitamin C levels in over 15,000 Caucasian participants from five longitudinal studies (Timpson et al. 2010). They identified a rare variant (rs33972313) in *SLC23A1* that was statistically significantly associated with circulating blood vitamin C concentration. In our study, variant rs33972313 (GA genotype) was a predictor of lower plasma vitamin C level, which is in agreement with the much larger study of Timpson et al. This variant lies in exon 8, results in a missense change, and has MAF of 3 % in Caucasians; thus, it is relatively uncommon.

Only one previous study of *SLC23A1* and *SLC23A2* SNP markers and GC risk has been published, a population-based case–control study from Poland (Wright et al. 2009). In that study, the authors analyzed 279 GC cases and 414 population-based controls and identified an association between *SLC23A2* rs12479919 and GC risk with no effect modification by vitamin C intake and no associations with markers in *SLC23A1*. *SLC23A2* rs12479919 is not in LD with rs6116569 for which we observed an association with GC. Further, none of the *SLC23A2* markers evaluated in the Polish study are in LD with rs6116569 (all  $r^2 < 0.4$ ). Although the evaluated SNP markers were not the same, the Polish study and our study suggest that variation in *SLC23A1* does not influence GC risk, but that variation in *SLC23A2* could play a limited role in GC susceptibility.

The functions of *SLC23A1* and *SLC23A2* are to encode and express SVCT1 and SVCT2, key proteins for the transport and accumulation of vitamin C in mammalian

cells and tissues (Savini et al. 2008). SVCTs are surface glycoproteins that show distinct functions and tissue distributions, with SVCT1 having a role in whole organism vitamin C homeostasis and SVCT2 playing a role in oxidative stress management in metabolically active tissues (Savini et al. 2008). Interestingly, SVCT2 has been detected in rat gastric tissue, but not SVCT1, the protein product of *SLC23A1* (Tsukaguchi et al. 1999). Our study identified one SNP marker (and haplotypes) in association with GC risk from the *SLC23A2* gene, the homologous gene found in rat gastric tissue. Little is known about the distribution of these proteins in humans, but the genes are highly conserved in humans and rodents, indicating a common ancestral gene (Eck et al. 2004; Savini et al. 2008). Little is known about the possible functions of the SNP markers for which we and others have observed associations (Chen et al. 2009; Erichsen et al. 2006; Wright et al. 2009; Skibola et al. 2008), but discrepancies across studies could also reflect different expression patterns for these genes in different tissues.

Potential mechanisms for a protective effect of vitamin C in gastric carcinogenesis include its antioxidant properties, as well as inhibition of *N*-nitroso compound formation in the stomach (often elevated in persons with high intakes of processed meat) (Drake et al. 1996; Sasazuki et al. 2008; Tannenbaum et al. 1991). In our data, we did not observe a statistically significant interaction between dietary intake of vitamin C (or plasma concentration of vitamin C) and processed meat intake. Another potential mechanism is that vitamin C may act by inhibiting or slowing the infection and growth of *H. pylori* in the gastric mucosa (Pal et al. 2011; Zhang et al. 1997). Weaknesses of our study include potential misclassification of dietary intake of foods containing vitamin C (and lack of information on use of dietary supplements) and imprecision in plasma vitamin C measurements. Our sample size was relatively small, especially when evaluating plasma vitamin C levels and genotypes together as these data were available only in a subset of genotyped participants; thus, we may have missed subtle effects of some *SLC23A1* and *SLC23A2* markers. Further, some of the associations we report may have been due to chance. Our study had limited precision for evaluating rare markers or risks within anatomic subtypes (i.e., non-cardias). Since over 80 % of our GC cases were positive for *H. pylori* infection, we were unable to evaluate interactions between *H. pylori* and vitamin C intake or plasma levels. Strengths of our study were that blood collection and dietary assessment occurred at baseline; thus, our study avoided many of the biases inherent to retrospective study designs.

In conclusion, we identified two markers in *SLC23A1* and two in *SLC23A2* that were predictors of plasma vitamin C concentration in controls and one additional marker

in *SLC23A2* (and corresponding haplotype) that was moderately associated with risk for non-cardia GC in the EPIC cohort. Additional prospective studies of common and rare variants in the vitamin C pathway and GC risk in larger populations and consortia are warranted.

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