Gene polymorphisms in the ornithine decarboxylase–polyamine pathway modify gastric cancer risk by interaction with isoflavone concentrations

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Abstract

Background The study aimed to examine the association between genes encoding molecules in the ornithine decarboxylase (ODC)–polyamine pathway (*ODC1*, *AMD1*, *NQO1*, *NOS2A*, and *OAZ2*) and gastric cancer risk and whether the gene–phytoestrogen interaction modifies gastric cancer risk.

Methods Among 76 gastric cancer cases and their 1:4 matched controls within the Korean Multi-center Cancer Cohort, a total of 30 SNPs in five genes involved in the

ODC pathway were primarily analyzed. The second-stage genotyping in 388 matched case–control sets was conducted to reevaluate the significant SNPs interacting with phytoestrogens during the primary analysis. The summary odds ratios (ORs) [95 % confidence intervals (CIs)] for gastric cancer were estimated. Interaction effects between the SNPs and plasma concentrations of phytoestrogens (genistein, daidzein, equol, and enterolactone) were evaluated.

Results In the pooled analysis, *NQO1* rs1800566 showed significant genetic effects on gastric cancer without heterogeneity [OR 0.83 (95 % CI 0.70–0.995)] and a greater decreased risk at high genistein/daidzein levels [OR 0.36 (95 % CI 0.15–0.90) and OR 0.26 (95 % CI 0.10–0.64),

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respectively; $p_{\text{interaction}} < 0.05$]. Risk alleles of *AMD1* rs1279599, *AMD1* rs7768897, and *OAZ2* rs7403751 had a significant gene–phytoestrogen (genistein and daidzein) interaction effect to modify the development of gastric cancer. They had an increased gastric cancer risk at low isoflavone levels, but a decreased risk at high isoflavone levels ($p_{\text{interaction}} < 0.01$).

Conclusions Our findings suggest that common variants in the genes involved in the ODC pathway may contribute to the risk of gastric cancer possibly by modulating ODC polyamine biosynthesis or by interaction between isoflavones and *NQO1*, *OAZ2*, and *AMD1*.

Keywords Gastric cancer · Ornithine decarboxylase · SNPs · Phytoestrogen · Polyamine · *NQO1* · *OAZ2* · *AMD1*

Introduction

Helicobacter pylori is a major carcinogen for gastric cancer [1]. *H. pylori* infection results in inflammation and induces innate immunity in the host, which can be eradicated by host immune responses, such as nitric oxide (NO) production in macrophages. However, *H. pylori*-induced immune responses can fail to eradicate the bacterium by polyamine synthesis through the sequential ornithine decarboxylase (ODC)–polyamine pathway [12]. Elevated polyamine levels through the sequential ODC–polyamine pathway are associated with gastric cancer [2]. The ODC–polyamine pathway could contribute to gastric cancer by controlling chronic inflammation and host innate immune reaction, persistent *H. pylori* infection, and DNA damage (Fig. 1).

Since increased polyamine levels are associated with increased gastric cancer risk [2], decreases in polyamine levels may be key to reducing gastric cancer risk. Polyamine synthesis is stimulated by ODC, a rate-limiting

enzyme, and *S*-adenosylmethionine decarboxylase (AMD), another key enzyme [3, 4], and thus inhibition of ODC and AMD may be of major importance. Previous studies showed that the irreversible inhibition of ODC suppressed cancer formation [5, 6]. Therefore, changes in these enzymes and dysfunction of homeostasis to control ODC and polyamine synthesis could be of paramount importance in gastric carcinogenesis. The sequential processes in the ODC–polyamine pathway can be regulated by their host genes, and therefore, it has been hypothesized that polymorphic variants of genes in the ODC–polyamine pathway may affect cancer susceptibility by altering their encoded enzymes, through either expression or function, to modulate polyamine synthesis.

Isoflavones have also been shown to affect ODC. The isoflavone genistein participates directly in inhibition of ODC activity and decreases polyamine levels [7]. It has been suggested that the interaction between genetic variants of enzymes in the ODC–polyamine synthesis pathway and isoflavone might be involved in modifying gastric cancer risk.

The objective of this study is twofold. First, the role of gene polymorphisms related to the ODC–polyamine pathway in the development of gastric carcinogenesis was investigated through a two-phase study that included (1) a discovery phase, which was a candidate gene approach focusing on five genes involved in the ODC pathway (*OAZ2*, *ODC1*, *AMD1*, *NQO1*, and *NOS2A*) and (2) an extension phase, which further examined the significant single-nucleotide polymorphisms (SNPs) identified in the discovery genetic and interaction analysis. Second, the risk modification by gene–environment interaction between gene polymorphisms in the ODC–polyamine pathway and host serum phytoestrogen levels for gastric cancer was examined.

Materials and methods

Ethics statement

All participants signed an informed consent form before entering the studies. The study protocols for the Korean Multi-center Cancer Cohort (KMCC) study and the current nested case–control study were approved by the institutional review boards of Seoul National University Hospital and the National Cancer Center of Korea (H-0110-084-002, C-0907-044-2861-170), and Hanyang University Hospital (2003–2004).

Study population

In the discovery phase, the population-based nested case–control study population was recruited from the KMCC, a

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Fig. 1 The ornithine decarboxylase (ODC)–polyamine biosynthesis pathway. *AdoMet* S-adenosylmethionine, *AMD1* S-adenosylmethionine decarboxylase 1, *dcAdoMet* decarboxylated S-adenosylmethionine,

iNOS inducible nitric oxide synthase, *NQO1* NAD(P)H dehydrogenase, quinone 1, *OAZ1* ornithine decarboxylase antizyme 1, *SMO* spermine oxidase

community-based prospective cohort of participants recruited from four urban and rural areas in Korea (Haman, Chungju, Uljin, and Youngil) from 1993 through 2004 [8]. Participants signed informed consent forms and completed a detailed standardized interview-based questionnaire on general lifestyle, medical history, physical activity, diet, reproductive factors, pesticide exposure, and additional environmental factors. Blood and spot urine samples were collected and stored at -70 and -20 °C, respectively.

On December 31, 2002, 136 gastric cancer cases in the KMCC were identified through computerized record linkages to the national cancer registry, national death certificates, and health insurance medical records. The passive follow-up methods were reported to be 99 % efficient, and completeness was assured [9]. Case patients diagnosed before recruitment ($N = 36$) and without blood samples ($N = 16$) were excluded. Cancer-free controls were randomly selected from the KMCC population. Four controls were matched to each gastric cancer case by incidence density sampling on the basis of age (± 5 years), sex, residential district, and enrollment. Additionally, eight cases and 14 controls were excluded owing to insufficient DNA

or poor genotyping. Finally, 76 cases and 322 controls were included in the discovery phase.

In the extension phase, 388 gastric cancer case–control sets were selected as follows. There were 95 new gastric cancer cases and 52 prevalent cases in December 2008 and 52 additional case patients whose blood samples were later obtained from the KMCC. In addition, from March 2002 to September 2006, 490 newly diagnosed gastric cancer cases from two university hospitals in Korea (Chungnam University Hospital and Hanyang University GURI Hospital) were identified. Epidemiological data and venous blood samples were collected at the time of diagnosis or prior to gastric cancer surgery. Of the newly diagnosed gastric cancer cases, 189 patients with sufficient DNA samples and who had provided informed consent were included. Community-based controls matched by age (± 5 years), sex, and enrollment year from 2001 to 2005 were randomly selected from the KMCC. Two cases and 40 controls were excluded owing to poor genotyping and insufficient sample. Finally, 386 cases and 348 controls were included in the extension phase. Pooled analysis and meta-analysis included 462 cases and 670 controls.

Candidate gene and SNP selection

In the discovery phase, five genes involved in the ODC pathway were selected as follows: *ODC1*, which encodes ODC1; *AMD1*, which encodes AMD1; *OAZ2*, which encodes ornithine decarboxylase antizyme 2; *NQO1*, which encodes NAD(P)H dehydrogenase, quinone 1 (NQO1); and *NOS2A*, which encodes inducible nitric oxide synthase (iNOS).

SNPs from the genes were selected according to the following criteria: (1) SNPs that were reported to have a possible functional significance in previous studies; (2) SNPs with a minor allele frequency greater than 0.05 in Asian databases such as SNP500Cancer and CGAP using dbSNP IDs (<http://www.ncbi.nlm.nih.gov/SNP>); (3) concurrently, SNPs with a minor allele frequency greater than 0.05 in HapMap Japanese (JPT). Finally, 30 SNPs with a design score of 1.1, $r^2 > 0.8$, were selected: 20 SNPs located in the intron region, seven SNPs located in the promoter region, and three SNPs located in the coding region.

In the extension phase, SNPs were selected as follows. In the discovery analysis of the single SNP association, no SNPs were found to be significant. In the gene–environment interaction analysis, *NQO1* rs1800566 and rs1437135, *OAZ1* rs7403751, and *AMD1* rs1279599, rs7768897, and rs811921 were selected owing to a significant p value for interaction ($p < 0.05$) and a lower raw p value ($p < 0.05$) at low or high phytoestrogen level by classification of the median. Of the three *AMD1* SNPs and four *NQO1* SNPs, tagging SNPs such as rs1279599 and rs7768897 in *AMD1* and rs740375 in *NQO1* were selected. For *OAZ2*, rs1800566 was selected.

Genotyping

Genotyping in the discovery phase was performed in 20 using the GoldenGate™ assay (Illumina®, San Diego, CA, USA). Of the 30 SNPs in the ODC pathway, five SNPs were deemed unusable owing to failure of genotyping (rs7208775, rs1137933) and a SNP call rate below 90 % (rs6494486, rs2872753, rs2248814) and were excluded from the analysis. Finally, we analyzed 25 SNPs in five genes in the ODC pathway (genotyping rate of 99.5 %). To ensure quality control and evaluate the intrasubject concordance rate, 52 duplicate samples were randomly distributed in the genotyping plate. Concordance rates for all assays were greater than 99 %.

Genotyping in the extension phase was performed in 20 using the Illumina VeraCode GoldenGate assay with BeadXpress according to the manufacturer's protocol (Illumina®, San Diego, CA, USA) [16]. To ensure the reliability of the two different genotyping methods, 135

samples (59 cases and 76 controls) were genotyped twice by both the genome-wide human SNP Array 5.0 and the Illumina VeraCode GoldenGate assay, and the concordance rate was greater than 98.2 %. DNA concentration was determined using a NanoDrop ND-1000 spectrophotometer for the primary quality controls.

Measurement of phytoestrogen biomarkers

Plasma concentrations of four phytoestrogen biomarkers—genistein, daidzein, and equol (isoflavones) and enterolactone (a lignan)—were measured using time-resolved fluoroimmunoassay kits (Labmaster, Finland) in 2010. Free phytoestrogen biomarkers were extracted from 200 μ L of plasma, and the time-resolved fluorescence was measured using a VICTOR3™ 1420 multilabel counter (PerkinElmer). Full details of the measurement method are described elsewhere [10]. Among the total genetic study population, the phytoestrogen concentrations of 406 case patients and 417 controls were measured, and these were analyzed in the gene–environment interaction analysis.

Statistical analysis

χ^2 and Student t tests were conducted to compare selected characteristics between gastric cancer cases and controls. Differences in selected characteristics of sex, age, *H. pylori* infection, CagA and VacA seropositivity, cigarette smoking, alcohol drinking, and gastritis history between cases and controls were determined by $p = 0.05$.

Hardy–Weinberg equilibrium (HWE) in the control group was evaluated using the χ^2 test or Fisher's exact test with a cut-off level of HWE < 0.0001 . In the discovery analysis, the association between individual SNPs and gastric cancer risk was evaluated on the basis of raw and permuted p values using the likelihood ratio test with one degree of freedom in the additive, dominant, and recessive models. The additive model assumes a dose–response effect with an increasing number of variant alleles. The dominant and recessive models are tests for the minor allele. If d is the minor allele and D is the major allele, the dominant model is DD vs $dd + Dd$ and the recessive model is dd vs $DD + Dd$. Permuted p values were estimated by 100,000 permutation tests. Gastric cancer risk was calculated as odds ratios (ORs) and 95 % confidence intervals (CIs) using an unconditional logistic regression model with adjustment for potential risk factors of age, smoking status (ever vs never), *H. pylori* infection (positive vs negative), and CagA seropositivity (positive vs negative) in the additive, dominant, and recessive models. Haploblocks were created and tag-SNPs were identified in haplotype analysis.

In the extension phase, the most significant SNPs in the discovery phase were reanalyzed. On the basis of the

additive and/or recessive models, gastric cancer risk was estimated as ORs and 95 % CIs using an unconditional logistic regression model with adjustment for the aforementioned risk factors. Stratified analysis by high and low levels of phytoestrogen biomarkers where the cut-off levels were determined by the median level of controls in the discovery phase and splicing analysis in the pooled analysis was conducted using unconditional logistic regression models. Recessive models of the SNPs that have the most significant effect on gastric cancer risk were computed as ORs (95 % CIs) adjusted for the same covariates.

All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA), PLINK version 1.06 (<http://pngu.mgh.harvard.edu/purcell/plink>) [11], and Haploview 4.1 (<http://www.broadinstitute.org/haploview/haploview>).

Results

There was no significant difference in the selected characteristics between cases and controls in the discovery and extension phases ($p > 0.05$). In the total population, a significantly greater number of case patients were sero-positive for CagA and VacA (Table 1).

In the single SNP analysis for genes involved in the ODC pathway and gastric cancer in the discovery phase, there were no significant SNPs associated with gastric cancer ($p > 0.05$) (Table S1). Four phytoestrogen biomarkers were measured in a total of 823 subjects (406

cases and 417 controls). The overall serum concentrations of genistein, daidzein, and enterolactone in case patients were significantly lower than in controls ($p < 0.001$), whereas the serum concentration of equol did not reach statistical significance ($p = 0.0977$) (Table S2).

Table 2 shows minor allele frequencies in the discovery phase, extension phase, and pooled analyses and gastric cancer risk of four SNPs in three genes in the ODC pathway that were reanalyzed in the extension phase. The minor allele frequencies were similar in the discovery and extension phases. *NQO1* rs1800566 was found to be significantly associated with a decreased risk of gastric cancer in the pooled analysis and meta-analysis [OR 0.83 (95 % CI 0.70–0.995), $p = 0.047$, and OR 0.83 (95 % CI 0.69–1.00), $p = 0.049$, respectively] (Table 2).

The gene–environment interaction analysis in the discovery phase showed an interaction effect between SNPs in the ODC pathway and phytoestrogens. We found that $p_{\text{interaction}}$ was significant for the following SNPs and phytoestrogens: genistein/daidzein and *NQO1* rs1800566; genistein/daidzein and *NQO1* rs1437135; daidzein/enterolactone and *AMD1* rs1279599; daidzein/enterolactone and *AMD1* rs7768897; daidzein/enterolactone and *AMD1* rs811921; equol and *OAZ2* rs7403751 (Table 3).

Table 4 shows gene–environment interactions between four SNPs in three genes in the ODC pathway that were reanalyzed in the extension phase and four phytoestrogen concentrations. In the gene–environment pooled analysis, *NQO1* rs1800566 significantly interacted with genistein and daidzein to modify gastric cancer risk

Table 1 Selected characteristics of gastric cancer cases and controls in the gene–environment interaction analysis

	Discovery phase ^{a,f}		Extension phase ^{b,f}		Total		<i>p</i>
	Cases (<i>n</i> = 76) <i>N</i> (%)	Controls (<i>n</i> = 322) <i>N</i> (%)	Cases (<i>n</i> = 386) <i>N</i> (%)	Controls (<i>n</i> = 348) <i>N</i> (%)	Cases (<i>n</i> = 462) <i>N</i> (%)	Controls (<i>n</i> = 670) <i>N</i> (%)	
Age ^c	64.5 (8.6)	62.8 (8.4)	61.5 (10.5)	63.1 (8.4)	62.0 (10.3)	63.0 (8.4)	0.18
Female	20 (26.3)	98 (30.4)	130 (33.7)	110 (31.6)	150 (32.5)	208 (31.0)	0.91
<i>Helicobacter pylori</i> infection positive	64 (84.2)	271 (84.2)	342 (88.6)	299 (85.9)	406 (87.9)	570 (85.0)	0.38
CagA positive	65 (85.5)	273 (84.8)	355 (92.0)	308 (88.5)	420 (90.9)	581 (86.7)	0.06
VacA positive	44 (57.9)	171 (53.1)	271 (70.2)	233 (67.0)	315 (68.2)	404 (60.6)	0.01
Ever smoker ^d	52 (68.4)	183 (56.8)	238 (61.7)	191 (54.9)	290 (62.8)	374 (55.8)	0.11
Ever drinker ^e	46 (60.5)	184 (57.1)	239 (62.1)	206 (59.2)	285 (61.8)	390 (58.2)	0.20
Gastric ulcer history	8 (10.5)	25 (7.8)	59 (17.6)	50 (18.7)	67 (17.8)	75 (16.8)	0.51

^a Merged data from genetic study results from the discovery phase and phytoestrogen measurement experiments conducted in 2008

^b Merged data from genetic study results from the discovery and extension phases and phytoestrogen measurement experiments conducted in 2008 and 2011

^c Mean age in years; median age for all cases and controls was 62.9 years. The age ranged from 29 to 85 years.

^d Ever smoker defined as former and current smoker

^e Ever drinker defined as former and current drinker

^f $p > 0.05$ for all selected characteristics in the discovery and extension phases.

Table 2 Association between gastric cancer risk and single-nucleotide polymorphisms (SNP) of genes involved in the ornithine decarboxylase (ODC) pathway in the pooled analysis and the meta-analysis

Gene	Chromosome	SNP	Genotype	Genotype frequency			OR (95 % CI)	
				Discovery phase	Extension phase	Pooled analysis	Pooled analysis ^a	Meta-analysis ^b
<i>AMD1</i>	6	rs1279599	TT vs CC + TC	0.20	0.20	0.20	1.0	1.0
				0.80	0.80	0.80	1.01 (0.84–1.22)	1.00 (0.82–1.22)
		rs7768897	GG vs AA + GA	0.21	0.20	0.21	1.0	1.0
				0.79	0.80	0.80	1.01 (0.84–1.22)	1.02 (0.84–1.25)
<i>OAZ2</i>	15	rs7403751	CC vs GG + CG	0.51	0.50	0.50	1.0	1.0
				0.49	0.50	0.50	1.12 (0.83–1.52)	1.12 (0.81–1.54)
<i>NQO1</i>	16	rs1800566	CC vs TT + CT	0.13	0.13	0.13	1.0	1.0
				0.87	0.87	0.87	0.83 (0.70–0.995)	0.83 (0.69–1.00)

Bold values indicate $p < 0.05$

CI confidence interval, OR odds ratio

^a Adjusted for age, smoking status, CagA seropositivity, and *H. pylori* in the dominant model.

^c No heterogeneity (Cochran Q test, $p_{\text{heterogeneity}} > 0.05$)

Table 3 Selected SNPs of the genes involved in the ODC pathway interacting with phytoestrogens to modify gastric cancer risk in 67 gastric cancer cases and 209 controls (discovery scan)

Phytoestrogen	Gene	rs number	Risk allele	Low level ^a		High level ^a		$P_{\text{interaction}}$
				p^b	OR (95 % CI) ^c	p^b	OR (95 % CI) ^c	
Genistein	<i>AMD1</i>	rs1279599	T	0.21	1.94 (0.81–4.64)	0.39	1.42 (0.59–3.43)	0.77
		rs7768897	G	0.17	1.98 (0.83–4.70)	0.39	1.42 (0.59–3.43)	0.74
		rs811921	T	0.17	1.99 (0.83–4.70)	0.39	1.42 (0.59–3.43)	0.74
	<i>OAZ2</i>	rs7403751	C	0.03	5.32 (1.60–17.7)	0.80	0.99 (0.23–4.23)	0.11
	<i>NQO1</i>	rs1800566	T	0.19	1.53 (0.57–4.11)	0.03	0.36 (0.15–0.90)	0.04
		rs1437135	G	0.19	1.53 (0.57–4.11)	0.03	0.36 (0.15–0.90)	0.04
Daidzein	<i>AMD1</i>	rs1279599	T	0.36	1.79 (0.74–4.35)	0.06	0.21 (0.14–1.03)	0.03
		rs7768897	G	0.36	1.79 (0.74–4.35)	0.06	0.22 (0.25–1.03)	0.03
		rs811921	T	0.36	1.79 (0.74–4.35)	0.06	0.22 (0.25–1.03)	0.03
	<i>OAZ2</i>	rs7403751	C	0.05	6.26 (1.66–23.63)	0.44	1.33 (0.36–4.96)	0.18
	<i>NQO1</i>	rs1800566	T	0.06	2.28 (0.82–6.30)	<0.01	0.26 (0.10–0.64)	<0.01
		rs1437135	G	0.06	2.28 (0.82–6.30)	<0.01	0.26 (0.10–0.64)	<0.01
Equol	<i>AMD1</i>	rs1279599	T	0.26	1.67 (0.66–4.25)	0.35	1.41 (0.61–3.29)	0.88
		rs7768897	G	0.26	1.67 (0.66–4.25)	0.30	1.46 (0.63–3.39)	0.90
		rs811921	T	0.26	1.67 (0.66–4.25)	0.30	1.46 (0.63–3.39)	0.90
	<i>OAZ2</i>	rs7403751	C	<0.01	6.49 (1.78–23.70)	1.00	0.92 (0.21–3.97)	0.04
	<i>NQO1</i>	rs1800566	T	0.54	1.37 (0.50–3.77)	0.21	0.42 (0.17–1.04)	0.12
		rs1437135	G	0.54	1.37 (0.50–3.77)	0.21	0.42 (0.17–1.04)	0.12
Enterolactone	<i>AMD1</i>	rs1279599	T	0.01	3.13 (1.29–7.61)	0.68	0.85 (0.35–2.08)	0.03
		rs7768897	G	0.01	3.22 (1.33–7.80)	0.68	0.85 (0.35–2.08)	0.03
		rs811921	T	0.01	3.22 (1.33–7.80)	0.68	0.85 (0.35–2.08)	0.03
	<i>OAZ2</i>	rs7403751	C	0.40	2.06 (0.59–7.17)	0.06	0.24 (0.07–0.89)	0.57
	<i>NQO1</i>	rs1800566	T	0.90	0.91 (0.37–2.26)	0.47	0.53 (0.21–1.34)	0.52
		rs1437135	G	0.90	0.91 (0.37–2.26)	0.47	0.53 (0.21–1.34)	0.52

Bold values indicate $p < 0.05$

^a According to the median blood phytoestrogen concentration among controls (low concentration, below the median; high concentration, the median or above). The median concentration of each phytoestrogen was as follows: 225.85 nmol/L for genistein, 134.20 nmol/L for daidzein, 37.19 nmol/L for equol, and 54.20 nmol/L for enterolactone.

^b Wald p value in the dominant model

^c Adjusted for age, CagA seropositivity, *H. pylori*, and smoking status in the dominant model.

Table 4 Pooled analysis (406 gastric cancer cases and 417 controls) for the interaction between selected SNPs of genes involved in the ODC pathway and phytoestrogen concentrations to modify gastric

cancer risk in a nested case–control study within the Korean Multi-center Cancer Cohort

Phytoestrogen	Gene	rs number	Risk allele	Low level ^a OR (95 % CI) ^b	High level ^a OR (95 % CI) ^c	<i>P</i> _{interaction}
Genistein	<i>AMD1</i>	rs1279599	T	1.27 (0.84–1.93)	0.97 (0.75–1.26)	0.32
		rs7768897	G	0.31 (0.87–1.97)	0.95 (0.74–1.24)	0.23
	<i>OAZ2</i>	rs7403751	C	1.13 (0.61–2.09)	0.72 (0.46–1.12)	0.25
	<i>NQO1</i>	rs1800566	T	1.46 (0.98–2.19)	0.68 (0.53–0.88)	0.002
Daidzein	<i>AMD1</i>	rs1279599	T	1.59 (1.08–2.32)	0.84 (0.64–1.11)	0.006
		rs7768897	G	1.59 (1.08–2.32)	0.84 (0.64–1.11)	0.006
	<i>OAZ2</i>	rs7403751	C	1.52 (0.86–2.68)	0.58 (0.36–0.92)	0.008
	<i>NQO1</i>	rs1800566	T	1.19 (0.83–1.72)	0.73 (0.56–0.96)	0.005

Merged data of genetic study results from discovery and extension phases and phytoestrogen experiments conducted in 2008 and 2011

Bold values indicate $p < 0.05$ ^a The selected SNPs were also replicated with significant association with gastric cancer in the extension phase.^b According to the median blood phytoestrogen concentration among controls (low concentration, below the median; high concentration, the median or above). The median concentration of each phytoestrogen was as follows: 225.85 nmol/L for genistein, 134.20 nmol/L for daidzein, 37.19 nmol/L for equol, and 54.20 nmol/L for enterolactone^c Adjusted for age, CagA seropositivity, *H. pylori*, and smoking status in the dominant model

($p_{\text{interaction}} = 0.002$ and 0.005 , respectively). The gastric cancer risk of *NQO1* rs1800566 changed according to the genistein and daidzein concentrations: the T allele of *NQO1* rs1800566 was associated with a reduced risk of gastric cancer at high genistein and daidzein levels [OR 0.68 (95 % CI 0.53–0.88) and OR 0.73 (95 % CI 0.56–0.96), respectively], whereas it was nonsignificantly associated with an increased risk of gastric cancer at low genistein levels [OR 1.46 (95 % CI 0.98–2.19)] and was not associated with an increased risk of gastric cancer at low daidzein levels [OR 1.19 (95 % CI 0.83–1.72)]. Major alleles of *AMD1* rs1279599, *AMD1* rs7768897, and *OAZ2* rs7403751 had significant gene–daidzein interaction effects to modify gastric cancer risk ($p_{\text{interaction}} = 0.006$, 0.006 , and 0.008 , respectively). At high daidzein concentration, *OAZ2* rs7403751 showed a significantly decreased gastric cancer risk [OR 0.58 (95 % CI 0.36–0.92)]. At low daidzein levels, both *AMD1* rs1279599 and *AMD1* rs7768897 had significant increased risks [both ORs 1.59 (95 % CIs 1.08–2.32)], whereas at high daidzein levels, no associations were observed [both ORs 0.84 (95 % CIs 0.64–1.11)] (Table 4).

Discussion

NQO1 rs1800566 showed a significant association with gastric cancer risk, and an interaction with genistein and daidzein could modify gastric cancer risk. Gene–environment analysis demonstrated significant interaction effects between genes controlling ODC such as *NQO1*,

AMD1, and *OAZ2*, and isoflavones such as genistein and daidzein.

The ODC–polyamine biosynthesis pathway is illustrated in Fig. 1. *H. pylori* stimulates ODC in host macrophages and arginase II. ODC metabolizes L-ornithine to polyamines (spermine, spermidine, putrescine). The presence of *H. pylori* induces macrophage iNOS for host immunity activation, whereas spermine inhibits expression of *NOS2A* (encoding iNOS). Then iNOS converts L-arginine into citrulline and NO, where the latter possess antimicrobial and proinflammatory properties [12], leading to killing of *H. pylori*. Spermine to spermidine back-conversion by spermine oxidase, also induced by the presence of *H. pylori* [13], produces aldehyde (3-aminopropanal) and H₂O₂ [14]. Both by-products play a role in gastric carcinogenesis through DNA, RNA, protein, and membrane damage caused by reactive oxygen radicals [15]. Polyamine synthesis is stimulated by ODC and AMD [3, 4]. ODC is controlled by *NQO1* and ornithine decarboxylase antizyme 1 (*OAZ1*), and polyamines stimulate *OAZ1*. *NQO1* induces ODC by binding to and stabilizing ODC [16], whereas *OAZ1* inactivates ODC by degradation [17]. The sequential processes in the ODC–polyamine pathway can be regulated by their host genes, thereby affecting cancer susceptibility by altering expression or function of enzymes to modulate polyamine synthesis.

NQO1 is a member of the NAD(P)H dehydrogenase (quinone) family that reduces quinones to hydroquinones, and participates in reducing the levels of free radicals. It binds to and stabilizes ODC, which attenuates the immune response through elevated levels of polyamines [16]. In this

study, *NQO1* rs1800566 alone showed an association with gastric cancer risk, and an interaction with genistein and daidzein modified gastric cancer risk according to isoflavone concentration. Previous studies support our results. Ikeda et al. [18] reported the T allele of *NQO1* rs1800566 was associated with a reduced risk of gastric cancer, although Hamajima et al. [19] did not find a statistical significance with gastric cancer risk. *NQO1* rs1800566 is a functional SNP in *NQO1* [C609T (Pro187Ser)]. The T allele has null enzyme activity, whereas the C allele has full enzyme activity [20], and the enzyme activity of the CT genotype decreases to approximately one third of that of the wild genotype (CC) [21, 22]. In our study, the T allele of *NQO1* rs1800566 was associated with reduced risk of gastric cancer. On the basis of previous studies, the *NQO1* T allele encodes the null *NQO1* enzyme activity, which is reduced in the ODC pathway, and thus is involved in decreasing polyamine synthesis. The decrease in polyamine levels does not inhibit iNOS function to produce NO. Thus, the *NQO1* T allele could be indirectly related to less persistent *H. pylori* infection. Another study indirectly supports our results: Goto et al. [23] showed the CC genotype of the same *NQO1* gene variant was associated with *H. pylori* seropositivity. Therefore, our result that the *NQO1* rs1800566 T allele is associated with low risk of gastric cancer is biologically plausible.

Our study showed an interaction effect between *NQO1* rs1800566 and isoflavones (genistein and daidzein) to modify gastric cancer risk. An in vivo study supports our interaction result. Wiegand et al. [24] reported that genistein significantly increased activity levels of *NQO1*. Although the need for *NQO1* at high isoflavone concentration was increased, the *NQO1* rs1800566 T allele encoded low activity of *NQO1*, and *NQO1* encoded by the T allele reduces the function of ODC, leading to decreased polyamine synthesis. Moreover, isoflavone is directly involved in decreasing ODC activity [7]. ODC inhibition by isoflavone alone and low *NQO1* enzymatic activity caused by the rs1800566 T allele can result in decreased polyamine synthesis. Therefore, interaction between *NQO1* rs1800566 and isoflavone could modify gastric cancer risk.

Other mechanisms to control polyamine synthesis are by *OAZ1* and *AMD1*. Although it is not known whether isoflavones control *OAZ1* and *AMD1*, it has been hypothesized that if isoflavones stimulate *OAZ1*, and the *OAZ1* C allele is a high-activity allele, the amount of highly active *OAZ1* increases, leading to degradation of ODC and decreased polyamine synthesis. In contrast, if isoflavones induce *AMD1* and the major allele of *AMD1* is related to low enzyme activity, the low-activity *AMD1* could decrease polyamine synthesis. Future investigations should assess whether the interaction with isoflavones observed for gastric cancer could be explained by biological

interaction between *AMD1* and *OAZ1* and isoflavones and should study functional *AMD1* and *OAZ1* SNPs.

Limitations should be noted. In the extension phase, hospital and community-based cases were matched with community-based controls, which may introduce bias. However, information bias is minimized since people are born with their genes and changes in genes are not common. Although this was a two-phase study that aimed to increase the power of the study, we were not able to stratify the study with regard to important factors such as type (cardiac and noncardiac) and variants (intestinal and diffuse). Also, selection bias was minimized because cases were matched with controls according to important risk factors in the initial study design stage.

The study is a two-phase population-based nested case-control genetic association study that is free of many biases common in retrospective designs. Confounding factors were adjusted for in the multivariate models. In the gene-environment analysis, phytoestrogen biomarkers were measured from blood samples, which are less subject to recall bias compared with food frequency questionnaires.

In conclusion, we report that *NQO1* rs1800566 involved in the ODC pathway can be a genetic susceptibility factor for gastric cancer. Genes involved in the ODC pathway (*NQO1*, *AMD1*, and *OAZ1*) and isoflavones (genistein and daidzein) showed a significant interaction to modify gastric cancer risk. Replication and validation genetic studies with a greater number of subjects conducted in other ethnic populations in addition to in-depth in vitro and animal studies on the real metabolic function and cellular events of the gene variants in gastric cancer tissue will elucidate and further clarify the biological pathways, causal mechanisms, and genetic and environmental individual and interactive effects involved in gastric carcinogenesis.

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Conflict of interest The authors declare no competing interests.

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