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Polymorphic variation of CYP1A1 is associated with the risk of gastric cardia cancer: a prospective case–cohort study of cytochrome P-450 1A1 and GST enzymes

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Abstract

Objective: To determine if genetic polymorphisms of CYP1A1, GSTM1, GSTP1, or GSTT1 are associated with an increased risk of developing esophageal squamous cell carcinoma (ESCC), gastric cardia cancer (GCC), or either in a high-risk Asian population.
Methods: We conducted a case-cohort analysis with 5 years of prospective follow-up. The analytical cohort contained 642 individuals who participated in either the Dysplasia Trial (DT) or the General Population (GPT) of the Nutrition Intervention Trials conducted in Linxian, China, and included 131 cases of ESCC and 90 cases of GCC. Genotyping analysis was performed on DNA extracted from red blood cells using a PureGene kit (Gentra Systems, Inc., Minneapolis, MN) and real-time PCR analysis amplification (Taq-Man). Relative risks and 95% confidence intervals were estimated using the case – cohort estimator for the Cox proportional hazards models. *p*-values from nested models with genotyping variables came from score tests.
Results: The relative risks for developing ESCC, GCC, or either cancer were calculated in the entire analytic cohort for GSTM1, P1*B (*A*313*G*), and T1 and CYP1A1*2A (*T*3801*C*) and *2C (*A*2455*G*) genotypes, and no significant associations were identified. However, because of the difference in cancer risks between the DT (9.3 cases per 1000 person years) and the GPT (5.3 cases), the analytical cohort was stratified by trial; the DT participants who were heterozygous or homozygous for the variant-allele at CYP1A1*2A had a reduced risk for developing GCC (adjusted RR (95%CI) 0.47 (0.23–1.00) $p = 0.037$).
Conclusions: This study found an association for the CYP1A1*2A variant allele and a reduced risk of GCC in people at high risk for development of this disease. This finding is consistent with previous studies suggesting that substrates for the cytochrome P-450 1A1 metabolic pathway, such as polycyclic aromatic hydrocarbons, may be etiologically significant in this high-risk region.

Introduction

Regional differences in esophageal squamous cell carcinoma rates may, in part, result from a combination of inherited genetic factors and environmental influences such as carcinogen exposure [1–6]. This hypothesis is supported by studies of the association between cancer risk and polymorphisms in genes that metabolize known and potentially carcinogenic environmental exposures [7–12]. Polymorphisms in these genes affect enzymatic activities and may alter an individual's ability to metabolize pro-carcinogenic and related compounds, which may change the biologic effect of a given exposure [13].

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Linxian, China, currently known as Linzhou, has some of the highest rates of esophageal squamous cell carcinoma (ESCC) and gastric cardia adenocarcinoma (GCC) with a combined incidence of approximately 100 per 10^5 person years [1, 2]. Recent studies have suggested that environmental exposure to carcinogenic polycyclic aromatic hydrocarbons (PAHs) may be etiologically related to these high rates. Supporting this possibility are the finding of histologic changes in esophageal cancer resections consistent with high exposure to PAH associated air pollution [14]; high concentrations of benzo(a)pyrene (B(a)P), a carcinogenic PAH, in local staple foods [15]; high concentrations of 1-hydroxypyrene glucuronide, a PAH metabolite in the urine of local residents [16]; and the extensive use of domestic coal-burning stoves from which a variety of PAHs in soot extracts were recovered [17].

The cytochrome P-450 and glutathione-S-transferase enzymes metabolize PAHs formed during the combustion of organic material and, thus, are among the pathways with the greatest potential to modify the effect of PAH exposure [18]. Cytochrome P-450 1A1 is the Phase-I enzyme that is most important in metabolically activating B(a)P and other PAHs, and glutathione-S-transferase M1 (GSTM1) and glutathione-S-transferase P1 (GSTP1), are the most important enzymes involved in detoxifying reactive PAH metabolites [13, 19, 20].

The current study was conducted to determine if genetic polymorphisms in CYP1A1, GSTM1, GSTP1, or GSTT1 alone, or in combination, are associated with an increased risk of developing ESCC, GCC, or either in the high-risk population of Linxian, China. The study's analytical cohort represents a sub-cohort of the Linxian Nutrition Intervention Trials (NIT), including both the Dysplasia Trial (DT) and the General Population Trial (GPT) [21]. Both trials were randomized, double-blind, placebo-controlled trials which tested the effects of vitamin/mineral supplements on the rates of ESCC and GCC in Linxian. DT participants (n = 3318) had a pretrial balloon cytology diagnosis of dysplasia, a neoplastic precursor lesion for the development of ESCC [22, 23], whereas GPT participants (n = 29,584) had no cytologic evidence of dysplasia (10%) or were not tested [21]. We analyzed the most common polymorphic variants of CYP1A1, GSTM1, GSTP1 and GSTT1.

Material and methods

A detailed description of the methods and results of the DT and GPT Trials has been reported [21, 24, 25]. In May 1991, at the conclusion of the interventions, whole blood was collected from approximately 6000 NIT participants. We successfully extracted ≥ 1.5 μg DNA from RBCs of 4005 subjects and these were considered eligible for participation in the current study. A stratified case-cohort design [26–28] was used to select individuals for inclusion from the cohort of all eligible participants. All eligible incident cases of ESCC (n = 131) and GCC (n = 90) that occurred between May 1991 and April 1996 were included in the study. During the study period, mortality from all causes and incidence of all cancers were ascertained by monthly contact with village health workers, with little or no loss to follow up. Diagnostic materials for cancers, including X-ray films and cytological, pathological and surgical specimens, were collected from local hospitals and from a study medical team that provided clinical and diagnostic services, and cancers were confirmed by a committee of international experts [21]. An age- and sex-stratified random sample of all eligible trial participants irrespective of case status was selected to serve as the reference control group termed the sub-cohort (n = 454). The six strata included both sexes and three age categories (defined as age at the start of the trial intervention), < 50, 50–59, and > 60. The within-strata ratios of control subjects to case subjects for the incident site-specific cancers ranged from 2.3 to 5.8 for ESCC, 3.7–7.0 for GCC, and 1.6–2.5 for the combined endpoint (ESCC or GCC).

Genotyping analysis was performed at a commercial laboratory (BioServe Biotechnologies Ltd., Laurel, MD), using a PureGene kit (Gentra Systems Inc., Minneapolis, MN) and real-time PCR analysis amplification (Taq-Man; PE Biosystems, Foster City, CA) on DNA extracted from frozen blood and the CYP1A1, GSTM1, GSTT1, and GSTP1 genotyping was successful in at least 90% of the cases (Table 1). PCR primers and dual-labeled allele discrimination probes were designed using Primer Express software (version 1.5; Perkin-Elmer). All laboratory personnel were blinded to case–control status. A blinded repeat genotyping of 10% of the DNA samples yielded 100% concordance for all four genes. Both GSTM1 and GSTT1 were coded as having the gene either absent or present, while GSTP1 was coded as homozygous for the common allele, heterozygous, or homozygous variant. Because of the reported altered functionality for the B variant allele [29, 30] and the small number of individuals possessing the variant allele, the GSTP1 analysis compared homozygous for the common allele *versus* the combination of heterozygotes and homozygous variants. The relatively rare GSTP1*C and *D alleles were not evaluated.

Table 1. Genotype polymorphisms with variant alleles

Allele	Nucleotide changes	Effect
CYP1A1*1A	None	No change
CYP1A1*2A	3801T > C (*Msp1*)	No change
CYP1A1*2C	2455A > G	I462V
CYP1A1*3	3205T > C	No change
GSTP1*A	None	No change
GSTP1*B	313A > G	I105 V

Portions adapted from the human cytochrome P450 (CYP) allele nomenclature committee [http://www.imm.ki.se/CYPalleles/].

Table 2. Linzhou NIT case–cohort subject characteristics

	Subcohort	Esophageal cancer	Gastric cardia cancer
Number	454	131	90
Age (year (SD))	58.8 (7.7)	57.4 (6.9)	60.4 (6.8)
Male (%)	55.5	50.4	41.1
Smoking (%)	39.3	36.6	47.8
Drinking (%)	26.4	22.1	23.3
GP trial (%)	63.7	41.2	46.7
Follow-up time (year (SD))	4.6 (1.0)	3.2 (1.6)	3.0 (1.7)

Covariate definitions

Smoking was defined as a dichotomous variable, never *versus* ever smoking for ≥6 months. Drinking was defined as a dichotomous variable, none *versus* any drinking in the previous 12 months. Follow-up time was defined as days from May 1, 1991 to incident cancer or April 30, 1996.

Statistical analysis

Pearson correlation coefficients, Tajima's D-prime, *t*-test and and chi-square tests for association were calculated and Hardy-Weinberg equilibrium was tested with the chi-square in the subcohort. All *p*-values were 2-sided tests.

When analyzing cancers at a specific site we treated persons with cancers at other sites as censored at the time of cancer occurrence. We estimated relative risks (RR) and 95% confidence intervals (CI) using the case-cohort estimator for the Cox proportional hazards models [26–28, Preston Epicure 1998 Computer Program]. All estimates came from models stratified on the six sex-age sampling strata. Additional stratum-specific age terms for continuous age were used to adjust for variation within age strata. *p*-values from nested models with genotyping variables came from score tests. We tested the proportional hazards assumption for each main effect (genotype) with each of the three case definitions (esophagus, gastric cardia, or combined) using a time-dependent covariate (genotype*follow-up time). This test was non-significant ($p > 0.05$) in all cases.

The statistical analyses include the following: (1) tabulation of subject personal characteristics (Table 2), (2) tabulation of genotype frequencies by case status (Table 3), (3) correlation of genotypes with other covariates, (4) calculation of 'crude' and adjusted relative risks and 95% confidence intervals for esophageal, gastric cardia, and combined cancer (Table 4).

For some genotype-cancer site comparisons we tested whether the cancer RRs varied by the covariates age, sex, tobacco use, alcohol use (*i.e.*, interactions). We did this by comparing a model with the main effect of a covariate (*e.g.*, sex) and a single risk parameter for the analyte to a model with the main effect term for the covariate and separate risk parameters for each subgroup (*e.g.*, females and males) defined by the covariate. Since all models were stratified on sex and age, there was no main effect term when testing and estimating a sex interaction. Cases and the entire subcohort were included in analyses stratified by trial.

A model was also fit with variables for all three GST genotypes simultaneously, with variables for the two

Table 3. Percent of each genotype by case status

	Subcohort			Esophageal cancer			Gastric cardia cancer		
	Present	Absent		Present	Absent		Present	Absent	
GST M1	68.0	32.0		68.3	31.2		73.4	26.1	
GST T1	46.4	53.6		41.5	58.5		52.3	47.7	
	Wt[b]	Ht[b]	Hv[b]	Wt	Ht	Hv	Wt	Ht	Hv
GST P	62.4	31.2	6.3	65.4	29.2	5.4	62.2	30.0	7.8
Cyp 1A1*2A	34.3	48.5	17.2	38.8	42.2	19.0	41.8	39.2	19.0
Cyp 1A1*2C	59.3	33.0	7.7	61.5	32.0	6.6	59.3	34.6	6.2

[a] The Hardy-Weinberg equilibrium assumption was tested for each genotype using a chi-square test with the known stratum-specific sampling weights. *p*-values were as follows GST *p*: 0.062, Cyp 1A1*2A = 0.269, Cyp 1A1*2C = 0.040.

[b] Wt: Homozygous common allele; Ht: Heterozygous; Hv: Homozygous variant.

Table 4. Association between genotype and esophageal squamous cell carcinoma (ESCC) and gastric cardia adenocarcinoma (GCC) for the entire NIT sub-cohort, General Population Trial (GPT) and the Dysplasia Trial (DT) in Linzhou, China

		All cases[c]	p[b]	GPT[c]	p[b]	DT[c]	p[b]
GST M1	RR[a]						
	ESCC	1.15 (0.73–1.81)	0.48	0.79 (0.41–1.53)	0.39	1.39 (0.72–2.69)	0.29
	GCC	0.83 (0.49–1.40)	0.45	0.72 (0.34–1.51)	0.36	0.86 (0.39–1.88)	0.63
GST P	RR[a]						
	ESCC	0.93 (0.60–1.42)	0.61	1.28 (0.70–2.32)	0.40	0.68 (0.36–1.29)	0.23
	GCC	0.98 (0.61–1.58)	0.80	1.04 (0.53–2.07)	0.81	0.94 (0.47–1.89)	0.82
GST T1	RR[a]						
	ESCC	1.43 (0.93–2.19)	0.094	1.17 (0.63–2.19)	0.47	1.55 (0.86–2.82)	0.13
	GCC	0.80 (0.50–1.28)	0.33	0.93 (0.47–1.84)	0.71	0.72 (0.37–1.41)	0.31
CYP1A1*2A	RR[a]						
	ESCC	0.92 (0.59–1.43)	0.38	0.64 (0.35–1.19)	0.13	1.23 (0.66–2.30)	0.47
	GCC	0.69 (0.41–1.15)	0.13	1.05 (0.48–2.26)	0.47	0.48 (0.23–1.00)	0.037
CYP1A1*2C	RR[a]						
	ESCC	0.98 (0.64–1.51)	0.45	0.71 (0.37–1.36)	0.22	1.34 (0.73–2.47)	0.25
	GCC	1.02 (0.62–1.67)	0.73	1.70 (0.85–3.41)	0.11	0.56 (0.26–1.19)	0.12

[a] Relative risks and 95%CI calculated using a Cox model stratified on sex and adjusted for continuous age, smoking, drinking, and trial.
[b] p-values are from score tests for the addition of the main effect term to the base model.
[c] Range of cases available for analysis after exclusion for missing PCR or covariate data, All ESCC cases: n = 122–131, GPT n = 49–54, DT n = 69–76; All GCC cases: n = 81–90, GPT n = 37–42, DT n = 42–48.

CYP1A1 genotypes, or with all five variables simultaneously.

Results

The analytical cohort consisted of 642 individuals, including 347 (54%) males and 295 (46%) females. 275 (43%) participated in the DT and 367 (57%) participated in the GPT.

The 131 cases of ESCC were similar in age, gender, smoking and drinking exposure to the sub-cohort (Table 2). The 90 cases of GCC included more females and more smokers than the other groups. Both the ESCC and GCC cases were more likely than the sub-cohort members to be in the DT, a reflection of the higher rate of cancer in the DT participants (ESCC DT 47% (77/165) *versus* GPT 19% (54/289)); GCC 29% (48/165) *versus* 14% (42/289)). The subcohort was comprised of 64% GPT participants and 36% DT participants.

Genotype analysis showed that the most common genotypes in the sub-cohort were GSTM1*1 (68%), GSTT1*0 (54%), GSTP1 homozygous for the common allele (62%), CYP1A1*2A heterozygotes (48%) and CYP1A1*2C homozygous for the common allele (59%) (Table 3). In addition, a test for Hardy-Weinberg Equilibrium showed CYP1A1*2C to be marginally not in allelic equilibrium ($p = 0.040$) due to under-representation of the variant allele. This population was not polymorphic for CYP1A1*3; consequently, further analysis of this genotype was not conducted.

Association between genotypes and with covariates of age, sex, smoking, drinking, and trial were examined and an association between the CYP1A1*2A and *2C genotypes was identified ($D = 0.64$). No other strong associations were identified.

The crude and adjusted relative risks for developing ESCC, GCC, or either cancer were calculated in the entire analytic cohort for each of the GSTM1, P1, T1 and CYP1A1*2A and *2C genotypes and no significant associations were identified. Because of the different risks for cancer in the two trials (Cancers per 1000 person-years, DT 9.3 *versus* GPT 5.3; (24,25)) we stratified our analytic cohort by trial. In the lower risk GPT participants, we found no significant associations between cancer risk and genotype. In the higher risk DT, however, the participants who were heterozygous or homozygous for the variant-type allele at CYP1A1*2A appeared to have a reduced risk of developing GCC (adjusted RR (95%CI) 0.47 (0.23-1.00) $p = 0.037$, Table 4). No other significant genotype associations between ESCC risk or risk of the combined cancer variable were found in subjects from either trial.

A model with variables for all three GST genotypes simultaneously was fit and produced estimates for each variable that were similar to the estimates produced by the simpler models. The same result occurred when fitting the two CYP1A1 variables or when fitting all 5 genotype variables simultaneously (data not shown).

Discussion

We performed a case-cohort study to determine whether genetic polymorphisms of CYP1A1, GSTM1, GSTP1, or GSTT1 were associated with an increased risk of developing ESCC, GCC, or either in a high-risk Asian population during 5 years of prospective follow-up. This is the first prospective study of P-450 related genetic polymorphisms and cancer of the upper aerodigestive tract in this high-risk region. These polymorphic enzymes were selected based on evidence that environmental exposures such as PAHs that are metabolized by the P-450 1A1 pathway and GST enzymes may contribute to carcinogenesis of upper gastrointestinal cancers in this high-risk region [14–17].

Differences in enzymatic activity between various genotypes are seen in biomarkers of exposure and may help explain why, for a given exposure, some individuals have an increased risk for developing cancers of the upper aerodigestive tract [19, 31, 32]. For instance, the number of PAH-DNA adducts formed in the bronchial tissues of smokers lacking GSTM1 (*i.e.*, GSTM1*0) can be up to 100-fold higher than smokers possessing GSTM1 (GSTM1*1) [33]. In addition, studies of GSTP1 allelotypes find isoenzymes with a valine in position 105 (GSTP1*B) to more effectively metabolize the diol epoxides of polycyclic aromatic hydrocarbons and those with a A114V transition (GSTP1*C) in the presence of V105 to more efficiently metabolize benzo(a)pyrene [29, 30]. GSTT1 is also in the metabolic pathway for several PAHs [20]. This is illustrated by the association of GSTT1*1 with increased levels of excreted 1-hydroxyprene glucuronide [34] in addition to an inverse association between erythrocytic activity and DNA adducts in mononuclear leukocytes [35].

The current study results show an association between CYP1A1*2A genotype and risk of GCC among the higher-risk participants of the Linxian Dysplasia Trial (adjusted RR (95%CI) 0.47 (0.23–1.00)). This finding is consistent with *in vitro* studies that identify differences among the catalytic activity of the CYP1A1 variants, with the highest activity identified for the wild-type enzyme, followed by CYP1A1*4 (T461N) (approx. 60%) and CYP1A1*2 (I462V) (approx. 40%) [36–39]. However, our results show no effect of the polymorphisms measured here on ESCC risk or on GCC in the total analytic group.

This prospective study's negative GSTM1 results contrast with those from a case-control study conducted in the same population by Tan *et al.* [40]. Tan *et al.* found the GSTM1 non-null (+/+ and +/0) genotype to be over-represented in cancer cases. However, similar to our results, Tan did not identify an association between GSTT1 and GSTP1 genotypic frequencies and esophageal cancer. In addition, in a small cross-sectional study of asymptomatic Linxian inhabitants with a biopsy-proven diagnosis of mild or moderate squamous dysplasia, we found that individuals with a GSTM1*0 genotype had a tendency for an increased risk of esophageal squamous dysplasia [12]. Potential explanations for the lack of an association between GSTM1 and cancer in the current study include the fact that not all dysplastic foci progress to cancer and the finite number of cases identified during the follow-up period [41].

The strength of this study's findings stem from its incorporation of many of the prerequisites considered necessary for an informative analysis of genetic polymorphisms as set forth by Bartsch *et al.* [19]. These include a clear definition of the representative study population and controls, avoidance of confounding created by the use of study subjects of mixed ethnic background, and the inclusion of gene polymorphisms shown to lead to altered phenotypic expressions. In addition, the current study's potential for selection bias is limited by its prospective design and subsequent use of incident rather than prevalent cases.

However, as with any study, these results must be considered in the context of potential design related weaknesses. In the current setting, these include the limited number of cases identified during the follow-up period and the inability to assess cytochrome P-450 and GST related factors, other than genotype, that may modify an individual's risk of disease. For example, possessing an inducible form of the CYP1A1 enzyme can result in an increase in the biologically effective dose for a given exposure and has been associated with an increased risk for bronchial, laryngeal, and oral cavity tumors in smokers [19, 31]. Similarly, some exogenous chemicals (*e.g.*, NSAIDS, some pesticides) can induce and locally increase the expression of GSTs [20, 42, 43] and this induction may also affect an individual's risk [44, 45]. This inducibility underlies the importance of quantifying the relevant exposures and is exemplified by Lan *et al.* [46] population based case – control study of lung cancer in a coal using, high-risk lung cancer region in China in which a 2.4-fold increased risk was found only among the heavy coal users (>130 tons) with the GSTM1*0 genotype. The potential significance of such characterization in Linxian, given its extensive use of coal for heating and cooking, is that identification of the heavy coal users in Linxian may be necessary to identify a genotype associated with elevation in risk. Yet, the current study, and the original NIT upon which it is based, were not designed to exhaustively characterize amount (dose) and length (duration) of potentially significant exposures, and, therefore, is unable to

accurately evaluate these potential factors for heterogeneity in its assessment of risk. It is possible that people at highest risk, as reflected in a cytology finding of dysplasia, may be the most exposed to PAHs, but this remains to be shown.

In summary, there continues to be considerable interest in the interaction between polymorphisms of xenobiotic metabolizing enzymes and exposure to environmental carcinogens and their effect on disease risk. For those hoping to lessen the morbidity and mortality of high-risk populations this interest stems from the promise of successfully identifying etiologic factors so that they can be avoided and identifying individuals at increased risk for disease. The current study represents the first prospective examination of the association between esophageal and gastric cardia cancer and polymorphisms in CYP1A1 and GST enzymes in the high-risk population of Linxian. This study finds the CYP1A1*2A variant allele may reduce the risk of GCC in people at highest risk for developing this disease. This is consistent with previous studies that suggest that substrates for the cytochrome P-450 1A1 metabolic pathway, such as PAHs, may be etiologically significant in this high-risk region. Future larger studies including a comprehensive and quantitative environmental analysis in conjunction with a genotypic evaluation are still needed to identify potential variability in the induction of these genes and an individual's level of relevant exposures.

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