RESEARCH ARTICLE

Lack of Associations between Genetic Polymorphisms in GSTM1, GSTT1 and GSTP1 and Pancreatic Cancer Risk: A Multi-Institutional Case-Control Study in Japan

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Abstract

Background: We aimed to evaluate the role of genetic polymorphisms in tobacco carcinogen-metabolizing genes and their interactions with smoking in a hospital-based case-control study of Japanese subjects. Materials and Methods: We examine the associations of pancreatic cancer risk with genetic polymorphisms in GSTM1, GSTT1 and GSTP1, phase II enzymes that catalyze the conjugation of toxic and carcinogenic electrophilic molecules. The study population consisted of 360 patients and 400 control subjects, who were recruited from several medical facilities in Japan. Unconditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between genotypes and pancreatic cancer risk. Results: Among the control subjects, the prevalence of the GSTM1-null genotype and the GSTT1-null genotype was approximately 56% and 48%, respectively. Cases and controls were comparable in terms of GSTM1 and GSTT1 genotype distributions. Neither of the deleted polymorphisms in GSTM1 and GSTT1 was associated with the risk of pancreatic cancer, with an age- and sex-adjusted OR of 0.99 (95%CI: 0.74-1.32) for the GSTM1-null genotype, and 0.98 (95%CI: 0.73-1.31) for the GSTT1-null genotype. The OR was 0.97 (95%CI: 0.64-1.47) for individuals with the GSTM1 and GSTT1-null genotypes compared with those with the GSTM1 and GSTT1-present genotypes. No synergistic effects of smoking or GST genotypes were observed. Conclusions: Our results indicate no overall association between the GSTM1 and GSTT1 deletion polymorphisms and pancreatic cancer risk in the Japanese subjects in our study.

Keywords: GSTM1 - GSTT1 - GSTP1 - pancreatic cancer - risk

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Introduction

The etiology of pancreatic cancer remains largely unknown. Epidemiologic studies have consistently shown positive associations between pancreatic cancer with cigarette smoking and long-standing diabetes (Duell, 2012; Ben et al., 2011). According to a 2008 meta-analysis, current smokers had approximately double the risk of pancreatic cancer relative to nonsmokers (Iodice et al., 2008). Although the exact mechanisms underlying the smoking-pancreatic cancer association remain to be clarified, similar to tobacco-induced cancers, a DNA adduct is thought to play a crucial role in pancreatic carcinogenesis. The accumulation of unrepaired genetic mutations due to tobacco-derived carcinogen-DNA adducts can cause disruption of cell cycle checkpoints

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and chromosomal instability (Hecht, 2008).

The process of carcinogen metabolism involves phase I metabolic activation and phase II detoxification [5], with a variety of enzymes involved in each phase. Cytochrome 450 (CYP1A1) is a phase I enzyme that initiates metabolic activation of carcinogens (Nebert et al., 2006). Glutathione S-transferases (GSTs) are the principal phase II enzymes that catalyze the conjugation of toxic and carcinogenic electrophilic molecules (Hayes et al., 2005). Three common polymorphisms in the GSTM1, GSTT1 and GSTP1 genes have been extensively studied in molecular epidemiologic studies due to their varied effects on enzyme activity (Moyer et al., 2007; Moyer et al., 2008). Variants in the GSTM and GSTT1 genes have attracted the most attention because inherited homozygous deletions of the GSTT1 and GSTM1 genes lead to an absence of enzyme activity, therefore increasing disease susceptibility. For GSTP1, a single nucleotide substitution (A→G) at position 313 of the GSTP1 gene (rs1695) substantially diminishes GSTP1 enzyme activity (Moyer et al., 2006). Loss-of-function (LoF) deletion polymorphisms in the GSTM1 and GSTT1 genes have been linked to an increased risk for many cancers, including head and neck, lung, liver, colon and pancreatic cancers (Geisler et al., 2001; Moore et al., 2005; White et al., 2008; Carlsten et al., 2008; Cote et al., 2009; Jang et al., 2012). Compared with GSTT1 and GSTM1 genetic polymorphisms (Bartsch et al.,1998; Liu et al., 2000; Duell et al., 2002; Jiao et al., 2007; Vrana et al., 2009; Jang et al., 2012), very few studies have studied GSTP1 genetic polymorphisms and their associations with pancreatic cancer risk. Only one previous study reported that GSTP1 polymorphisms were significantly associated with pancreatic cancer survival (Jiao et al., 2007)

Because the frequencies of GST genotypes vary across populations and ethnicities (Di Pietro et al., 2010), large inter-individual differences might exist in the metabolic response to carcinogen exposure. As a result, the risk of pancreatic cancer could be partly determined by these factors. In this study, we examined the associations of pancreatic cancer risk with genetic polymorphisms in GSTM1, GSTT1 and GSTP1 in Japanese subjects, using a hospital-based case-control study.

Materials and Methods

Study subjects

We aimed to clarify the roles of genetic polymorphisms and gene-environment interaction in the development of pancreatic cancer, using data obtained from an ongoing multi-institutional case-control study. The details of our case-control study have been described elsewhere (Lin et al., 2013). Briefly, the eligible cases were patients who were newly diagnosed with pancreatic cancer at five hospitals from April 1, 2010, through May 15, 2012. Imaging modalities and pathologic reports (if available) were used for pancreatic cancer diagnosis. During the same time period, we enrolled control subjects from the following three sources: 1) inpatients and outpatients from the same participating hospitals where the cases were enrolled; 2) relatives of inpatients from the same participating hospitals where the cases were enrolled; and 3) individuals who were undergoing medical checkups at one of the participating hospitals. All of the control subjects who were recruited from among inpatients and outpatients had no prior diagnoses of cancer at the time of enrollment. The diagnoses for control subjects included a variety of diseases, such as anemia, gastric ulcers and irritable bowel syndrome. We achieved a response rate of 85% (441/516) for cases and 98% (525/534) for control subjects as of July 1, 2012. The control subjects were frequency matched to the case patients by sex and age (within 10-year categories). As a result, the data from 360 case patients and 400 control subjects were included in the present analysis.

We obtained written, informed consent from all of the study subjects. The ethical board of Aichi Medical University and all of the participating hospitals approved this study.

Data collection

The study participants completed a self-administered questionnaire covering information on demographic characteristics, medical history and lifestyle factors, such as cigarette smoking, alcohol consumption and dietary intake. Information on cigarette smoking included smoking status (never, former or current smokers), average number of cigarettes smoked per day, age at starting and quitting smoking and duration of smoking. In addition to lifestyle information, a 7-mL venous blood sample was collected from all of the consenting participants.

Genomic DNA was extracted from peripheral lymphocytes in the blood at SRL Hachioji Laboratory and was stored at -30°C until genotyping.

Genotyping assays

All of the genotyping was conducted in the laboratory of Aichi Cancer Center Research Institute in Nagoya, Japan, with the laboratory staff blinded to case or control status. For GSTM1 and GSTT1, the genotyping was performed using the Taqman SNP Genotyping assay. Two quality control samples were included in each assay. The assays were undertaken independently using 30-50 ng of genomic DNA in a 10-μL reaction. The reactions were performed in a 96-well plate format. The GSTM1 and GSTT1 real-time assays were conducted using 4 μL of the 2×Genotyping Master Mix Universal (ABI). The thermocycling conditions were as follows: 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles 95°C for 15 seconds and 56°C for 1 minute and 30 seconds. Real-time fluorescence was monitored during PCR amplification, and the results were analyzed using Applied Biosystems 7500 Real-Time PCR systems. The GSTP1 rs1695 polymorphism was genotyped using Fluidigm SNPtype assays.

Statistical analysis

Deviation from Hardy-Weinberg equilibrium (HWE) in the control subjects was evaluated using the chi-squared test. Unconditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between GST genotypes and pancreatic cancer risk. All of the analyses were adjusted

for age (continuous) and sex (male or female). The interaction of genotype and smoking with regard to pancreatic cancer risk was assessed using a likelihood ratio test.

The statistical tests were two-sided, and a P-value less than 0.05 was considered statistically significant. All of the statistical analyses were performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Table 1 shows the distributions of selected characteristics and risk factors for pancreatic cancer. The cases were more likely to be current smokers and to have a history of diabetes, compared to the controls. The OR was 2.86 (95%CI: 1.79-4.57) for current smokers after adjustment for age, sex, BMI and history of diabetes. Subjects who reported a history of diabetes had a 2.9-fold increased risk of pancreatic cancer (OR=2.94; 95%CI: 1.90-4.57).

As shown in Table 2, the prevalence of the GSTM1-null genotype and GSTT1-null genotype among the control subjects was approximately 56% and 48%, respectively. The cases and controls were comparable

Table 1. Characteristics of Case Patients and Control Subjects

Characteristics	Case patients (N=360)	Control subjects (N=400)	OR (95% CI)
Mean age±SD	67.8±8.8	64.8±9.5	
Male (%)	215 (59.7)	226 (56.5)	
Body mass index (kg/m^2)			
<25	278 (77.2)	312 (78.0)	1.00
25.0-29.9	64 (17.8)	75 (18.7)	0.96 (0.65-1.43)
≥30	16 (4.4)	12 (3.0)	1.21 (0.53-2.77)
Unknown	2 (0.6)	1(0.3)	
Smoking status			
Non-smokers	145 (40.2)	202 (50.5)	1.00
Former smokers	119 (33.1)	140 (35.0)	1.23 (0.82-1.85)
Current Smokers	96 (26.7)	58 (14.5)	2.86 (1.79-4.57)
History of diabetes			
No	269 (74.7)	362 (90.5)	1.00
Yes	87 (24.2)	35 (8.7)	2.94 (1.90-4.57)
Unknown	4 (1.1)	3 (0.8)	

*OR; odds ratio, CI; confidence interval, SD; standard deviation, OR was adjusted for sex, age, body mass index, history of diabetes, and cigarette smoking. The numbers shown in parentheses are percentages

Table 2. Association of Pancreatic Cancer with GSTM1 and GSTT1 Polymorphisms

	Case patients (n=360)	Control subjects (n=400)	OR (95% CI)
GSTM1			
Present	160 (44.4)	175 (43.8)	1.00
Null	200 (55.6)	225 (56.2)	0.99 (0.74-1.32)
GSTT1			
Present	193 (53.6)	209 (52.3)	1.00
Null	167 (46.4)	191 (47.7)	0.98 (0.73-1.31)
*GSTP1 (rs1695)			
AA	266 (73.9)	284 (71.0)	1.00
AG	88 (24.4)	113 (28.3)	0.83 (0.60-1.16)
GG	6 (1.7)	3 (0.7)	2.41 (0.58-9.98)
AG+GG	94 (26.1)	116 (29.0)	0.87 (0.63-1.20)

**OR: odds ratio; CI: confidence interval, OR was adjusted for age and sex

Table 3. Joint Efects of GSTT1, GSTM1 Genotypes on Pancreatic Cancer Risk

GSTT1	GSTM1	Case patients	Control subjects	OR (95%CI)
Present	Present	85	87	1.00
Present	Null	108	122	0.95 (0.64-1.42)
Null	Present	75	88	0.93 (0.60-1.45)
Null	Null	92	103	0.97 (0.64-1.47)
				P for interaction=0.62

*OR: odds ratio; CI: confidence interval, OR was adjusted for age and sex

Table 4. Joint Effects of Smoking and GSTT1, GSTM1 Genotypes on Pancreatic Cancer Risk

GSTT1	Smoking	Case patients	Control subjects	OR (95%CI)
Present	Non-smokers	71	103	1.00
Present	Current smokers	47	28	3.22 (1.72-6.04)
Null	Non-smokers	74	99	1.08 (0.70-1.67)
Null	Current smokers	49	30	3.27 (1.76-6.06)
				P for interaction=0.79
GSTM1				
Present	Non-smokers	67	98	1.00
Presnt	Current smokers	44	23	3.67 (1.93-6.98)
Null	Non-smokers	78	104	1.08 (0.69-1.68)
Null	Current smokers	52	35	2.92 (1.63-5.25)
				P for interaction=0.39

*OR: odds ratio; CI: confidence interval, OR was adjusted for age and sex

in terms of GSTM1 and GSTT1 genotype distributions. Neither of the deleted polymorphisms in GSTM1 and GSTT1 was significantly associated with the risk of pancreatic cancer, with an age- and sex-adjusted OR of almost 1.0. The results remained unchanged after further adjustment for BMI, history of diabetes and cigarette smoking (data not shown). The distribution of GSTP1 rs1695 genotypes among the control subjects deviated from HWE (p=0.02). No significant associations were observed between rs1695 genotypes in GSTP1 and the risk of pancreatic cancer. Compared with individuals with the AA genotype, the age- and sex-adjusted OR was 0.87 (95%CI: 0.63-1.20) among those with the AG and GG genotype.

Table 3 shows the combined effects of GSTM1 and GSTT1 polymorphisms on pancreatic cancer risk. The OR was 0.97 (0.64-1.47) for individuals with the GSTM1 and GSTT1-null genotypes compared with those with the GSTM1 and GSTT1-present genotypes. No statistically significant interactions were noted (P=0.62). No synergistic effects of smoking or GST genotypes were observed (Table 4). The risk estimates were similar for current smokers with the GSTT1 or GSTM1-null genotypes, compared to current smokers with the GSTT1 or GSTM1-present genotypes.

Discussion

We evaluated the associations between genetic polymorphisms in GSTM1 and GSTT1 and pancreatic cancer risk in Japanese subjects. We found that neither the GSTM1-null genotype nor the GSTT1-null genotype was associated with increased pancreatic cancer risk. Furthermore, although smoking was significantly

associated in our study with an increased risk of pancreatic cancer, the results of the gene-environment interactions did not indicate a synergistic effect of smoking and GST-null genotypes on the risk.

The frequencies of GSTM1 and GSTT1-null genotypes vary widely across ethnicities. It has been shown that Asians and Caucasians display higher frequencies of GSTM1-null genotypes than African populations (Di Pietro et al., 2010). The prevalence of the GSTT1-null genotype is low in Caucasians, and it is significantly greater in Asian populations. In our control group, the GSTT1-null genotype represented approximately 48% of the subjects, which was greater than the percentage reported in Caucasians (Di Pietro et al., 2010). The allele frequencies for GSTT1 and GSTM1 in our study were similar to those reported in other Asian populations (Di Pietro et al., 2010).

Previous studies have yielded mixed results regarding the associations between GSTT and GSTM polymorphisms and pancreatic cancer. To date, at least six case-control studies have addressed this association (Bartsch et al., 1998; Liu et al., 2000; Duell et al., 2002; Jiao et al., 2007; Vrana et al., 2009; Jang et al., 2012). All the studies were conducted in Western countries, with the exception of a population-based case-control study in the San Francisco Bay area, in which a small number of Asian participants were included (Duell et al., 2002). No main effects of the GSTT1 and GSTM1-null genotypes on pancreatic cancer risk were noted in any of the studies, with the exception of a population-based case-control study conducted in Canada (Jang et al., 2012).

The lack of association between LoF variants, such as GSTM1 and GSTT1, and pancreatic cancer risk indicates that a common gene-disrupting variant alone might not confer major susceptibility. Two possibilities exist. First, given the high prevalence of null genotypes, such as GSTT1 and GSTM1, it is unlikely that any major effects exist, because natural selection is expected to prevent the most severely deleterious alleles from reaching high population frequencies (MacArthur et al., 2012). Another possibility is that the pancreas is not directly exposed to tobacco-derived carcinogens, suggesting that the effect of carcinogen-metabolizing enzymatic activity might be weaker than in other organs that are directly exposed to tobacco carcinogens, such as the lungs. Even for lung cancer, a meta-analysis of the GSTM1-null genotype showed a weakly positive association, with a summary OR of 1.22 (95%CI: 1.14-1.30) (Carlsten et al., 2008).

On the basis of a multiplicative interaction model, we observed no synergistic effect of smoking and GST-null genotypes on pancreatic cancer risk. Although the notion that smokers with GSTT1 or GSTM1-null genotypes had the highest risk compared with non-smokers with GSTT1 or GSTM1-present genotypes is biologically plausible, the clarification of genotype-environment interactions remains a challenge. This is due to a limited sample size and difficulty of obtaining accurate exposure information. In the population-based case-control study carried out in six San Francisco Bay areas, the OR was 5.0 (95%CI 1.8-14.5) for heavy smokers who had a deletion polymorphism in GSTT1, suggesting that inherited deletion polymorphisms in GSTT1 increase the susceptibility to smoking-related pancreatic cancer (Duell et al., 2002). The results, however, might have been due to chance because they were based on a very limited sample size.

We recognize several limitations of our study. First, as with other case-control studies, selection bias was an inherent limitation and should be addressed when interpreting the study results. Selecting an appropriate control group remains a major challenge, especially in hospital-based case-control studies. Ideally, the cases and controls should come from the same source population. However, the hospital controls did not necessarily represent the same population from which the cases were derived. The frequency of GST genotypes observed among the control subjects in this study was comparable to that obtained from other Asian populations, suggesting that our results regarding GST polymorphisms and pancreatic cancer were robust. Second, we were limited to detecting significant gene-environment interactions in the subgroups. For example, the numbers of cases and controls were small, especially after stratification by smoking status. Third, the genotyping methods, based on PCR techniques, used in our study and in other studies could not distinguish GSTM1 and GSTT1 homozygous wild-type +/+ from heterozygous +/- individuals. Only one previous study found phenotypic differences between these two groups based on a newly developed assay (Moore et al., 2005). Fourth, although we showed that a combination of GSTM1 and GSTT1-null genotypes was not associated with the risk of pancreatic cancer, the pathways of carcinogen metabolism are complex and are mediated by a variety of factors. These factors include the balance between metabolic activation and the detoxification of tobacco carcinogen compounds, as well as the efficiency of DNA repair. For example, it is likely that a deficiency in one class of GST enzymes due to a genetic polymorphism can be compensated for by the presence of other classes of GST enzymes. We genotyped rs1695 in GSTP1 and found no significant association between rs1695 polymorphisms and the risk of pancreatic cancer. It should be noted that the distribution of genotypes among control subjects was not in HWE. The reason for this fact is unclear, but genotyping error and population stratification are possible explanations (Pompanon et al., 2005). Further studies are needed to integrate genetic variations into different pathways, to define the risk of pancreatic cancer better.

In conclusion, our case-control study indicated no overall association between the GSTM1 and GSTT1 variants and pancreatic cancer risk in Japanese subjects. As common low-risk variants in different genes might act collectively to confer susceptibility to pancreatic cancer, further studies will be required to uncover the full spectrum of these variants and their effects on pancreatic cancer.

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