RESEARCH COMMUNICATION

Effect of Genetic Predisposition on the Risk of Gallbladder Cancer in Hungary

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Abstract

A CYP1A1 polymorphism has been associated with an increased risk for gallbladder cancer (GBC) in Japanese women. However, genetic risk factors for GBC in Hungary, where the population has a relatively high GBC incidence, has not been well studied. We therefore tested associations between CYP1A1 T3801C, CYP1A1 Ile462Val, GSTM1deletion, and TP53 Arg72Pro and GBC in Hungary. Genomic DNA was extracted from peripheral blood of 100 controls (52 men and 48 women) and from the tissue embedded in paraffin of 43 cases (6 men and 37 women). The case-control analysis was limited to females due to a small number of males. Of 37 female cases, 21 (56.8%) were diagnosed as adenocarcinoma, and the remaining 16 (43.2%) were classified as non-adenocarcinoma. The odds ratios (ORs) for the Ile/Val genotype and the Val allele were 8.9 (95% CI: 2.9-27.4) and 4.4 (95% CI: 1.7-11.1), respectively. The occurrence of the combined variant genotypes of CYP1A1 Ile462Val and GSTM1 (37.8% vs. 8.3%) or CYP1A1 Ile462Val and TP53 Arg72Pro (24.3% vs. 0%) was significantly higher in the cases than in the controls. The Ile/Val genotype was significantly associated with an increased risk of adenocarcinoma (OR 9.2; 95% CI: 2.6-32.6) and non-adenocarcinoma (OR 8.4; 95% CI: 2.2-32.4). Additionally, the Arg/Pro genotype increased risk of non-adenocarcinoma (OR 3.8; 95% CI: 1.2-12.8). The Val allele may contribute to the development of GBC not only in Japanese but also in Hungarian women. Our results provide a rationale for further studies of genetic variation on the risk of GBC in Hungary.

Key Words: Gallbladder cancer - single nucleotide polymorphism -CYP1A1-GSTM1-TP53-genetic susceptibility

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Introduction

There is a prominent worldwide geographic and ethnic variability of gallbladder cancer (GBC) incidence (Wistuba and Gazdar, 2004). Although a number of risk factors have been reported (Nagorney and McPherson, 1988; Henson et al., 1992; Strom et al., 1995; Carriaga and Henson, 1995; Orth, 2000; Pandey, 2003; Randi, 2006), geographically specific environmental and genetic susceptibility contributions have yet to be fully elucidated.

In light of this, we have conducted long-term epidemiological studies of mortality rates for biliary tract cancer (BTC), including GBC and extra-hepatic bile duct cancer, in Japan since the 1980s. Of 47 prefectures in Japan, Niigata Prefecture was the area with the highest standardized mortality rate from BTC during 1981 and 1986 (Endoh et al., 1993). Our studies determined several environmental (Yamamoto et al., 1993) and genetic (Tsuchiya et al., 2007) risk factors for GBC in Niigata, and the application of necessary preventive measures highlighted in our findings contributed to the subsequent decline of the GBC mortality rate in that area.

Hungary, in central Europe, is one of the countries with a relatively high GBC incidence rate (Zatonskí et al., 1993; Levi et al., 2003). Although risk factors for developing GBC in Hungary have not been identified, we anticipate that genetic and race-related characteristics are very important, and genetic factors clarified in Japan may also be associated with an increased risk for developing GBC in Hungary. Here we conducted a case-control study to clarify this possibility, with special emphasis on the genes for drug metabolizing enzymes and the p53 tumor suppressor gene (TP53).

Materials and Methods

Study subjects and their DNA extraction

A total of 43 patients (6 men and 37 women) with GBC who had been diagnosed by histological examination between 2000 and 2004 at the National Institute of

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Oncology, Budapest, Hungary, were eligible as cases for this case-control study. As a control group, 100 healthy subjects (52 men and 48 women) were randomly selected from among examinees undergoing an annual check-up by the National Institute of Oncology between 2004 and 2005.

The demographic and histologic characteristics of the cases and controls are shown in Table 1. Adenocarcinomas were in the majority. Other diagnoses, including squamous cell carcinoma, adenosquamous carcinoma, carcinosarcoma, undifferentiated carcinoma, and non-classified type, were classified as non-adenocarcinoma. However, the number of male cases was so small that only female cases and controls were selected as our study subjects.

Informed consent was obtained from each subject before enrollment in this study, which was approved by the Ethics Committee at the National Institute of Oncology.

Genomic DNA of the cases was extracted from the tissue embedded in paraffin using a standard commercial kit (DEXPAT, Takara Bio Inc., Tokyo, Japan). Genomic DNA of the controls was extracted from their blood samples using a standard phenol/chloroform extraction method (Poncz et al., 1982).

Table 1. Demographic and Histologic Characteristics

	Cases	Controls
Men		
Number of individuals	6	52
Age* (years)	72.7 ± 7.1	38.9 ± 10.3
Histologic types	Adenocarcinoma (66.7%)	
	Non-classified (33.3%)	
Women		
Number of individuals	37	48
Age* (years)	70.0 ± 9.1	37.2 ± 12.1
Histologic types	Adenocarcinoma (56.8%)	
	Squamous cell carcinoma (5.4%)	
	Adenosquamous carcinoma (2.7%)	
	Carcinosarcoma (2.7%)	
	Undifferentiated carcinoma (2.7%)	
	Non-classified (29.7%)	

*Age is represented as the mean ± standard deviation

Genetic analysis

The genotype of the cytochrome P450 1A1 (CYP1A1) T3801C polymorphism was evaluated using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Hayashi et al., 1991). The genotype of the CYP1A1 Ile462Val polymorphism was evaluated using the PCR-RFLP method (Coscorbi et al., 1996) and that of the glutathione S-transferase class-mu 1 (GSTM1) deletion polymorphism by allele-specific PCR analysis (Zhong et al., 1993). GSTM1 genotypes are divided into two categories in relation to enzymatic activity. Lack of activity is caused by the homozygous deletion of an intact gene (the null genotype). The non-null genotype is the wild type or heterozygote. The TP53 Arg72Pro genotypes were evaluated using the PCR-RFLP method (Kawajiri et al., 2001).

To ensure quality control in the assays, all samples were tested by two independent individuals, and the results were 100% concordant.

Statistical analysis

Statistical analysis in this study was performed using SAS (Release 6.12, SAS Institute Inc., Cary, NC, USA) and STATA software (SE 8.0, STATA Corporation, TX, USA).The chi-square test and Fisher's exact probability test were used to assess the association between the genotypes or their alleles and GBC risk, either alone or in combination. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated by logistic regression analysis. P values less than 0.05 were considered to indicate statistical significance. We used Pearson's chi square test to check for the Hardy-Weinberg equilibrium.

Table 2. Association of CYP1A1, GSTM1, and TP53 Polymorphisms with Gallbladder Cancer Risk in Women

	Cases		Controls		Odds Ratio (95% Confidence Interval)	p value
	n	%	n	%		
CYP1A1 T3801C						
T/T	31	83.8	37	77.1	1.0	
T/C	6	16.2	9	18.7	0.8 (0.3-2.5)	ns
C/C	0	0.0	12	14.2	Infinite	ns
T/C+C/C	6	16.2	11	22.9	0.7 (0.2-2.0)	ns
CYP1A1 Ile462Val						
Ile/Ile	18	48.6	42	87.5	1.0	
Ile/Val	19	51.4	5	10.4	8.9 (2.9-27.4)	p < 0.001
Val/Val	0	0.0	1	2.1	Infinite	ns
Ile/Val+Val/Val	19	51.4	6	12.5	7.4 (2.5-21.6)	p < 0.001
GSTM1						
Non-null	12	32.4	16	33.3	1.0	
Null	25	67.6	32	66.7	1.0 (0.4-2.6)	ns
TP53 Arg72Pro						
Arg/Arg	17	46.0	30	62.5	1.0	
Arg/Pro	18	48.6	13	27.1	2.4 (0.9-6.2)	ns
Pro/Pro	2	5.4	5	10.4	0.7 (0.1-4.0)	ns
Arg/Pro + Pro/Pro	20	54.0	18	37.5	2.0 (0.8-4.7)	ns

ns: no significant difference. Each genotype of 37 cases and 48 controls was analyzed by the PCR-RFLP method or allele-specific PCR analysis

Table 3. Gallbladder Cancer Risk Associated with Combined Mutant Genotypes of CYP1A1, GSTM1, and TP53 in Women

	Cases (%)	Controls (%)	OR	95% CI	P value
T/C + C/C and Ile/Val + Val/Val	4 (10.8)	6 (12.5)	0.8	0.2-3.3	ns
T/C + C/C and GSTM1 null	6 (16.2)	9 (18.8)	0.8	0.3-2.5	ns
T/C + C/C and Arg/Pro + Pro/Pro	3 (8.1)	1 (2.1)	4.0	0.4-40.4	ns
Ile/Val + Val/Val and GSTM1 null	14 (37.8)	4 (8.3)	6.7	2.0-22.7	p < 0.001
Ile/Val + Val/Val and Arg/Pro + Pro/Pro	9 (24.3)	0 (0.0)	Infinite		
GSTM1 null and Arg/Pro + Pro/Pro	15 (40.5)	13 (27.1)	1.8	0.7-4.4	ns

ns: no significant difference. Genotypes were analyzed by the PCR-RFLP method or allele-specific PCR. Associations between combined genotypes and risk for gallbladder cancer were evaluated by the chi-square test or Fisher's exact probability test

When the P values exceeded 0.05, we estimated that the sample was under the Hardy-Weinberg equilibrium.

Results

The genotypic frequencies of the CYP1A1, GSTM1deletion, and TP53 polymorphisms were compared between male and female controls. No significant differences in the genotypic frequencies were observed between the two groups. Additionally, the genotype distribution of the polymorphisms agreed with Hardy-Weinberg equilibrium.

Table 2 shows the GBC risk associated with the CYP1A1, GSTM1deletion, and TP53 polymorphisms. The frequency of the Ile/Val genotype was significantly higher in the cases than in the controls; the OR for the GBC risk was 8.9. Since the Val/Val genotype was not detected in the cases, we calculated the OR for the combined genotype of Ile/Val and Val/Val, and found it to be 7.4. Additionally, the frequency of the Val allele was significantly higher in the cases (25.7%) than in the controls (7.3%) (data not shown). The Val allele may be associated with an increased risk of GBC. Meanwhile, no significant differences in the frequencies of CYP1A1 T3801C, GSTM1, and TP53 Arg72Pro genotypes or alleles were found between the cases and controls.

Table 3 shows the risk of GBC associated with the combined variant genotypes of the CYP1A1, GSTM1 deletion, and TP53 polymorphisms. The frequency of the combined variant genotypes of CYP1A1 Ile462Val and GSTM1 was significantly higher in the cases compared with that in the controls, with an OR of 6.7(95% CI; 2.0-22.7). Moreover, the frequency of the combined variant genotypes of CYP1A1 Ile462Val and TP53 Arg72Pro was 24.3% (9 of 37) in the cases and 0% (0 of 48) in the controls. However, no significant differences in the combined variant genotypes between the CYP1A1 T3801C and CYP1A1 Ile462Val , CYP1A1 T3801C and GSTM1, CYP1A1 T3801C and TP53 Arg72Pro, or GSTM1 and TP53 Arg72Pro were found between the cases and controls.

Table 4 shows the distribution of the CYP1A1

Table 4. Distribution of CYP1A1 Ile462Val and TP53 Arg72Pro Genotypes in the Female Cases of Adenocarcinoma and Non-adenocarcinoma

	Cases		Controls		Odds Ratio (95% CI)		p value
	n	%	n	%			
Adenocarcinoma (n = 21)							
CYP1A1 Ile462Val							
Ile/Ile	10	47.6	42	87.5	1.0		
Ile/Val	11	52.4	5	10.4	9.2	2.6-32.6	p < 0.001
Val/Val	0	0.0	1	2.1	Infinite		
Ile/Val + Val/Val	11	52.4	6	12.5	7.7	2.3-25.8	p < 0.001
TP53 Arg72Pro							
Arg/Arg	11	52.4	30	62.5	1.0		
Arg/Pro	8	38.1	13	27.1	1.7	0.5-5.1	ns
Pro/Pro	02	19.5	5	10.4	1.1	0.2-6.5	ns
Arg/Pro + Pro/Pro	10	47.6	18	37.5	1.5	0.5-4.3	ns
Non-adenocarcinoma (n = 16)							
CYP1A1 Ile462Val							
Ile/Ile	8	50.0	42	87.5	1.0		
Ile/Val	8	50.0	5	10.4	8.4	2.2-32.4	p < 0.001
Val/Val	0	0.0	1	2.1	Infinite		ns
Ile/Val + Val/Val	8	50.0	6	12.5	7.0	1.9-25.7	p = 0.002
TP53 Arg72Pro							
Arg/Arg	6	37.5	30	62.5	1.0		
Arg/Pro	10	62.5	13	27.1	3.8	1.2-12.8	p = 0.024
Pro/Pro	0	0.0	5	10.4	Infinite		ns
Arg/Pro + Pro/Pro	10	62.5	18	37.5	2.8	0.9-8.9	ns

OR: odds ratio, 95% CI: 95% confidence interval ns: no significant difference. Non-adenocarcinoma includes squamous cell carcinoma, adenosquamous carcinoma, carcinosarcoma, undifferential carcinoma, and non-classified type of gallbladder cancer.

Ile462Val and TP53 Arg72Pro genotypes by histologic type, adenocarcinoma and non-adenocarcinoma. No significant differences in the frequency of the CYP1A1 Ile462Val, GSTM1 deletion, or TP53 Arg72Pro genotype were found between the cases of adenocarcinoma and non-adenocarcinoma. The frequency of the Ile/Val genotype was significantly higher in the cases with adenocarcinoma (52.4%) than in the controls (10.4%). No significant differences in the frequencies of the CYP1A1 T3801C, GSTM1, or TP53 Arg72Pro genotype were found between the cases and controls. However, the frequencies of the Ile/Val and Arg/Pro genotypes combined were significantly higher in the cases with non-adenocarcinoma (50.0% and 62.5%, respectively) than in the controls (10.4% and 27.1%, respectively). There were no significant differences in the frequencies of the CYP1A1 T3801C or GSTM1 genotypes between the cases and controls.

Discussion

Based on our 20-year-long epidemiological study of the etiology of GBC in Japan, we determined the genetical risk factors for the development of GBC in Niigata, which had the highest standardized mortality rate for BTC during 1981 and 1986 in Japan (Endoh et al., 1993). Individuals with the Ile/Val genotype of CYP1A1 Ile462Val polymorphism in women and with the Arg/Pro genotype of TP53 Arg72Pro polymorphism in men were found to be at high risk of developing GBC in Japanese people (Tsuchiya et al., 2007). Although environmental and genetic risk factors in the development of GBC in Hungary have not been identified yet, the CYP1A1 and TP53 gene mutations may also play a role in the development of GBC in Hungary. To explore this possibility, we conducted this case-control study to clarify the genetic risk factors in the development of GBC in Hungary.

Large ethnic differences in the allele frequencies or genotypic distributions of the CYP1A1 polymorphisms have been reported: Asians and South Amerindians show higher frequency, while Caucasians shows lower frequency (Garte et al., 2001). In addition to our study conducted in Japan, a previous study showed that high frequencies of the minor C and Val alleles of the CYP1A1 gene were found in the Mapuche, who are the indigenous inhabitants of central and southern Chile and southern Argentina (Muñoz et al., 1998). Interestingly enough, the highest mortality rates for GBC have been reported among Chilean Mapuche Indians and Bolivian and Chilean Hispanics (Lazcano-Ponce et al., 2001). These findings suggest that CYP1A1 gene polymorphisms may be associated with an increased risk of GBC. In the present study, the genotypic distributions of CYP1A1 polymorphisms were similar to those observed previously in Hungarian healthy subjects (Kiss et al., 2000; Schoket et al., 2001), and the frequency of the Ile/Val genotype in the cases was significantly higher than that in the controls. Although the Val/Val genotype was not observed in the cases, the frequency of the Ile/Val and Val/Val genotypes combined was significantly higher in the cases than in the controls (OR 7.4, 95% CI; 2.5-21.6, $p < 0.001$). Therefore, the development of GBC in Hungary may be associated with the presence of at least one minor allele.

Glutathione-S-transferase (GST) is a phase II drug metabolizing enzyme. Higher reactive intermediates of polycyclic aromatic hydrocarbons oxidized by CYP1A1 enzyme are metabolized by GSTs, so the GST genes have been studied thoroughly in relation to various cancer risks (Parl, 2005). The association between the GSTM1 deletion polymorphism and cancer risk was inconclusive, however. To the best of our knowledge, no study has thus far been reported regarding the null genotype and GBC risk in Hungary. In the present study, the presence of the null genotype alone was not associated with GBC risk. Moreover, a previous study showed that no association between the null genotype and colorectal cancer risk was found in Hungary (Kiss et al., 2000). GSTs have been considered important in the development of cancer because of their critical roles in providing protection of DNA against damage and adduct formation (Rojas et al., 2000), but the GSTM1 null genotype may be not linked to the development of cancer in Hungary. Other isoenzymes such as alpha, kappa, mu, omega, pi, sigma, theta, and zeta (Pemble et al., 1994; Board et al., 2000) may play a role in the development of GBC in Hungary.

The human TP53 tumor suppressor gene plays a central role in many cellular processes via DNA repair and apoptosis (Kastan et al., 1992; Caelles et al., 1994). Therefore, the TP53 Arg72Pro polymorphism involved in multiple steps of carcinogenesis may account for genetic differences in GBC susceptibility. A number of researchers have studied the association between the TP53 Arg72Pro polymorphism and cancer susceptibility, and they found this polymorphism was associated with the risk for the development of lung, esophageal, and cervical cancers (Zehbe et al., 1999; Fan et al., 2000; Lee et al., 2000). In the present study, the genotypic frequencies in the controls were similar to that in Hungarian healthy subjects reported previously (Hernádi et al., 2003). There was no significant relationship between the TP53 Arg72Pro polymorphism and GBC risk. This finding was consistent with our results obtained from Japanese female patients with GBC (Tsuchiya et al., 2007).

Significant differences of the combined variant genotypes of CYP1A1 Ile462Val and GSTM1, or CYP1A1 Ile462Val and TP53 Arg72Pro polymorphisms were observed between the cases and controls. Therefore, these polymorphisms may be associated with a higher risk of GBC. In particular, the Val allele had the greatest effect. Our data suggest that the presence of some environmental factors, such as chemical carcinogens, and CYP1A1-inducing chemicals, may be associated with the development of GBC. Of particular interest has been whether the risk of GBC associated with a particular environmental exposure differs with respect to functionally different polymorphisms of these genes, i.e. gene-environment interaction (Mucci et a., 2001). Studying gene-environment interactions in relation to risk of GBC may be valuable because positive findings would clearly implicate the chemicals with which the gene interacts as disease-causing exposures, clarify GBC etiology, and point to environmental modifications for GBC prevention.

We also examined the distribution of the genotypes in the cases of adenocarcinoma or non-adenocarcinoma to clarify the genetic characteristics in the histologic type-segregated patients. No significant difference of the TP53 Arg72Pro genotypes was found between the cases with adenocarcinoma and controls, so these cases might be severely dependent on the presence of the Val allele. The frequencies of Ile/Val and Arg/Pro genotypes were significantly higher in the cases with non-adenocarcinoma than in the controls. The development of non-adenocarcinoma GBC might be involved in a pathway associated with a particular carcinogen-metabolizing enzyme and multiple steps of carcinogenesis. Our results indicate that different genetic factors may be associated with the development of adenocarcinoma GBC and non-adenocarcinoma GBC. Or, the higher frequency of the Arg/Pro genotype in the cases of non-adenocarcinoma might have been caused by the small sample size, because no significant difference in the frequency of Arg/Pro plus Pro/Pro was observed between the cases and controls.

This study had several limitations. Our sample size of the cases and controls was small, and the cases had a strong bias in age distribution against the controls. Thus our results may have reduced statistical power for detecting possible associations between genetic factors and the risk of GBC, or they may have failed to reflect precisely an association between the two. Nonetheless, the Val allele was associated with an increased risk for GBC not only in Japan women but also in Hungarian women. Therefore, additional studies that are age-adjusted and include a greater number of cases and controls are needed to clarify whether this genetic predisposition is a common factor in developing GBC in different ethnic populations.

In summary, the presence of the Ile/Val genotype increased the risk of GBC in Hungarian women. This genetic predisposition appears to be especially important among those who have the combined variant genotypes of the CYP1A1 Ile462Val and GSTMl, or CYP1A1 Ile462Val and TP53 Arg72Pro polymorphisms. While our findings require additional confirmations, they provide evidence that the development of GBC in Hungarian women is associated with the CYP1A1 Ile462Val gene mutation. Our results provide a preliminary rationale for further studies on the roles of genetic susceptibility to developing GBC not only in Japanese women but also in Hungarian women.

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