their study and ours differ in terms of participants. It is known that pregnancy and lactation can cause disease regression and improvements in symptoms of endometriosis (30,31). To reflect the authentic phenotype of endometriosis at the time of surgical diagnosis, our study excluded women who had given birth or lactated. Finally, considerable intraobserver and interobserver variability is reported in the revised AFS classification (32). Although this variability is a concern in any case control study of endometriosis, it does not decrease the validity or reliability of the association found.

Our study provides evidence for an association between the *AhRR* codon 185 polymorphism and endometriosis, although the exact mechanism for this effect is still unknown. One possible explanation for this association is alteration of *AhR*-mediated signaling by the polymorphism. The *AhRR* C/G + G/G genotype may facilitate proliferation of endometrial cells through the diminished down-regulation of *AhR*-mediated signaling. Because of the small sample size, ours is not a conclusive study, and the results need to be investigated further in large studies that also consider ethnic variation.

The *AhRR* codon 185 polymorphism is associated with susceptibility to and severity of endometriosis in Japanese women, but *AhR* codon 554 and *ARNT* codon 189 polymorphisms appear not to be associated with endometriosis. The pathogenesis of endometriosis is still not clearly understood, so the *AhRR* codon 185 polymorphism could be a useful genetic marker in predicting endometriosis susceptibility and severity.

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Associations between serum levels of selected organochlorine compounds and endometriosis in infertile Japanese women ☆

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Abstract

Endocrine-disrupting chemicals (EDCs) have been proposed as risk factors for endometriosis. Persistent organochlorine compounds, a group of suspected EDCs, are present to some extent in almost all human adipose tissue and blood via the food chain. A few animal studies have confirmed that exposure to these compounds can increase the incidence of endometriosis. In this study, we examined the associations between endometriosis and exposure to selected organochlorine compounds, including 8 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 coplanar polychlorinated biphenyls (cPCBs), 36 *ortho*-substituted polychlorinated biphenyls (PCBs), and 13 chlorinated pesticides or their metabolites. The participants were 139 infertile Japanese women who were examined by laparoscopy and diagnosed as either endometriosis cases (Stages II–IV) or controls (Stages 0–I). The serum levels (lipid adjusted) of the targeted organochlorine compounds were in both 58 cases and 81 controls. There were very few differences in the various levels between endometriosis cases and controls. The total serum toxic equivalency (TEQ) value of PCDDs was significantly higher in the controls than in the cases ($P = 0.02$). No other total TEQ values differed between cases and controls. For PCDDs, PCDFs, cPCBs, and PCBs, the multivariate odds ratio was 0.38 [95% confidence interval (CI), 0.12–1.17] and 0.41 (95% CI, 0.14–1.27) for the third and highest quartiles, respectively, compared to the lowest quartile of total TEQ values. A weak, negative dose–response relationship was evident for total TEQs (P for trend of 0.06). The results of this study provide some evidence that serum levels of these organochlorine compounds are not associated with an increased risk of endometriosis in infertile Japanese women.

Keywords: Organochlorines; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans; Coplanar polychlorinated biphenyls; Endometriosis

1. Introduction

A number of industrial chemicals in the environment mimic (agonists) or antagonize (antagonists) endogenous hormones (Safe, 2000). These substances are referred to as endocrine-disrupting chemicals (EDCs)

☆All participants gave their written informed consent before the laparoscopic examination. The study protocol was approved by the Institutional Review Board of the Jikei University School of Medicine, the National Cancer Center, National Institute for Environmental Studies, and the US Centers for Disease Control and Prevention (CDC).

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because of their ability to interact with the endocrine system. Many of the EDCs are organochlorine compounds, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar polychlorinated biphenyls (cPCBs), *ortho*-substituted polychlorinated biphenyls (PCBs), and chlorinated pesticides. PCDDs and PCDFs are formed as byproducts of various industrial chemical reactions and combustion processes, including waste incineration. PCBs and chlorinated pesticides were widely used in industry and agriculture until the 1970s in Japan. Because these compounds are lipid soluble and resistant to metabolism, they tend to bioaccumulate through the food chain. They are also present in human adipose tissue and in the lipid components of blood, mainly because of food intake (Safe, 2000).

There is evidence that EDCs may adversely affect the health of wildlife and humans (Birnbaum and Fenton, 2003; Safe, 2000; Steenland et al., 2004). Some of these chemicals, such as dichlorodiphenyltrichloroethane (DDT) and certain PCBs, are considered to be estrogenic substances (Wolff and Toniolo, 1995), while others, including dioxins, are considered to be anti-estrogenic substances (Krishnan et al., 1995). A recent study revealed that dioxins enact estrogenic effects through estrogen receptor-mediated signaling modulated by the agonist-activated aryl hydrocarbon receptor (AhR) ligand complex (Ohtake et al., 2003). Endogenous estrogens enlarge the endometrial lining of the uterus, and estrogenic substances have been suggested as inducing endometriosis. A study of adult rhesus monkeys revealed that chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in feed (0, 5, or 25 ppt) was associated with a dose-dependent increase in the incidence and severity of endometriosis (Rier et al., 1993). In humans, a few case-control studies have been conducted on the association between serum levels of PCDDs, PCDFs, cPCBs, PCBs and chlorinated pesticides and endometriosis, but the results are inconsistent because of differences in study design, analytical methods, and the organochlorine compounds measured in each study (Boyd et al., 1995; Gerhard and Runnebaum, 1992; Lebel et al., 1998; Mayani et al., 1997; Pauwels et al., 2001). Recently, a cohort study of residents of Seveso, Italy, who were exposed to TCDD from the explosion of a trichlorophenol-manufacturing factory in 1976 revealed a doubled but nonsignificant risk for endometriosis among women with serum TCDD levels of 100 ppt or higher. No clear dose–response relation was found among 19 endometriosis cases, 277 nondiseased women, or 305 uncertain-status cases (Eskenazi et al., 2002). Although in vitro studies using certain organochlorine compounds have suggested many possible endocrine-disrupting mechanisms, such as estrogenic or anti-estrogenic properties, immune suppression, and enzyme induction through binding to the AhR (Nicolopoulou-Stamati and Pitsos, 2001), less is known about the effect of these organochlorine compounds on human endometriosis.

The purpose of this study was to determine the possible association between environmental exposures to selected organochlorine compounds and the prevalence of endometriosis among infertile Japanese women. In a hospital-based case-control study, the serum levels of 8 PCDDs, 10 PCDFs, 4 cPCBs, 36 PCBs, and 13 chlorinated pesticides were measured in laparoscopically confirmed endometriosis patients and in their corresponding references.

2. Materials and methods

2.1. Subjects and sample collection

Eligible women were aged 20–45 years, had complained of infertility, and had consulted doctors in the Department of Obstetrics and Gynecology of the Jikei University School of Medicine in 1999 and 2000. Women who had ever given birth or lactated were not eligible for this study. Sampling was consecutive, and a total of 159 women met these criteria. After we excluded 15 women who did not give their consent and 5 women who did not go through a blood screen or a laparoscopic examination, 139 women were available for analysis. Endometriosis was diagnosed laparoscopically according to the revised classification of the American Fertility Society (1985). Fifty-nine women (42.4%) were assigned to stage 0. Twenty-two (15.8%) were assigned to stage I, 10 (7.2%) to stage II, 23 (16.5%) to stage III, and 25 (18.0%) to stage IV. The 58 women with stage II or higher endometriosis were designated as "cases." Eighty-one women with stage 0 or I were designated as "controls." Among controls, some causal infertility conditions were confirmed laparoscopically: myoma of the uterus (39.5%), polycystic ovary (16.0%), and obstruction of the ovarian duct passage (23.5%). All participants gave their written informed consent before the laparoscopic examination. The study protocol was approved by the Institutional Review Board of the Jikei University School of Medicine, the National Cancer Center, National Institute for Environmental Studies, and the US Centers for Disease Control and Prevention (CDC).

A fasting blood sample was obtained before the laparoscopic examination. Serum was immediately collected by centrifugation, transferred into a stock tube, and stored at −80 °C until analyzed.

2.2. Questionnaire survey

Participants were interviewed face-to-face before the laparoscopic examination by a trained interviewer using

a structured questionnaire. Responses were elicited concerning demographic and anthropometric variables (age, height, and weight), occupation (primary industry, plant worker, office worker, specialist, merchant, housewife, no occupation, and other), education (junior high school, high school, junior college or vocational school, college, graduate school), marital status (married, never married, divorced, widowed, and other), family history of endometriosis, use of alcohol (rare, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, every day), and smoking habits (never, past, and current). We derived a menstrual history from responses to the following questions: (1) "At what age did you have your first menstrual period?" (2) "In the past year, were your menstrual periods regular? Irregular? Other? Don't know?" (3) "In the past year, what was the usual length of your menstrual cycle?" We also obtained information about potential sources of exposure to endocrine-disrupting compounds (e.g., tampons, oral contraceptives, and pesticides).

2.3. Analytical methods

Serum analyses were performed at the CDC by gas chromatography/high-resolution isotope-dilution mass spectrometry for a total of 71 compounds: 8 PCDDs [2,3,7,8-tetra-chlorodibenzo-*p*-dioxin (CDD), 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,6,7,8-hexaCDD, 1,2,3,7,8,9-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, 1,2,3,4,6,7,9-heptaCDD, 1,2,3,4,6,7,8,9-octaCDD], 10 PCDFs [2,3,7,8-tetra-chlorodibenzofuran (CDF), 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, 1,2,3,7,8,9-hexaCDF, 2,3,4,6,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, 1,2,3,4,7,8,9-heptaCDF, 1,2,3,4,6,7,8,9-octaCDF], 4 cPCBs [3,3′,4,4′-tri-chlorobiphenyl (CB), 3,4,4′,5-tetraCB, 3,3′,4,4′,5-pentaCB, 3,3′,4,4′,5,5′-hexaCB], 36 *ortho*-substituted PCBs (International Union of Pure and Applied Chemistry Nos. 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 105, 110, 118, 128, 138/158, 146, 149, 151, 153, 156, 157, 167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196/203, 201, 206, 209), and 13 selected persistent chlorinated pesticides or their metabolites [hexachlorobenzene, β-hexachlorocyclohexane, λ-hexachlorocyclohexane, aldrin, heptachlor epoxide, oxychlordane, *trans*-nonachlor, *p,p′*-1,1′-[2,2-dichloroethenylidene]-bis[4-chlorobenzene] (*p,p′*-DDE), dieldrin, endrin, *o,p′*-dichlorodiphenyltrichloroethane (*o,p′*-DDT), *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), and mirex]. The analytical methods and quality control procedures have been described previously (DiPietro et al., 1997; Patterson et al., 1987; Turner et al., 1994). Because the PCDDs, PCDFs, cPCBs, PCBs, and chlorinated pesticides are lipophilic and concentrate in the body's lipid stores, including the lipid in serum, the serum levels for these compounds were adjusted for serum lipid levels. Triglycerides and total cholesterol were used in calculating total lipid levels ($2.27 \times$ total cholesterol + triglycerides + 62.3) (Phillips et al., 1989). Limits of detection (LODs) on a lipid-adjusted basis were calculated for each sample. Because we could not measure PCB138 and 158, or PCB196 and 203, separately, combined values for PCB138/158 and PCB196/203 were reported. Dioxin toxic equivalency (TEQ) was assessed using a "toxic equivalency factor" (TEF) based upon the relative potency of each congener of the PCDDs, PCDFs, cPCBs, and mono-*ortho*-substituted PCBs compared with 2,3,7,8-TCDD, the most potent of the compounds (Van den Berg et al., 2000). To calculate the TEQ values of PCDDs, PCDFs, cPCBs, and PCBs in this study, the World Health Organization TEF values were assigned to all of the PCDDs, PCDFs, and cPCBs, except for 1,2,3,4,6,7,9-heptaCDD, and PCB105, 118, 156, 157, 167, and 189 (Van den Berg et al., 1998). Because of the small sample volume, the respective serum PCDDs/PCDFs/cPCBs and PCBs could not be measured for each participant. All targeted organochlorine compounds could not be measured for 1 control subject because of the poor conditions under which the serum was maintained.

2.4. Statistical analysis

Characteristics of cases and controls were compared using the χ^2-test and Student's *t*-test. To estimate the value of samples below the LOD for a comparison of serum organochlorine levels between cases and controls, the values < the LOD were assigned one-half of the LOD. Total levels of TCDDs/TCDFs/cPCBs, PCBs, and pesticides were calculated for the sum of the serum molar concentrations of TCDDs/TCDFs/cPCBs, PCBs, and pesticides. The serum organochlorine levels were normalized by log transformation because their distributions were confirmed to be skewed by the Kolmogorov–Smirnoff test. Differences in log-transformed serum organochlorine levels and total TEQs between 2 or more groups were established by Student's *t*-test or an analysis of variance. Odds ratios (ORs), 95% confidence intervals (CIs), and *P* values for trends were obtained by unconditional logistic regression analysis to estimate the association between total TEQ and endometriosis. Cases and controls were divided into 4 groups by the quartile of serum total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs among controls, and crude and adjusted ORs were calculated. Because menstruation was thought to be correlated with the risk for endometriosis (Cramer et al., 1986; Eskenazi and Warner, 1997), ORs for endometriosis were adjusted for menstrual regularity (regular or irregular) and average cycle (days). A *P* value of less than 0.05 (2-tail) was considered statistically significant. All analyses were

conducted using the SAS (Version 8.2) program (SAS Institute Inc., Cary, NC, USA).

3. Results

Mean ages of cases and controls were 32.4 and 32.9 years, respectively (Table 1). Differences in other risk factors for endometriosis between cases and controls were not statistically significant, except for irregularity in the menstrual cycle (11.3% of cases and 32.9% of controls, $P = 0.005$) and the duration of menstrual cycles (28.5 days for cases and 30.4 days in controls, $P = 0.03$).

In this study, 3 dioxin-like chemicals (1,2,3,4,6,7,9-heptaCDD, octaCDF, and 3,3′,4,4′-tetraCB), 4 PCBs (International Union of Pure and Applied Chemistry Nos. 18, 28, 149 and 177), and 2 pesticides (aldrin and endrin) could not be measured because of analytical conditions. Statistical analyses were performed on 7 PCDDs, 9 PCDFs, and 3 cPCBs; 32 PCBs; and 11 pesticides. An analysis of variance showed that the value of log-transformed 1,2,3,6,7,8-hexaCDD was significantly greater in controls than in cases ($P = 0.03$), although the other compounds showed no such significant differences (data not shown).

We analyzed the association between total TEQ values and endometriosis (Table 2). The serum total TEQ value of PCDDs was significantly higher in controls than in cases ($P = 0.02$). No other total TEQ values differed between cases and controls. Furthermore, we compared total levels of PCDDs, PCDFs,

Table 1
Baseline characteristics of women with and without endometriosis

Characteristic	Cases ($n = 58$)		Controls ($n = 81$)		P value
	No.[a]	(%)	No.[a]	(%)	
Age, mean (SD), yr	32.4	(3.4)	32.9	(3.9)	0.43[b]
Height, mean (SD), cm	159.0	(5.6)	158.1	(5.4)	0.34[b]
Weight, mean (SD), kg	51.1	(5.6)	52.5	(8.9)	0.33[b]
Education					
High school or less	9	(17.0)	13	(16.9)	
Junior college or vocational school	30	(56.6)	31	(40.3)	
College or graduate school	14	(26.4)	33	(42.9)	0.13[c]
Family history of endometriosis					
Yes	1	(2.0)	5	(6.6)	
No	49	(98.0)	71	(93.4)	0.24[c]
Age menses began, (SD), yr	12.5	(1.3)	12.4	(1.6)	0.66[b]
Oral contraceptive use					
Used	5	(9.4)	12	(15.6)	
Never used	48	(90.6)	65	(84.4)	0.31[c]
Regularity of menstrual cycle					
Regular	47	(88.7)	51	(67.1)	
Irregular	6	(11.3)	25	(32.9)	0.005[c]
Duration of menstrual cycles, mean (SD), days	28.5	(3.2)	30.4	(5.5)	0.03[b]
Tampon use					
Used	41	(77.4)	62	(80.5)	
Never used	12	(22.6)	15	(19.5)	0.66[c]
Smoking status					
Never	36	(69.2)	50	(64.9)	
Former	9	(17.3)	11	(14.3)	
Current	7	(13.5)	16	(20.8)	0.55[c]
Alcohol consumption					
Daily	10	(18.9)	15	(19.5)	
Weekly	22	(41.5)	24	(31.2)	
Occasionally/none	21	(39.6)	38	(49.4)	0.45[c]

Abbreviation: SD, standard deviation.
[a]Number of participants for each item varied because of missing information.
[b]Student's t-test.
[c]Chi-square test.

Table 2
Serum total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs among endometriosis cases and controls

Analyte	No.	TEQ values (pg TEQ/g lipid)				*P* value for difference
	Total (cases/controls)	Cases		Controls		
		Median	(Q_1, Q_3)	Median	(Q_1, Q_3)	
PCDDs	57/80	7.43	(5.17, 9.47)	8.63	(6.39, 10.75)	0.02
PCDFs	57/80	7.80	(6.07, 9.06)	7.51	(6.32, 9.00)	0.64
cPCBs	57/80	4.61	(3.41, 5.92)	5.14	(3.46, 7.41)	1.00
PCBs	57/80	3.40	(2.59, 4.22)	3.59	(2.37, 5.03)	0.79
PCDDs/PCDFs/cPCBs	57/80	19.40	(16.08, 25.26)	21.58	(17.40, 26.90)	0.23
Sum	56/80	22.76	(19.73, 29.14)	25.07	(20.27, 31.84)	0.23

Q_1 = 25th percentile; Q_3 = 75th percentile.

Table 3
Risk for endometriosis according to quartile of serum total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs

Variable	Total no.	Serum total TEQ values of PCDDs, PCDFs, cPCBs and PCBs (pg TEQ/g lipid)				*P* value for trend
		Quartile 1 ≤20.27	Quartile 2 >20.27–25.07	Quartile 3 >25.07–31.84	Quartile 4 >31.84	
No. of cases	56	17	17	12	10	
No. of controls	80	20	20	20	20	
Crude OR (95% CI)	56/80	1.00 (reference)	1.00 (0.40, 2.50)	0.71 (0.27, 1.85)	0.59 (0.22, 1.59)	0.23
No. of cases	51[b]	17	16	10	8	
No. of controls	70[b]	17	16	18	19	
Adjusted OR (95% CI)[a]	52/70	1.00 (reference)	0.97 (0.36, 2.63)	0.38 (0.12, 1.17)	0.41 (0.14, 1.27)	0.06

[a]OR was adjusted for menstrual regularity (regular or irregular) and average cycle length (days).
[b]Number of total participants varied because of missing information.

cPCBs, PCBs, and pesticides between the 2 groups. The serum level of PCDDs was significantly higher in controls than in cases ($P = 0.04$). No statistically significant differences were found in the total levels of PCDFs, cPCBs, PCBs, pesticides, and the sum of organochlorines between groups (data not shown).

We found nonsignificantly lower crude ORs for women in the third or fourth quartile of total TEQ compared to women in the first quartile (OR = 0.71, 95% CI, 0.27–1.85, and OR = 0.59, 95% CI, 0.22–1.59, respectively) (Table 3). Similarly, adjusted ORs for women in the third or fourth quartile of total TEQs were lower than those for the first, but not significantly so (OR = 0.38, 95% CI, 0.12–1.17, and OR = 0.41, 95% CI, 0.14–1.27, respectively). A weak inverse dose–response relationship was evident for total TEQs (*P* for trend of 0.06). We also analyzed the ORs by quartile of total serum levels of PCDDs/PCDFs/cPCBs, PCBs, and pesticides but found no significantly increased or decreased ORs. The serum organochlorine levels in women in whom stages I, II, III, and IV were diagnosed by laparoscopy did not differ significantly from the levels in women with stage 0 (data not shown).

4. Discussion

Our study found no evidence of an increased risk for endometriosis related to serum levels of several organochlorine compounds among infertile Japanese women. Furthermore, the serum level of 1,2,3,6,7,8-hexaCDD was significantly lower in endometriosis patients than in controls. This result might be attributed to multiple comparisons because 62 compounds were simultaneously analyzed in this study. However, the total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs were lower in cases than in controls, and the endometriosis risk for women with higher total TEQ values tended to be lower than for those with lower total TEQ values, although not significantly so. These results were not consistent neither with a previous animal study of Rier et al. (1993) nor with the cohort study in Seveso (Eskenazi et al., 2002), possibly because of the difference in exposure levels. Estimated TCDD exposure levels among Seveso residents were similar to those in monkeys in the 25-ppt group in an earlier study by Rier et al. (Bois and Eskenazi, 1994). The median serum TCDD levels reported in Seveso for the women with

endometriosis were 77.3 vs. 61.0 ppt for the controls, whereas the corresponding median total TEQ levels among our study participants were 17.8 ppt in cases vs. 19.2 ppt in controls. According to the animal studies of Foster et al. (1997) and Yang et al. (2000), high-dose exposure to TCDD increases the size of an endometrial fragment autotransplanted to the abdominal or pelvic cavity; in contrast, low-dose exposure decreases implant size. Thus, high TCDD exposure might stimulate endometrial tissue, but low exposure levels appear to be inhibitory, perhaps because of the anti-estrogenic mechanism of TCDD (Scialli, 2001). Pauwels et al. (2001) investigated the association between dioxin-like compounds and endometriosis in a human epidemiological study and reported no statistically significant associations between dioxin-like compounds and the occurrence of endometriosis. As in the study of Pauwels et al. (2001), we analyzed the risk of endometriosis by subdividing serum TEQ values into 4 categories (<20.0 pg TEQ/g, 20.1–60.0 pg TEQ/g, 60.1–100 pg TEQ/g, and >100 pg TEQ/g). Crude ORs of the groups with 20.1–60.0 pg TEQ/g, 60.1–100 pg TEQ/g, and >100 pg TEQ/g were 0.93 (95% CI, 0.29–3.30), 0.53 (95% CI, 0.14–1.94), and 3.6 (95% CI, 0.37–34.94) compared to the group with <20.0 pg TEQ/g. The risks of endometriosis were decreased with low TEQ values, but the risk of endometriosis was increased with high TEQ values, in the study of Pauwels et al. The total TEQ values in our study ranged from 1.38 to 44.7 TEQ/g; thus, our results were consistent with the results of Pauwels et al.

Our study has several strengths. First, we applied a clinical best definition of cases and controls. Endometriosis has been defined as the presence of endometrial glands and stroma outside of the uterine cavity, and the presence of an ectopic endometrium can be accurately determined only by inspection of the pelvic cavity during laparoscopy (Holt and Weiss, 2000; Zondervan et al., 2002). In the present study, cases and controls were diagnosed by laparoscopic examination. Moreover, we conducted consecutive sampling, so potential selection bias was excluded. Secondly, the detailed interviews were performed by the same trained interviewer before the laparoscopic examination. This interviewer validated each questionnaire so that misclassification of this information might be minimized. Because no participants knew whether they had endometriosis before the examination, recall bias can be ruled out. Detailed questionnaires were designed to detect possible confounding factors for the risk of endometriosis such as the regularity and duration of menstrual cycles. Adjustments for such variables were performed when calculating ORs for endometriosis in relation to the total TEQ values of serum levels of dioxin-like chemicals. Although in our study differences were not significant among age, smoking, and age at menarche between cases and controls, age and age at menarche have been reported to be risk factors for endometriosis, and smoking has been reported to be protective for endometriosis (Cramer et al., 1986; Eskenazi and Warner, 1997). Thus, we adjusted these factors in calculating the ORs for endometriosis in relation to the total TEQ values, but again no significant ORs were found.

On the other hand, our study has some limitations. Many participants showed values <LOD on some organochlorine compounds because of the low serum levels or small sample volume. Therefore, we evaluated the risk for total TEQ values. To assess the effects of dioxin mixtures, dioxin toxicity was assessed using a TEF that estimated the toxic potency of a compound relative to TCDD. The TEF values were consensus estimates based on an evaluation of all of the data (Van den Berg et al., 1998). In our study, the median total TEQ value of 19 PCDDs, PCDFs, cPCBs, and 6 PCBs was 25.07 pg TEQ/g lipid in the controls. This value is slightly higher than those previously reported for other Japanese populations by Arisawa et al. (2003). They measured 7 PCDDs, 10 PCDFs, and 12 PCBs among randomly selected groups of 131 men and 122 women who had no occupational exposure to dioxins, and the median total TEQs in men and women were 17 and 16 pg TEQ/g lipid, respectively. However, these values may be underestimates because the values <LOD were assigned to zero. When values <LOD were assigned to zero in our study, the median total TEQ serum levels of PCDDs, PCDFs, cPCBs, and PCBs were computed as 19.2 pg TEQ/g lipid in the controls, a value roughly consistent with that reported by Arisawa et al. (2003). We calculated the median total TEQ values of PCDDs and PCDFs to be 12.5 pg TEQ/g lipid in our controls, while the median total TEQ values of PCDDs and PCDFs previously reported were 9.8–22.9 pg TEQ/g lipid in Japan (Arisawa et al., 2003; Kumagai et al., 2000), 16.1–40.8 pg TEQ/g lipid in Germany (Papke, 1998; Wittsiepe et al., 2000), and 14.6–20.6 pg TEQ/g lipid in Canada (Ayotte et al., 1997; Ryan et al., 1997). The TEF exposure metric, which is based on Ah receptor induction, may not be appropriate for the estrogenic or anti-estrogenic activity of the chemicals measured. Some of the chemicals have been shown to have primarily anti-estrogenic characteristics (TCDD), while others have been shown to be estrogenic (PCBs, along with some of the organochlorine pesticides). Some of the DDT congeners are also anti-androgenic. It is difficult to propose an alternative metric that is more appropriate at this time, as no well-validated metrics have been developed for total estrogenic activity, anti-androgenic activity, and anti-estrogenic activity. However, it might be worthwhile to attempt to analyze these data using some alternative metrics. Another study limitation involves endogenous estrogen exposure.

Because the growth of endometrial cells depends on estrogen, the endogenous estrogen level is an important risk factor for endometriosis. Endogenous estrogen levels were not measured in the present study. Other related factors, such as menstrual regularity and average cycle, were adjusted in calculating the endometriosis risk from organochlorine exposure. Furthermore, participants of the present study had complained of infertility, and some of the controls had other estrogen-related diseases, such as myoma of the uterus (39.5%) and polycystic ovary (16.0%), according to the laparoscopic findings. Thus, the present controls may not reflect women in general. Finally, in our case-control study, endometriosis could have affected the serum levels of these organochlorine compounds because regularity and short menstrual cycles might increase the total menstrual flow, thus decreasing the women's serum levels.

Human exposure to PCDDs, PCDFs, and cPCBs occurs almost exclusively through food consumption, particularly of fish and meat. Many kinds of fish from several supermarkets in Japan reportedly contain elevated levels of PCDDs, PCDFs, and cPCBs, and the mean daily intake of these chemicals from the ingestion of fish and shellfish was higher than that of other foods (Tsutsumi et al., 2001). These findings suggest that people who eat fish often should have a higher level of PCDDs, PCDFs, and cPCBs in their system. Some studies have investigated the associations between the serum level of organochlorine compounds and the frequency of fish intake in Japan, and most of these results have shown that fish consumption is associated positively with serum levels of organochlorines (Arisawa et al., 2003; Kitamura et al., 2000; Kumagai et al., 2000). Among women in the present study, we analyzed the association between serum total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs and 6 items of food intake, and we also found that higher total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs correlate with an increased frequency of fish intake (unpublished data).

In summary, serum levels of these targeted organochlorine compounds did not differ between endometrial cases and controls except for 1,2,3,6,7,8-hexaCDD. The risk for endometriosis among participants with higher total TEQ values of PCDDs, PCDFs, cPCBs, and PCBs was nonsignificantly lower than that for participants with lower total TEQ values. These findings suggest that higher serum levels of these organochlorine compounds are not associated with an increased risk for endometriosis among infertile Japanese women.

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ORIGINAL PAPER

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Human glutathione S-transferase A1, T1, M1, and P1 polymorphisms and susceptibility to prostate cancer in the Japanese population

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Abstract *Purpose*: The incidence of prostate cancer is increasing in low-risk populations such as Japanese. One of the causes of this increase is considered to be associated with the Western diet, especially the high intake of red meat and fat. Glutathione S-transferase (GST) A1, T1, M1, and P1 are phase II enzymes that are important for activation and detoxification of chemical carcinogens. *Methods*: In this study, 190 Japanese male patients with prostate cancer and 294 healthy controls, frequency-matched for age, were compared for frequencies of *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes. *Results*: Among smokers, the frequency of the *GSTA1*A/*B* or **B/*B* genotype in patients with prostate cancer (27.8%) showed a statistically significant increase compared with the control group frequency (18.2%; odds ratio [OR] = 1.72; 95% CI, 1.01–2.94). In addition, the frequency of *GSTT1* nondeletion genotype was associated with prostate cancer among smokers (OR = 1.68; 95% CI, 1.06–2.68). The OR of carrying the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion was 2.08 (95% CI, 1.14–3.80) with the combined genotyping of *GSTA1*A/*A* and *GSTT1* null as a reference. On the other hand, no significant associations were observed for genotypes of *GSTM1* and *GSTP1 I105V*. *Conclusions*: These findings suggest that the *GSTA1* and *GSTT1* polymorphisms are associated with prostate cancer susceptibility, especially among smokers.

Keywords *GSTA1* · *GSTT1* · Polymorphism · Prostate cancer

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Introduction

Prostate cancer is the most common cancer in men in Europe, North America, and some parts of Africa. The incidence varies widely between ethnic populations and countries. The lowest rates are usually in Asia, and the highest are in North America and Scandinavia, especially in African-American people in the USA.

Migration studies have shown that when Japanese people move from Japan to the USA, the incidence of prostate cancer in these people increases; however, the increase is only to about 50% of the rate for Caucasian people and to 25% of that for African-American people in the USA (Gronberg 2003). These findings suggest that these differences are caused not only by exposure to external risk factors but also by a combination of underlying differences such as genetic susceptibility.

Several studies (Hayes et al. 1999, Veierod et al. 1997) suggest that prostate cancer is associated with a western lifestyle and, in particular, a diet that includes a high intake of red meat, which results in the formation of very potent carcinogens such as heterocyclic amines. For example, PhIP can induce a large increase in mutant frequencies in the rat prostate (Stuart et al. 2000), and ^{32}P-postlabeling analysis of DNA demonstrated that PhIP-DNA adducts are produced in all lobes of the prostates of rats receiving PhIP (Shirai et al. 1997).

Most environmental carcinogens are metabolized via complex enzymatic mechanisms involving activation and inactivation. There are genetic differences in some of these metabolisms, and these individual variations may modulate cancer risk. The glutathione S-transferases (GSTs) are a large family of phase II enzymes that facilitate the detoxification of various carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress.

GSTA1, an alpha class enzyme, is the most abundant GST found in the liver. It catalyzes the reduction of the carcinogenic *N*-acetoxy derivative back to the parent amine (Lin et al. 1994). Recently, a polymorphism of

GSTA1 gene was discovered, and the variant allele significantly lowers enzyme expression (Coles et al. 2001, Morel et al. 2002). *GSTM1* and *GSTT1* genes are polymorphic in humans, and the phenotypic absence of enzyme activity is due to the absence of a homozygous and inherited gene (Seidegard et al. 1998, Pemple et al. 1994). GSTM1, a mu class enzyme, detoxifies the reactive metabolites of benzo[a]pyrene and other polycyclic aromatic hydrocarbons (Ketterer et al. 1992). GSTT1 metabolizes various potential carcinogens, such as monohalomethanes, which are widely used as methylating agents, pesticides, and solvents (Guengerich et al. 1995). A polymorphic site at nucleotide 313 (an A-to-G substitution replacing Ile with Val) in the *GSTP1* gene was detected and found to modify the enzyme's specific activity and affinity for electrophilic substrates—for example, benzo[a]pyrene and diol epoxide (Ali-Osman et al. 1997, Watson et al. 1998).

This case control study was carried out to examine whether the genetic polymorphisms of major phase II enzymes *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* are associated with the risk of prostate cancer.

Materials and methods

Subjects

The demographic data of both case and control groups are presented in Table 1. The case groups comprised 190 prostate cancer patients (age 70.6 ± 5.9 years) from Kitakyushu City and Miyazaki Prefecture, Japan. The patients were consecutive cases presenting at the University of Occupational and Environmental Health Hospital and Miyazaki University Hospital and had been histologically diagnosed during the period of September 1992 to January 2002. None of the patients refused to participate.

The control group comprised 294 individuals who had visited local medical clinics in Kitakyushu City and Miyazaki City between September 1993 and September 2001 for regular medical health check-ups, including collection of blood and urine specimens (age 67.0 ± 10.4 years). Although no specific age-matching was carried out, the mean ages of the case individuals were similar to the control individuals. The control individuals had no current or previous diagnosis of cancer. All participants completed a questionnaire administered by a trained interviewer that covered medical, residential, occupational, and smoking status. Smoking status was summarized as smoker or never-smoker until the time of the interview. Data for prostate cancer risk factors, such as body mass index, cooking preferences, drug use, and physical activity, were not available. All participants were given an explanation of the nature of the study, and informed consent was obtained. This study was approved by the ethics committees of the University of Occupational and Environmental Health and the University of Miyazaki.

Table 1 Distribution of demographic variables for patients and controls

Variables Age (years)	Controls (n = 294)	Patients (n = 190)
Mean age (± SD)	67.0 ± 10.4	70.6 ± 5.9
Range	45–94	52–80
Smoking status		
Nonsmoker (%)	91 (31.0%)	57 (30.0%)
Smoker (%)	203 (69.0%)	133 (70.0%)

Genotype analysis

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol-chloroform extraction (Sambrook et al. 1989). The genotype of *GSTA1* (*GSTA1*A*-69C and *GSTA1*B*-69T) was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to Coles et al. (2001). Briefly, the primers used in the PCR were sense primer (5'-TGT TGA TTG TTT GCC TGA AAT T-3') and antisense primer (5'-GTT AAA CGC TGT CAC CCG TCC T-3'). The amplification was performed by denaturing at 94°C for 5 min, followed by 35 cycles at 94°C for 60 s, annealing at 64°C for 60 s, and extending at 72°C for 60 s in a Perkin-Elmer 9700 (Norwalk, CT, USA). The amplification products (20 µl) were digested by 10 U of restriction endonuclease EarI at 37°C for 12 h (Fig. 1).

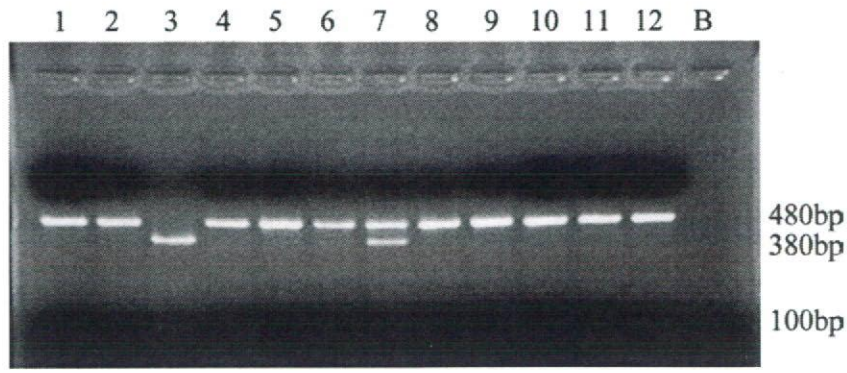

Fig. 1 Examples of restriction fragment length polymorphism (RFLP) of *GSTA1*-specific polymerase chain reaction (PCR) products. The gel shows lanes 1, 2, 4, 5, 6, 8, 9, 10, 11, and 12 homozygous *GSTA1*A* genotype samples; lane 7 a heterozygous genotype sample; lane 3 a homozygous *GSTA1*B* genotype sample; and lane B PCR reagent blank.

A multiple PCR method was used to detect the presence or absence of the *GSTT1* and *GSTM1* genes (Katoh et al. 1996). Briefly, this PCR method had both *GSTT1*- and *GSTM1*-specific primer pairs in the same amplification mixture, and included a third primer pair for β-globin.

The genotype of *GSTP1* exon5 (Ile105Val) was determined by the PCR-RFLP method according to Watson et al. (1998). Briefly, the primers used in the PCR were sense primer (5'-GTA GTT TGC CCA AGG TCA AG-3') and antisense primer (5'-AGC CAC CTG AGG GGT AAG-3'). The amplification products were digested by the restriction endonuclease Alw26I. All digest patterns were determined by resolution on a 2% agarose gel.

Statistical analysis

We used a chi-square test to compare the *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* gene polymorphisms in the prostate cancer patients with the expected gene distribution from the healthy control individuals. Crude odds ratios and 95% confidence intervals (CI) were calculated for *GATA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes. Odds ratios (OR) were adjusted for age and smoking status by using multiple logistic regression analysis. All statistical analyses were based on two-tailed probabilities. Values of $p < 0.05$ were considered statistically significant. SPSS II for Windows software (version 11.0 J, SPSS Japan, Tokyo, Japan) was used for statistical analysis.

Results

The frequencies of *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes are shown in Table 2. The distribution of *GSTA1*A/*B* genotypes were in good agreement with those expected in a Hardy-Weinberg equilibrium. The frequency of *GSTA1*A/*B* or **B/*B* genotype individuals among prostate cancer cases increased to 26.3% compared with the control groups (19.0%); however, this difference did not reach statistical significance (OR = 1.49; 95% CI, 0.96–2.32) after adjustment for age and smoking status. The *GSTT1* nondeletion genotype was weakly associated with increased incidence of prostate cancer (OR = 1.39; 95% CI, 0.95–2.03). There was no association of the *GSTM1* or *GSTP1 I105V* variant with the risk of prostate cancer.

Based on a hypothesized role for GSTs in modulating the effects of carcinogens present in tobacco smoke, we investigated the combined role of smoking and GSTs. Table 3 outlines the relationship between the *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes and prostate cancer by stratifying by smoking status. Among smokers, the frequency of *GSTA1*A/*B* or **B/*B* genotype was significantly higher in prostate cancer cases (27.8%) compared with the controls (18.2%). The OR of the individuals with *GSTA1*A/*B* or **B/*B* genotype to develop prostate cancer was 1.72 (95% CI, 1.01–2.94). Similarly, the frequency of *GSTT1* nondeletion genotype was significantly higher in prostate cancer cases (63.6%) compared with the controls (51.2%) among smokers (OR = 1.68; 95% CI, 1.06–2.68). No significant associations were observed for genotypes of *GSTM1* and *GSTP1 I105 V* variant with the risk of prostate cancer for either never-smokers or smokers.

To evaluate the interaction between the genotypes, we similarly analyzed the combined genotypes in subgroups (Table 4). The adjusted OR of carrying the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion was 2.08 (95% CI, 1.14–3.80), with the combined genotyping of *GSTA1*A/*A* and *GSTT1* null as a reference.

Discussion

This study presents the first data on the frequency of the *GSTA1* polymorphism at *GSTA1*A* (-567T, -69C, -52G) and *GSTA1*B* (-567G, -69T, -52A) in a Japanese population. The prevalence of the *GSTA1*A/*A*, **A/*B*, and **B/*B* genotypes in the control population ($n = 294$)

Table 2 Relationship between the *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes and prostate cancer (*OR* odds ratio, *CI* confidence interval)

		Controls % (*n*)	Prostate cancer % (*n*)	OR[a] (95% CI)
GSTA1	**A/*A*	81.0% (238)	73.7% (140)	1
	**A/*B*	17.0% (50)	23.7% (45)	1.48 (0.94–2.35)
	**B/*B*	2.0% (6)	2.6% (5)	1.33(0.39–4.51)
	**A/*B* or **B/*B*	19.0% (56)	26.3% (50)	1.49 (0.96–2.32)
GSTT1	Null genotype	48.3% (139)	39.8% (74)	1
	Nondeletion genotype	51.7% (149)	60.2% (112)	1.39 (0.95–2.03)
GSTM1	Nondeletion genotype	45.5% (131)	50.0% (93)	1
	Null genotype	54.5% (157)	50.0% (93)	0.76 (0.52–1.12)
GSTP1	105 Ile/Ile	72.9% (212)	76.5% (143)	1
	105 Ile/Val	23.7% (69)	20.9% (39)	0.86 (0.55–1.36)
	105 Val/Val	5.4% (10)	2.7% (5)	1.01 (0.32–3.12)
	105 Ile/Val or *105 Val/Val*	27.1% (79)	23.5% (44)	0.87 (0.57–1.35)

[a]ORs were adjusted for age and smoking status; $p < 0.05$

Table 3 Relationship between the *GSTA1*, *GSTT1*, *GSTM1*, and *GSTP1* genotypes and prostate cancer (OR odds ratio, CI confidence interval)

		Controls % (*n*)	Prostate cancer % (*n*)	OR[a] (95% CI)	
	GSTA1	**A/*A*	79.1% (72)	77.2% (44)	1
		**A/*B* or **B/*B*	20.9% (19)	22.8% (13)	1.10 (0.49–2.46)
Never smokers	GSTT1	Null genotype	46.6% (41)	47.4% (27)	1
		Nondeletion genotype	53.4% (47)	52.6% (30)	0.95 (0.49–1.86)
	GSTM1	Nondeletion genotype	37.5% (33)	56.1% (32)	1
		Null genotype	62.5% (55)	43.9% (25)	0.46 (0.23–1.06)
	GSTP1	105 Ile/Ile	71.9% (64)	71.9% (41)	1
		105 Ile/Val or *105 Val/Val*	28.1% (25)	28.1% (16)	1.00 (0.47–2.09)
	GSTA1	**A/*A*	81.8% (166)	72.2% (96)	1
		**A/*B* or **B/*B*	18.2% (37)	27.8% (37)	1.72 (1.01–2.94)[b]
Smokers	GSTT1	Null genotype	48.8% (104)	36.4% (47)	1
		Nondeletion genotype	51.2% (109)	63.6% (82)	1.68 (1.06–2.68)[b]
	GSTM1	Nondeletion genotype	49.3% (105)	47.3% (61)	1
		Null genotype	50.7% (108)	52.7% (68)	0.96 (0.61–1.51)
	GSTP1	105 Ile/Ile	73.3% (148)	78.5% (102)	1
		105 Ile/Val or *105 Val/Val*	26.7% (54)	21.5% (28)	0.84 (0.49–1.44)

[a]ORs were adjusted for age
[b]$p < 0.05$

was 81.0% ($n = 238$), 17.0% ($n = 50$), and 2.0% ($n = 6$), respectively. The distribution of the *GSTA1* polymorphism among different ethnic groups in the literature is as follows: African-American ($n = 70$) **A/*A* 61%, **A/*B* 26%, **B/*B* 13%, and Caucasian ($n = 278$) **A/*A* 38%, **A/*B* 48%, **B/*B* 14% (Coles et al. 2001). Japanese male genotype frequencies were significantly different from each of these other populations. The comparative genotype frequencies suggest that there may be racial differences in the metabolism of chemicals detoxified by GSTA1, such as activated heterocyclic aromatic amine carcinogen N-acetoxy-PhIP.

In this study, we present the first evidence of an association between *GSTA1*B* (-567G, -69T, -52A) and smoking status among prostate cancer patients. Some reports have shown an association between the incidence of prostate cancer and tobacco smoking (Hickey et al. 2001). We analyzed the prostate cancer risk in relation to *GSTA1* and *GSTT1* genotype and smoking status. Our results showed that *GSTA1*A/*B* or **B/*B* genotypes were associated with a 49% higher but nonstatistically significant increased risk of prostate cancer (OR = 1.49; 95% CI, 0.96–2.32). However, among smokers, the OR of the individuals with these genotypes to develop prostate cancer was 1.72 (95% CI, 1.01–2.72). GSTA1 has been reported to be most efficient in detoxifying N-acetoxy-PhIP, and its presence in tobacco smoke is 22.9 ng/cig (Smith et al. 2001). Therefore, we considered that GSTA1 might play an important role in protecting DNA from tobacco-derived PhIP. Although this observation needs further study, the effect of smoking may be more important for susceptible populations such as those with *GSTA1*A/*B* or **B/*B* genotypes.

Table 4 Combined effects of *GSTA1* and *GSTT1* genotypes among Japanese prostate cancer patients and control individuals (*OR* odds ratio, *CI* confidence interval)

GSTA1	GSTT1	Controls	Cases	OR[a] (95% CI)
**A/*A*	Null	112	56	1
	Nondeletion	120	80	1.36 (0.88–2.10)
**A/*B* or **B/*B*	Null	27	18	1.45 (0.73–2.89)
	Nondeletion	29	32	2.08 (1.14–3.80)[b]

[a]Odds ratios were calculated by comparing control individuals and prostate cancer groups, adjusted for age and smoking status
[b]$p = 0.018$

Rebbeck's group reported the *GSTT1* nondeletion genotype to be associated with prostate cancer risk (OR = 1.83; 95% CI, 1.19–2.80) (Rebbeck et al. 1999). Murata's group also reported similar results without statistical significance (OR = 1.6; 95% CI, 0.99–2.51) (Murata et al. 2001). Furthermore, Kelada's group reported a significant interaction between *GSTT1* nondeletion genotype and smoking that elevates the risk of prostate cancer (Kelada et al. 2000). Our results are similar to theirs (OR = 1.39; 95% CI, 0.95-2.03, and for smokers OR = 1.68, 95% CI, 1.06–2.68). These findings are consistent with the knowledge that GSTT1 produces genotoxic metabolites in response to specific exposure such as methyl chloride in cigarette smoke and dichloromethanes (Hallier et al. 1994). GSTT1 is expressed at high levels in the prostate, suggesting that GSTT1 may play a role in prostate carcinogenesis, especially among smokers.

To evaluate the interaction between the genotypes, we analyzed combined genotypes of *GSTA1* and *GSTT1*. The OR of carrying the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion was 1.36, 1.45, and 2.08 with the combined genotyping of *GSTA1*A/*A* and *GSTT1* nondeletion, *GSTA1*A/*B* or **B/*B* and *GSTT1* null, *GSTA1*A/*A* and *GSTT1* null as a reference. These results suggest that the combined genotyping of *GSTA1*A/*B* or **B/*B* and *GSTT1* nondeletion may be strongly linked to prostate cancer. We considered that this interaction may be caused by

different chemical carcinogens, such as PhIP and methyl chloride, but that the most important and common origin of the chemicals associated with this interaction is tobacco smoke.

On the other hand, no significant association was observed for genotypes of *GSTM1* and *GSTP1 I105V*. Rebbeck's group and Jeronimo's groups reported similar results (Rebbeck et al. 1999, Jeronimo et al. 2002). GSTM1 and GSTP1 metabolize a variety of potential carcinogens, including cigarette smoke-derived chemicals such as benzo[a]pyrene. Nelson et al. reported that GSTP1 has been shown to inhibit the adduction of activated PhIP metabolites to DNA in cell-free systems (Nelson et al. 2001); however, GSTP1 did not play an important role in prostate carcinogenesis in our study.

In conclusion, our data show a significant relationship between prostate cancer and genetic polymorphism of *GSTA1* and *GSTT1*, especially among smokers. These findings may be helpful for researching the risk for, and identifying individuals susceptible to, prostate cancer.

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Human glutathion S-transferase A1 polymorphism and susceptibility to urothelial cancer in the Japanese population

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Abstract

GSTA1 has been reported to be most efficient in detoxifying N-acetoxy PhIP. In this study, 341 Japanese urothelial cancer patients and 457 healthy controls were compared for frequencies of *GSTA1* genotype. We present the first evidence of an association between *GSTA1*B* (-567G, -69T, -52A) and urothelial cancer among never smokers. The frequency of *GSTA1 *A/*B* or **B/*B* genotype was 24.3% in urothelial cancer cases, compared with 21.2% in the control groups (OR = 1.22; 95%CI 0.87–1.72) after adjustment for age, gender and smoking status. But among never smokers, the *GSTA1 *A/*B* or **B/*B* genotype was significantly higher in urothelial cancer cases (31.2%) compared with the controls (19.9%) (OR = 1.73; 95%CI 1.01–2.97). This study suggests that exposure to food-derived PhIP could be one of the risk factors in the incidence of urothelial cancer in never smokers.

Keywords: *GSTA1*; Polymorphism; Urothelial cancer

1. Introduction

Approximately 6600 people in Japan die of urothelial cancer per year [1]. Tobacco smoking and occupational exposure to arylamines are two of the leading causes of this malignancy [2]. Studies of molecular epidemiology have suggested that the excess of urothelial cancer in smokers could be attributed to arylamines [3]. In general, all types of urothelial cancer have a similar histology of transitional cell carcinoma and are considered to have the same etiology. For this reason, we analyzed the four types (bladder, renal pelvis, ureter and overlap) as one category.

Most environmental carcinogens are metabolized via complex enzymatic mechanisms involving activation and inactivation. In some of these metabolisms, there are genetic differences and these individual variations may modulate cancer risk. The glutathione *S*-transferase (GSTs) are a large family of phase II

Abbreviations: GST, Glutathione *S*-transferase; PhIP, 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

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enzymes which facilitate the detoxification of various carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress.

GSTA1, an alpha class enzyme, catalyses the glutathione-dependent detoxification of the activated heterocyclic aromatic amine carcinogen N-acetoxy-PhIP [4]. A polymorphism of *GSTA1* gene was recently discovered. This polymorphism consist of three, apparently linked, single nucleotide polymorphisms (SNPs): -567T>G, -69C>T, -52G>A. GSTA1 activity is due to differences in *GSTA1*A* (-567T, -69C,-52G) and *GSTA1*B* (-567G, -69T, -52A) polymorphism [5].

This case control study was carried out to examine whether the genetic polymorphism of a major phase II enzyme, GSTA1, is associated with the risk of urothelial cancer among Japanese people in relation to their smoking status.

2. Materials and methods

2.1. Subjects

The case groups comprised 341 urothelial cancer patients (bladder $n=265$, renal pelvis $n=11$, ureter $n=23$, overlap $n=42$) (265 men, 76 women; age 69.6 ± 10.5) from Kitakyushu City and Miyazaki City, Japan. The patients were consecutive cases presenting at the University of Occupational and Environmental Health Hospital and Miyazaki University Hospital, and had been histologically diagnosed during the period of September 1992 to January 2002. None of the patients refused to participate. All the pathological types of urothelial cancer were transitional cell carcinoma.

The control group comprised 457 individuals who had visited local medical clinics in Kitakyushu City and Miyazaki City between September 1993 and April 2002 for regular medical health check-ups (including collection of blood and urine specimens). The individuals with hematuria were excluded from the control group. There were 284 men and 173 women (age 67.2 ± 12.2 years). While no specific age matching was carried out, the mean ages of the cases were similar to the control individuals. The control individuals had no current or previous diagnosis of cancer. All participants completed a questionnaire administered by a trained interviewer, covering medical, residential, occupational and smoking status. A few patients suspected occupational urothelial cancer were excluded from the case group. Smoking status was summarized as smoker or never-smoker up to the time of the interview. Data for urothelial cancer risk factor, such as cooking preferences, drug use and physical activity, were not available. All participants were given an explanation of the nature of the study and informed consent was obtained. This study was approved by the ethics committees of the University of Occupational and Environmental Health and the University of Miyazaki.

2.2. Genotyping

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/-chloroform extraction [6]. The genotype of *GSTA1* was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) according to Coles et al. [5]. Briefly, the primers used in the PCR were sense primer (5′-TGT TGA TTG TTT GCC TGA AAT T-3′) and antisense

Table 1
GSTA1 genotype frequencies among Japanese urothelial cancer patients and control individuals

	Age (mean±SD)	*GSTA1 *A/*A*	*GSTA1 *A/*B*	*GSTA1 *B/*B*	*GSTA1 *A/*B* or **B/*B*	Odds ratio[a] (95% confidence interval)
Control individuals ($n=457$)	67.2 ± 12.2	78.8% (360)	19.7% (90)	1.5% (7)	21.2% (97)	1
Urothelial cancer ($n=341$)	69.6 ± 10.5	75.7% (258)	22.6% (77)	1.8% (6)	24.3% (83)	1.22 (0.87–1.72)

[a] Odds ratio(95% confidence interval). Odds ratios were calculated by comparison of control individuals and cancer groups for *GSTA1 *A/*B* or *GSTA1 *B/*B* versus *GSTA1 *A/*A*, adjusted for age, gender and smoking status. $^{*}P<0.05$.

Table 2
Odds ratio of urothelial cancer by smoking habit

	Urothelial cancer, odds ratio (95%CI)[a]
Smoking status	
Never-smokers	1.00[b]
Smokers	1.59 (1.12–2.24)[c]

[a] Odds ratio was adjusted for age and gender.
[b] Reference category.
[c] $P=0.009$.

primer (5′-GTT AAA CGC TGT CAC CCG TCC T-3′). Thirty nanograms of DNA was amplified in a total volume of 30 μl containing 16.7 pmol of each primer, 0.8 U of Taq polymerase, 2 mM $MgCl_2$, and PCR buffer. The amplification was performed by denaturing at 94 °C for 5 min, followed by 35 cycles at 94 °C for 60 s, annealing at 64 °C for 60 s and 72 °C for 60 s in a Perkin–Elmer 9700 (Norwalk USA). The amplification products (20 μl) were digested by 10 U of restriction endonuclease EarI at 37 °C for 12 h. Digest patterns were determined by resolution on a 2% agarose gel.

2.3. Statistical analysis

We used a χ^2-test to compare the *GSTA1* gene polymorphism in the urothelial cancer patients with the expected gene distribution from the healthy control individuals. Crude odds ratios and 95% confidence intervals (95%CI) were calculated for *GATA1* genotype. Odds ratios (OR) were adjusted for age, gender and smoking status using multiple logistic regression analysis. All statistical analyses were based on two-tailed probabilities. Values of $P<0.05$ were considered statistically significant. SPSS II for Windows software (version 11.0J, SPSS Japan, Tokyo, Japan) was used for statistical analysis.

3. Results

The frequency of the *GSTA1* genotype in relation to the urothelial cancer is shown in Table 1. The distribution of *GSTA1 *A/*B* genotypes were in good agreement with those expected in a Hardy-Weinberg equilibrium. The frequency of *GSTA1 *A/*B* or **B/*B* genotype was 24.3% in urothelial cancer cases, compared with 21.2% in the control groups (OR= 1.22; 95%CI 0.87–1.72) after adjustment for age, gender and smoking status. There was no significant difference in this frequency between the cancer cases and controls.

Based on a hypothesized role for GSTs in modulating the effects of carcinogens present in tobacco smoke, we investigated a combined role of smoking and *GSTA1* polymorphism. Table 2 outlines the risk of urothelial cancer by smoking habit. The age-adjusted OR of smoking was 1.59 (95%CI 1.12–2.24). Table 3 outlines the relationship between the *GSTA1* genotypes and urothelial cancer by stratifying for smoking status. Among never smokers, the *GSTA1 *A/*B* or **B/*B* genotype was significantly higher in urothelial cancer cases (31.2%) compared with the controls (19.9%). The OR of the individuals with *GSTA1 *A/*B* or **B/*B* genotype to get urothelial cancer was 1.73 (95%CI 1.01–2.97). On the other

Table 3
Relationship between the *GSTA1* genotype and smoking among study group

	Age (mean ± SD)	*GSTA1* genotype		Risk
		**A/*A* genotype	**A/*B *B/*B* genotype	Odds ratio 95% confidence interval[a]
Never-smoker				
Control (216)	69.3 ± 11.9	80.1% (173)	19.9% (43)	1
Urothelial cancer (109)	71.4 ± 10.9	68.8% (75)	31.2% (34)	1.73 (1.01–2.97)[b]
Smoker				
Control (241)	65.2 ± 12.2	77.6% (187)	22.4% (54)	1
Urothelial cancer (232)	68.7 ± 10.2	78.9% (183)	21.1% (49)	0.99 (0.63–1.55)

[a] Odds ratio (95% confidence interval). Odds ratio were calculated by comparison of control individuals and cancer group for *GSTA1 *A/*B* or **B/*B* versus *GSTA1 *A/*A*, adjusted for age and gender category.
[b] $P=0.045$.

hand, there was no association of the GSTA1 genotypes with the risk of urothelial cancer among smokers (OR = 0.99; 95%CI 0.63–1.55).

4. Discussion

In this study, we present the first evidence of an association between *GSTA1*B* (-567G, -69T, -52A) and urothelial cancer among never smokers.

GSTA1 is the most abundant GST found in the human liver. GSTA1 activity is due to differences in *GSTA1*A* (-567T, -69C, -52G) and *GSTA1*B* (-567G, -69T, -52A). A significant decrease of luciferase activity was observed *GSTA1*B* (-567G, -69T, -52A) and the base change at position -52 might explain the different activities of *GSTA1*A* and *GSTA1*B* promoters [7].

Smoking is the greatest risk factor for urothelial cancer. Smokers get bladder cancer twice as often as people who do not smoke, and the dose–response relationship between smoking and urothelial cancer was observed [8]. Our data also show that the age-adjusted OR of smoking was 1.59 (95%CI 1.12–2.24). Several studies reported [2,9] that arylamines, including 4-aminobiphenyl (ABP) in tobacco smoke, play an important role in urothelial cancer etiology. Also, it has been recognized that genetic polymorphism of *N-acetyltransferase (NAT)1, NAT2* and *GSTM1*, which detoxifies the reactive metabolites of arylamine and benzo[a]pyrene, are associated with urothelial cancer susceptibility, especially among smokers [10–12].

Our results showed that *GSTA1 *A/*B* or **B/*B* genotypes were not associated with an increased risk of urothelial cancer (OR = 1.22 95%CI 0.87–1.72), but among never smokers the OR of the individuals with these genotypes to get urothelial cancer was 1.73 (95%CI 1.01–2.97). GSTA1 has been reported to be most efficient in detoxifying N-acetoxy PhIP. Various heterocyclic amines have been suggested as potential dietary factors increasing the risk of bladder cancer [13,14]. Therefore we hypothesized that urothelial cancer in smokers may be mainly attributed to the arylamines and benzo[a]pyrene contained in tobacco smoke. However, in never smokers exposure to food-derived PhIP could be one of the risk factors in the incidence of urothelial cancer.

In conclusion, our data show a significant relation between urothelial cancer and genetic polymorphism of *GSTA1* among never smokers. These findings may be helpful for researching the risk and identifying individuals susceptible to urothelial cancer.

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Fish intake and serum levels of organochlorines among Japanese women

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Abstract

This study evaluates background serum levels of selected organochlorine compounds among Japanese women of reproductive age and investigates whether lifestyle factors, especially dietary factors, may be associated with these levels. A cross-sectional study was performed on 80 Japanese women, aged 26–43 years, who complained of infertility and were confirmed not to have endometriosis. The serum levels of total toxic equivalency (TEQ), 18 polychlorinated dibenzo-*p*-dioxins (PCDDs)/polychlorinated dibenzofurans (PCDFs), 4 coplanar polychlorinated biphenyls (cPCBs), 36 *ortho*-substituted polychlorinated biphenyls (PCBs), and 13 chlorinated pesticides or their metabolites were measured and data were collected on the women's age, residence, occupation, body mass index (BMI), smoking and alcohol habit and 6 dietary intakes (fish, meats, rice, vegetables, fruits and dairy products). The serum median level of total TEQ was 25.1 pg TEQ/g lipid, that of PCDDs/PCDFs/cPCBs was 11.5 pmol/g lipid, that of PCBs was 0.46 nmol/g lipid, and that of total pesticides was 1.32 nmol/g lipid. The serum levels of total TEQ, PCDDs/PCDFs/cPCBs, PCBs and pesticides were positively associated with age (P for trend=0.003, 0.01, 0.005 and 0.01, respectively) and frequent fish consumption (P for trend=0.002, 0.003, 0.0003 and 0.006, respectively). Other lifestyle factors were not associated with serum organochlorine levels. The present study suggests that Japanese women who consume fish frequently in their reproductive period tend to accumulate organochlorines in their bodies.

Keywords: Fish intake; Organochlorines; Dioxins; Polychlorinated biphenyls; Pesticides; DDT

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