นิพนธ์ต้นฉบับ • Original Article

ความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของจีน *Glutathione S-transferase theta-1* (*GSTT1*) ร่วมกับการสูบบุหรี่ของคู่นอนกับความเสี่ยงต่อการเกิดมะเร็งปากมดลูก

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The Correlation between *Glutathione S-Transferase Theta-1* (*GSTT1*) Polymorphism in Relation to Partners' Smoking and Cervical Cancer Risk

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หลักการและวัตถุประสงค์: ความหลากหลายทางพันธุกรรมของจีน *glutathione S-transferases theta-1* (*GSTT1*) เป็นปัจจัยหนึ่งที่ทำให้การทำลายสารก่อมะเร็งที่พบในบุหรี่แตกต่างกันซึ่งอาจส่งผลต่อการเกิดโรคมะเร็งในแต่ละบุคคลที่แตกต่างกันด้วย การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาบทบาทของความหลากหลายทางพันธุกรรมของจีน *GSTT1* ต่อความเสี่ยงในการเกิดมะเร็งปากมดลูกในสตรีที่มีคู่นอนสูบบุหรี่

วิธีการศึกษา: ศึกษาความหลากหลายทางพันธุกรรมของจีน *GSTT1* ด้วยวิธี multiplex PCR ในกลุ่มผู้ป่วยมะเร็งปากมดลูกชนิด squamouscell carcinoma จำนวน 204 ราย และเปรียบเทียบกับกลุ่มสตรีที่มีสุขภาพดีที่มีอายุใกล้เคียงกัน จำนวน 204 ราย

ผลการศึกษา: การศึกษานี้ ไม่พบความแตกต่างระหว่างจีโนไทป์ของ *GSTT1* ในกลุ่มผู้ป่วยมะเร็งปากมดลูกและกลุ่มควบคุม อย่างไรก็ตาม ในกลุ่มสตรีที่มีคู่นอนที่มีระยะเวลาการสูบบุหรี่ตั้งแต่ 40 ปีขึ้นไป พบว่าสตรีที่มีจีโนไทป์แบบ heterozygous present (+/-) จะมีความเสี่ยงต่อการเกิด

Background and Objectives: Genetic polymorphism in *glutathione S-transferases theta-1* (*GSTT1*) gene has involved in detoxification of carcinogens derived from tobacco smoke and considered as a potential modifier of individual cancer susceptibility. The present study was to investigate the role of *GSTT1* polymorphism and partners' smoking status on cervical cancer risk.

Methods: The *GSTT1* polymorphism was determined by multiplex PCR analysis in 204 SCCA patients compared with 204 age-matched healthy controls.

Results: No significant difference was observed in the distribution of *GSTT1* genotypes in both patients and controls. Among subjects who had partners with 40 years and over of smoking duration, a significant increase in cervical cancer risk was observed in women carrying heterozygous (+/-) genotype with adjusted OR of 9.54 (95%CI = 1.19-76.51, p = 0.034).

Conclusion: This result demonstrates the interaction effect between *GSTT1* polymorphism and timing of

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มะเร็งเพิ่มขึ้น 9.54 เท่า เมื่อเทียบกับสตรีที่มีจีโนไทป์แบบ homozygous present (+/+) (95%CI = 1.19-76.51, p = 0.034)

สรุป: การศึกษานี้ได้แสดงให้เห็นถึงบทบาทของหลากหลายทางพันธุกรรมของจีน *GSTT1* ร่วมกับระยะเวลาการสูบบุหรี่ของคู่นอนมีผลเพิ่มความเสี่ยงต่อการเกิดมะเร็งปากมดลูกในสตรีไทย

tobacco smoke exposure on cervical cancer increased susceptibility among Thai population.

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Introduction

Cervical cancer is a second most common cancer in Thai female[1]. The known risk factors involved in cervical carcinogenesis are human papillomavirus (HPV) infection, multiparity, use of oral contraceptives, smoking and genetic variations[2,3]. Partners' smoking habits has also been found to be associated with cervical cancer due to inhalation of carcinogen in tobacco smoke or exposure to tobacco-related carcinogens in seminal fluid[4]. Carcinogens present in tobacco smoke such as polycyclic aromatic hydrocarbons (PAHs), ethylene oxide,1,3 butadiene and nicotin require metabolic activation and detoxification by phase I and/or phase II enzymes[5,6]. Thus, the determining individual susceptibility to smoking-related cancer may be depending on carcinogen metabolizing genes variations.

Phase II *glutathione S-transferases* (*GSTs*) genes encode GST enzymes involved in the detoxification of potential carcinogens derived from tobacco smoke[7]. These enzymes detoxify electrophilic metabolites through conjugation with reduced glutathione[8]. There are several polymorphisms present in the genes encoding GSTs. *Glutathione S-transferase theta-1* (*GSTT1*), located on chromosome 22 (22q11.23) is partially explain inter- individual's susceptibility to cancer[9]. Polymorphism in *GSTT1* exhibits a deletion polymorphism with two alleles, one functional and the other nonfunctional alleles[10]. The genotypes are divided into homozygous present (+/+), heterozygous present (+/-) and homozygous deletion (-/-); null genotypes. Individuals with null genotype lead to the complete lack of phenotypic enzyme activity whereas heterozygous present genotype carriers result in low enzymatic activity that are considered to be at elevated risk for carcinogenesis due to reduced efficiency in protection against environmental carcinogens[5, 10-12].

Several studies have been suggested complex interaction between environmental and genetic factors on the development of cancers[8,12,13]. There are evidences concerning the association between *GSTT1* and susceptibility to smoking-related cancers such as cervical cancer[12,13], lung cancer[14] and laryngeal and pharyngeal cancer[10]. Nevertheless, most of studies did not distinguish between the genotypes of homozygous and heterozygous present[15]. Identification analysis between individuals with two, one, or no functional *GSTT1* alleles may provide more useful information for understanding of GSTT1 enzymatic activity[16]. Therefore, the purpose of this study was to evaluate the influence of *GSTT1* polymorphism independently or in combination with partners' smoking duration on cervical cancer susceptibility among Thai population.

Materials and Methods

Study population

The study population was conducted in Khon Kaen hospital and Srinagarind hospital, Khon Kaen province, northeastern Thailand that included 204 patients with pathological examinations confirmed squamous cell carcinoma of the cervix (SCCA) and 204 healthy women without cervical cancer and/or pre-invasive lesion of the cervix confirmed by cytological and histological examination. The controls were matched to the cases on

age within 5 year interval. All subjects were interviewed by a questionnaire to obtain information on smoking status. The study was approved by the Ethic Committee of Khon Kaen university (Reference No. 561382) and written informed consent was obtained from all subjects who participated in the study.

Detection of *GSTT1* polymorphism

Genomic DNA (gDNA) was extracted from buffy coat using GF-1 Blood DNA Extraction Kit (Vivantis, USA). *GSTT1* polymorphism was done by multiplex polymerase chain reaction (PCR) method with following 2 primer pairs: 5'-CAGTTGTGAGCCACCGTACCC-3', 5'-CGATAGTTGCTGGCCCCCTC-3', 5'-CCAGCTCAC-CGGATCATGGCCAG-3' and 5'-CCTTCCTTACTGGTC-CTCACATCTC-3'. The PCR amplification was performed in a reaction mixture of 25 µl containing 1 µl of DNA template, 12.5 µl of KOD buffer, 5 µl of dNTP, 0.2 mM of each primer, 0.5 unit of KOD FX (Toyobo, Japan). The cycling condition included initial denaturation at 94°C for 2 min, followed by 30 cycles of 98°C for 10 sec and 68°C for 90 sec. PCR product (1460 bp and/or 466 bp) was analyzed by electrophoresis on 1.5% agarose gels.

Statistical Analyses

Statistical analyses were performed using the STATA software. The differences of genotype and allele frequencies between cases and controls were examined by using chi-square test. The association between genotypes and risks for SCCA was performed by using uni- and multi-variate logistic regression analyses. The odds ratio (OR) and 95 % confidence interval (95%CI) were calculated to estimate the strength of the association between *GSTT1* polymorphism and cervical cancer risk. A p-value less than 0.05 was considered to be statistically significant.

Results

The genotype and allele frequencies of *GSTT1* polymorphism are described in Table 1. Genotypic frequencies of homozygous present (+/+), heterozygous present (+/-) and homozygous deletion genotypes (-/-) were 17.17%, 46.97% and 35.86% in controls and 17.17%, 50.51% and 32.32% in cases. Allelic frequencies of *GSTT1* between cervical cancer patients and healthy controls were not significantly different. Furthermore, there was no significant association between *GSTT1* polymorphism and cervical cancer susceptibility ($p > 0.05$).

Interaction effect between partners' smoking status and the *GSTT1* polymorphism on cervical cancer risk is presented in Table 2. Interestingly, who had partners with 40 years and over of smoking duration found a significant increase in cervical cancer risk in women having heterozygous (+/-) genotype with adjusted OR of 9.54 (95%CI = 1.19-76.51, p = 0.034).

Table 1 Association between *GSTT1* polymorphism and risk for cervical cancer

GSTT1 genotypes	Control n (%)	Case n (%)	Crude OR [95%CI, *p*]	Adjusted OR[a] [95%CI, *p*]
+/+	34(17.17)	34(17.17)	1	1
+/-	93(46.97)	100(50.51)	1.08[0.60-1.94, 0.7970]	1.43[0.62-3.29, 0.401]
-/-	71(35.86)	64(32.32)	0.90[0.48-1.68, 0.7272]	1.26[0.52-3.04, 0.603]
+/- and -/-	164(82.83)	164(82.83)	1.00[0.57-1.75, 1.0000]	1.36[0.62-3.00, 0.447]
Allele distribution				
Null allele	0.59	0.58	1	NA
Present allele	0.41	0.42	0.93[0.69-1.25, 0.6137]	NA

OR: odds ratio, CI: confidence interval

[a]adjusted multiple logistic regression for partners' smoking, oral contraceptive use and HPV infection

NA: not applicable

Table 2 Association between *GSTT1* polymorphism, duration of partners' smoking and risk for cervical cancer

Duration of partners' smoking		*GSTT1* genotypes	Control n (%)	Case n (%)	Crude OR [95%CI, *p*]	Adjusted OR[a] [95%CI, *p*]
No		+/+	15 (7.58)	6 (3.03)	1	1
		+/-	38 (19.19)	33 (16.67)	1.17 [0.76-6.24, 0.150]	2.32 [0.55-9.77, 0.250]
		-/-	38 (19.19)	16 (8.08)	1.05 [0.35-3.20, 0.928]	0.70 [0.16-3.14, 0.644]
		+/- and -/-	76 (38.38)	49 (24.75)	1.61 [0.59-4.44, 0.355]	1.39 [0.37-5.31, 0.626]
Yes	0 - <20 years	+/+	3 (1.52)	6 (3.03)	1	1
		+/-	15 (7.58)	10 (5.05)	0.33 [0.07-1.65, 0.178]	0.08 [0.00-2.58, 0.154]
		-/-	6 (3.03)	11 (5.56)	0.92 [0.17-5.05, 0.920]	0.49 [0.02-10.88, 0.652]
		+/- and -/-	21 (10.61)	21 (10.61)	0.50 [0.11-2.27, 0.369]	0.22 [0.01-4.06, 0.307]
	20 - <40 years	+/+	8 (4.04)	14 (7.07)	1	1
		+/-	31 (15.66)	31 (15.66)	0.57 [0.21-1.56, 0.273]	0.84 [0.16-4.28, 0.830]
		-/-	14 (7.07)	25 (12.63)	1.02 [0.34-3.03, 0.971]	2.14 [0.35-12.96, 0.408]
		+/- and -/-	45 (22.73)	56 (28.28)	0.71 [0.27-1.84, 0.483]	1.14 [0.25-5.43, 0.866]
	≥ 40 years	+/+	8 (4.04)	8 (4.04)	1	1
		+/-	9 (4.55)	26 (13.13)	2.88 [0.84-9.97, 0.093]	9.54 [1.19-76.51, 0.034*]
		-/-	13 (6.57)	12 (6.06)	0.92 [0.26-3.24, 0.901]	4.11 [0.47-36.25, 0.203]
		+/- and -/-	22 (11.11)	38 (19.19)	1.73 [0.57-5.25, 0.335]	6.96 [0.99-49.19, 0.052]

OR: odds ratio, CI: confidence interval, * p< 0.05

[a]adjusted multiple logistic regression for oral contraceptive use and HPV infection

Discussion

The most common *GSTT1* genotype in our population is heterozygous genotype which is consistent with previous studies in German population[14], and Danish population[16] but differ from Japanese population[17].No significant association was observed between *GSTT1* polymorphisms independently and cervical cancer susceptibility in Thais. This finding is consistent with the reported in other populations, Chinese, Thai, Pakistan and Turkish populations[18-21]. Thus, our results denote that genetic polymorphism in *GSTT1* gene alone is not sufficient to cervical cancer susceptibility. Nevertheless, we found evidence of interaction between *GSTT1* polymorphism and smoking duration of their partners on cervical cancer susceptibility. Heterozygous present genotype carriers who had partners with 40 years and over of smoking duration were significantly increased risk for cervical cancer compared to homozygous present genotype. The mechanisms of this association may be described by the role of enzymatic activity of GST on detoxification of tobacco carcinogens[5,8,22,23]. There are many carcinogens found in tobacco smoke, especially PAHs and ethylene oxide that are mostly metabolized by cytosolic GST enzymes[6]. Low activity of GSTT1 enzyme in women carrying only one functional allele of *GSTT1* would be more susceptible to cervical cancer than those with homozygous present *GSTT1* allele if they were exposed to carcinogens due to impairment of detoxification capacity[10]. Interestingly, an increased risk for cervical cancer was observed only in heterozygous *GSTT1* carriers with long-term smoke exposure (≥40 years). It seems to be the impact of timing exposure leading to different accumulative dose of exposed carcinogens[24,25]. These results are in agreement with those obtained in other studies, an elevated risk for cervical cancer was observed in carriers of *GSTT1* null and heterozygous genotypes[12, 26]. Nevertheless, there was no interaction of *GSTT1* null genotype and passive smoking on cervical cancer that may be explained by compensatory role of the other GST superfamily members[27].

Conclusion

In conclusion, our results suggest the interaction effect between *GSTT1* polymorphism and timing of tobacco smoke exposure on cervical cancer susceptibility. In passive smoker, heterozygous *GSTT1* carriers are at higher risk of developing cervical cancer compared to homozygous present individuals.

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