RESEARCH ARTICLE

Lack of Participation of the *GSTM1* Polymorphism in Cervical Cancer Development in Northeast Thailand

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Abstract

The potential association between the *GSTM1* deletion polymorphism and risk of cervical cancer was investigated in Northeastern Thailand. DNA was extracted from buffy coat specimens of 198 patients with squamous cell carcinoma of the cervix and 198 age-matched healthy controls. Genotyping of the *GSTM1* was conducted by using two PCR methods, a short- and a long-PCR. Distribution of the *GSTM1* genotypes in between the cases and the controls was not significantly different ($p>0.5$ by χ^2 test). The results suggest that the *GSTM1* deletion polymorphism is not a risk factor for squamous cell carcinoma of the cervix in the northeast Thai women.

Keywords: Cervical cancer - *GSTM1* genotype - genetic susceptibility - Northeast Thailand

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Introduction

Glutathione S-transferases (GSTs) belong to a group of the phase II enzymes, which play roles in the detoxification of exogenous substrates (Schnakenberg et al 2000, Sanyal et al 2004). Among a variety of GST genes, an allele with a deletion of the whole *GSTM1* gene (*GSTM1*Δ) was identified (Duell et al., 2002). Homozygous for the *GSTM1*Δ known as *GSTM1-null* resulting in the lack of enzyme activity has been investigated with a special reference to the susceptibility to various cancers such as oral cancer, nasopharyngeal carcinoma, lung cancer and others (Schnakenberg et al., 2000; Tiwawech et al., 2005, Liu et al., 2014). However, the conventional PCR assay identifying the *GSTM1-null* (Δ/Δ) is unable to distinguish the heterozygous genotype (W/Δ) from the homozygous for the wild-type allele (W/W) and the susceptibility to cancers by genotype was unpredictable; in fact, risks for cancer development of the *GTM1Δ* are still unknown and related reports are also controversial (Singh et al., 2008; Matic et al., 2013; Safarinejad et al., 2013).

In our previous study, we employed the conventional PCR to evaluate risk of the *GSTM1-null* for the squamous cell carcinoma of the cervix (SCCA) development in the northeast Thai women and found no relation between *GSTM1-null* and SCCA development (Settheetham-Ishida et al., 2009) however, presence of the genotype contribution could not be rejected. To make it clear whether the *GSTM1*Δ can be a risk for SCCA development, we here employed a set of PCR assays, conventional (short) and long PCR, for genotyping *GSTM1*.

Materials and Methods

Study subjects

This case-control study comprised 198 cases of pathologically defined squamous cell carcinoma of the cervix (SCCA) and 198 age-matched healthy controls with normal cytology (Pap smear test) and histology. All the subjects aged 26-81 years were recruited at Khon Kaen General Hospital and Srinagarind (university) Hospital, Khon Kaen Province, Northeast Thailand, between February 2009 and August 2011. The controls and cases were matched within 5-year age groupings. Each subject was informed of the methodology and objectives of the research and signed a consent form. This study was reviewed and approved by the Ethics Committee of both Khon Kaen University (HE 450333) and Khon Kaen Hospital (No. 03/02/2554).

Genotyping of the GSTM1

Genomic DNA was extracted from buffy coat using GF-1 Blood DNA Extraction Kits (Vivantis, USA). Genotyping of the *GSTM1* was performed by using two steps (a short- and a long-PCR method) as following: 1) the *GSTM1-null* allele was identified by the short-PCR

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method (Tiwawech et al., 2005). The sequences for the primer pairs were: 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and 5'-GTT GGG CTC AAA TAT ACG GTG G-3'. Co-amplification of the human β-globin gene using primers 5'-AAC TTC ATC CAC GTT CAC C-3' and 5'-GAA GAG CCA AGG ACA GGT AC-3' was used as the internal control. PCR amplifications were conducted in 25 μl PCR and the conditions consisted of an initial denaturation at 95°C for 5 min, followed by 40 cycles of 98°C 10 sec, 60°C for 20 sec and 72°C for 45 sec, and a final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis on agarose gels 2.5%. and the *GSTM1* heterozygous allele was identified by the long-PCR method (Roodi et al., 2004) performed with the following primers to amplify the exon 4 of *GSTM1*: primer M3, 5'-CCT GTT GAA GGA GCT TAT GCT GAA-3' and M4, 5'- TTC TGA GGA CTG GAC TGA TGA TC-3'. The long PCR with KOD FX (Toyobo, Japan) was conducted in a total volume of 25 μl, and cycle condition was as follows: 94°C for 2 min, followed by 30 cycles of 98°C for 14 sec and 62°C for 30 sec, and a final extension at 68°C for 14 min. PCR product (14kb) was analyzed by electrophoresis on 0.5% agarose gels.

Statistical analyses

The χ^2-test was used for the analyses. Differences were considered statistically significant when the *p*-value was<0.05.

Results

Distribution of the *GSTM1-null* among cases and controls is shown in Table 1 where the *GSTM1-null* prevalence in the cases was not different between 2002-3 and 2009-11 (p=0.36 by Fisher's exact test). No significant deviation from Hardy-Weinberg equilibrium in the genotype distribution was observed both in cases and controls (p>0.5 by χ^2 test). Distribution of the *GSTM1* genotypes between the cases and the controls was not significantly different (p>0.5 by χ^2 test) (Table 2).

Table 1. *GSTM1* Presence and Cervical Cancer

	Type of *GSTM1*	
	Present	Null
Present study*		
Case	68 (34.3)	130 (65.7)
Control	73 (36.9)	125 (63.1)
Settheetham-Ishida et al. (2009)**		
Case	36 (40.0)	54 (60.0)
Control	38 (40.4)	56 (59.6)

*based on the subjects recruited in 2009-2011; **based on the subject recruited in 2002-2003

Discussion

Contribution of the *GSTM1-null* to the development of various types of cancer has been documented with controversial conclusions (Duell et al., 2002; Roodi et al., 2004; Tiwawech et al., 2005; Liu et al., 2014; Stosic et al., 2014). Our present study identifying *GSTM1* genotypes in the age matched case-control study could not find the effect of *GSTM1* polymorphism on cervical carcinogenesis; the result rejects the association between genetic polymorphism of *GSTM1* and increased risk for cervical cancer (Table 2). This genotyping study confirmed our previous report on the *GSTM1-null* and cervical cancer (Settheetham-Ishida et al., 2009) and agreed with the previous finding in Caucasian (Chen et al., 1999), Japanese (Niwa et al., 2005), Indian (Sobti et al., 2006, Singh et al., 2008), Sicilian (Agodi et al., 2010) and Italian (Palma et al., 2010) women. In contrast, an association between the *GSTM1* polymorphism and increased risk of cervical cancer was observed in central Serbia and suggested a possible important role of the *GSTM1* deletion in the development of early stage of precancerous lesions (Stosic et al., 2014). Furthermore, a meta-analysis found *GSTM1-null* genotype showed an increased risk of uterine cervical lesions in Chinese and Indian but no risk for Japanese, European and American (Gao et al., 2011; Zhang et al., 2012). The *GSTM1* gene deletion may promote a development of cervical dysplasia by inhibiting the detoxification of polycyclic hydrocarbons and other compounds that modulate oxidative stress and DNA adduct formation (Parl, 2005), while ethnic backgrounds may influence on the cancer susceptibility and result in the controversial conclusions (Gao et al., 2010; Zhang et al., 2012).

The *GSTM1* deletion is caused by homologous recombination, which results in a 16-kb deletion containing the entire *GSTM1* gene (Roodi et al., 2004). There is a substantial difference in the baseline frequency of the *GSTM1-null* genotype in different ethnic groups (Roodi et al., 2004, Singh et al 2008). The frequency of the *GSTM1-null* genotype has been reported 53.1% (42.0-60.0%) in Caucasians, 52.9% (42.0-54.0%) in Asians, and 26.7% (16.0-36.0%) in Africans (Garte et al., 2001). An association between the *GSTM1* and elevated breast cancer risk was observed in Caucasian but not in African-American (Roodi et al., 2004). Also, different effects on significantly increased risk of cervical neoplasia were observed in Asian populations but not in Caucasian and mixed populations (Gao et al., 2011; Zhang et al., 2012).

Table 2. *GSTM1* Genotype Distribution and Cervical Cancer

GSTM1 polymorphism	Cases n (%)	Controls n (%)	OR [95% CI, *p*-value]	Adjusted OR[a] [95% CI, *p*-value]
W/W and W/Δ	68 (34.34)	73 (36.87)	1	1
Δ/Δ	130 (65.66)	125 (63.13)	1.12 [0.72-1.72, 0.60]	0.85 [0.45-1.60, 0.62]
W/W	15 (7.58)	15 (7.58)	1	1
W/Δ	53 (26.77)	58 (29.29)	0.91 [0.38-2.22, 0.83]	1.25 [0.63-2.47, 0.52]
Δ/Δ	130 (65.66)	125 (63.13)	1.04 [0.45-2.39, 0.92]	0.90 [0.27-2.99, 0.87]

*W: wild-type allele; Δ: null allele; [a] adjusted for HPV status, smoking status and age

To clarify the role of the *GSTM1* in cervical carcinogenesis, *GSTM1* genotype and the development of cervical abnormality should be examined. At present, we conclude that *GSTM1*Δ itself is not a risk for cervical cancer development in northeast Thai women.

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