RESEARCH ARTICLE

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

Sitakan Natphopsuk1,2, Wannapa Settheetham-Ishida1*, Dariwan Settheetham3, Takafumi Ishida4

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 χ² 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 (GSTM1Δ) was identified (Duell et al., 2002). Homozygous for the GSTM1Δ 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 GSTM1Δ 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 GSTM1Δ 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 AGC 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 G-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 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 containing the entire gene (Roodi et al., 2004). The χ²-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 χ² test). Distribution of the GSTM1 genotypes between the cases and the controls was not significantly different (p>0.5 by χ² test) (Table 2).

### 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 (Setthetheetham-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 (Purl, 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 1. GSTM1 Presence and Cervical Cancer

<table>
<thead>
<tr>
<th>Type of GSTM1</th>
<th>Present</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>68 (34.3)</td>
<td>130 (65.7)</td>
</tr>
<tr>
<td>Control</td>
<td>73 (36.9)</td>
<td>125 (63.1)</td>
</tr>
<tr>
<td>Setthetheetham-Ishida et al. (2009)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>36 (40.0)</td>
<td>54 (60.0)</td>
</tr>
<tr>
<td>Control</td>
<td>38 (40.4)</td>
<td>56 (59.6)</td>
</tr>
</tbody>
</table>

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

### Table 2. GSTM1 Genotype Distribution and Cervical Cancer

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

*W: wild-type allele; Δ: null allele; * 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 GSTM1Δ itself is not a risk for cervical cancer development in northeast Thai women.

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