

GSTM1 Null Genotype is Associated with an Increased Risk of Cutaneous Malignant Melanoma in the Japanese Population

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Abstract

Variations in the Glutathione S-transferase (GST) supergene family have been reported to influence cancer susceptibility in Caucasian. However the genetic backgrounds and skin types are quite different between Caucasian and non-Caucasian. We therefore investigated the distribution of GST gene polymorphism in a Japanese population to ascertain the role of this polymorphism in melanomagenesis. Forty-six patients with cutaneous malignant melanoma and 92 healthy individuals who visited Kobe University Hospital between April 2004 and November 2010 were enrolled in this study. Genotype of GST gene was determined by using polymerase chain reaction-restriction fragments length polymorphism analysis. The frequencies of GSTM1 null genotype were significantly different between malignant melanoma and controls (adjusted odds ratio=2.25, 95% confidence interval 1.06-4.77). The polymorphism of GSTM1 locus could be associated with cutaneous malignant melanoma risk among Japanese population.

Keywords: Glutathione S-transferase; Cutaneous malignant melanoma; Polymorphism; SNP; Skin cancer

Introduction

Members of the Glutathione S-transferase (GST) supergene family, which consists of nine gene subfamilies (GSTA, GSTD, GSTK, GSTM, GSTO, GSTP, GSTT, GSTZ, MGST), are known to metabolize toxic products produced by ultraviolet (UV)-induced oxidative stress [1], mostly by conjugation of glutathione to electrophiles but also by other specific mechanisms [2,3].

Certain genes within the GSTM and GSTT (GSTM1 and GSTT1) subfamilies exhibit deletion polymorphisms which have been involved in cancer susceptibility [2,4]. Genetic variations at the GSTM1 loci have been shown to alter the susceptibility to basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM) in the United Kingdom [5]. And more recent additional studies have implicated polymorphisms status of GSTM1 and GSTT1 as relevant for the development of non melanoma skin cancers (NMSCs) among Caucasian population [6-8].

On the other hand, differences between ethnicities were observed in published reports of meta-analysis studies concerning GST genotypes at risk of gastric cancer and acute leukemia [9,10]. And also GSTM1 locus could be an important factor in susceptibility to SCC in a Japanese population [11]. Moreover there has been no report focusing on susceptibility to MM among a Japanese population. In this study, we analyzed the relationship between polymorphisms of GST supergene families and cutaneous MM in a Japanese population, and proposed to establish a simple screening method of identifying the high risk group for cutaneous MM.

Materials and Methods

Study population

Forty-six patients with cutaneous MM (mean age \pm standard deviation (SD), 63.6 ± 13.3 years; age range, 35-89 years) (14 acral lentiginous melanoma (ALM), 3 lentigo maligna melanoma (LMM), 7 nodular melanoma, 2 superficial spreading melanoma (SSM) and 20 undetermined melanoma) who visited the dermatology clinic in Kobe University Hospital between April 2004 and November 2010 were enrolled in this study. Clinical diagnosis was confirmed by

	Control	Malignant melanoma
Male	49	24
Female	43	22
Total	92	46
Mean age: Year \pm SD	65.7 \pm 14.0	63.6 \pm 13.3

SD, standard deviation

Table 1: Clinical characteristics and number of cases and controls.

histopathological analysis in all cases. The control group consists of 92 age- and sex-matched non affected unrelated Japanese individuals (mean age \pm SD, 65.7 ± 14.0 years; age range, 28-89 years) from the same area in Japan with minor fungi, bacterial infections or seborrheic keratosis (Table 1).

We subcategorized the patients according to the location of cancer lesions whether on sun exposure site (head, neck and hand) or not. The frequencies of MM were: sun exposure site, 17.4% and not-sun exposure site, 82.6%. We handled these patients as anonymous samples about which nobody could know their personal information other than their genotypes. Written informed consent was also obtained from all participants. The Medical Ethics Committee of Kobe University approved this work that was conducted in accordance with the Declaration of Helsinki principles.

Identification of genotypes

Genomic DNA was extracted from peripheral mononuclear cells using a Qiagen FlexiGene DNA kit (Qiagen, Tokyo, JAPAN) according

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to the manufacturers' protocols. The *GSTM1* null or positive genotype and the *GSTT1* null and expressing subjects were identified by using a PCR approach. The *GSTP1* polymorphism (p.105 Ile>Val) (rs 1695) were determined by PCR-restriction fragment length polymorphisms (RFLP) technique. Each sequence of the primers, restriction enzymes and details of genotyping were described elsewhere [3]. In some cases, determination of allelic polymorphism in *GSTM1* fragments length polymorphisms (RFLP) technique. Each sequence of the primers, restriction enzymes and details of genotyping were described elsewhere [3]. In some cases, determination of allelic polymorphism in *GSTM1* and *GSTP1*, PCR product was purified by a Qiagen PCR purification kit and DNA direct sequences analysis was performed using Applied Biosystems Model 377A Automated DNA Sequencer (Applied Biosystems, Foster City, CA).

Statistical analysis

For statistical analysis, we used SPSS for Windows, version 17.0 (SPSS Japan Inc.) to calculate the adjusted odds ratio (AOR) and 95% confidence interval (CI). All adjusted models included age and sex.

Results

Polymorphisms of *GSTM1*, *GSTP1* and *GSTT1* and malignant melanoma risk

The *GST* gene polymorphisms *GSTM1*, *GSTP1* and *GSTT1* were investigated. Their genotype distributions in MM and controls are shown in table 2. We found a significantly increased risk of MM was associated with *GSTM1* null genotype (AOR = 2.25, 95% CI 1.06-4.77).

Genotype	Controls (%)	Malignant melanoma		
		Cases (%)	OR (95% CI)	AOR (95% CI)
<i>GSTM1</i>				
positive	46 (50.0)	14 (30.4)	1.0 (-)	1.0 (-)
null	46 (50.0)	32 (69.6)	2.29 (1.08-4.84)	2.25 (1.06-4.77)
<i>GSTP1</i>				
Ile/Ile	66 (71.7)	34 (73.9)	1.0 (-)	1.0 (-)
Ile/Val + Val/Val	26 (28.3)	12 (26.1)	0.90 (0.40-1.99)	0.88 (0.39-1.99)
<i>GSTT1</i>				
positive	53 (57.6)	28 (60.9)	1.0 (-)	1.0 (-)
null	39 (42.4)	18 (39.1)	0.87 (0.42-1.80)	0.86 (0.41-1.83)

OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

Table 2: Distribution of Glutathione S-transferase genotypes in patients and controls.

Since *GSTM1* and *GSTT1* protect from oxidized lipid and DNA, epoxide, and cytotoxic reagents, individuals null at both *GSTM1* and *GSTT1* loci would be expected to be at a greater risk than those lacking only one gene [12]. Thus our results were further analyzed to determine whether combinations of genotypes of *GST* genes were involved in the MM development, however, there were no significant correlation between different genotypic combination of null genes and increased risk of MM (data not shown).

Polymorphisms of *GSTM1*, *GSTP1* and *GSTT1* and risk of malignant melanoma on sun- or less-exposed area

To assess the effect of sun-exposure, we subdivided the MM whether the lesion was on the sun-exposed area or not (Table 3). No statistical significance was found on the analysis of subcategorized data.

Discussion

In our study, *GSTM1* null genotype was seen in 50% of the control population, 69.7% of MM cases. Marked differences in the distribution of *GSTM1* genotype were found between MM and control. The frequencies of homozygous deletions of *GSTM1* and *GSTT1* in the controls were similar to those previously reported in other studies [13], while *GSTM1* polymorphism was associated with MM, not consistent with the findings of other groups [14]. It could be possible that the different gene expression is associated with different types of MM in the MM growth. Most of our samples are MM originated from covered area (Table 3), consisting of acral lentiginous melanoma and nodular melanoma, whereas most MM in Australian [14] originate from sun-exposed area, mainly superficial spreading type. *GSTs* detoxify reactive oxygen species that are generated in the skin following exposure to UV and can directly attack DNA and can cause DNA damage [15]. UV exposure is one of the important factors involved in the process of skin carcinogenesis. Excessive oxidative stress induced by large amount of UV exposure could overcome the effect of detoxification of *GST*-encoding enzymes in case of melanoma of sun-exposed area. On the other hand, continual mechanical stimulation also could cause chronic inflammation, which also might overcome the effect of detoxification of *GST*-encoding enzyme. As far as our data are concerned, sun-exposure had nothing to do with the *GSTM1* null genotype in MM (Table 3).

Like that of the *GSTM1* gene, *GSTT1* also has a null genotype. According to the previous data, the null genotype was seen in approximately 20% of the cases and controls in the Caucasian population [6,15]. In our study the distributions of *GSTT1* null genotype were quite different from the data among Caucasian population both in cases (39.1%) and controls (42.4%). The frequency of *GSTT1* null type did not differ between patients and controls.

Genotype	Controls (%)	MM with sun-exposed			MM without sun-exposed		
		Cases (%)	OR (95% CI)	AOR (95% CI)	Cases (%)	OR (95% CI)	AOR (95% CI)
<i>GSTM1</i>							
positive	46 (50.0)	1 (14.3)	1.0 (-)	1.0 (-)	13 (33.3)	1.0 (-)	1.0 (-)
null	46 (50.0)	6 (85.7)	6.00 (0.70-51.8)	5.89 (0.68-51.3)	26 (66.7)	2.00 (0.92-4.37)	1.96 (0.90-4.30)
<i>GSTP1</i>							
Ile/Ile	66 (71.7)	5 (71.4)	1.0 (-)	1.0 (-)	29 (74.4)	1.0 (-)	1.0 (-)
Ile/Val + Val/Val	26 (28.3)	2 (28.6)	1.02 (0.19-5.57)	1.09 (0.19-6.12)	10 (25.6)	0.88 (0.37-2.05)	0.84 (0.35-1.99)
<i>GSTT1</i>							
positive	53 (57.6)	4 (57.1)	1.0 (-)	1.0 (-)	24 (61.5)	1.0 (-)	1.0 (-)
null	39 (42.4)	3 (42.9)	1.02 (0.22-4.82)	0.91 (0.18-4.57)	15 (38.5)	0.85 (0.40-1.83)	0.86 (0.38-1.93)

OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

Table 3: Distribution of Glutathione S-transferase in malignant melanoma with or without sun-exposed lesion Cases.

The homozygous variant genotype of *GSTP1* (p.105 Val/Val) has been seen in other study to be involved in an increase in susceptibility of other cancer types. Therefore expression of wild type allele type (Isoleucine) has been shown to be protective against tumor progression. Although previous study indicated that polymorphism at the *GSTP1* locus would be an important factor in susceptibility to bladder and testicular cancer [4], no significant difference in the frequencies of *GSTP1* wild type (p.105 Ile/Ile) and variant types (p.105 Ile/Val and p.105 Val/Val) could be demonstrated between controls and malignant melanoma groups in our analysis. The reason why we combined the *GSTP1* p.105 Ile/Val and Val/Val was that only two cases (1 MM and 1 control) of *GSTP1* p.105 Val/Val genotype were seen in the population.

Although the reason for the discrepancy between the result of our study and that of the studies in Caucasian population remains unknown, we could hypothesize that the genetic factors involved in melanomagenesis vary among races and also skin types [16,17]. Curtin et al. also reported that genetic backgrounds which correlate with the mechanisms of melanomagenesis are different between ALM melanoma and the other types of MM such as LMM and SSM [18]. Indeed, the involvement of *BRAF* gene mutation in melanoma cases in a Japanese population is also less frequent than that of Caucasian [19]. In this regard, there could be various other pathways through which other genes can act, in particular genes involved in cell cycle regulation, DNA repair and other anticancer immune mechanisms, these would be expected to act independently of *GSTM1* pathway. Since the number of the cases and controls in our study were insufficient, future studies should explore the association between *GST* gene polymorphisms and skin cancer susceptibility in a Japanese population.

Conclusions

In summary, we conclude that the polymorphism at the *GSTM1* locus could be an important factor in susceptibility to MM in Japanese population. Therefore, a better understanding of the factors that predispose to MM will enable identification of causative factors and development of prevention strategies, especially identification of high risk group for MM. High risk patients could be included into skin cancer surveillance program to promote earlier detection of MM.

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