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Polymorphisms of Glutathione S-Transferase in Skin Cancers in a 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 non-Caucasian population to ascertain the role of this polymorphism in skin carcinogenesis. One-hundred and fifteen patients with skin cancers and 92 controls 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 positive genotype were significantly higher in squamous cell carcinomas than those in controls (adjusted odds ratio = 3.09, 95% confidence interval 1.04 - 9.21). The polymorphism of GSTM1 locus could be an important factor in susceptibility to squamous cell carcinoma among Japanese population.

INTRODUCTION

Members of the Glutathione S-transferase (GST) supergene family, which consists of eight gene subfamilies (GSTA, GSTK, GSTM, GSTO, GSTP, GSTT, GSTZ and MGST), are known to protect against chemical toxins and carcinogens by catalyzing the conjugation of glutathione to electrophiles in substrate (1 - 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 in the United Kingdom (5). Recent additional studies have implicated polymorphisms status of GSTM1 and T1 as relevant for the development of nonmelanoma skin cancers (NMSC) among Caucasian population (6 - 8).

On the other hand, differences between ethnic populations were observed in published reports of meta-analysis studies concerning GST genotypes at risk of gastric cancer and acute leukemia (9, 10). Moreover there has been no report focusing on susceptibility to skin cancers among non Caucasian population.

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In this study, we analyzed the relationship between polymorphisms of GST supergene families and skin cancers in non-Caucasian population, and proposed to establish a simple screening method of identifying the high risk group for skin cancers.

MATERIALS AND METHODS

Study population.

One-hundred and fifteen patients with skin cancers (mean age ± standard deviation (SD), 75.3 ± 10.6 years; age range, 43 - 95 years) who visited the dermatology clinic in Kobe University Hospital between April 2004 and November 2010 were enrolled in this study. Skin cancers included 20 actinic keratoses (AK), 41 BCC, 25 SCC and 29 Bowen's diseases (BD). Bowenoid type actinic keratoses were included in AK. Clinical diagnosis was confirmed by histopathological analysis in all cases. The control group consists of 92 age- and sex-matched non affected unrelated Japanese individuals (mean age ± SD, 65.7 ± 14.0 years; age range, 28 - 89 years) from the same area in Japan with slight fungal or bacterial infections, or seborrheic keratosis. (Table I)

Table I. Clinical characteristics and number of cases and controls

	Control	Actinic keratosis	Basal cell carcinoma	Squamous cell carcinoma	Bowen's disease
Male	49	6	22	12	11
Female	43	14	19	13	18
Total	92	20	41	25	29
Mean-age: year±SD	65.7 ± 14.0	79.8 ± 7.2	72.8 ± 11.4	79.0 ± 9.1	72.7 ± 11.1

SD, standard deviation

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 to the manufacturers' protocols. The GSTM1 null or positive (includes A, B and AB) genotype and GSTP1 Ile/Ile, Ile/Val and Val/Val polymorphism were determined by PCR-restriction fragment length polymorphisms (RFLP) technique. GSTT1 null and expressing subjects were identified by using a PCR approach. 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 P1, 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 skin cancer risk.

The polymorphisms in GST supergene family, GSTM1, GSTP1 and GSTT1 were investigated for NMSC. Their genotype distributions in skin cancers and controls are shown in Table II. We found a significantly increased risk of squamous cell carcinomas was associated with GSTM1 positive genotype (AOR = 3.09, 95% CI 1.04 - 9.21).

Table II. Distribution of Glutathione S-transferase genotypes in skin cancer patients and controls

Genotype	Controls (%)	Actinic keratosis			Bowen's disease		
		Cases (%)	OR (95% CI)	AOR (95% CI)	Cases (%)	OR (95% CI)	AOR (95% CI)
GSTM1							
null	46 (50.0)	7 (35.0)	1.0 (-)	1.0 (-)	13 (44.8)	1.0 (-)	1.0 (-)
positive	46 (50.0)	13 (65.0)	1.86 (0.68-5.08)	1.75 (0.57-5.37)	16 (55.2)	1.23 (0.53-2.85)	1.12 (0.47-2.66)
GSTP1							
Val/Val + Val/Ile	26 (28.3)	3 (15.0)	1.0 (-)	1.0 (-)	7 (24.1)	1.0 (-)	1.0 (-)
Ile/Ile	66 (71.7)	17 (85.0)	2.23 (0.60-8.26)	1.77 (0.41-7.70)	22 (75.9)	1.24 (0.47-3.25)	1.15 (0.42-3.11)
GSTT1							
null	39 (42.4)	10 (50.0)	1.0 (-)	1.0 (-)	13 (44.8)	1.0 (-)	1.0 (-)
positive	53 (57.6)	10 (50.0)	0.74 (0.28-1.94)	0.88 (0.29-2.63)	16 (55.2)	0.91 (0.39-2.10)	0.95 (0.39-2.32)

Genotype	Controls (%)	Basal cell carcinoma			Squamous cell carcinoma		
		Cases (%)	OR (95% CI)	AOR (95% CI)	Cases (%)	OR (95% CI)	AOR (95% CI)
GSTM1							
null	46 (50.0)	21 (51.2)	1.0 (-)	1.0 (-)	6 (24.0)	1.0 (-)	1.0 (-)
positive	46 (50.0)	20 (48.8)	0.95 (0.46-1.99)	0.88 (0.41-1.90)	19 (76.0)	3.17 (1.16-8.65)	3.09 (1.04-9.21)
GSTP1							
Val/Val + Val/Ile	26 (28.3)	11 (26.8)	1.0 (-)	1.0 (-)	7 (28.0)	1.0 (-)	1.0 (-)
Ile/Ile	66 (71.7)	30 (73.2)	1.07 (0.47-2.46)	0.92 (0.39-2.18)	18 (72.0)	1.01 (0.38-2.71)	0.57 (0.18-1.78)
GSTT1							
null	39 (42.4)	22 (53.7)	1.0 (-)	1.0 (-)	9 (36.0)	1.0 (-)	1.0 (-)
positive	53 (57.6)	19 (46.3)	0.64 (0.30-1.33)	0.61 (0.28-1.34)	16 (64.0)	1.31 (0.52-3.27)	1.39 (0.51-3.84)

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

Polymorphisms of GSTM1, GSTP1 and GSTT1 risk of SCC on sun-exposed or less-exposed skin area.

To assess the effect of sun-exposure, we subdivided the SCCs whether the lesion was on the sun-exposed area or not (Table III). The GSTM1 positive genotype may be associated with SCC risk according to non-sun-exposed lesion (AOR = 5.79, 95% CI 1.15 - 29.1).

Table III. Distribution of Glutathione S-transferase in squamous cell carcinoma on sun-exposed or less-exposed skin area

Genotype	Controls (%)	SCC on sun-exposed area			SCC on less-exposed area		
		Cases (%)	OR (95% CI)	AOR (95% CI)	Cases (%)	OR (95% CI)	AOR (95% CI)
GSTM1							
null	46 (50.0)	4 (33.3)	1.0 (-)	1.0 (-)	2 (15.4)	1.0 (-)	1.0 (-)
positive	46 (50.0)	8 (66.7)	2.00 (0.56-7.11)	1.76 (0.43-7.12)	11 (84.6)	5.50 (1.16-26.2)	5.79 (1.15-29.1)
GSTP1							
Val/Val + Val/Ile	26 (28.3)	3 (25.0)	1.0 (-)	1.0 (-)	4 (30.8)	1.0 (-)	1.0 (-)
Ile/Ile	66 (71.7)	9 (75.0)	1.18 (0.30-4.71)	0.66 (0.13-3.32)	9 (69.2)	0.89 (0.25-3.13)	0.60 (0.15-2.37)
GSTT1							
null	39 (42.4)	6 (50.0)	1.0 (-)	1.0 (-)	3 (23.1)	1.0 (-)	1.0 (-)
positive	53 (57.6)	6 (50.0)	0.74 (0.22-2.46)	0.69 (0.18-2.67)	10 (76.9)	2.45 (0.63-9.51)	2.36 (0.58-9.66)

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

DISCUSSION

In our study, marked differences in the distribution of GSTM1 genotype were found between SCC and control (Table II). In addition, the frequency of homozygous deletions of GSTM1 was surprisingly reduced the risk of SCC on less-exposed area in Japanese population.

In general, GST null genotype is reported to susceptibility of skin cancer (7, 11-13), and also the enzymatic activity is lower than GST positive genotype (14, 15). On the view point of carcinogenesis, lower activity of GST enzymes is likely to induce cell damage that finally causes production of skin cancer.

GSTs detoxify reactive oxygen species (ROS) which can directly attack DNA and can cause DNA damage (11). UV exposure is one of the major factors involved in the process of skin carcinogenesis. UV generates ROS and oxidative DNA damage caused by UV is involved in skin carcinogenesis. Thus, it could be possible that insufficient function of GST might cause oxidative stress related skin cancers. In fact Carless et al. reported that the GSTM1 null genotype confers an increased risk for solar keratosis development in an Australian Caucasian population (6).

Nevertheless, our results did not indicate that null type of GST is involved in the susceptibility of AK or SCC on the sun-exposed area. Rather, SCCs on both sun-exposed skin area and less-exposed skin area were closely related to the GSTM1 positive genotypes (Table III).

The reason of these conflicting results is unknown. The difference in the acute sunburn inflammation among the ethnic group could be one of the reasons for this discrepancy. The studies of polymorphism in the susceptibility of carcinogenesis were sometimes competing. Different ethnic, racial, environmental factor and so on could be related to the results.

In Japanese population, GSTM1 null genotypes may be alter the enzymatic activity but induce another pathway of protecting cancer development, further analysis are needed.

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, 11). In our study the distributions of GSTT1 null genotype were quite different from the data among Caucasian population both in cases (48.9%) and controls (42.4%). The frequency of GSTT1 null type did not differ between patients and controls.

The homozygous variant genotype of GSTP1 (Val/Val) has been seen in other study to be involved in an increase in susceptibility of other cancer types. 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 (Ile/Ile) and variant types (Val/Val, Val/Ile) could be demonstrated between controls and skin cancer groups in our analysis. Since GSTM1 and T1 protect against oxidized lipid and oxidized DNA, epoxide, and cytotoxic reagents, individuals nulled at both GSTM1 and T1 loci would be expected to be at a greater risk than those lacking only one gene (16). In our study, there was no correlation between SCC susceptibility and null type of both GSTM1 and T1.

One possible explanation for our results is that GSTM1 positive phenotype suppresses cell apoptosis that might allow the survival of mutated cells, since GSTP1 suppresses cell apoptosis and over expression of GSTP1 is reported in many types of cancers (17). In addition 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 (18).

Since the number of the cases and controls in our study were insufficient, further studies should explore the association between GST gene polymorphisms and skin cancer susceptibility in non-Caucasian population.

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

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REFERENCES

1. **Strange, R.C. and Fryer, A.A.** The glutathione S-transferase: influence of polymorphism on cancer susceptibility. In Boffetta, P., Caporaso, N., Cuzick, J., Lang, M., Vineis, P. eds. Metabolic polymorphisms and cancer. IARC Scientific Publications, IARC, Lyon, pp. 231-249.
2. **Zhong, S., Wyllie, A.H., Barnes, D., Wolf, C.R. and Spurr, N.K.** Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis 1993; **14:** 1821-1824.
3. **Curran, J.E., Weinstein, S.R. and Griffiths, L.R.** Polymorphisms of glutathione S-transferase genes (GSTM1, GSTP1 and GSTT1) and breast cancer susceptibility. Cancer letters 2000; **153:** 113-120.
4. **Harries, L.W., Stubbins, M.J., Forman, D., Howard, G.C. and Wolf, C.R.** Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to testicular, breast and prostate cancer. Carcinogenesis 1997; **18:** 641-644.
5. **Heagerty, A.H., Fitzgerald, D., Smith, A., Bowers, B., Jones, P., Fryer, A.A., Zhao, L., Alldersea, J. and Strange, R.C.** Glutathione S-transferase GSTM1 phenotypes and protection against cutaneous tumours. Lancet 1994; **343:** 266-268.
6. **Carless, M.A., Lea, R.A., Curran, J.E., Appleyard, B., Gaffney, P., Green, A. and Griffiths, L.R.** The GSTM1 null genotype confers an increased risk for solar keratosis development in an Australian Caucasian population. J Invest Dermatol 2002; **119:** 1373-1378.
7. **Ramsay, H.M., Harden, P.N., Reece, S., Smith, A.G., Jones, P.W., Strange, R.C. and Fryer, A.A.** Polymorphisms of glutathione S-transferase are associated with altered risk of nonmelanoma skin cancer in renal transplant recipients: A preliminary analysis. J Invest Dermatol 2001; **117:** 251-255.
8. **Fryer, A.A., Ramsay, H.M., Lovatt, T.J., Jones, P.W., Hawley, C.M., Nicol, D.L., Strange, R.C. and Harden, P.N.** Polymorphisms in glutathione S-transferase and non-melanoma skin cancer risk in Australian renal transplant recipients. Carcinogenesis 2005; **26:** 185-191.
9. **Ye, Z. and Song, H.** Glutathione S-transferase polymorphisms (GSTM1, GSTP1 and GSTT1) and the risk of acute leukemia: a systemic review and meta-analysis. Eur J Cancer 2005; **41:** 980-989.
10. **La Torre, G., Boccia, S. and Ricciardi, G.** Glutathione S-transferase M1 status and

gastric cancer risk: a meta-analysis. Cancer lett 2005; **217:** 53-60.

11. **Mossner, R., Andres, N., Konig, I.R., Kruger, U., Schmidt, D., Berking, C., Ziegler, A., Brockmoller, J., Kaiser, R., Volkenandt, M., Westphal, G.A. and Reich, K.** Variations of the melanocortin-1 receptor and the glutathione S-transferase T1 and M1 genes in cutaneous malignant melanoma. Arch Dermatol Res 2007; **298:** 371-379.
12. **Shanley, S.M., Chenevix-Trench, G., Palmer, J. and Hayward, N.** Glutathione S-transferase GSTM1 null type in Australian patients with nevoid bacal cell carcinoma syndrome or sporadic melanoma. Carcinogenesis 1995; **16:** 2003-2004.
13. **Lira, M.G., Provezza, L., Malerba, G., Naldi, L., Remuzzi, G., Boschiero, L., Forni, A., Rugiu, C., Piaserico, S., Alaibac, M., Turco, A., Girolomoni, G. and Tessari, G.** et al. Glutathione S-transferase and CYP1A1 gene polymorphisms and non-melanoma skin cancer risk in Italian transplanted patients. Exp Dermatol 2006; **15:** 958-965.
14. **Hayes, J.D. and Strange, R.C.** Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology. 2000; **61:** 154-166.
15. **Stewart, R.K., Smith, G.B.J., Donnelly, P.J., Reid, K.R., Petsikas, D., Conlan, A.A. and Massey, T.E.** Glutathione S-transferase-catalyzed conjugation of bioactivated aflatoxin B1 in human lung: differential cellular distribution and lack of significance of the GSTM1 genetic polymorphism. Carcinogenesis. 1999; **20:** 1971-1977.
16. **Elexpuru-Camiruaga, J., Buxton, N., Kandula, V., Dias, P.S., Cambell, D., McIntosh, J., Broome, J., Jones, P., Inskip, A., Alldersea, J., Fryer, A.A. and Strange, R.C.** Susceptibility to astrocytoma and meningioma: Influence of alleism at glutathione S-transferase (GSTT1 and GSTM1) and Cytochrome p-450 (CYP2D6) loci. Cancer Res 1995; **55:** 4237-4239.
17. **Mutallip, M., Nohata, N., Hanazawa, T., Kikkawa, N., Horiguchi, S., Fujimura, L., Kawakami, K., Chiyomaru, T., Enokida, H., Nakagawa, M., Okamoto, Y. and Seki, N.** Glutathione S-transferase P1 (GSTP1) suppresses cell apoptosis and its regulation by miR-133α in head and neck squamous cell carcinoma (HNSCC). Int J Mol Med 2010; Epub ahead of print.
18. **Lear, J.T., Smith, A.G., Strange, R.C. and Fryer, A.A.** Detoxifying enzyme genotypes and susceptibility to cutaneous malignancy. Br J Dermatol 2000: **142:** 8-15.