Truncal Tumor Site Is Associated with High Risk of Multiple Basal Cell Carcinoma and Is Influenced by Glutathione *S*-Transferase, GSTT1, and Cytochrome P450, CYP1A1 Genotypes, and Their Interaction

John T. Lear,* Andrew G. Smith,* Bill Bowers,† Adrian H.M. Heagearty,* Peter W. Jones,‡ Janice Gilford,§ Julie Alldersea,§ Richard C. Strange,§ and Anthony A. Fryer§

*Department of Dermatology, Central Outpatients Department, North Staffordshire Hospital, Stoke-on-Trent, Staffordshire, U.K.; †Department of Dermatology, Royal Cornwall Hospitals, Truro, U.K.; ‡Department of Mathematics, Keele University, Staffordshire, U.K.; and §Center for Pathology and Molecular Medicine, School of Postgraduate Medicine, Keele University, North Staffordshire Hospital, Stoke-on-Trent, Staffordshire, U.K.

Basal cell carcinoma (BCC) places increasing burdens on clinicians; incidence is rising and patients may develop multiple primary tumors. Although UV exposure is critical, many patients develop tumors at less-exposed sites, such as the trunk, suggesting a genetic predisposition. We previously showed that polymorphism in loci encoding the detoxifying enzymes, glutathione *S*-transferase (GSTM1, GSTM3, GSTT1) and cytochrome P450 (CYP2D6, CYP1A1) influences susceptibility to BCC. We now describe a case–control approach in 345 patients with BCC that examines the role of these polymorphisms and patient characteristics (age, gender, skin type, hair color, eye color, smoking, occupation) in determining susceptibility to truncal tumors. GST and CYP genotypes were identified using polymerase chain reaction-based methods. Patients with one or more truncal tumors were significantly younger (p = 0.0170) than those with no truncal tumors. Male gender also appeared more common in the truncal tumor group, although this did not achieve significance (p = 0.0925). Patients whose first tumor was truncal had significantly more tumors (p = 0.0297). GSTT1 null (p = 0.0245, odds ratio 2.24) and CYP1A1 Ile/Ile (p = 0.0386, odds ratio 2.86) were associated with truncal site after correction for age and gender. The combination, GSTT1 null and CYP1A1 Ile/Ile, was particularly significant (p = 0.0059, odds ratio = 2.95). These effects were present after correction for tumor numbers. These data show first, patients with truncal tumors constitute a high-risk group for BCC, second, a significant genetic influence on BCC site, and third, a significant interaction between GSTT1 and CYP1A1 genotypes. ***Key words: genetic predisposition/detoxifying enzymes/polymorphism. J Invest Dermatol 108:519–522, 1997***

Basal cell carcinoma of skin (BCC) is the commonest cancer in Caucasians, accounting in 1994 for about 35% of all newly diagnosed neoplasms in the United States (Boring *et al*, 1993, 1994; Miller and Weinstock, 1994). A remarkable feature of this pathology is the risk suffered by patients of developing further tumors at different sites. Importantly, this risk depends on the number of lesions already present; 27% of patients with one tumor will suffer a further tumor within 5 y compared with 90% in those with 10 or more lesions (Kricker *et al*, 1993). Exposure to ultraviolet radiation (UV) is recognized as a critical factor in the pathogenesis of BCC (Kricker *et al*, 1993; Kripke, 1994; Karagas and Greenberg, 1995), although the relationship between amount, timing, and nature of exposure and risk is complex and poorly understood. Indeed, compared with cutaneous squamous cell cancer, BCC are more common on generally less-exposed sites, especially the trunk (Kricker *et al*, 1993; Karagas and Greenberg, 1995), with lesions infrequently found on the forearms or backs of the hands. Also, whereas the incidence of BCC is increasing, it is the proportion of tumors on the trunk that demonstrates the greatest increase (Kricker *et al*, 1993). Together, these data suggest that susceptibility to BCC is dependent not merely on UV exposure but also on host genetic factors. This view is supported by data showing susceptibility to UVB-induced inhibition of contact hypersensitivity appears to be a better indicator of non-melanoma skin cancer risk than cumulative UV exposure (Schmieder *et al*, 1992).

Because UV exposure is a critical factor in the pathogenesis of BCC (Kripke, 1994; Karagas and Greenberg, 1995), factors that mediate individual response to the pleiotrophic effects of radiation are candidates for susceptibility to BCC. Thus, skin type and male gender are recognized as risk factors (Kricker *et al*, 1993). The concept of susceptibility, however, is complex because genetic factors, as well as influencing BCC risk in individuals without tumors, may also influence tumor numbers, rate of appearance of

Manuscript received August 28, 1996; revised December 10, 1996; accepted for publication January 7, 1997.

Reprint requests to: Dr. Anthony A. Fryer, School of Postgraduate Medicine, University of Keele, North Staffordshire Hospital, Stoke-on-Trent, Staffordshire, ST4 7PA. U.K.

Abbreviations: BCC, basal cell carcinoma; GST, glutathione *S*-transferase; CYP, cytochrome P-450; CI, confidence interval.

0022-202X/97/$10.50

tumors (accrual), and their site. We have studied genetic predisposition to BCC using each of these risk parameters. Some promising candidates for BCC risk are identified, polymorphism in genes involved in repair of UV-damaged DNA and detoxification of the products of oxidative stress being significant (Heagerty *et al*, 1994; Wei *et al*, 1994; Heagerty *et al*, 1996; Lear *et al*, 1996). Thus, we have shown that polymorphism at detoxifying enzyme gene loci such as members of the glutathione *S*-transferase (GSTM1 null, GSTM3 AA, and GSTT1 null genotypes) and cytochrome P450 (CYP2D6 EM and CYP1A1 m1 m1 genotypes) supergene families, as well as patient characteristics such as skin type 1, mediate susceptibility to increased numbers of primary tumors and their rate of accrual (Lear *et al*, 1996; Yengi *et al*, 1996). GSTM1, GSTM3, and GSTT1 appear attractive candidates, because these enzymes utilize the products of oxidative stress-induced damage to DNA and lipids. Both CYP1A1 and its ligand-dependent transcription factor, the Ah receptor, are widely expressed in extrahepatic tissues including skin (Raunio *et al*, 1995), and although the gene has largely been studied in the context of environmental pollutants (e.g., polycyclic aromatic hydrocarbons), there is evidence that its products utilize endogenous ligands and participate in defense against oxidative stress (Nebert, 1994; Smith *et al*, 1995). The role of CYP2D6 is unclear (Ingelman-Sundberg and Johansson, 1995). Its expression is mainly hepatic and although found in brain and intestine, it has not been detected in skin (Raunio *et al*, 1995). The association between CYP2D6 PM and susceptibility to Parkinson's disease, however, suggests *in vivo* substrates that include endogenous neurotoxins and molecules containing amine or guanidino groups (Guengerich, 1995; Smith *et al*, 1995). Further, because systemic agents such as arsenic predispose to multiple BCC (Yeh *et al*, 1968), CYP2D6-mediated hepatic detoxification of photosensitizing agents may be important.

Because the trunk is less exposed, or at least intermittently exposed, to UV, it could be hypothesized that patients with truncal tumors represent a high-risk group because they are less able to handle the products of UV-induced damage. Little research has addressed this issue or investigated possible differences in individuals with and without truncal tumors, although it is known that exposure to arsenic-containing tonics predisposes to truncal tumors (Yeh *et al*, 1968), supporting the view that factors other than UV alone may be important. We hypothesize that the presence of truncal tumors is more strongly associated with genetic susceptibility. Accordingly, we now describe studies to investigate genetic differences and individual characteristics in patients with and without truncal tumors.

MATERIALS AND METHODS

Patients The influence of genotypes and characteristics on tumor site was studied in 345 unrelated Northern European Caucasians with histologically proved BCC. They were recruited from Dermatology clinics in the North Staffordshire Hospital, Stafford District General Hospital, and Royal Cornwall Hospitals. Of first tumors, 80.1% were on the head/neck, 11.4% were truncal, 6.5% were lower limbs, and the remaining 2.0% were on the upper limbs; 170 patients suffered one tumor and 175 patients between two and 30 tumors. Recurrences were excluded from the total number of primary BCC. No patients with basal cell nevus syndrome, xeroderma pigmentosum, or BCC and another malignancy (cutaneous or internal) were included. None of those approached refused to participate. All patients were examined and interviewed by a trained dermatologist (J.T.L., A.S., A.H.M.H.) to obtain information on hair (blonde/red and brown/black) and eye color (blue/green and brown) at 21 y of age, skin type (types 1–4), occupation (indoor/outdoor), smoking history (ever/never smokers) (Lear *et al*, 1996), as well as the time between tumor appearance and presentation to a physician. Patients were also questioned regarding ingestion of arsenic-containing tonics and use of drinking water from potentially contaminated wells. No arsenic-exposed patients were identified.

Identification of GSTM1, GSTM3, GSTT1, CYP2D6, and CYP1A1 Genotypes Blood (5 ml) was taken with Ethics Committee approval into ethylenediamine tetraacetic acid and stored at −50°C. GSTM1 null, A, B, and A/B were identified using a polymerase chain reaction approach (Lear *et al*, 1996). GSTM3 genotypes were identified using primers to exon 6/7 (Yengi *et al*, 1996). *GSTM3*B* was differentiated from *GSTM3*A* by digestion with MnlI. GSTT1 null and expressers were also identified by polymerase chain reaction (Lear *et al*, 1996). Two mutant CYP2D6 alleles (G-A transition at intron 3/exon 4, base pair deletion in exon 5) were identified (Lear *et al*, 1996). Together these assays are about 90% predictive of phenotype in European Caucasians (Lear *et al*, 1996). Two mutant CYP1A1 alleles (exon 7 Ile-Val and 3′-flanking region *Msp*I mutations) were detected using polymerase chain reaction (Lear *et al*, 1996; Yengi *et al*, 1996).

Table I. Patient Demographics

	Patients with No Truncal Tumors	Patients with at Least One Truncal Tumor
Mean age (y)	68.3 ± 12.2 (SD) (n = 263)	66.2 ± 11.9 (SD) (n = 74)
Males	54.1% (n = 268)	64.9% (n = 77)
Mean BCC no.	1.99 ± 1.81 (SD) (n = 270)	5.40 ± 6.03 (SD) (n = 75)
Blue and green eyes	70.1% (n = 148)	75.8% (n = 47)
Brown eyes	29.9% (n = 63)	24.2% (n = 15)
Skin type 1	16.4% (n = 34)	16.9% (n = 10)
Skin type 2–4	83.6% (n = 173)	83.1% (n = 49)
Ever smoker	65.1% (n = 142)	62.9% (n = 39)
Never smoker	34.9% (n = 76)	37.1% (n = 23)
Brown and black hair	74.8% (n = 101)	64.1% (n = 25)
Blonde and red hair	25.2% (n = 34)	35.9% (n = 14)
Outdoor occupation	16.7% (n = 13)	19.2% (n = 5)
Indoor occupation	83.3% (n = 65)	80.8% (n = 21)

Statistical Analysis T tests were used to assess differences in ages and number of BCCs between the two groups. χ^2-tests were used to examine for homogeneity between cases with and without at least one truncal tumor. As some genotype frequencies were small, the StatXact-Turbo statistical package was used to obtain exact p values. As various factors (GSTM1, GSTM3, GSTT1, CYP2D6, CYP1A1, gender, age, skin type, eye color, hair color, smoking status, occupation) were studied, the influence on tumor site of each alone and in combination was studied by logistic regression analysis. Combinations of genotypes and characteristics were studied in the presence of the main effects, and only those in which the interactive term was more significant than either of the main effects were included. For example, the combination GSTT1 null+CYP1A1 Ile/Ile was considered in the presence of GSTT1 null alone and CYP1A1 Ile/Ile alone. Because age and gender were significant confounding factors, analysis of the influence of genotypes and other patient characteristics were corrected for these factors. Since patients with at least one truncal tumor suffer more BCC, the associations with tumor site were further analyzed by correction for BCC number as well as age and gender.

RESULTS

Comparison of Patient Characteristics between Truncal and Nontruncal Groups **Table I** shows the characteristics of the study groups. The mean age at presentation of patients with at least one truncal tumor was lower than those without truncal tumors [p = 0.0170, χ^2_1 = 5.70, odds ratio = 0.975, 95% confidence interval (CI) = 0.955, 0.995], and the proportion of males was greater although this did not reach significance (p = 0.0925, χ^2_1 = 2.83, odds ratio = 1.571, 95% CI = 0.928, 2.659). The number of BCC in patients with at least one truncal tumor was greater than the nontruncal tumor group because the larger the number of tumors, the greater the probability that one will be truncal. The mean number of primary tumors in patients whose *first* tumor was truncal (n = 40; mean tumor number ± SD = 3.96 ± 4.74), however, was also significantly greater than those whose *first* tumor was not truncal (n = 312; mean tumor number ± SD = 2.58 ± 3.32; p = 0.0297, χ^2_1 = 4.73, odds ratio = 1.079, 95% CI = 1.008, 1.156). Other patient characteristics (skin type, eye color, smoking, occupation, hair color) were not significantly different between the two groups, although the presence of red or blonde

Table II. Factors Demonstrating Significant Differences between Patients with at Least One Truncal Tumor and Those with No Truncal Tumors[a]

	p Value	χ^2	Odds Ratio	95% CI
GSTT1 null	0.0245	5.06	2.24	1.11–4.53
CYP1A1 Ile/Ile	0.0386	4.28	2.86	1.06–7.72
GSTT1 null + CYP1A1 Ile/Ile	0.0059	18.70	2.95	1.37–6.39

[a] Data are corrected for age and gender.

hair, corrected for age and gender, approached significance (p = 0.0974).

Genetic Factors Associated with the Presence of Truncal Tumors **Table II** shows the genotypes, corrected for imbalances in patient age and gender, associated with tumor site. By logistic regression, the age-corrected proportion of GSTT1 null and CYP1A1 Ile/Ile genotypes was significantly greater in patients with at least one truncal tumor. The importance of GSTT1 and CYP1A1 genotypes is further emphasized by the increased significance of the interaction (the combination of both GSTT1 null and CYP1A1 Ile/Ile). In order to further assess the importance of these genotypes as determinants of tumor site, the associations with tumor site were also corrected for BCC number as well as age and gender, because patients with at least one truncal tumor suffer more BCC **(Table I)**. This demonstrated that individually, both GSTT1 null and CYP1A1 Ile/Ile remained significant but with reduced odds ratios (p = 0.0316, odds ratio = 1.44, 95% CI = 1.24, 1.66, and p = 0.0130, odds ratio = 1.51, 95% CI = 1.29–1.77, respectively). The combination of these genotypes (both GSTT1 null and CYP1A1 Ile/Ile), however, remained highly significant, with a decreased p value and increased odds ratio (p = 0.0035, odds ratio = 3.56, 95% CI = 1.52–8.34) despite this rigorous correction. None of the other genotypes examined (GSTM1 null, GSTM3 AA, CYP2D6 EM, and CYP1A1 m1 m1) was associated with tumor site, either alone or in combination with other genotypes or patient characteristics.

DISCUSSION

We have studied the influence of detoxifying enzyme genotypes and patient characteristics on the development of truncal BCC by comparing these factors in patients with at least one truncal BCC and those with no truncal tumors. BCC are a major burden to health care agencies, with an incidence in the United States as high as 300 per 100,000 people and reported annual increases of about 10% (Karagas and Greenberg, 1995). In view of this high and increasing incidence, and because mortality is low, it is predicted the prevalence of this tumor will be greater than that for all other cancers combined (Boring *et al*, 1994). Thus, the lifetime risk of BCC for an American child born in 1994 is estimated to be 28–33% (Miller and Weinstock, 1994). Further, although exposure to UV is a recognized risk factor, our data show that about 20% of patients with BCC develop at least one tumor at sites generally believed to suffer relatively little exposure.

The mean age at first presentation of patients with at least one truncal tumor was lower than in those with no truncal tumors. Furthermore, patients whose first tumor is truncal are at increased risk of further tumors, suggesting that patients with truncal tumors represent a high-risk group. The increased prevalence of males in the truncal compared with the nontruncal tumor group supports our local clinical impression, although this failed by a small margin to achieve statistical significance. This is consistent with the association of both male gender and truncal tumor site with increased risk of multiple tumors (Lear *et al*, 1996). Outdoor occupation was not a significant factor in our analysis, suggesting that males do not have increased chronic UV exposure compared with females. Other explanations for the increased risk associated with male gender, such as less effective melanization than females (McLeod *et al*, 1994), are worthy of investigation.

Since both age and gender were significant confounding factors, data on the influence of genotypes and patient characteristics were corrected for imbalances in these factors. Following correction, none of the patient characteristics studied were associated with tumor site. In particular, skin type 1 was not significantly different between the two groups, suggesting that patients with truncal tumors are no more likely to burn on UV exposure than patients with nontruncal tumors.

Analysis of the role of detoxifying enzymes in determining tumor site showed that GSTT1 and CYP1A1 are important, even after correction for BCC number as well as age at first presentation and gender. Although the level of significance of these factors was relatively low, the influence of the highly significant interactive term (both GSTT1 null and CYP1A1 Ile/Ile) suggests the effects are real. We have previously shown that GSTT1, whose enzyme substrates include the products of oxidative stress–induced damage to DNA, is significantly associated with rate of accrual of BCC in patients with multiple tumors (Lear *et al*, 1996). The data presented here support the view that GSTT1 null genotypes are associated with faster appearance of further tumors and the presence of truncal tumors. Thus, it appears that this genotype exerts its effect on the rate of BCC appearance, because it predisposes to tumors on both chronically and intermittently exposed sites. These data suggest that individuals deficient in the ability to repair UV-derived oxidative stress–induced damage to DNA and/or lipids are genetically predisposed to BCC and are more likely to develop subsequent tumors. GSTT1 null individuals may be more susceptible to UV-induced BCC following even relatively little UV exposure, resulting in an increased number of tumors at a younger age and the development of lesions on intermittently exposed sites such as the trunk. Unlike GSTT1 null, however, no GSTM1 null effect was identified, complementing data showing that the products of these loci have some differences in substrate specificities (Norppa *et al*, 1995).

Data showing that UV-oxidized tryptophan binds to the CYP1A1 ligand-dependent Ah receptor transcription factor and UV induces CYP1A1 expression in skin suggest a role for allelism at this locus in skin carcinogenesis (Gonzalez, 1995). The influence of CYP1A1 Ile/Ile on tumor site shown here is more difficult to interpret than the GSTT1 effect, because we have shown that this genotype was associated with slower BCC accrual, although this effect was relatively weak. It is not known whether CYP1A1 is uniformly expressed in skin; UV is known to induce expression, suggesting that differential effect in chronically and intermittently exposed skin is possible.

Although interactions between GSTM1 and CYP1A1 have been identified in mediating risk of lung cancer (Anttila *et al*, 1994), this report describes an interaction between GSTT1 and CYP1A1. Indeed, particularly after correction for age, gender, and BCC number, the odds ratio for this effect (3.56) was relatively high. This study presents further evidence that patients with truncal tumors represent a high-risk group and that factors other than UV exposure are important in the pathogenesis of these tumors.

We gratefully acknowledge the support of the Cancer Research Campaign [project grant (SP2207/0201)].

REFERENCES

Anttila S, Hirvonen A, Husgafvel-Pursianen K, Karjalainen A, Nurminen T, Vainio H: Combined effect of CYP1A1 inducibility and GSTM1 polymorphism on histological type of lung cancer *Carcinogenesis* 15:1133–1135, 1994

Boring CC, Squires TS, Tong T, Montgomery S: *Cancer Statistics, CA 1994* 44:7–26, 1994

Boring CC, Squires TS, Tong T: *Cancer Statistics, CA 1993* 43:7–26, 1993

Gonzalez F: Role of cytochrome P-450 1A1 in skin cancer. In: Mukhtar H (ed.) *Skin Cancer: Mechanisms and Human Relevance.* CRC, Boca Raton, FL, 1995, pp 89–97

Guengerich FP: Cytochromes P450 of human liver. Classification and activity profiles of the major enzymes. In: Pacifici GM, Fracchia GN (eds.) *Advances in Drug Metabolism in Man.* Directorate-General Science, Research and Development, European Commission, Brussels, Belgium, 1995, pp 181–231

Heagerty A, Smith A, English J, Lear J, Perkins W, Bowers B, Jones P, Gilford J, Alldersea J, Fryer AA, Strange RC: Susceptibility to multiple cutaneous basal cell carcinomas: significant interactions between glutathione S-transferase GSTM1 genotypes, skin type and male gender. *Br J Cancer* 73:44–48,1996

Heagerty AHM, Fitzgerald D, Smith A, Bowers B, Jones P, Fryer A, Zhao L, Alldersea J, Strange RC: Glutathione S-transferase GSTM1 phenotypes and protection against cutaneous malignancy. *Lancet* 343:266–268, 1994

Ingelman-Sundberg M, Johansson I: The molecular genetics of the human drug metabolizing cytochrome P450s. In: Pacifici GM, Fracchia GN (eds.) *Advances in Drug Metabolism in Man*. Directorate-General Science, Research and Development, European Commission, Brussels, Belgium, 1995, pp 545–585

Karagas MR, Greenberg ER: Unresolved issues in the epidemiology of basal cell and squamous cell skin cancer. In Mukhtar H (ed.) *Skin Cancer: Mechanisms and Human Relevance*. CRC, Boca Raton, FL, 1995, pp 79–86

Kricker A, Armstrong BK, Jones ME, Burton RC: Health, solar UV radiation and environmental change. *International Agency for Research on Cancer*. Technical Report no 13. Lyon, France, 1993, pp 52–61

Kripke ML: Ultraviolet radiation and immunology: something new under the sun. *Cancer Res* 54:6102–6105, 1994

Lear JT, Heagerty AHM, Smith A, Bowers B, Rowland Payne C, Smith CAD, Jones PW, Gilford J, Yengi L, Alldersea J, Fryer AA, Strange RC: Multiple cutaneous basal cell carcinomas: glutathione S-transferase (GSTM1, GSTT1) and cytochrome P450 (CYP2D6, CYP1A1) polymorphisms influence tumor numbers and accrual. *Carcinogenesis* 17:1891–1896, 1996

McLeod SD, Ranson M, Mason RS: Effects of estrogens on human melanocytes *in vivo*. *J Steroid Biochem Mol Biol* 49:9–14, 1994

Miller DL, Weinstock MA: Nonmelanoma skin cancer in the United States: incidence. *J Am Acad Dermatol* 30:774–778,1994

Nebert DW: Drug-metabolizing enzymes in ligand-modulated transcription. *Biochem Pharmacol* 47:25–37, 1994

Norppa H, Hirvonen A, Jarventaus H, Uuskula M, Tasa G, Ojajarvi A, Sorsa M: Role of GSTT1 and GSTM1 genotypes in determining individual sensitivity to sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes. *Carcinogenesis* 16:1261–1264, 1995

Raunio H, Pasanen M, Maenpaa J, Hakkola J, Pelkonen O: Expression of extrahepatic cytochrome P450 in humans. In: Pacifici GM, Fracchia GN (eds.) *Advances in Drug Metabolism in Man*. Directorate-General Science, Research and Development, European Commission, Brussels, Belgium, 1995, pp 233–287

Schmieder GJ, Yoshikawa T, Mata SM, Streilein JW, Taylor, JR: Cumulative sunlight exposure and the risk of developing skin cancer in Florida. *J Dermatol Surg Oncol* 18:517–522, 1992

Smith G, Stanley LA, Sim E, Strange RC, Wolf CR: Metabolic polymorphisms and cancer susceptibility. *Cancer Surv* 25:27–65, 1995

Wei Q, Mutanoski GM, Farmer ER, Hedayati MA, Grossman L: DNA repair related to multiple skin cancers and drug use. *Cancer Res* 54:437–440, 1994

Yeh S, How SW, Lin CJ: Arsenical cancer of skin. Histologic study with special reference to Bowen's disease. *Cancer* 21:312–319, 1968

Yengi L, Inskip A, Gilford J, Alldersea J, Bailey L, Smith A, Lear JT, Heagerty AHM, Bowers B, Hand P, Hayes JDH, Jones PW, Strange RC, Fryer AA: Polymorphism at the glutathione S-transferase GSTM3 locus: interactions with cytochrome P450 and glutathione S-transferase genotypes as risk factors for multiple basal cell carcinoma. *Cancer Res* 56:1974–1977, 1996.