Presentation with Multiple Cutaneous Basal Cell Carcinomas: Association of Glutathione *S*-Transferase and Cytochrome P450 Genotypes with Clinical Phenotype[1]

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Abstract

We previously reported associations between numbers of basal cell carcinomas (BCCs) and glutathione *S*-transferase (*GSTM1* and *GSTT1*) and cytochrome P450 (*CYP2D6*) genotypes. Thus, although *GSTM1 AB* is protective, *GSTM1 null*, *GSTT1 null*, and *CYP2D6 EM* are associated with increased numbers of lesions. Here, we examine the hypothesis that these genotypes are associated with high-risk subgroups. The subgroup studied comprised 119 patients with more than one previously unidentified BCC at first or later presentations [multiple presentation phenotype (MPP)]. These patients were part of a group of 773 BCC patients that also included 567 patients with one BCC and 87 patients with only one lesion at each presentation [single presentation phenotype (SPP)] but who developed multiple BCCs. The number of tumors in the MPP was significantly greater than that in the SPP groups. In the MPP but not SPP patients, *GSTM1 AB*, *GSTT1 null*, and *CYP2D6 EM* were significantly associated with BCC numbers, suggesting that previously observed associations reflect the influence of these genes only in the MPP cases. There was no evidence that MPP patients had received more UV exposure. We also determined whether the increased numbers of BCC in the MPP cases reflects an association with the truncal tumor phenotype. The values of the rate ratios indicated that the MPP is a marker for the risk of many BCCs, although the combination of MPP and a truncal tumor is a higher-risk phenotype. The data demonstrate the heterogeneity in BCC patients, which reflects differences in genetic factors that determine skin response to UV.

Received 7/13/98; revised 10/20/98; accepted 10/26/98.
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[1] Supported by the Cancer Research Campaign (Project Grant SP2207/0201).
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Introduction

Cutaneous BCCs[3] are a major burden to health care agencies worldwide. The cancer is the most common in Caucasians; the estimated lifetime risk for a North American child born in 1994 is ~30% (1–4). A further, striking characteristic of this cancer is the considerable phenotypic variation demonstrated by patients. For example, the number (from 1–30 lesions in our series) and accrual of tumors varies widely between patients (1–4). The factors that determine such differences remain unclear, although a better understanding of why some patients have many lesions would be useful if it allowed identification of such cases at first presentation for entry into surveillance program. The resultant earlier detection and easier removal of smaller, new lesions should help reduce morbidity.

Although UV radiation is a critical causative factor in the pathogenesis of BCC, obtaining accurate measurements of total sun exposure is difficult, and the magnitude of its effect on risk is unclear. Thus, although there is evidence of a positive association between BCC risk and increasing exposure, estimates of the magnitude of this increase vary markedly (5, 6). The relationship between UV exposure and outcome, assessed as numbers of primary tumors, is also unclear (7). Risk of further BCC appears to depend on host factors; 27% of patients with 1 tumor developed a further tumor within 5 years, compared with 90% of those with 10 or more lesions (8, 9). BCC site is also important; patients with a tumor on the less commonly exposed trunk have more lesions than other patients (10). Such studies indicate that the phenotypic heterogeneity seen in patients is not just the result of differences in exposure but reflects the presence of subgroups that are defined by particular host characteristics (10–12).

Several studies, using BCC numbers as outcome, have shown that skin type 1 (burning on exposure to UV without tanning), male sex, and DNA repair phenotype are associated with risk of further lesions (3, 13–15). Some relevant genes have also been identified. These include the polymorphic loci that encode enzymes involved with cellular defense against UV-induced oxidative stress (14, 16). Thus, the heterozygote *GSTM1 AB* genotype is associated with reduced risk, and the homozygous *GSTT1 null* genotype is associated with increased risk of many tumors (14, 16). *CYP2D6* is also relevant, with homozygosity for wild-type alleles (*CYP2D6 EM*) being associated with BCC numbers, although the mechanism is unclear (14). It is noteworthy that, although an unknown number of other genes must also influence risk of further lesions, the quantitative effect of the *GST* and *CYP* genotypes is similar to that of skin type and sex (14).

[3] The abbreviations used are: BCC, basal cell carcinoma; MPP, multiple presentation phenotype; SPP, single presentation phenotype.

Data showing associations between tumor numbers and allelic variants are largely based on studies in nonselected BCC cases, although such polymorphisms are likely to be most significant in high-risk subgroups (17, 18). For example, in patients who first present with a truncal tumor, *GSTT1 null* is associated with decreased time to next presentation (18). Patients with a truncal lesion comprise ~20% of BCC cases, suggesting the presence of other relevant subgroups. In this context, patients with two or more primary lesions at a single presentation (MPP) comprise a further, potentially interesting subgroup. Thus, we found their median time from first to next tumor presentation was 3.6 years, compared with 5.8 years in other patients (17). Accrual of tumors is very variable, even in individual patients, and it is not known whether these cases develop more lesions than other BCC patients. In particular, it is not known whether the MPP cases are a high-risk subgroup, distinct from those BCC patients who have only a single lesion at any presentation (SPP) but who develop many lesions over repeated presentations. Indeed, little is known of the factors that are associated with tumor numbers in these groups. Here, we describe studies (a) to determine whether MPP patients demonstrate more primary BCC than SPP cases with more than one BCC; (b) to determine whether the phenotype was associated with skin type 1, male sex, or *GSTM1 null*, *GSTT1 null*, or *CYP2D6 EM* genotype, because we found that the MPP patients developed more lesions; and (c) to compare the influence of skin type 1, male sex, and the genotypes on BCC numbers in the MPP patients with that in patients with a truncal lesion, because these cases have been reported to suffer increased numbers of lesions. We selected *GSTM1*, *GSTT1*, and *CYP2D6* for study because previous findings showed allelic variants at these loci are associated with BCC numbers in the total case group (14, 16–18).

Materials and Methods

Patients. A total of 773 unrelated Northern European Caucasians with one or more histologically proven BCC were recruited from general dermatology outpatient clinics in North Staffordshire Hospital, Stafford District General Hospital, and Royal Cornwall Hospital with ethics committee approval and informed consent. These cases were recruited between 1991 and 1995 and represented ~50% of the cases seen in the participating centers during this period. Their clinical characteristics (age at diagnosis, sex, and skin type) were typical for BCC patients seen in English Hospital clinics. None of those approached refused to participate. We excluded recurrences from the total number of primary BCCs and patients with basal cell nevus syndrome, xeroderma pigmentosum, or BCC combined with another malignancy (cutaneous or internal). Otherwise, we attempted to recruit all BCC patients. Thus, the patients not recruited were inadvertently missed in busy clinics and do not represent a subgroup. We recruited patients at their first presentation, as well as those returning with further tumors or for routine follow-up. All cases were examined and interviewed by a dermatologist to obtain information on tumor site (head and neck, upper limbs, lower limbs, and trunk) and numbers at each presentation. We also recorded hair (blonde, red, brown, and black) and eye (blue, green, and brown) colors at 21 years of age and smoking history. Skin type (types 1–4) was determined using the Fitzpatrick scale (19), which is based on past reaction to UV exposure. Although classification into types 1 and 4 appears straightforward, categorizing into types 2 and 3 is less reliable because it depends on the impressions of often elderly patients (3). Patients were also interviewed to obtain data on sun exposure, as reported previously (10). A record of occupation (outdoor work was defined as >75% of work time spent outside) and time spent outside the United Kingdom in hot climates was obtained. Patients were also questioned regarding ingestion of arsenic-containing medicines or drinking water from potentially contaminated wells. No arsenic-exposed patients were identified. Similarly, patients were specifically asked whether they had suffered conditions that required treatment with radiotherapy. No such patients were identified. In a few patients, radiotherapy was used to treat primary BCC; in no cases were further lesions identified at the same site. Patients were classified as having the MPP if they had more than one BCC at first presentation or more than one BCC at a later presentation, these particular tumors not being present at an earlier visit to the dermatologist. This group included patients who had more than one lesion, as noted by themselves or their general practitioners, as well as patients who were unaware of additional lesions. These were identified by the clinician at the same clinic visit.

Determination of Genotypes. PCR conditions and the primers used in the genotyping assays were described previously (14). *GSTM1* genotypes were identified using a PCR assay that identifies *GSTM1*0* homozygotes and *GSTM1*A/GSTM1*B* heterozygotes and the *GSTM1 A* and *GSTM1 B* phenotypes. It does not distinguish *GSTM1*0/GSTM1*A* and *GSTM1*A* homozygote or the equivalent *GSTM1 B* genotypes. *GSTT1 null* and expressing subjects were also identified using PCR. Homozygotes or compound heterozygotes for two mutant *CYP2D6* alleles, *CYP2D6*4* (G→A transition at intron 3/exon 4) and *CYP2D6*3* (bp deletion in exon 5) and heterozygotes for these alleles and *CYP2D6*5* (gene deletion) were classified as poor metabolizers (*PM*), heterozygotes for either *CYP2D6*4* or *CYP2D6*3* and the wild-type allele were classified as *HET*, and homozygotes for the wild-type allele and heterozygotes for this allele and *CYP2D6*5* were classified as *EM*. Thus, some subjects classed as wild-type homozygotes could be heterozygotes for uncommon, variant alleles such as *CYP2D6*5* (20).

Statistical Analysis. Stata Release 5 for Windows 95 (Stata Corp., College Station, TX) was used for statistical analysis. It has the advantage of allowing numerical codes to be factorized in a field, which simplifies comparison of more than two genotypes at one locus. χ^2 analysis was used to compare the frequencies of the *GST* and *CYP* genotypes, smoking, and occupation in the BCC patient groups. To determine the factors associated with a risk phenotype (with a discrete dichotomous outcome), logistic regression was performed. The age and sex of the patient at phenotype classification were also included in the logistic regression models. This compensates for the potential influence that age and sex may have on classification. Odds ratios and *P*s were calculated. To determine the factors associated with the number of BCC, Poisson regression analysis was used, normalized for follow-up time and corrected for age and sex, with BCC number as outcome (14). The model assumes the Poisson rate parameter (mean number BCC) may be expressed as a linear function of a set of covariates: skin type 1, sex, and genotypes. A rate ratio, defined as the multiplicative effect of a change of a covariate by 1 was calculated (for this data, usually being a change from 0 to 1). Thus, the rate ratio for males (1) against females (0) is mean number of BCCs in males/mean number of BCCs in females when sex alone (*i.e.*, not in the presence of other covariates) is considered. In the Poisson regression, this will change in the presence of other covariates. $P \leq 0.05$ was considered statistically significant. Because there are dangers in making inferences on individual

factors in multiple testing, we have corrected the *P*s obtained in the main analysis of the association of genotypes with BCC numbers using Holm's procedure (21).

Results

Numbers of MPP and SPP Patients. The total BCC group of 773 patients included 119 MPP patients (Table 1). Seventy-nine of these patients demonstrated more than one BCC at first presentation (10.2%), and a further 40 patients (5.2%) presented with more than one BCC after first presentation with a single lesion. Because tumor numbers (mean ± SD; 4.7 ± 3.9 and 6.8 ± 4.2, respectively) and other recorded characteristics (sex, skin type, occupation, hair and eye color, and smoking history) in these two groups were similar, we combined them to form the MPP group. Mean follow-up time (± SD) in the MPP patients was 7.9 ± 6.9 years. The remaining 654 patients demonstrated the SPP; 567 patients had a single lesion, and 87 patients demonstrated multiple BCC but only presented with single lesions at each clinic visit. Mean follow-up time (± SD) in the SPP patients was 8.8 ± 5.3 years.

Comparison of BCC Numbers in the MPP and SPP Cases. To confirm that the MPP patients had more lesions than other BCC patients, we compared BCC numbers in these patients with those in SPP patients. Descriptive data showing the mean and maximum numbers of tumors (uncorrected for follow-up time) in the MPP and SPP groups are shown in Table 1. The mean number of lesions in the 87 SPP patients with more than one BCC was lower than in those with the MPP. We used a Poisson regression model (corrected for sex and age at first presentation and normalized for follow-up time) to show that this difference in BCC numbers was significantly different ($P < 0.001$). We also determined the proportion of patients with particular numbers of lesions; most of the 87 SPP patients had two or three BCCs; none had more than six tumors. In the MPP group, 27 patients (22.7%) had more than six BCCs (Table 1).

Comparison of Characteristics and Genotypes in MPP and SPP Cases. We used logistic regression (with correction for age at classification and sex) to identify differences in the frequencies of characteristics in the MPP and SPP groups. Skin type 1 was significantly more common in the MPP group than in either the total SPP group ($P = 0.009$) or 87 SPP patients with more than one BCC ($P = 0.046$; Table 1). The frequencies of skin type 1 in the 567 SPP patients with one BCC and in the 87 SPP patients with more than one lesion was not significantly different. The frequency of the other recorded characteristics, including hair color, eye color, and smoking history, were not different in the MPP group, the total SPP group, or the 87 SPP patients with more than one BCC. We also analyzed the data to determine whether the MPP cases had spent more time in hot climates outside the United Kingdom or had received more occupational exposure. Eleven of 773 patients (8 males and 3 females) reported significant sun exposure (all before 25 years of age) outside the United Kingdom for >6 months; 7 cases developed 1 lesion, 1 case developed 2 BCCs, 2 cases developed 3 BCCs, and 1 patient developed 15 BCCs. Only one of these cases (female; three BCCs) had the MPP. Thirty-seven MPP patients, 23 SPP patients with more than one BCC, and 133 SPP patients with one lesion were classified as having outdoor occupations. These proportions were not significantly different. The mean numbers of BCC (± SD) in the MPP patients with outdoor (6.6 ± 5.6) and indoor occupations (4.0 ± 1.5) were not significantly different ($P = 0.23$).

GSTM1, *GSTT1*, and *CYP2D6* genotype frequencies in the MPP cases and SPP cases with one and more than one BCC are shown in Table 1. We used logistic regression analysis (corrected for classification age and sex) to determine whether the frequencies in these groups were significantly different. We found no differences in *GSTM1* and *GSTT1* genotype frequencies. The frequency of *CYP2D6 EM* was significantly greater in the MPP group compared with the total SPP group ($P = 0.013$) and 87 SPP patients with more than one BCC ($P = 0.003$). The frequency of *CYP2D6 EM* in the SPP patients with one BCC was not different from that in those with more than one lesion (Table 1). These findings indicate that of the characteristics studied, only *CYP2D6 EM* and skin type 1 are factors associated with the MPP.

Table 1 Characteristics of patients with the SPP and the MPP

	SPP with 1 BCC	SPP with >1 BCC	MPP
No. of patients	567	87	119
No. of tumors ± SD[a]	1.0	2.4 ± 0.8	5.4 ± 4.9
Minimum no./maximum no. of BCCs	1/1	2/6	2/30
No. of males (%)	306 (54.1)	50 (57.5)	75 (63.0)
No. of females (%)	260 (45.9)	37 (42.5)	44 (37.0)
Skin type (%)			
1[b]	47 (12.7)	6 (10.0)	19 (21.4)
2	117 (31.5)	21 (35.0)	31 (34.8)
3	118 (31.8)	27 (45.0)	23 (25.8)
4	89 (24.0)	6 (10.0)	16 (18.0)
Hair color (%)			
Red	18 (7.4)	2 (5.4)	7 (12.3)
Blonde	40 (16.5)	11 (29.7)	9 (15.8)
Brown	166 (68.6)	22 (59.5)	35 (61.4)
Black	18 (7.4)	2 (5.4)	6 (10.5)
Eye color (%)			
Brown	112 (28.6)	11 (17.5)	26 (28.6)
Blue	233 (58.6)	47 (74.6)	56 (61.5)
Green	46 (11.8)	5 (7.9)	9 (9.9)
No. of cigarette smokers (%)	176 (44.2)	29 (44.6)	41 (42.8)
No. of nonsmokers (%)	141 (35.9)	22 (33.9)	38 (39.6)
No. of ex-smokers (%)	68 (17.3)	12 (18.5)	16 (16.0)
No. of pipe/cigar smokers (%)	7 (1.8)	2 (3.1)	1 (1.0)
No. of ex-pipe/ex-cigar smokers (%)	1 (0.3)	0	0
GSTM1 (%)			
null	283 (51.9)	45 (56.3)	57 (52.3)
A	59 (29.3)	23 (28.8)	30 (27.5)
B	83 (15.3)	12 (15.0)	20 (18.4)
AB	19 (3.5)	0	2 (1.8)
GSTT1 (%)			
null	81 (17.1)	16 (23.5)	18 (19.8)
A	394 (83.0)	52 (76.5)	73 (80.2)
CYP2D6 (%)			
EM[c]	282 (62.2)	36 (51.4)	65 (73.9)
HET	139 (30.6)	31 (44.3)	21 (23.9)
PM	33 (7.3)	3 (4.3)	2 (2.3)

[a] Poisson regression model with correction for age at first presentation and sex and normalized for follow-up time was used to compare BCC numbers in the MPP and SPP patients with more than one lesion (reference category): $P < 0.001$, rate ratio = 2.4, 95% CI = 2.1–2.8.

[b] Logistic regression with correction for age at classification and sex was used to compare skin type frequencies: skin type 1 in the MPP group *versus* 654 SPP patients: $P = 0.009$, odds ratio = 2.28, 95% CI = 1.2–4.2. Skin type 1 in the MPP group *versus* 87 SPP patients with multiple BCC: $P = 0.046$, odds ratio = 2.8, 95% CI = 1.04–7.7. Skin type 1 in 567 SPP patients with one BCC *versus* 87 SPP patients with multiple lesions: $P > 0.28$.

[c] Logistic regression analysis, after correction for classification age and sex, was used to show the *CYP2D6 EM* frequency was greater in the MPP group *versus* the 654 SPP cases ($P = 0.013$, odds ratio = 1.94, 95% CI = 1.15–3.27) and 87 SPP patients with >1 BCC ($P = 0.003$, odds ratio = 2.77, 95% CI = 1.45–5.5). Frequencies of *CYP2D6 EM* in the SPP patients with 1 BCC *versus* >1 BCC were not significantly different ($P = 0.11$).

Table 2 Association of genotypes and characteristics with BCC numbers in patients with the MPP and total BCC group

	Total BCC group				Patients with MPP			
	Patients (%)	RR[a]	95% CI	Mean BCC[b]	MPP patients (%)	RR[a]	95% CI	Mean BCC[b]
Male	431 (55.8)	1.3	1.1–1.5	3.0	75 (63.0)	1.4	1.2–1.7	6.0
Female	341 (44.2)	Reference		2.1	44 (37.0)	Reference		4.5
Skin type 1	72 (13.8)	1.3	1.1–1.4	2.4	19 (21.3)	1.2	0.9–1.4	5.5
Skin types 2–4	448 (86.2)	Reference		1.8	70 (78.7)	Reference		5.1
GSTM1								
null	425 (53.5)	Reference		2.1	57 (52.3)	Reference		5.5
A	225 (28.3)	1.0	0.9–1.2	1.8	30 (27.5)	0.9	0.7–1.1	4.2
B	123 (15.5)	1.2	1.0–1.4	2.4	20 (18.4)	1.1	0.9–1.3	8.0
AB	22 (2.8)	0.9	0.5–1.3	1.4	2 (1.8)	0.4	0.2–0.95	2.5
GSTT1								
null	127 (18.6)	1.2	1.0–1.4	2.7	18 (19.8)	1.7	1.4–2.1	6.7
A	556 (81.4)	Reference		1.9	73 (80.2)	Reference		4.8
CYP2D6								
EM	418 (63.2)	1.4	1.2–1.6	2.2	65 (73.9)	1.5	1.2–1.9	5.7
HET	202 (30.6)	1.0	0.8–1.6	1.9	21 (23.9)	1.0	0.5–1.9	4.1
PM	41 (6.2)	Reference		1.5	2 (2.3)	Reference		4.5

[a] RR, rate ratio obtained using a Poisson regression analysis (corrected for sex and age at first presentation and normalized for follow-up time) to identify individual associations between male sex (reference category, female sex), skin type 1 (reference category, skin types 2–4), and genotypes (reference categories, *GSTM1 null*, *GSTT1 A*, and *CYP2D6 PM*) and tumor numbers in the total BCC group and MPP patients.
[b] Mean numbers of BCC in cases with particular characteristics and genotypes (uncorrected data).

Because *in vitro* substrates for *GSTM1*, *GSTT1* and *CYP2D6* enzymes include tobacco-derived carcinogens and genotypes at these loci have been considered to be candidates for susceptibility to tobacco-related cancers, we inspected the data to determine whether these genotypes were associated with smoking. Using a χ^2 test, we found that, in both the total group of 773 cases and MPP patients, genotype frequencies in current and previous smokers were not significantly different from those in patients who has never smoked ($P > 0.17$). We also examined genotype frequencies in patients with outdoor and indoor occupations. In both the total case group and MPP patients, these frequencies were not significantly different ($P > 0.28$).

Genotypes and Characteristics Associated with BCC Numbers in the Total BCC Group. Poisson regression analysis (corrected for age and sex and normalized for follow-up time) was used to confirm previous data (14) showing that skin type 1 and male sex are associated with more tumors than other skin types or females in the 773 patients in the total BCC group (Table 2). *GSTM1 B*, *GSTT1 null*, and *CYP2D6 EM* were also associated with greater numbers of BCC. Table 2 shows the mean numbers of BCC in patients with and without particular genotypes (descriptive data uncorrected for follow-up time). Having confirmed our previous findings, we further analyzed the data by correcting the Poisson regression model for the MPP. Following correction for this factor, the significant influence of the genotypes was lost. This suggests that the effect of these genotypes on BCC numbers is particularly associated with the MPP patients.

We also analyzed the data to determine whether combinations of genotypes and smoking or genotypes and outdoor occupation were associated with risk of many lesions. We used a Poisson regression model to determine whether the interaction terms of smoking and *GSTM1 B*, *GSTT1 null*, or *CYP2D6 EM* or these genotypes and outdoor occupation were associated with increased numbers of BCC in the total BCC group. Using a Poisson regression model that included the interaction term of the main effects (individual genotypes and smoking/occupation) with correction for sex and age at first presentation and normalized for follow-up time, we found no significant associations between the interaction term and numbers of BCC. We also examined the data to determine whether the association between factors and genotypes and BCC numbers was observed in patients stratified by smoking history or occupation. The rate ratios obtained for male sex; skin type 1; *GSTM1 A*, *B*, and *AB*; *GSTT1 null*; and *CYP2D6 EM* and *HET* in smoking and nonsmoking subjects were similar, as were those in patients with indoor and outdoor occupations.

Genotypes and Characteristics Associated with BCC Numbers in MPP Patients. We next analyzed the data from the MPP group for associations between genotypes and numbers of BCC (normalized for follow-up time). Table 2 shows that male sex, *GSTM1 AB*, *GSTT1 null*, and *CYP2D6 EM* but not skin type 1 were associated with BCC numbers. *GSTM1 AB* was associated with a reduced risk of further lesions, although, because of the relatively low frequency of this genotype, significance was lost after correction of the *P* for multiple testing. The significance of the associations between male sex and the other genotypes and increased numbers of BCC remained after correction of *P*s for multiple testing. We further analyzed the data from MPP patients to determine whether the association of BCC numbers with genotypes and host factors was influenced by smoking or occupation. Stratifying the 119 MPP patients by smoking or occupation would have resulted in numbers of patients that were too small for reliable inferences. Accordingly, we first corrected the Poisson regression model for smoking and occupation. The rate ratios obtained were similar to those shown for MPP patients in Table 2. Then, we used a Poisson regression model to determine whether the combination of smoking and either *GSTT1 null* or *CYP2D6 EM* or outdoor occupation and these genotypes were associated with increased numbers of BCC in the MPP patients. Using a model that included the interaction term of the main effects (individual genotypes and smoking/occupation) with correction for sex and age at first presentation and normalized for follow-up time, we found no significant associations between the interaction term and numbers of BCC ($P > 0.18$).

None of the genotypes were associated with BCC numbers

in the SPP patients including the 87 patients with more than one lesion (*GSTM1 AB*, $P \geq 0.37$, rate ratio = 1.35; *GSTT1 null*, $P \geq 0.46$, rate ratio = 0.91; *CYP2D6 EM*, $P \geq 0.59$, rate ratio = 1.05). This supports the view that the associations between BCC numbers and genotypes found in the total BCC group reflect the influence of these genes only in the patients with the MPP.

BCC Numbers in Patients with Truncal Tumors and the MPP. Inspection of the characteristics of the MPP patients showed that a high proportion (45 of 119; 37.8%) had truncal tumors (Table 3). Because such patients are reported to develop more lesions than nontruncal tumor patients (9, 18), we further analyzed the data to determine whether the increased numbers of BCC found in the MPP cases reflect an association with the truncal tumor phenotype. The Poisson regression model was corrected for sex and age at first presentation and normalized for follow-up time and used the patients who did not have a truncal BCC or the MPP as the reference category. Table 3 shows that SPP patients with a truncal lesion were not at risk of more BCC. However, patients who had the MPP but no truncal lesion or who had the MPP and a truncal lesion were at significantly higher risk of more BCCs. The values of the rate ratios as well as that of the 95% CI, which do not overlap, indicate that the MPP (rate ratio = 1.6, 95% CI = 1.4–1.9) is a marker for risk of many BCCs, although the combination of MPP and a truncal tumor is the higher-risk phenotype (rate ratio = 3.0; 95% CI = 2.6–3.6).

Because the MPP and truncal tumor phenotype were associated with different risks of increased numbers of BCC, we examined the data for associations between *GSTM1 null*, *GSTT1 null*, and *CYP2D6 EM* and tumor numbers in three patient groups: (a) MPP and a truncal tumor, (b) MPP but no truncal tumor, and (c) SPP and a truncal tumor (Table 3). This analysis showed that *GSTT1 null* was associated with increased BCC numbers in MPP patients, both those with and without truncal lesions. *GSTT1 null* was not associated with BCC numbers in SPP patients with a truncal tumor. *CYP2D6 EM* was associated with the number of lesions in patients with a truncal lesion, both SPP and MPP. The genotype was not associated with BCC numbers in nontruncal MPP cases. No significant associations between *GSTM1* genotypes and BCC numbers were identified.

Table 3 Association of SPP, MPP, truncal phenotype, and *GSTT1* and *CYP2D6* genotypes with BCC numbers

	Nontruncal/SPP (n = 591)	Truncal/SPP (n = 63)	Nontruncal/MPP (n = 74)	Truncal/MPP (n = 45)
BCC mean ± SD[a]	1.1 ± 0.4	1.7 ± 1.1	4.2 ± 2.5	7.4 ± 6.8
Rate ratio	Reference	1.0	1.6	3.0
95% CI		0.8–1.3	1.4–1.9	2.6–3.6
GSTT1 null[b]				
Rate ratio	0.9	0.9	1.6	1.5
95% CI	0.5–1.4	0.5–1.5	1.1–2.2	1.1–1.9
CYP2D6 EM[c]				
Rate ratio	1.0	1.5	1.3	1.7
95% CI	0.8–1.2	1.0–2.3	0.9–1.7	1.2–2.4

[a] Poisson regression analysis (corrected for sex and age at first presentation and normalized for follow-up time) was used to compare BCC numbers in the truncal/MPP, nontruncal/MPP, and truncal SPP patients. Patients who were nontruncal/SPP serve as the reference category.
[b] Poisson regression analysis (corrected as shown above) was used to compare BCC numbers in the truncal/MPP, nontruncal/MPP, and truncal SPP patients with the *GSTT1 null* genotype *versus* those with the reference category, *GSTT1 A.*
[c] Poisson regression analysis (corrected as above) was used to compare BCC numbers in the truncal/MPP, nontruncal/MPP, and truncal SPP patients with *CYP2D6 EM versus* those in the reference category, other *CYP2D6* genotypes.

Discussion

Although host characteristics, including age, male sex, response to UV, and allelic variants, are associated with susceptibility to many primary BCC (3, 6, 13–17), our ability to predict, at first presentation, which patients are at most risk of further lesions is uncertain. Recent studies suggest that particular clinical phenotypes such as a truncal tumor may help identify subgroups of BCC patients who are at risk of a worse outcome (17). Certain *GST* genotypes are also associated with this risk (17). Patients with the MPP have not been investigated previously, and in this study, we have shown that they demonstrate markedly more tumors than SPP cases who developed more than one lesion (17). We have also shown that the association between BCC numbers and *GST* and *CYP* genotypes, identified in an nonselected group of cases, largely results from the presence of MPP patients.

The 119 MPP patients were recruited in three hospitals over ~5 years. This may not be an accurate incidence of this phenotype because only ~50% of the potentially available patients were recruited. Indeed, we may have overestimated the incidence of the phenotype because those patients with the characteristics under investigation were favored. However, with an average follow-up of 8 years, a considerable number of patient-years have been accumulated, and it is not surprising that relatively uncommon phenotypes are identified. Interestingly, our estimate of the frequency of the MPP is similar to that expected from studies showing that the highest risk of further BCC occurs within 1 year of first tumor presentation (13, 22, 23). Thus, Hoy (13) reported that 14.8% of the BCCs found in 4958 cases over 28 years were identified on the same date as the first tumor. In our analyses, we combined patients who presented with multiple BCCs at first or a later presentation because we did not identify differences in the frequency of characteristics or genotypes. Classification of patients was based only on their presentation phenotype. MPP patients were either aware of more than one lesion or they were unaware and the additional lesion(s) were identified by a physician. All of the dermatologists were aware of the study aims and were, therefore, actively looking for lesions. We used the time of presentation with BCC rather than attempting to estimate time of appearance of lesions because, in our clinical experience, the former is more reliable because it does not rely on the accurate memories of often elderly people. Clearly, it is unlikely that the separate lesions appeared at precisely the same time; rather, the MPP reflects the relatively fast rate of development of lesions in at least some cases (13, 22, 23). In other MPP cases, the appearance of each BCC may be separated in time but been unnoticed until the patient was examined. Unfortunately, clinical inspection does not usually allow determination of the time since the appearance of individual lesions. It was not possible, therefore, to include only patients who could be sure that the individual lesions at a particular presentation appeared within a short time period. Our impression at interview was that, in most cases, lesions had been noticed and patients believed them to be fairly contemporaneous. Clearly, these data are subjective. Importantly, the MPP group did not comprise significantly more males than the SPP groups because males may leave lesions unattended for longer periods than females (24).

However, although we have emphasized the uncertainties regarding the time of appearance of lesions, the MPP patients do appear to be a high-risk group. Thus, previous studies

showed they demonstrated decreased times to next tumor presentation (17), and data from this study show that they developed markedly more tumors than non-MPP patients. We first attempted to identify characteristics and/or genotypes that define the MPP group. We found that only skin type 1 and *CYP2D6 EM* were associated with the phenotype. The finding of these factors provides reassurance regarding the classification of these patients but clearly does not define them. We next considered the influence of the selected genes on BCC numbers. We found that male sex, *GSTM1 AB, GSTT1 null*, and *CYP2D6 EM* were associated with BCC numbers in MPP patients. Indeed, the previously reported associations between these genotypes and BCC numbers (14, 16) appear to largely result from their influence in the MPP cases. Interestingly, many MPP patients demonstrated lesions on the trunk, a phenotype that is associated with many BCCs (18). It was possible, therefore, that the association of the MPP with BCC numbers results from the presence of cases with truncal tumors. Our analysis, however, did not support this suggestion. Non-MPP patients with a truncal lesion did not have more BCC than non-MPP patients without a truncal BCC. The combination of the MPP and a truncal tumor was associated with the highest risk of many BCC. The associations of *GSTT1* and *CYP2D6* genotypes with tumor numbers appeared to be rather different; *GSTT1 null* was associated with BCC numbers in MPP patients, and *CYP2D6 EM* was associated with truncal lesions.

The mechanism for the association between BCC numbers and genotypes is unclear, although the glutathione *S*-transferase enzymes are implicated in the detoxication of lipid and DNA products of UV-derived oxidative stress (25). Thus, allelic variants at *GSTM1* and *GSTT1* are associated with outcome in various oxidative stress-related diseases (25). The basis for the association of BCC numbers with *CYP2D6 EM* is unclear because there is no evidence that this gene is expressed in skin. *CYP2D6* allelic variants have been linked to cancer risk, and there is evidence that smokers with *CYP2D6 EM* are at increased risk of lung cancer (26), although other studies are contradictory (27, 28). We found no significant interaction between smoking and *CYP2D6 EM* (or *GST* genotypes) and BCC numbers. It is also suggested that the *CYP2D6* protein has no physiological function (27). This implies that any influence of this gene results from linkage dysequilibrium with an unidentified locus on chromosome 22q13 (29).

The data presented demonstrate the heterogeneity in the total BCC group and the value of using a molecular epidemiological approach to study subgroups. Thus, the various presentations (MPP, truncal, and SPP) reflect differences in host factors that may lead to altered responses to UV. It is noteworthy that the genotypes studied appear to be markers of outcome rather than susceptibility. It is possible, therefore, that these and other detoxicating enzymes influence outcome and that between-study discrepancies in the effect of genotypes on susceptibility reflect variation in the clinical grades of the patients studied (30). The extent of UV exposure is a further factor that may influence the association of genotypes with BCC numbers, although the data we obtained did not indicate that MPP patients had received more UV than SPP patients. Surprisingly, few of the 773 patients admitted to significant exposure, and only one of these was in the MPP group. Many retrospective studies are limited where exposure is concerned, because it is dependent on accurate recollection by often elderly patients (3, 4, 31). Outdoor occupations did not appear to be associated with the MPP.

BCC patients demonstrate marked differences in the numbers and rate of appearance of lesions, and defining high-risk subgroups who perhaps should be targeted for regular follow-up is not simple. The MPP is defined by an obvious phenotype and appears to include many of the patients with numerous lesions. Our data indicate that this is a risk group with those MPP patients who also have a truncal lesion being at particular risk of many tumors. Accordingly, they might be considered for entry into surveillance program. We interpret our data as indicating that the MPP largely comprises individuals who demonstrate rapid formation of tumors. This characteristic appears to be relatively common in BCC patients (13, 22, 23). The identification of subgroups such as the MPP patients will allow a better assessment of the influence of other polymorphic genes on tumor numbers. Relevant genes include those encoding other proteins involved in protection against oxidant stress and those involved in DNA repair, melanization, and immune modulation. Such studies will help determine the association between phenotypes and allelic variants. They will also allow determination of the number of genes that mediate outcome in this cancer as well as which gene-gene interactions are most important. The latter concept is of particular interest because the combined effect of genes may not be predictable from their individual effects (32). Ultimately, genes identified in molecular epidemiological studies as influencing outcome must be linked with a mechanism. Studies in other cancers have shown that *GST* genotypes are associated with loss of function of tumor suppressor genes. Thus, in women with ovarian cancer, *GSTM1 null* is associated with overexpression of *p53* (33). Similar studies have not been reported in BCC though clearly *p53* and *ptch* are relevant tumor suppressor genes (34).

Acknowledgments

We thank our colleagues Drs. W. E. Farrell and P. R. Hoban for helpful comments in preparing the manuscript.

References

1. Chuang, T. Y., Popescu, A., and Su, W. P. D. Basal cell carcinoma. A population based incidence study. J. Am. Acad. Dermatol., *22:* 413–417, 1990.

2. Hunter, D. J., Colditz, G. A., Stampfer, M. J., Rosner, B., Willett, W. C., and Speizer, F. E. Risk factors for basal cell carcinoma in a prospective cohort of women. Ann. Epidemiol., *1:* 13–23, 1990.

3. Kricker, A., Armstrong, B. K., Jones, M. E., and Burton, R. C. Health, Solar UV Radiation and Environmental Change, Technical Report No. 13, pp. 52–61. Lyon, France: IARC, 1993.

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

5. Rosso, S., Zanetti, R., Martinez, C., Tormo, M. J., Schraub, S., Sanchogarnier, H., Franceschi, S., Gafa, L., Perea, E., Navarro, C., Laurent, R., Schrameck, C., Talamini, R., Tumino, R., and Wechsler, J. The multicentre south European study "Helios" II: different sun exposure patterns in the aetiology of basal cell and squamous cell carcinomas of the skin. Br. J. Cancer, *73:* 1447–1454, 1996.

6. Kricker, A., Armstrong, B. K., and English, D. R. Sun exposure and non-melanocytic skin cancer. Cancer Causes Control, *5:* 367–392, 1994.

7. Kricker, A., Armstrong, B. K., English, D. R., and Heenan, P. J. A dose-response curve for sun exposure and basal cell carcinoma. Int. J. Cancer, *60:* 482–488, 1995.

8. Karagas, M. R., Stukel, T. A., Greenberg, R., Baron, J. A., Mott, L. A., and Stern, R. S. Risk of subsequent basal cell carcinoma and squamous cell carcinoma of the skin among patients with prior skin cancer. J. Am. Med. Assoc., *267:* 3305–3310, 1992.

9. Karagas, M. R. Occurrence of cutaneous basal cell and squamous cell malignancies among those with a prior history of skin cancer. J. Invest. Dermatol., *102* (Suppl.)*:* 10S–13S, 1994.

10. Lear, J. T., Tan, B. B., Smith, A. G., Bowers, W., Jones, P. W., Heagerty, A. H., Strange, R. C., and Fryer, A. A. Risk factors for basal cell carcinoma in the UK: case-control study in 806 patients. J. R. Soc. Med., *90:* 371–374, 1997.

11. Schmieder, G. J., Yoshikawa, T., Mata, S. M., Streilein, J. W., and Taylor, J. R. Cumulative sunlight exposure and the risk of developing skin cancer in Florida. J. Dermatol. Surg. Oncol., *18:* 517–522, 1992.

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

13. Hoy, E. E. Nonmelanoma skin carcinoma in Albuquerque, New Mexico. Cancer (Phila.), *77:* 2489–2495, 1996.

14. Lear, J. T., Heagerty, A. H. M., Smith, A., Bowers, B., Payne, C., Smith, C. A. D., Jones, P. W., Gilford, J., Yengi, L., Alldersea, J., Fryer, A. A., and Strange, R. C. Multiple cutaneous basal cell carcinomas: glutathione *S*-transferase (GSTM1, GSTT1) and cytochrome P450 (CYP2D6, CYP1A1) polymorphisms influence tumour numbers and accrual. Carcinogenesis (Lond.), *12:* 1891–1896, 1996.

15. Wei, Q., Mutanoski, G. M., Farmer, E. R., Hedayati, M. A., and Grossman, L. DNA repair related to multiple skin cancers and drug use. Cancer Res., *54:* 437–440, 1994.

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

17. Lear, J. T., Smith, A. G., Heagerty, A. H. M., Bowers, B., Jones, P. W., Gilford, J., Alldersea, J., Strange, R. C., and Fryer, A. A. Truncal site and detoxifying enzyme polymorphisms significantly reduce time to presentation of further primary cutaneous basal cell carcinoma. Carcinogenesis (Lond.), *18:* 1499–1503, 1997.

18. Lear, J. T., Smith, A. G., Bowers, B., Heagerty, A. H. M., Jones, P. W., Gilford, J., Alldersea, J., Strange, R. C., and Fryer, A. A. 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. J. Invest. Dermatol., *108:* 519–522, 1997.

19. Fitzpatrick, T. B. The validity and practicality of sun reaction skin types I through VI. Arch. Dermatol., *124:* 869–871, 1988.

20. Marez, D., Legrand, M., Sabbagh, N., Lo Guidice, J-M., Lafitte, J-J., Meyer, U. A., and Broloy, F. Polymorphism of the cytochrome P450 *CYP2D6* gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. Pharmacogenetics, *7:* 193–202, 1997.

21. Rosenberger, W. F. Dealing with multiplicities in pharmacoepidemiologic studies. Pharmacoepidemiol. Drug Safety, *5:* 95–100, 1996.

22. Miller, D. L., and Weinstock, M. A. Nonmelanoma skin cancer in the United States: incidence. J. Am. Acad. Dermatol., *30:* 774–778, 1994.

23. Schreiber, M. M., Moon, T. E., Fox, S. H., and Davidson, J. The risk of developing subsequent nonmelanoma skin cancers. J. Am. Acad. Dermatol., *23:* 1114–1118, 1990.

24. Robinson, J. K., Altman, J. S., and Rademaker, A. W. Socio-economic and attitudes of 51 patients with giant basal and squamous cell carcinoma and paired controls. Arch. Dermatol., *131:* 428–431, 1995.

25. Strange, R. C., and Fryer, A. A. The glutathione *S*-transferases: influence of polymorphism on susceptibility to cancer. *In:* P. Boffetta, N. Caporaso, J. Cuzick, M. Lang, and P. Vineis (eds.), Metabolic Polymorphisms and Cancer, IARC Scientific Publication. Lyon, France: IARC, in press, 1999.

26. Bouchardy, C., Benhamou, S., and Dayer, P. The effect of tobacco on lung cancer risk depends on CYP2D6 activity. Cancer Res., *56:* 251–253, 1996.

27. Rannug, A., Alexandrie, A-K., Persson, I., and Ingelman-Sundberg, M. Genetic polymorphism of cytochromes P450 1A1, 2D6 and 2E1: regulation and toxicological significance. J. Occup. Environ. Med., *37:* 25–36, 1995.

28. Smith, G., Stanley, L. A., Sim, E., Strange, R. C., and Wolf, C. R. Metabolic polymorphisms and cancer susceptibility. Cancer Surv., *25:* 27–65, 1995.

29. Wilhelmsen, K., Mirel, D., Marder, K., Bernstein, M., Naini, A., Leal, S. M., Cote, L. J., Tang, M-X., Freyer, G., Graziano, J., and Mayeux, R. Is there a genetic susceptibility locus for Parkinson's disease on chromosome 22q13? Ann. Neurol., *41:* 813–817, 1997.

30. Worall, S. F., Corrigan, M., High, A., Starr, D., Matthias, C., Wolf, C. R., Jones, P. W., Hand, P., Gilford, J., Farrell, W. E., Hoban, P., Fryer, A. A., and Strange, R. C. Susceptibility and outcome in oral cancer: preliminary data showing an association with polymorphism in cytochrome P450 CYP2D6. Pharmacogenetics, *8:* 433–439, 1998.

31. Lear, J. T., and Smith, A. G. Basal cell carcinoma. Postgrad. Med. J., *73:* 538–542, 1997.

32. Frankel, W. N., and Schork, N. J. Who's afraid of epistasis? Nat. Genet., *14:* 371–373, 1996.

33. Sarhanis, P., Redman, C., Perrett, C., Brannigan, K., Clayton, R. N., Hand, P., Musgrove, C., Suarez, V., Jones, P., Fryer, A. A., Farrell, W. E., and Strange, R. C. Epithelial ovarian cancer: influence of polymorphism at the glutathione *S*-transferase *GSTM1* and *GSTT1* loci. Br. J. Cancer, *74:* 1–5, 1996.

34. Gailani, M. R., Stähle-Böckdahl, M., Leffell, D. J., Glynn, M., Zaphiropoulos, P. G., Pressman, C., Undën, A. B., Dean, M., Brash, D. E., Bale, A. E., and Toftgärd, R. The role of the human homologue of *Drosophila patched* in sporadic basal cell carcinomas. Nat. Genet., *14:* 78–81, 1996.