(29) Santoro M, Rosati R, Grieco M, Berlingieri MT, D'Amato GL, de Franciscis V, et al. The ret proto-oncogene is consistently expressed in human pheochromocytomas and thyroid medullary carcinomas. Oncogene 1990;5: 1595–8.
(30) Worby CA, Vega QC, Zhao Y, Chao HH, Seasholtz AF, Dixon JE. Glial cell line-derived neurotrophic factor signals through the RET receptor and activates mitogen-activated protein kinase. J Biol Chem 1996;271: 23619–22.
(31) Santoro M, Wong WT, Aroca P, Santos E, Matoskova B, Grieco M, et al. An epidermal growth factor receptor/ret chimera generates mitogenic and transforming signals: evidence for a ret-specific signaling pathway. Mol Cell Biol 1994;14:663–75.
(32) Vega QC, Worby CA, Lechner MS, Dixon JE, Dressler GR. Glial cell line-derived neurotrophic factor activates the receptor tyrosine kinase RET and promotes kidney morphogenesis. Proc Natl Acad Sci U S A 1996;93: 10657–61.
(33) Trupp M, Arenas E, Fainzilber M, Nilsson AS, Sieber BA, Grigoriou M, et al. Functional receptor for GDNF encoded by the c-ret proto-oncogene. Nature 1996;381:785–8.
(34) Klein RD, Sherman D, Ho WH, Stone D, Bennett GL, Moffat B, et al. A GPI-linked protein that interacts with Ret to form a candidate neurturin receptor. Nature 1997;387:717–21.

Notes

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UV-Radiation-Specific p53 Mutation Frequency in Normal Skin as a Predictor of Risk of Basal Cell Carcinoma

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Background: **A strong association has been found between skin cancer and exposure to UV radiation. The p53 tumor suppressor gene (also known as TP53), which is frequently mutated in human cancers, is believed to be an early target in UV radiation-associated skin carcinogenesis. We have previously developed a sensitive, polymerase chain reaction-based method capable of detecting and quantifying a UV radiation-specific mutation in the p53 gene (codons 247 and 248: AAC CGG → AAT TGG) in normal skin. We have used this method to examine whether UV radiation-specific mutation frequency is associated with risk of basal cell carcinoma (BCC) and with sun exposure.** ***Methods:*** **This case–control study in Australia involved 53 case subjects with BCC and 75 control subjects. DNA was isolated from normal skin (mirror-image anatomic site to the cancer site for case subjects and a randomly selected site for control subjects) and assayed for p53 mutation. Relationships between p53 mutation frequency and risk of BCC, sun sensitivity, or sun exposure were estimated by use of odds ratios (ORs) and 95% confidence intervals (95% CIs).** ***Results:*** **Case subjects were more likely to have a p53 mutation than control subjects (OR = 3.1; 95% CI = 1.3–7.1). In addition, the odds of BCC increased monotonically with increasing frequency of p53 mutation. No statistically significant associations could be demonstrated between p53 mutation frequency and age, sex, sensitivity to the sun, pigmentary characteristics, total lifetime sun exposure, or sun exposure to the biopsy site.** ***Conclusions:*** **Our results indicate that tandem CC → TT mutations involving codons 247 and 248 of the p53 gene are associated with an increased risk of BCC but cannot be used as an accurate measure of total UV-radiation exposure. [J Natl Cancer Inst 1998;90:523–31]**

Carcinogenesis is a complex multistage process associated with the accumulation of critical genetic alterations. Some of these alterations can be directly linked to exposure to specific carcinogens; these carcinogen-specific gene changes are called ''molecular signatures'' of carcinogens. We have proposed that such molecular signatures may serve as dosimeters of cumulative exposure to carcinogens and as predictors of cancer risk *(1)*. Skin cancer provides an ideal experimental model to pursue such an idea because it is largely associated with one agent (UV radiation) *(2)*, and UV radiation-specific gene changes have been detected *(3)*.

The tumor suppressor gene p53 (also known as ATP53) is mutated frequently in human cancers. These mutations can cause the loss of p53's tumor suppressor functions, which include regulation of the cell cycle, apoptosis, and DNA repair *(4–6)*. Mutation of p53 is considered to occur early in the process of UV radiation-associated skin carcinogenesis *(7–12)*. We have previously developed a sensitive, allele-specific polymerase chain reaction (AS-PCR) assay *(13,14)* to detect UV-specific mutations at two distinct hotspots of the p53 gene *(3,15)*. We chose to detect CC to TT tandem base mutations, especially at the boundary of p53 codons 247 and 248 (i.e., AAC CGG → AAT TGG) because of the

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See ''Notes'' following ''References.''

following: 1) there is evidence that this tandem-base mutation is specifically induced by UV radiation *(3,13,15)*; 2) tandem mutation (CC to TT) is more specifically detected by PCR than single-base substitution mutations (e.g., C to T), leading to a high sensitivity of detection; and 3) the arginine encoded by codon 248, which is one of the most frequently altered genetic sites in human cancers, is critical for the DNA-binding capacity of p53 *(16)*, a key property to achieve its tumor suppressor function *(17,18)*.

We have recently further refined our AS-PCR method so that it provides more quantitative data on p53 mutation frequency and have confirmed its validity, using biopsy specimens of normal skin from Japanese patients *(14)*. Tandem mutations involving codons 247 and 248 of the p53 gene were detected at frequencies as low as 10^{-5} to 10^{-6}. Thus, cumulative p53 mutations can be measured reliably and used in the molecular epidemiologic analysis of UV-associated skin cancer.

We report here the use of quantitative AS-PCR (QAS-PCR) to analyze the frequency of UV-specific CC to TT tandem base mutations involving codons 247 and 248 of the p53 gene in biopsy specimens of normal skin in a case–control study of basal cell carcinoma (BCC) in Geraldton, Western Australia, an area of high skin cancer incidence *(19)*.

Subjects, Materials, and Methods

Subjects

The Geraldton Skin Cancer Prevention Survey is a longitudinal, population-based study that began in 1987. It has been described in detail elsewhere *(19,20)*. Surveys of the prevalence of skin cancer, in which participants were examined by dermatologists, were conducted in 1987 and 1992. All 5475 persons aged 40–64 years whose names were registered on the electoral roll in Geraldton were eligible to participate in the 1987 survey; 82% did participate. In 1988, all people found to have a nonmelanocytic skin cancer during the 1987 survey, or in the year prior, were invited to take part in a case–control study relating to the amount and patterns of sun exposure *(19–21)*. A random sample of people without skin cancer also participated in that case–control study. Of those invited to participate in the study, 89% were interviewed.

The study reported herein was restricted to case subjects of BCC and to control subjects. To facilitate the analysis of the relationship between the frequency of UV-specific p53 mutations and sun exposure and related risk factors, eligible subjects were those who participated in the 1988 case–control study. Participants in that study were asked questions about sun exposure to a specific anatomic site (hereafter called the designated site). Designated sites included the scalp, ears, face, front of the neck, back of the neck, shoulders, back, chest and abdomen, arms, forearms, backs of the hands, thighs, legs, and the feet. Areas covered by underpants were not included. For individual case subjects, the designated site was the site of their skin cancer (or one of the affected sites if the person had multiple skin cancers); whereas for control subjects, the site was chosen at random. Because it was our intention to take a biopsy specimen from the skin of the mirror image site to the designated site of all subjects and because we did not intend to take specimens from the face or ears, individuals whose designated site was the face or ears were not considered for this secondary study. We also excluded subjects who were born outside Australia or who were of southern European ethnic origin to ensure that the study subjects were as homogeneous as possible with respect to the adequacy of measurement of lifetime sun exposure and sun sensitivity *(22)*. Finally, because this study was conducted in 1993, people who did not participate in the 1992 survey were not considered.

All 114 people in the cohort who met each of these criteria and who had at least one BCC during the period of follow-up were selected as case subjects. One control subject was selected for each case subject, matched by age (in 5-year groups), sex, and designated anatomic site. They were selected at random from those who did not develop skin cancer during the observation period from 1987 to 1992. If a selected control subject did not participate, another was selected to take his or her place; a total of 170 control subjects were thus invited. Approval to conduct this study was granted by the Committee for Human Rights at the University of Western Australia. All subjects gave written, informed consent.

Skin Biopsy and DNA Extraction

A full-thickness, 3 mm-punch biopsy specimen of normal skin was taken from the mirror image site to the designated site of each subject. Specimens were frozen in ethanol cooled by dry ice and kept at −80 °C until use. The biopsy specimens were incubated overnight at 37 °C in lysis buffer (4 *M* urea, 0.5% *N*-lauroylsarcosine, 10 m*M* EDTA, 0.2 *M* NaCl, 100 m*M* Tris–HCl [pH 8.0]) that contained 5 U proteinase K/mL, followed by phenol–chloroform extraction for DNA preparation as described *(13)*. Samples were identified only by a code number, and, thus, p53 mutation assays were carried out blinded to an individual's case or control status.

Quantitative Mutant Allele-Specific PCR (QAS-PCR)

QAS-PCR, carried out as described previously *(14)*, is a nested PCR assay that consists of two successive PCR steps. In the first PCR step, the entire exon 7 of the p53 gene is amplified, using the primers 5′-ACT GGC CTC ATC TTG GGC CT-3′ and 5′-TGT GCA GGG TGG CAA GTG GC-3′. In the second, nested PCR step, a short sequence containing the CC to TT mutation at codons 247 and 248 is then selectively amplified, using the primers 5′-CTG CAT GGG CGG CAT GAA TT-3′ (specific for CC to TT tandem-base mutations) and 5′-CAA GTG GCT CCT GAC CTG GA-3′, which are 5′-end labeled with $^{32}PO_4$.

For the first PCR step, each reaction mixture (100 μL) contained 5 μg genomic DNA, 1 × PCR buffer (Boehringer Mannheim, Mannheim, Germany), 200 μ*M* each of the four standard deoxynucleoside triphosphates (dNTPs), 1 μ*M* each of the unlabeled primer, and 2 U *Taq* DNA polymerase (Boehringer Mannheim). Amplification was initiated by denaturation at 94 °C for 2 minutes and by the hot-start method (70 °C), followed by 35 cycles each of 94 °C for 30 seconds, 60 °C for 1 minute, and 72 °C for 30 seconds. The amplified exon 7 of the p53 gene was purified by column chromatography (Microcon 30; Amicon, Beverly, MA) and lyophilized to be used as the template for the second PCR step. The reaction mixture for this second, allele-specific amplification step contained the purified p53 exon 7 (first PCR product), 1 × PCR buffer, 330 μ*M* each dNTP, 0.17 μ*M* each of the $^{32}PO_4$ end-labeled primers, and 2 U of *Taq* DNA polymerase. PCR was performed by DNA denaturation at 94 °C for 2 minutes and by the hot-start method at 70 °C, followed by 30 cycles each of 94 °C for 30 seconds, 64 °C for 1 minute, and 72 °C for 30 seconds. Amplified DNAs were lyophilized and subjected to 6% polyacrylamide gel electrophoresis.

A calibration reaction assay, with a series of known amounts of mutant DNA, was included with each assay of samples. For the calibration assay, we used DNA extracted from a BCC obtained from a patient with xeroderma pigmentosum; this patient's DNA contained the AACCGG −> AATTGG tandem-base mutation of codons 247 and 248 of the p53 gene, corresponding to an Asn-Arg −> Asn-Trp amino-acid change *(23)*. The DNA was diluted serially (from 10^{-1} to 10^{-7}) with normal DNA extracted from lymphocytes, isolated from the peripheral blood of healthy donors by use of the ficoll density gradient method (Ficoll-Paque Research Grade solution; Pharmacia Biotech AB, Uppsala, Sweden).

After electrophoresis, the polyacrylamide gels were scanned with a phosphorimager (Molecular Dynamics, Sunnyvale, CA), employing the Scanner control program, and the images were analyzed by the use of the Image-Quant program (version 1.1; Molecular Dynamics). In this way, signal intensities of the gel bands deriving from the calibration standard dilutions and from the investigated samples were analyzed. From the calibration values (10^{-7} to 10^{-5}), the p53 mutation frequency for each sample was determined. We could detect one mutant allele in 10^6 wild-type alleles *(14)*.

Statistical Analysis

The reproducibility of the QAS-PCR assay was examined by comparing the results from two different assays on the same 33 DNA samples. This analysis is restricted to samples that had at least 3 μg of DNA remaining for the second assay.

The relationship between the mutation frequency of p53 and the risk of skin cancer was assessed by use of unconditional logistic regression analysis to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs) *(24)*, with adjustment for age (in 5-year groups), sex, and body site (grouped as head and neck, trunk, upper limbs, and lower limbs). The original pairwise matching by age, sex, and body

site had to be broken because not all case and control subjects who were eligible participated. The quantitative relationship between the risk of BCC and p53 mutation frequency was examined by analyzing the latter as a continuous variable. For both analyses, two transformations of the concentration values were used to allow for the minimum level of mutation frequency detectable by the assay. Because the reaction tubes contained the DNA equivalent of about 0.8 million cells, mutation frequencies greater than 10^{-6} should have been detectable on most occasions, and mutation frequencies greater than 0.5×10^{-6} should have been detectable on about one occasion in three. Thus, our first transformation involved setting all mutation frequencies less than 0.5×10^{-6} to a frequency of 0.5×10^{-6}, and the second involved setting all mutation frequencies less than 1.0×10^{-6} to a frequency of 1.0×10^{-6}.

We also examined the relationship between the risk of BCC and the presence or absence of p53 mutation defined in two ways (mutation frequency $<0.5 \times 10^{-6}$ versus $\geqslant 0.5 \times 10^{-6}$ and $<1.0 \times 10^{-6}$ versus $\geqslant 1.0 \times 10^{-6}$).

We also used logistic regression analysis when examining the relationship between p53 mutation and sun exposure, other biologic measures of sun exposure, and measures of the skin's sensitivity to sunlight. For this analysis, the sample was considered to be positive for a UV-radiation-induced p53 mutation if the mutation frequency was at least 0.5×10^{-6}. Results were adjusted for case or control status to allow for the way in which subjects were selected for study. There were too few subjects with p53 mutations to examine case and control subjects separately, although we did fit interactions between case or control status and the sun exposure variables. Pigmentary risk factors included hair and skin color. Sensitivity to sunlight was measured by the ability to tan on prolonged exposure and the propensity to burn on initial exposure to sunlight. Biologic measures of sun damage to the skin included cutaneous microtopography of the dorsum of the hand, facial telangiectasia, solar elastosis of the neck, and solar keratoses. The survey staff recorded hair and skin color in 1987. A reflectometer was used to measure the skin reflectance at 650 nm on two anatomic sites, the inner surface of the arm (representing an unexposed site or the constitutional skin color) and the forearm (representing an exposed site). Previously, we have taken forearm skin color, after adjustment for color of the unexposed site, to be a measure of tanning. We now prefer to measure the tan more directly. A simple linear regression of forearm color on inner arm color was performed. The residuals from this analysis were considered to estimate the relative degree of tanning. Subjects with large negative residuals had more tanning than expected, whereas those with large positive residuals had less tanning. This measure has the advantage of being uncorrelated with the color of the unexposed site. The variable was divided into three groups, based on approximate tertiles.

All of the biologic markers of exposure, except for cutaneous microtopography of the hands, were recorded by the examining dermatologist by use of ordinal scales. Cutaneous microtopography is a measure of solar elastosis of the hands. A silicon mold was placed on the dorsum of the hand, and the reticular pattern in the mold was later scored against a standard; a loss of fine markings is associated with sun damage. Details of the measurement and categorization of these variables is provided elsewhere *(22)*. The anatomic sites of the biopsy specimens were divided into two groups, usually exposed sites and occasionally exposed sites. Usually exposed sites included the neck, hands, forearms, and, for males only, the scalp. Occasionally, exposed sites included the arms, shoulders, back, the lower limbs, and the chest, excluding the breasts in females. No subject in the study had a biopsy on a rarely exposed site. The medians of the average daily hours of exposure were 2.3 hours for usually exposed sites and 0.5 hours for occasionally exposed sites. All reported *P* values are two-sided.

Results

Study Participation

One hundred ninety-two subjects had biopsy specimens taken (68% of 284 invited). The response proportions were higher for case subjects (83 [73%] of 114) than for control subjects (109 [64%] of 170). PCR results were obtained for 173 (90%) of the respondents, including 74 subjects with BCC and 99 control subjects. However, the PCR assay for the first 43 individuals tested had to be discarded because the assays had been carried out before we established optimal protocols and the results were too variable to be plausible; results for another two individuals were also discarded because of the appearance of unusual, nonspecific PCR products, possibly due to the inadequate quality of the extracted DNA. Thus, the final analysis was restricted to 53 subjects with BCC and 75 control subjects.

The demographic characteristics of these subjects are shown in Table 1. Also shown in Table 1 are ORs for variables that we previously found to be strongly associated with risk of BCC *(20–22)*, namely, the ability to tan, the number of solar keratoses, solar elastosis of the neck, the hours of sun exposure to the trunk, and the proportion of total time spent outdoors on nonworking days between the ages of 15 and 19 years. ORs found in the original case–control study *(20–22)* are also included. The similarity of patterns in the two sets of ORs indicates that, at least for these variables, the loss of subjects has not resulted in substantial selection bias.

Validation of QAS-PCR Assay

To examine the reproducibility of our QAS-PCR method, we analyzed 33 samples in two independent assays. We divided the mutation frequencies into three groups ($<0.5 \times 10^{-6}$, between 0.5×10^{-6} and 1.0×10^{-6}, and $>1.0 \times 10^{-6}$). For 26 samples, the mutation frequency was less than 0.5×10^{-6} in both assays; for one sample, it was between 0.5×10^{-6} and 1.0×10^{-6} in both assays; and, for five samples, the frequency was greater than 1.0×10^{-6} in both assays. Only for one sample was there disagreement; on the first occasion, the mutation frequency was less than 0.5×10^{-6}, and, on the second occasion, it was between 0.5×10^{-6} and 1.0×10^{-6}. Because only six of the 33 samples gave detectable mutation frequencies (i.e., $\geqslant 0.5 \times 10^{-6}$) on both occasions, no formal analysis of the quantitative agreement was possible. For these six samples, the mutation frequencies from the first and second assays were as follows: 5.25 and 6.07×10^{-7}, 2.59 and 2.38×10^{-6}, 4.65 and 4.35×10^{-6}, 3.36 and 1.04×10^{-6}, 1.61 and 1.53×10^{-6}, and 1.22 and 1.12×10^{-6}. Thus, in all assay pairs except one, the two values were close. Examination of the results of all assays by the order in which the samples were processed revealed no patterns suggestive of an effect of order.

Relationship Between CC to TT p53 Mutation and Risk of BCC

We first grouped the mutation frequency into five categories as follows: less than 0.5×10^{-6}. $0.5–0.99 \times 10^{-6}$, $1.0–1.49 \times 10^{-6}$, $1.5–2.49 \times 10^{-6}$, and greater than or equal to 2.5×10^{-6}. The numbers of subjects contained in the various frequency groups were as follows: less than 0.5×10^{-6}, 29 (55%) case subjects and 58 (77%) control subjects; $0.5–0.99 \times 10^{-6}$, eight (15%) case subjects and eight (11%) control subjects; $1.0–1.49 \times 10^{-6}$, nine (17%) case subjects and four (5%) control subjects; $1.5–2.49 \times 10^{-6}$, three (6%) case subjects and two (3%) control subjects; and greater than or equal to 2.5, four (8%) case subjects and three (4%) control subjects.

The median mutation frequencies were 0.23×10^{-6} for both groups, while the maximum mutation frequencies were 5.96×10^{-6} for case subjects and 4.35×10^{-6} for control subjects. We used Wilcoxon's rank sum test to assess the difference between the continuous mutation frequencies in case and control subjects. After setting values less than 0.5×10^{-6} to 0.5×10^{-6}, the *P* value was .005; after seeing values less than 1.0×10^{-6} to 1.0×10^{-6}, the *P* value was .01. When values above

Table 1. Demographic characteristics of case subjects and control subjects and results for variables previously shown to be strongly related to risk of basal cell carcinoma*

Characteristic	Present analysis						Previous analysis†	
	Case subjects (n = 53)	%	Control subjects (n = 75)	%	OR	95% CI	OR	95% CI
Age, y								
45–49	7	13	14	19				
50–54	14	26	11	15				
55–59	9	17	16	21				
60–64	12	23	16	21				
65–69	11	21	18	24				
Sex								
Female	23	43	31	41				
Male	30	57	44	59				
Anatomic site of biopsy								
Head or neck	6	11	15	20				
Trunk	34	64	45	60				
Upper limb	4	8	6	8				
Lower limb	9	17	9	12				
Ability to tan								
Deep tan‡	9	17	26	35	1.0		1.0	
Moderate tan	17	32	37	49	1.2	0.46–3.3	1.9	1.3–2.8
Mild tan	23	43	10	13	8.3	2.6–26	3.2	2.1–4.9
No tan	4	8	2	3	6.1	0.82–46	3.7	1.9–7.3
Solar elastosis of the neck								
None‡	2	4	6	8	1.0		1.0	
Mild	13	25	25	33	1.9	0.30–11	2.2	0.97–5.0
Moderate	27	51	29	39	6.1	0.84–45	3.8	1.6–8.8
Severe	11	21	15	20	4.0	0.50–31	6.0	2.5–15
No. of solar keratoses								
None‡	10	19	22	29	1.0		1.0	
1–5	3	6	17	23	0.4	0.08–1.7	2.1	1.3–3.4
6–14	9	17	10	13	2.3	0.65–8.0	2.8	1.6–4.6
15–39	21	40	19	25	3.2	1.0–10	5.3	3.3–8.6
⩾40	10	19	7	9	4.2	1.1–17	10	5.8–19
Hours of sun exposure to the trunk§								
None‡	9	26	14	31	1.0		1.0	
<5200	3	9	10	22	0.57	0.09–3.5	0.62	0.24–3.6
5200–12 999	12	35	7	16	4.3	0.91–20	1.0	0.51–2.1
⩾13 000	10	29	14	31	2.0	0.44–8.9	2.4	1.2–4.8
Proportion of total time spent outdoors on nonworking days between ages of 15 and 19 years								
0%–40%‡	8	15	14	19	1.0		1.0	
41%–58%	13	25	25	33	0.60	0.18–2.1	1.5	0.88–2.5
59%–99%	10	19	20	27	0.71	0.19–2.7	1.8	1.0–3.3
100%	22	42	16	21	3.0	0.81–11	3.9	1.97–7.8

*OR = odds ratio; 95% CI = 95% confidence interval.

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‡Reference category.

§Analysis restricted to subjects interviewed about exposure to their trunk.

0.5×10^{-6} were considered positive for p53 mutation, 24 (45%) of the case subjects were found to have CC to TT p53 mutations involving codons 247 and 248 compared with 17 (23%) of the control subjects, and the OR for a mutation was 3.1 (95% CI = 1.3–7.1) (likelihood ratio test $P = .007$). When values above 1.0×10^{-6} were considered positive for mutation, 16 (30%) of the case subjects and nine (12%) of the control subjects had a p53 mutation, and the OR was 3.5 (95% CI = 1.4–9.2) ($P = .008$).

When the mutation frequency was analyzed as a continuous variable in logistic regression, the logarithm of the frequency appeared to give the best fit to the data, since it resulted in a higher log-likelihood than models containing either the actual mutation frequency or a quadratic function of the frequency. The P value after adding the log of the mutation frequency to a model containing age, sex, and body site was .01 when mutation frequencies of less than 0.5×10^{-6} were set to 0.5×10^{-6}. From this model, the risk of BCC was predicted to increase 7.5 times (95% CI = 1.5–38.3) for each 10-fold increase in mutation frequency. When mutation frequencies of less than 1×10^{-6} were set to 1×10^{-6}, the P value was .10, and the risk was predicted to increase 9.0 times (95% CI = 0.6–131.8) for each 10-fold increase in mutation frequency. However, the apparent strength of the relationship should be viewed with caution, since the slopes had wide confidence intervals and were strongly influenced by two case subjects with high mutation frequencies.