Relationship Between p53 Codon 72 Polymorphism and Susceptibility to Sunburn and Skin Cancer

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Upregulation of p53 protein induces either growth arrest or apoptosis in response to cellular injury This is signaled from a highly conserved p53 domain between codons 64 and 92, where a functional polymorphism results in either a proline (p53-72P) or an arginine (p53-72R) at codon 72. Preliminary studies suggest that p53-72R may be a risk factor for cervical cancer and, consistent with this, preferential mutation and retention of the p53-72R allele has also been demonstrated in other cancers of squamous cell origin. Here we examine the relationship between allelic forms of p53 and nonmelanoma skin cancer, by determining the correlation with susceptibility to sunburn, which is a known risk factor, and then by p53 sequence analysis of a large series of tumors. We found a significant positive association between p53-72R and susceptibility to sunburn, as assessed by skin phototype and minimal erythemal dose following solar-simulated radiation ($p = 0.0001$ for trend). We also found a significant association between p53-72R homozygosity and nonmelanoma skin cancer in renal transplant recipients (basal cell carcinoma, $p < 0.01$; squamous cell carcinoma, $p < 0.05$) but not in immunocompetent patients compared with skin type matched controls. p53 sequence data revealed mutations in 30 of 70 (42.9%) nonmelanoma skin cancers, 28 (93%) of which were in the p53-72R allele. Loss of heterozygosity occurred more frequently in p53-72RP than in p53-72RR tumors ($p = 0.0001$) with preferential loss of p53-72P in heterozygotes ($p = 0.016$), irrespective of the mutant status of the concomitant allele. Together these data infer functional differences between polymorphic forms of p53 that are likely to be relevant to skin carcinogenesis. *Key words: human papillomavirus/immunosuppression/nonmelanoma skin cancer/renal transplantation/ultraviolet radiation. J Invest Dermatol 119:84–90, 2002*

The p53 tumor suppressor protein plays a crucial part in maintaining cellular integrity and tissue homeostasis through its ability to orchestrate the transcriptional activation of other genes (May, 1999). These protein–protein complexes activate downstream p53 target genes such as p21/Waf-1, resulting in either G_1 arrest or apoptosis, both of which play important parts in preventing tumorigenesis. p53 belongs to a recently recognized family of homologous proteins that includes p73 (encoded by TP73) and p63 (also known as Ket, p40, p73L, and p51; encoded by TP63) (Kaelin, 1999). A critical region of p53 for signaling apoptosis lies between codons 64 and 92, encoding a proline-rich region of the gene homologous to a SH3 binding domain (Walker and Levine, 1996; Sakamuro *et al*, 1997; Venot *et al*, 1998; Zhu *et al*, 1999) in which there is a common polymorphism resulting in either an arginine (CGC) or a proline (CCC) at codon 72 of exon 4. This is a nonconservative amino acid change and results in a structural change in the protein as the p53Pro variant migrates more slowly than the p53Arg variant in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Matlashewski *et al*, 1987). Whereas the existence of this polymorphism has been known since wild-type p53 complementary DNA were first cloned (Lamb and Crawford, 1986), its clinical and functional significance in such a critical domain has remained largely unexplored until very recently.

It is now known that the arginine (p53-72R) and proline (p53-72P) polymorphic alleles have distinct biochemical and functional properties, including their ability to signal apoptosis following DNA damage, with its attendant potential for influencing cancer risk (Thomas *et al*, 1999). The two allelic forms also differ in their relative susceptibility to human papillomavirus (HPV)-mediated E6 degradation, with implications for influencing the risk of HPV 16/18-associated anogenital cancers in particular (Storey *et al*, 1998). Several groups have now shown a possible association between p53-72R and susceptibility to HPV-associated cervical

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Abbreviations: RTR, renal transplant recipient; NMSC, nonmelanoma skin cancer; EV, epidermodysplasia verruciformis; SSR, solar-simulated radiation.

cancer, suggesting that the increased susceptibility of the p53-72R allele to E6-mediated degradation may be clinically important (Storey *et al*, 1998; Zehbe *et al*, 1999; Agorastos *et al*, 2000; van Duin *et al*, 2000); however the risk of cervical cancer attributable to p53-72R remains controversial, with other groups failing to find any obvious association (Helland *et al*, 1998; Hildesheim *et al*, 1998; Josefsson *et al*, 1998; Lanham *et al*, 1998; Minagichi *et al*, 1998; Rosenthal, 1998; Bertorelle, 1999; Klaes *et al*, 1999). To what extent this reflects differences in ethnicity, HPV exposure, and interlab variation in p53 genotyping remains to be established (Makni *et al*, 2000).

More recently, it has also been shown that the p53-72R allele may act as an intragenic modifier of mutant p53 behavior (Marin *et al*, 2000). Certain conformational p53 mutants can bind to and inactivate the p73 protein, with loss of p73-induced apoptosis, a property that is enhanced by the presence of arginine at codon 72. The subsequent demonstration of preferential mutation and retention of the p53-72R allele in some human cancers, including head and neck, vulval, and a small series of skin cancers (Marin *et al*, 2000), and in esophageal squamous cell carcinoma (Kawaguchi *et al*, 2000) indicates that this interaction may be clinically relevant, at least in squamous cell epithelia.

In this study we have evaluated the relationship between the p53 codon 72 polymorphism and skin cancer in more detail. First, we have examined the association of the polymorphism with susceptibility to sunburn [a known risk factor for nonmelanoma skin cancer (NMSC)] and with the presence of skin cancer independent of skin type. Secondly, a subset of tumors were investigated for evidence of an influence of the polymorphism on HPV DNA infection, p53 mutational frequency, and/or loss of heterozygosity (LOH). These studies were undertaken in both immunocompetent individuals and high-risk immunosuppressed organ transplant recipients, a population who represent an accelerated model of skin carcinogenesis (McGregor and Proby, 1996).

MATERIALS AND METHODS

Sources and selection of the cases, controls, and healthy volunteers Cases were selected consecutively from among patients attending either dedicated dermatology clinics for organ transplant recipients or general dermatology clinics at two centers in London (Barts and the London NHS Trust and St Thomas' Hospital). Skin type matched controls were also recruited from these two centers and consisted of: individuals attending general dermatology or medical clinics for problems unrelated to skin cancer; renal transplant recipients attending dedicated dermatology clinics for screening (but who had been transplanted for at least 10 y and had not developed skin cancer); and healthy volunteers working in the laboratories of both hospitals. Few of the individuals approached refused to participate (< 10 individuals across all groups), such that this was unlikely to have influenced the final composition of the groups. Volunteers for minimal erythemal dose (MED) measurements were selected randomly from each of the skin phototype groups.

Skin typing and MED assessment Skin type was assessed in NMSC patients and healthy volunteers by a detailed questionnaire using the Fitzpatrick skin typing system (Fitzpatrick, 1988), which ranges from skin type I/II (burns easily and tans with difficulty), III/IV (burns occasionally, tans readily) through to skin type V (Asian) and IV (black). The minimal dose of solar-simulated radiation (SSR) required to induce a just perceptible erythema at 24 h (MEDjp) was determined for 77 healthy volunteers on previously unexposed buttock skin, by geometric exposure series at six sites using $\sqrt{2}$ incremental doses (Diffey and Farr, 1989). SSR was generated by a 1 kW xenon arc solar simulator (Oriel, Stratford, CT). The doses cited represent the full SSR spectrum, including visible radiation, as determined by a wide-band thermopile radiometer (Medical Physics, Dryburn Hospital, Durham, U.K.). Spectroradiometric analysis (Bentham Instruments, Reading, U.K.) showed that the ultraviolet (UV) radiation (≈ 295–400 nm) component represents 45% of the total SSR dose.

Tissue samples and nucleic acid extraction Basal cell carcinoma and squamous cell carcinoma were excised using local anesthetic following informed consent. A radial section that included both normal and lesional skin was taken from each specimen, snap frozen at harvest, and stored in liquid nitrogen prior to nucleic acid isolation. The remainder was fixed in formol saline and stained with hematoxylin and eosin for histopathologic diagnosis and confirmation of excision margins. Genomic DNA was isolated by proteinase K digestion and RNA using RNAzol B according to the manufacturer's instructions (Biogenesis Ltd, Poole, U.K.). Whole blood for germline genotyping was venesected from controls and NMSC patients, and DNA extracted using a Nucleon DNA extraction kit (Scotlab, Nucleon Biosciences, Coatbridge, Lanarkshire, U.K.).

Polymerase chain reaction amplification of germline polymorphic sequences p53-codon 72 proline sequences were detected by polymerase chain reaction using the primer pair $p53Pro^{+}/p53^{-}$ ($p53Pro^{+}$: 5′GCCAGAGGCTGCTCCCCC; $p53^{-}$: 5′CGTGCAAGTCACAGACTT), and p53-codon 72 arginine by the primer pair $p53^{+}/Arg^{-}$ ($p53^{+}$: 5′TCCCCCTTGCCGTCCCAA; $p53Arg^{-}$: 5′CTGGTGCAGGGGCCACGC) as previously described (Storey *et al*, 1998).

LOH and p53 sequence analysis Samples for LOH assessment were chosen randomly from among those tumors for which frozen tissue had been collected at the time of surgery over a period of 10 y. LOH at 17p was performed using three microsatellite markers D17S520, TP53, and D17S513 (Jones and Nakamura, 1992). To identify mutation-containing exons in p53, single strand conformation polymorphism (SSCP) analysis was performed as previously described (Oliphant *et al*, 1991).

For analysis of p53 sequence, RNA was subjected to reverse transcription using the Stratagene first-strand cDNA synthesis kit (Stratagene, Europe). Total cellular RNA was extracted from frozen cancer biopsies with a commercially supplied phenol/guanidinium reagent according to the manufacturer's instructions (RNAzolB; Biogenesis Ltd). RNA was treated with DNAse (Genehunter Corporation), then used for first strand cDNA synthesis using the Prostar system (Stratagene). The p53 coding sequence was then amplified from cDNA as a 1.3 kb fragment using Pfx DNA polymerase (Life Technologies Ltd, Paisley, UK) and cloned using the Zero-Blunt system (Invitrogen Ltd, Paisley, UK). Mutations in p53 were identified by sequencing multiple clones in the proposed region of p53 suggested by abnormal SSCP mobility. In cancers where insufficient tissue was available to allow RNA isolation, a 2.8 kb genomic DNA fragment containing exons 4–9 of p53 were amplified from genomic DNA with Pfx DNA polymerase (Life Technologies) using primers as previously described (Nigro *et al*, 1989) and again cloned with the Zero-Blunt system. Mutations were then identified by sequencing of multiple plasmid clones. To identify in individual cancers the genotype at codon 72 in mRNA with either wild-type or mutant p53, plasmid clones were identified that contained either the mutation or were wild type. The same plasmid clones were then sequenced in both directions through codon 72 using additional sequencing primers.

HPV DNA detection HPV DNA was sought in a subset of 24 samples by a degenerate and nested polymerase chain reaction-based method with a panel of oligonucleotide primers located within the highly conserved L1 open reading frame of the HPV genome, as previously described (Harwood *et al*, 1999).

Statistical analysis Comparisons between specified groups were performed using a Pearson's chi-square test or Fisher's exact test for small numbers of observations. Genotype/skin type correlation was analyzed using a chi-squared test for trend in proportions. MED/skin type and MED/genotype correlation was assessed by Wilcoxon rank sum test. Preferential loss of p53-72P in RP heterozygotes was tested using an exact binomial test. All analyses were carried out using S-plus.

RESULTS

Characteristics of the cases, controls, and healthy volunteers Skin cancer cases and controls were adults aged between 18 and 75 y, matched for skin types I–III and derived from a north-west European white population. Within the extended healthy volunteer control population, skin types I–IV (all of whom were of north-west European origin) and those of skin types V and VI were included (comprising individuals of oriental/Asian and African/Afro-Caribbean origin, respectively). As the numbers of individuals with type V and VI skin was small, it was not possible to stratify for each ethnic group individually, although in future studies this would be desirable.

Patients in the two skin cancer populations (transplant and immunocompetent groups) were deemed broadly representative of

Table I. Relationship between p53-72 genotype and skin phototype in 227 healthy individuals

Skin photoype[a]	Total number of healthy volunteers	P523 codon 72 polymorphism status			OR for RR genotype[b]	
		RR (%)	RP (%)	PP (%)	OR	95% CI
I/II	69	42 (61)	26 (38)	1(1)	1	-
III/IV	69	33 (48)	32 (46)	4 (6)	1.7	0.9-3.3
V	48	16 (33)	16 (33)	16 (33)	3.1	1.5-6.7
VI	41	3 (7)	20 (49)	18 (44)	19.7	6.8-56.7

[a]According to Fitzpatrick classification (see text).
[b]OR, odds ratio; CI, confidence interval.

the skin cancer population in general as they were recruited consecutively; however, it is possible that among the transplant population, those with multiple cancers might be over-represented as they were reviewed more frequently. There were no obvious selective factors that we are aware of that might have influenced recruitment of the control populations, or in selection of samples for LOH assessment.

Relationship between p53-72R, skin type, and MED (Tables I and II) In our control population p53 genotypes were in Hardy–Weinberg equilibrium for all skin phototypes except V. There was strong correlation between skin phototype and susceptibility to sunburn as assessed by SSR MED in 77 individuals, skin types I/II having significantly lower MED than skin types III/IV ($p < 0.0001$) and similarly types III/IV and V/VI ($p < 0.0001$). We also found an association between p53-72R and susceptibility to sunburn, as determined both by skin type and MED phototesting. Among 227 volunteers, the proportion of individuals homozygous for p53-72R was found to be 61% in skin types I/II, decreasing sequentially to 7% in those of skin type VI ($p = 0.0001$ for trend) (**Table I**). Median MED scores similarly reflected this trend, increasing from 8.0 to 9.6 and 24 J per cm^2 for genotypes RR, RP, and PP, respectively, with a significant difference in MEDjp between genotypes RR/RP and PP ($p = 0.04$), although not between RR and RP.

Relationship between p53-72R and skin cancer (Table III) To test whether homozygous p53-72R was a risk factor for NMSC independent of skin type, the germline p53-72 polymorphism status of 182 skin cancer patients was compared with that of 156 skin-type matched healthy volunteers. Skin cancer patients comprised 92 renal transplant recipients, from a population at very high risk of multiple skin cancers (McGregor and Proby, 1996), and 90 otherwise healthy patients with skin cancer. We found that homozygous p53-72R was significantly over-represented in transplant patients with skin cancer ($p = 0.01$; odds ratio 2.1, 95% confidence interval 1.22–3.68), for both basal cell carcinoma ($p = 0.002$; odds ratio 3.76 95%, confidence interval 1.64–8.61) and squamous cell carcinomas ($p = 0.03$; odds ratio 2.14, 95% confidence interval 1.14–4.01). There was no evidence of a significant association between p53-72R homozygosity and either basal cell carcinoma or squamous cell carcinoma in immunocompetent patients. The odds ratios and confidence intervals, however, overlap for renal transplant recipients and immunocompetent patients, and it is possible that a larger series could show an effect.

HPV status and p53 codon 72 genotype (Table IV) As an excess of the RR genotype in transplant patients with skin cancer might reflect the HPV status of the tumors, as previously proposed for cervical cancer (Storey *et al*, 1998) and esophageal tumors (Kawaguchi *et al*, 2000), HPV DNA was sought in a subset of NMSC, chosen at random from both transplant and immunocompetent patients, using a combination of degenerate and nested polymerase chain reaction primers capable of detecting all known HPV, including mucosal, cutaneous, and epidermodysplasia verruciformis (EV) types (Harwood *et al*, 1999); however we found no significant relationship between HPV positivity and RR genotype in this series, where both HPV typing and p53 data were available (**Table III**).

Table II. Relationship of minimal erythemal dose to skin phototype and p53 codon-72 polymorphism in 77 healthy individuals

	Number of individuals	Minimal erythemal dose (J per cm^2)	
		Median	Range
Skin phototype[a]			
I/II	42	8	2.8–11.2
III/IV	28	11.2	8–16
V/VI	7	32	16–43
Genotype[b]			
RR	41	8	2.8–32
RP	30	9.6	4.16
PP	6	24	5.6–43

[a]Assessed by Fitzpatrick's classification (see text).
[b]Assessed by solar simulated radiation (see text).

P53 sequence analysis and LOH studies in NMSC (Tables IV and V) Finally, we examined the relationship between p53 polymorphism status, p53 mutation, and LOH at the p53 locus in 70 NMSC (**Table IV**). These comprised 25 tumors from immunocompetent patients and 45 from renal transplant recipients, each randomly selected without prior knowledge of the p53 polymorphism status. A subset of these tumors have been previously described (Marin *et al*, 2000; **Table IV**). p53 mutations were identified in 30 of 70 (43%) of all skin tumors, with no significant difference between either transplant and immunocompetent tumors [16 of 45 (36%) *vs* 14 of 25 (56%); $p = 0.13$], or RR and RP genotypes [22 of 56 (39%) *vs* eight of 14 (57%); $p = 0.24$]. Twenty-eight of 30 tumors with p53 mutations (93%) were mutant in the R allele but as the majority of these were RR homozygotes this did not reach statistical significance. Of the eight RP tumors that had a mutation, this was in the R allele in six (75%) cases compared with four expected ($p = 0.3$). Despite a lack of power to demonstrate preferential mutation of the R allele (Marin *et al*, 2000) we were able to show that LOH was significantly more prevalent in RP tumors, being present in two of 56 RR (3.6%) compared with seven of 14 RP cases (50%; $p = 0.0001$). There was also evidence for preferential loss of p53-72P in RP heterozygotes (all seven tumors that showed LOH lost the 72P allele *vs* 3.5 expected; $p = 0.016$), which occurred both with and without concomitant mutation in the paired allele. These data are summarized in **Table III**.

Table III. Germline frequencies of codon 72 p53 polymorphic forms in 182 skin cancer patients and 156 skin type-matched healthy controls

		p53 codon 72 *polymorphism status* (number of individuals [%])			OR for RR genotype	
Study group	NMSC status	RR	PP	RP	OR	95% CI
1. Controls[a]		85 (55)	5 (3)	66 (42)	1	–
2. RTRs	BCC	36[d] (82)[d]	0 (0)	8 (18)[d]	3.76	1.64–8.61
	SCC	46[b] (72)[c]	0 (0)	18 (28)	2.14	1.14–4.01
	NMSC (total)	66 (72)[d]	0 (0)	26 (28)[c]	2.12	1.22–3.68
3. ICPs	BCC	30 (67)	0 (0)	15 (33)	1.67	0.83–3.35
	SCC	28 (62)	0 (0)	17 (38)	1.38	0.70–2.72
	NMSC (total)	58 (64)	0 (0)	32 (36)	1.51	0.89–2.58

[a]Controls comprised 18 renal transplant recipients (RTRs) and 138 immunocompetent patients (ICPs) with RR genotype frequencies of 0.56 and 0.54 respectively.
[b]Sixteen patients had both BCC and SCC.
[c]Denotes significance at $p < 0.05$.
[d]$p<0.01$ between cases and controls.

Table IV. Loss of heterozygosity (LOH) analysis, p53 mutation analysis, HPV genotyping and p53 codon 72 allelotyping data for 25 NMSC from immunocompetent patients (ICP) and 45 NMSC from renal transplant recipients (RTR)

	NMSC (ICP)			NMSC (RTR)			NMSC (All)		
	RR (n = 15)[%]	RP (n = 10)[%]	Total (n = 25)[%]	RR (n = 41)[%]	RP (n = 4)[%]	Total (n = 45)[%]	RR (n = 56)[%]	RP (n = 14)[%]	Total (n = 70) [%]
LOH present	1 [6.7]	6 [60]	7[28]	1[2.3]	1 [25]	2 [4.4]	2[3.6]	7 [50]	9 [12.9]
p53 mutation	7 [47]	7 [70]	14 [56]	15 [37]	1 [25]	16 [36]	22 [39]	8 [57]	30 [43]
HPV positive	1/7 [14]	3/4 [75]	4/11 [36]	6/11 [55]	2/2 [100]	8/13 [62]	7/18 [39]	5/6 [83]	12/24 [50]

DISCUSSION

In this study we demonstrate an association between the arginine and proline allelic forms of p53 codon 72 and skin phototype, as determined by both Fitzpatrick clinical scoring and objectively by MED assessment. The p53-72R allele was significantly more prevalent in individuals with fair skin susceptible to both burn easily in the sun and, in the longer term, skin cancer. After allowing for skin type, we also found an association between p53-72R and NMSC in renal transplant patients, a group at particularly high risk of both basal and squamous cell cancers (McGregor and Proby, 1996). The association was not present in immunocompetent individuals with skin cancer, but this was not clearly attributable to the differing HPV status of the tumors in the two patient groups. Finally, p53 sequence data revealed that mutations were present in 43% of all the skin tumors, the majority (93%) occurring in the arginine allele, as previously described in other malignant squamous epithelia (Marin *et al*, 2000). There was a significant increase in LOH at the p53 locus in RP compared with RR tumors, with preferential loss of p53-72P in RP heterozygotes, irrespective of the mutant status of the concomitant allele. Together these data infer potentially important functional differences between polymorphic forms of p53 at codon 72, which are likely to be relevant to skin carcinogenesis.

The p53 codon 72 arginine/proline polymorphism has been recognized for almost 15 y (Lamb and Crawford, 1986). Population-based studies indicate that the proline allelic form is most prevalent in black-skinned races and least prevalent in those with white skin, with a clear and consistent decline in the prevalence of the proline allele with increasing latitude (Själander *et al*, 1995). Evidence suggests that the polymorphism is balanced and maintained at different levels in humans across the world population, which, in a functional domain of p53 that is otherwise so highly conserved, even through different species (Soussi *et al*, 1987), suggests clinically important differences between arginine and proline at codon 72 on which natural selection pressures are acting (Beckman *et al*, 1994). Studies of human fetal tissue indicate that these are not likely to be occurring prenatally (Beckman *et al*, 1994), so it remains to be determined what these might be.

P53 codon 72 genotype, skin type, and erythemal response One hypothesis is that the association between p53-72R frequency and increasing latitude has evolved as an adaptation to UV radiation exposure (Beckman *et al*, 1994). This is clearly speculative but our data would at least be consistent with this. There was an association of p53-72R with both fair skin type and burn easily in the sun as measured by SSR cutaneous erythemal response and, conversely, the p53-72P allele was most prevalent in dark-skinned races originating from areas with high ambient UV levels. It was not possible in this study to dissociate skin type from ethnic background. Whether p53-72P is actually protective against acute UV-induced DNA damage remains to be established but certainly it was associated in our study with higher median MED than the p53-72R genotype. In addition, in skin tumor material we found evidence for preferential loss of p53-72P, suggesting that this may be a necessary step in skin carcinogenesis, again implying a possible protective effect of p53-72P. Skin cancer is an unlikely endpoint for selection pressures to act on, however, as tumors predominantly occur after reproductive age. Another possibility is that the polymorphism may be linked to susceptibility to UV-induced immunosuppression; this is also skin type related (Kelly *et al*, 2000) and could influence susceptibility to infection, exerting potentially more important selective pressures. Whatever the basis of selection, there appear to be pressures to maintain p53-72P in black-skinned populations, and for the emergence of the p53-72R allele in those resident in successively more temperate climates.

P53 codon 72 status and skin cancer susceptibility As p53-72R is associated with skin types that are susceptible to burn easily in the sun, it might also be independently associated with the

Table V. Details of p53 codon 72 status, p53 mutational status, p53 LOH and HPV positivity in 25 NMSC from immunocompetent patients (ICP) and 45 NMSC from renal transplant recipients (RTR)

Immune status	Patient type	NMSC 72 status	p53 codon	LOH (allele lost)	P53 mutation status	Mutant allele	HPV status
ICP	1	SCC[a]	RP	Y(P)	236 TAC>AAC (Tyr>Asn)	R	ND
	1	SCC[a]	RP	Y(P)	287 GAG>AAG (Glu>Lys)	R	ND
	2	SCC[a]	RR	N	Wild type		ND
	3	SCC	RR	N	Wild type		ND
	4	SCC[a]	RP	Y(P)	Wild type		ND
	5	SCC[a]	RR	N	224 GAG>TAG (Glu>Ter)	R	ND
	6	BCC	RP	N	178 CAC>TAC (His>Tyr)	P	ND
	7	BCC	RR	N	137 GCC>GAC (Ala>Asp)	R	ND
	8	SCC	RP	N	Wild type		Neg
	9	SCC[a]	RR	N	248 CGG>TGG (Arg>Trp)	R	Neg
	10	SCC[a]	RR	Y(R)	Wild type		Neg
	11	SCC[a]	RR	N	Wild type		ND
	12	SCC[a]	RP	Y(P)	248 CGG>TGG (Arp>Trp)	R	+
	13	SCC[a]	RP	Y(P)	161 GCC>GTC (Ala>Val)	R	ND
	14	BCC	RP	N	138 GCC>GAC (Ala>Asp)	P	ND
	15	BCC	RR	N	Wild type		ND
	16	BCC	RR	N	216 GTG>GGG (Val>Gly)	R	Neg
	17	SCC[a]	RR	N	179 CAT>TAT (His>Tyr)	R	Neg
	18	SCC	RP	Y(P)	173 GTG>CTG (Val>Leu)	R	+
	19	SCC	RR	N	Wild type		Neg
	10	SCC	RP	N	Wild type		+
	20	BCC	RR	N	290 CGC>TGC (Arg>Cys)	R	+
	21	BCC	RR	N	Wild type		Neg
	22	BCC	RR	N	241 TCC>CCC (Ser>Pro)	R	ND
	23	BCC	RR	N	Wild type		ND
RTR	24	SCC	RR	N	Wild type		ND
	24	SCC	RR	N	Wild type		ND
	24	SCC	RR	N	Wild type		ND
	24	SCC	RR	N	170 ACG>ACA (Thr>Ala)	R	ND
	24	SCC	RR	N	80 CCT>CTT (Pro>Leu)	R	ND
	25	SCC	RR	N	Wild type		ND
	26	SCC	RR	N	295 CCT>CTT (Pro>Leu)	R	ND
	27	SCC	RR	N	Wild type		ND
	28	SCC	RR	N	243 ATG>AGG (Met>Arg)	R	ND
	29	SCC	RR	N	Wild type		ND
	29	SCC	RR	N	Wild type		ND
	29	SCC	RR	N	Wild type		ND
	29	SCC	RR	N	245 GGC>GTC (Trp>Val)	R	ND
	29	SCC	RR	N	248 CGG>CAG (Arg>Gln)	R	ND
	30	SCC	RP	N	Wild type		ND
	31	SCC	RR	N	Wild type		ND
	32	SCC	RR	N	Wild type		ND
	32	SCC	RR	N	135 TGC>TGA (Cys>Ter)	R	ND
	33	SCC	RR	Y(R)	85 CCT>CTT (Pro>Leu)	R	ND
	33	SCC	RR	N	Wild type		ND
	34	SCC	RP	Y(P)	Wild type		ND
	35	SCC	RR	N	104-105 CAGGGC>CAGGGGC (frameshift)	R	ND
	36	BCC	RR	N	250 CCC>CTC (Pro>Leu)	R	ND
	37	BCC	RR	N	117 GGG>GGGG (frameshift)	R	ND
	38	BCC	RR	N	Wild type		ND
	39	BCC	RR	N	Wild type		ND
	40	SCC	RR	N	Wild type		+
	41	SCC	RR	N	Wild type		+
	42	SCC	RR	N	Wild type		+
	43	SCC	RR	N	177 CCC>CTC (Pro >Leu)	R	Neg
	44	SCC	RR	N	Wild type		Neg
	45	SCC	RR	N	Wild type		+
	46	SCC	RR	N	Wild type		ND
	47	SCC	RR	N	Wild type		ND
	48	SCC	RP	N	Wild type		+
	49	SCC	RP	N	309 ccC>TCC (Pro>Ser)	R	+
	50	SCC	RR	N	Wild type		ND
	51	BCC	RR	N	219 CCC>CTC (Pro>Leu)	R	Neg
	52	BCC	RR	N	Wild type		ND
	53	BCC	RR	N	Wild type		+
	54	BCC	RR	N	149 TCC>TTC (Ser>Phe)	R	+
	55	BCC	RR	N	Wild type		Neg
	*56	BCC	RR	N	Wild type		Neg
	41	BCC	RR	N	175 CTG>CAG (Arg>His)	R	ND
	57	BCC	RR	N	Wild type		ND

[a]Tumors previously described (Marin *et al*, 2000).
[b]Samples typed with degenerate and nested primers (Hanvood *et al*, 1999).

development of skin cancer, a long-term consequence of cumulative UV exposure. To examine this, we compared the polymorphism status of skin cancer patients, from a high-risk immunosuppressed transplant population and an immunocompetent population, with skin type-matched healthy controls. We found a significant association between p53-72R and the development of both basal cell carcinoma and squamous cell carcinoma in transplant recipients, suggesting that it might indeed be a risk factor for skin cancer in this group. Another study found a similar trend in a small number of renal transplant patients with skin cancer but this did not reach statistical significance (Marshall *et al*, 2000); however, we found no such relationship in immunocompetent patients with skin cancer, although the trend was in the same direction. This might reflect a lack of power to detect weaker associations, particularly as there are multiple environmental and host factors, in particular UV exposure, which might influence skin cancer risk that the current model does not account for. Nonetheless, having identified p53-72R as a possible risk factor for skin cancer, the hypothesis needs to be tested prospectively in a multivariate analysis of a larger series of patients.

P53 codon 72 genotype, immune status, and HPV status in skin cancer patients If p53-72R is a risk factor for the development of skin cancer, it is not clear why a difference should exist between transplant and nontransplant patients since tumors from both groups appear otherwise to behave similarly with respect to p53 mutation prevalence and LOH at the p53 locus (McGregor *et al*, 1997). It has been proposed that transplant-associated tumors may have a different etiology, in particular that they may be causally associated with HPV infection, which is more prevalent in organ transplant recipients (Proby *et al*, 1996). Detection of HPV DNA is significantly higher in transplant than in nontransplant tumors, and often includes multiple HPV types of a broad phylogenetic spectrum within single lesions (Harwood *et al*, 2000). Although we found no overall relationship between HPV status and genotype in the present study, it may be found to exist for specific HPV types which we would not have the statistical power to detect, in a small series of all HPV-positive skin tumors from 29 patients were compared with 69 healthy controls, p53-72R homozygosity was significantly associated with NMSC even in the immunocompetent population (Dokianakis *et al*, 2000). At a mechanistic level, whether preferential degradation of p53-72R by the diverse HPV types present in skin tumors could account for susceptibility of homozygous p53-72R transplant patients to skin cancer remains to be investigated. Similarly, p53-72R and p53-72P alleles appear to differ in ability to signal apoptosis (Thomas *et al*, 1999) and a differential effect of HPV-encoded E6 proteins on their apoptotic capacity could potentially account for the apparent excess risk of skin cancer in homozygous p53-72RR transplant patients and needs to be tested. The recently described ability of cutaneous HPV types to inhibit UV-induced apoptosis in cells containing wild-type p53 by degradation of Bak (Jackson, 2000a; Jackson, 2000b) may also be relevant in this context is also unclear.

Preferential mutation of R allele and loss of P allele p53 gene mutations were present in 30 of 70 (43%) of all skin tumors examined, approximately equally in transplant and nontransplant tumors, as previously reported (McGregor *et al*, 1997), and in RR and RP tumors alike. The majority of all mutations occurred in the p53-72R allele, although we were unable to show that this was statistically significant as there were so few RP tumors in the series. Nonetheless, p53-72R was mutant in six of the eight RP heterozygotes where a p53 mutation was present and these data accord with previous p53 sequence data in squamous cell epithelia from predominantly anogenital tumors where significant preferential mutation of p53-72R is demonstrable (Marin *et al*, 2000).

We also found that LOH was significantly more common in RP than RR tumors, with preferential loss of p53-72P, irrespective of the mutant status of the concomitant p53-72R allele. Together these data are consistent with the hypothesis that p53-72R is preferentially mutated and retained in squamous cell tumors where it confers a selective advantage for tumorigenesis by neutralizing p73-induced apoptosis (Marin *et al*, 2000). Conversely, selection at the tumor level for loss of p53-72P suggests a possible protective effect of the proline-containing allele against skin cancer.

Potential confounding factors Our descriptive study of p53 codon 72 genotypes in skin cancer patients and healthy controls indicates that p53-72R is associated with fair skin type and that it may independently confer susceptibility to skin cancer in high-risk immunosuppressed patients. Its conclusions are limited by sample size and by possible confounders, including ethnicity, UV exposure, gender, and HPV status of tumors. Some of these can be addressed in future studies, employing multivariate analysis, but ethnicity in particular is difficult to dissociate from skin type. For example, we cannot exclude the possibility that our data reflect an association between p53-72R and specific ethnic groups, rather than skin type. The parallel decline in p53-72P alleles with increasing latitude makes this conclusion less likely, however, particularly as populations with similar skin types but marked ethnic and genetic dissimilarities, e.g., Indian and African populations, nonetheless display close similarities in codon 72 genotype frequencies (Beckman *et al*, 1994).

To dissociate, as far as is possible, ethnicity from skin type, the relationship between p53 genotype frequency and erythemal response should be confirmed in individuals of different skin type within a single ethnic population. The ethnicity of individuals with skin types I–IV in this study was of broadly similar north-west European origin, although some degree of ethnic heterogeneity cannot be entirely excluded. If these individuals are stratified within skin type groups I–IV, the trend with respect to codon 72 status remains, but there is insufficient power to show statistical significance. This is largely because individuals of skin type IV are poorly represented in our population. Another group, however, have recently reported just such a significant association between p53-72R genotype frequency and skin type within an apparently homogeneous caucasian population of presumed uniform ethnicity (Bastiaens *et al*, 2001).

In summary, we report a relationship between p53-72R and susceptibility to burn easily in the sun. In addition, in high-risk immunosuppressed groups we found that p53-72R was associated with the development of both basal and squamous cell carcinomas, although this relationship was not demonstrable in immunocompetent patients with skin cancer. In squamous cell tumors there is evidence of preferential mutation and retention of the p53-72R allele, together with selective loss of p53-72P. Binding of mutant p53-72R to p73 with subsequent loss of p73-induced apoptosis provides a plausible mechanism for the susceptibility to skin cancer that the p53-72R genotype may confer, but other explanations may emerge. Conversely, the demonstration that p53-72P is frequently lost in tumors suggests a possible protective effect of this allele against skin cancer. This would be consistent with the high prevalence of p53-72P alleles found in dark-skinned races living close to the equator and exposed to high levels of UV radiation.

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