Melanocortin-1 Receptor Genotype is a Risk Factor for Basal and Squamous Cell Carcinoma

Neil F. Box, David L. Duffy,* Rachel E. Irving,† Anne Russell,* Wei Chen, Lyn R. Griffiths,† Peter G. Parsons,* Adele C. Green,* and Richard A. Sturm

Center for Functional and Applied Genomics, Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia; *Queensland Institute of Medical Research and Joint Genetics Program University of Queensland, Brisbane, Queensland, Australia; †Genomics Research Center, Griffith University, Gold Coast, Queensland, Australia

MC1R gene variants have previously been associated with red hair and fair skin color, moreover skin ultraviolet sensitivity and a strong association with melanoma has been demonstrated for three variant alleles that are active in influencing pigmentation: Arg151Cys, Arg160Trp, and Asp294His. This study has confirmed these pigmentary associations with MC1R genotype in a collection of 220 individuals drawn from the Nambour community in Queensland, Australia, 111 of whom were at high risk and 109 at low risk of basal cell carcinoma and squamous cell carcinoma. Comparative allele frequencies for nine MC1R variants that have been reported in the Caucasian population were determined for these two groups, and an association between prevalence of basal cell carcinoma, squamous cell carcinoma, solar keratosis and the same three active MC1R variant alleles was demonstrated [odds ratio = 3.15 95% CI (1.7, 5.82)]. Three other commonly occurring variant alleles: Val60Leu, Val92Met, and Arg163Gln were identified as having a minimal impact on pigmentation phenotype as well as basal cell carcinoma and squamous cell carcinoma risk. A significant heterozygote effect was demonstrated where individuals carrying a single MC1R variant allele were more likely to have fair and sun sensitive skin as well as carriage of a solar lesion when compared with those individuals with a consensus MC1R genotype. After adjusting for the effects of pigmentation on the association between MC1R variant alleles and basal cell carcinoma and squamous cell carcinoma risk, the association persisted, confirming that presence of at least one variant allele remains informative in terms of predicting risk for developing a solar-induced skin lesion beyond that information gained through observation of pigmentation phenotype. ***Key words: genetic epidemiology/hair color/pigmentation/polymorphism/skin color. J Invest Dermatol 116:224–229, 2001***

The major environmental causative factor for basal and squamous cell carcinomas (BCC and SCC) is solar ultraviolet (UV) irradiation, which is important both in terms of total cumulative lifetime exposure and in terms of exposure profiles for these two types of tumors (IARC Monograph, 1992; Rosso *et al*, 1996; English *et al*, 1998b). It has been speculated that tumorigenic transformation of epithelial cells requires significantly less UV for BCC than for SCC and where tanning of the skin is able to develop and exposure continues, subjects tend to develop SCC rather than BCC (Rosso *et al*, 1996). UV may only be one factor for BCC, other influences may include diet (Wei *et al*, 1994) and the basal rate of tumors. Solar keratoses (SK) are premalignant UV-induced skin lesions that rarely transform to become SCC, but which often spontaneously regress (Frost *et al*, 2000). Presence of an SK in individuals strongly predicts their potential to develop both BCC and SCC and is regarded as an indicator of excessive sun exposure (Marks *et al*, 1988; Marks, 1990).

Constitutional factors also determine an individual's potential to develop BCC and SCC, with the skin's reaction to sunlight or "skin type" an important determinant of skin cancer risk. Although other factors such as skin thickness or DNA repair ability may influence the skin response to UV and, therefore, risk of skin cancer, pigmentation is clearly a central element in the interaction of the skin with UV light. Pigmentary traits such as fair skin, lack of tanning ability, and propensity to freckle have been identified as risk factors (Green and Battistutta, 1990; Kricker *et al*, 1991; Green *et al*, 1996; Zanetti *et al*, 1996; English *et al*, 1998a). The associations between light eye color and red or fair hair observed in these studies indicate that these traits are probably risk indicators by virtue of an interrelationship with a poor skin response to UV.

Given that pigmentation influences nonmelanocytic skin cancer (NMSC) risk, the identification of gene variants at the melanocortin 1 receptor (MC1R), which control the production of red pigmentation in Caucasian individuals, suggests that allelic variation within this gene should likewise be associated with skin cancer risk (Valverde *et al*, 1995; Box *et al*, 1997; Smith *et al*, 1998; Sturm *et al*, 1998). MC1R is a seven-pass transmembrane G-protein coupled receptor expressed by skin melanocytes that activates adenylate cyclase to elevate cyclic adenosine monophosphate levels upon

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Reprint requests to: Dr. Richard A. Sturm, Center for Functional and Applied Genomics, Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland 4072, Australia. Email: R.Sturm@mailbox.uq.edu.au

Abbreviations: BCC, basal cell carcinoma; MC1R, melanocortin-1 receptor; NMSC, nonmelanocytic skin cancer; RHC, red hair color; SK, solar keratosis.

stimulation by the proopiomelanocortin-derived peptides α-melanocyte-stimulating hormone and adrenocorticotropic hormone (Thody and Graham, 1998). Hormonal stimulation of MC1R leads to eumelanogenesis and is central to the tanning response of human melanocytes following UV irradiation (Suzuki *et al*, 1999). It has already been shown that three common MC1R variant alleles, Arg151Cys, Arg160Trp, and Asp294His are associated with an increased incidence of UV-induced melanoma within a large population of south-east Queensland cases and controls, with the conclusion that the effect that MC1R variant alleles have on melanoma is mediated predominantly by an effect on pigmentation phenotype (Palmer *et al*, 2000).

At least two studies have attempted to address the issue of MC1R variant allele predisposition to BCC and SCC: the first demonstrated an over-representation of the Asp294His variant in 46 BCC and eight SCC cases *vs* 25 controls, where Asp294His was the only variant examined (Smith *et al*, 1998), whereas a second study examining the Asp294His and Val92Met variant alleles in 311 BCC cases and 190 controls failed to observe any association between either of these variants and BCC (Ichii-Jones *et al*, 1999). The intention of the our study was to examine the relationship between nine MC1R variants identified in a south-east Queensland community (Box *et al*, 1997) and the prevalence of skin cancer or SK using allele-specific oligonucleotide screening of high-risk and low-risk groups selected from the Nambour Skin Cancer Study (Green *et al*, 1994, 1996).

MATERIALS AND METHODS

Study samples and DNA extraction Data collection for the Nambour skin cancer studies began with an original skin cancer survey in 1986, with subsequent whole-body dermatologic exams conducted in 1992, 1994, and 1996, which recorded numbers of sun-induced lesions in addition to a large selection of other data (Green *et al*, 1988, 1994, 1996, 1999; Green and Battistutta, 1990). A 10 ml blood sample was also collected and frozen down for the majority of participants in the 1996 skin exam. Of the 1311 participants in the Nambour Study of skin cancer who were observed in the 1996 skin exam, individuals were selected and DNA was extracted (Miller *et al*, 1988) from blood samples obtained from those who were regarded as being the most and least likely to develop BCC or SCC (Lea *et al*, 1998). The high-risk group was drawn on the basis of presence of at least one clinically confirmed BCC, SCC, or SK, as well as a high level of facial elastosis. An individual only required a single SK to be classed as high risk for developing future solar lesions. Conversely, the low-risk group comprised a subset of individuals from the same study population who were free from any BCC, SCC, or SK and who have had low levels of UV exposure represented by low facial elastosis. Using these groupings a total of 348 individuals were selected, of whom 306 had blood samples collected and frozen during the 1996 exam; however, only 111 individuals from the high-risk group and 109 individuals from the low-risk group were successfully DNA extracted and amplified using the polymerase chain reaction, giving a final study number of 220 individuals. There were no first-degree relatives included in the final selection of 220 individuals.

Measures The samples used in this study were drawn primarily with the aid of information collected during or before the 1996 skin exam. Numbers of BCC, SCC and solar keratoses were recorded and levels of UV-induced skin damage indicated by the measures of facial elastosis (none, +, ++, +++) and back freckling (none, +, ++, +++). Subjects were asked to report their hair color at age 21 y (blonde, light brown, red/ginger, auburn, dark brown and black), their skin response to strong sun after a long period without exposure (always burn, never tan; burn then tan; only tan). Natural skin color of nonexposed sites was assessed as a self-report using questionnaires described elsewhere and included the measures fair, medium and olive (Green *et al*, 1994).

MC1R allele-specific oligonucleotide genotyping Polymerase chain reaction was performed in 96 well plates with the MC1R coding region amplified using 25–50 ng of template DNA, with dot blotting performed as described previously to allow for allele-specific oligonucleotide detection of nine different commonly occurring MC1R variant alleles (Box *et al*, 1997; Palmer *et al*, 2000). Both consensus and variant probes were designed for the Val60Leu, Asp84Glu, Val92Met, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp, Arg163Gln, and Asp294His allele positions. Probes were designed as 15 base allele-specific oligonucleotides with the mismatch centrally positioned to provide maximal binding stability to the complementary sequence and instability of a mismatch. 100 ng of each oligonucleotide were radiolabeled with γ-^{32}P-adenosine triphosphate using polynucleotide kinase (NEB) and fractionated from unincorporated nucleotide by chromatography on Sephadex G-25 (Amersham, Uppsala, Sweden, NAP 5 columns). Identically blotted pairs of filters were prehybridized at 42°C for at least 2 h in the presence of tetramethylammonium chloride (Wood *et al*, 1985) in 10 ml of 3 M tetramethylammonium chloride, 0.1% sodium dodecyl sulfate, 1 mM ethylenediamine tetraacetic acid, 25 mM Na_3PO_4 pH 6.8, 5 × Denhardt's solution, 0.1 mg herring sperm DNA per ml (Shuber *et al*, 1993). Fifty microliters of radiolabeled consensus or variant probe, equating to no less than 2.75×10^5 cpm per ml of hybridization solution, was added directly to the prehybridizing solution of each pair of filters and left to hybridize overnight at 42°C. The probe was denatured by heating to 70°C for 5 min then quenched on ice before addition. Filters were rinsed in 3 M tetramethylammonium chloride, 0.1% sodium dodecyl sulfate, 1 mM ethylenediamine tetraacetic acid, Na_3PO4 pH 6.8 wash solution at room temperature for 5 min followed by a single 20 min wash at 50°C before autoradiographic exposure. Under these conditions probes specifically designed to the consensus and variant sequence will detect these sequences alone, but will not remain bound in the heteroduplex form resulting in an ability to specifically detect both consensus and variant alleles.

Statistical analysis Most analyses were performed using SAS, version 7.0 (SAS Institute, 1998), including log-linear and multinomial regression modeling done by means of SAS PROC CATMOD. Pairwise linkage disequilibrium was tested using ASSOCIATE 2.33 (Ott, 1996) and was quantitated as the standardized pairwise disequilibrium coefficient (D′) (Weir, 1996).

RESULTS

Phenotypic characteristics of high and low BCC and SCC risk groups The presence of a UV-induced lesion was used as the primary criterion for distinguishing between the high- and low-risk groups in this study (**Table I**). Individuals in the high-risk group all had a medium level of facial elastosis and a significantly increased level of back freckling ($p < 0.0001$) when compared with the low-risk group, which had only limited or absent facial elastosis. Both of these are indicators of environmental UV exposure as well as innate sun sensitivity. All of the SCC cases and 93% of BCC cases exhibited concurrent solar keratoses. Furthermore, of the 20 SCC cases, 11 exhibited at least one BCC.

The other major risk factor for BCC and SCC, which was evident in the distinction of the high- and low-risk groups, was pigmentation phenotype. Individuals in the high-risk group were significantly more likely to have light brown or red colored hair. There were too few individuals in this study with fair hair for the difference between study groups to be significant. The small numbers of individuals in the fair hair group was balanced by the excess of individuals reported as light brown when compared with estimates of hair color frequencies in different Caucasian populations (Bliss *et al*, 1995; Palmer *et al*, 2000). This indicates probable reporting differences for fair hair colors between this and other studies. Fair constitutive skin color was also significantly associated with the high-risk grouping ($p < 0.0001$) as was an increased skin propensity to sunburn.

The presence or absence of a solar lesion as the distinguishing feature between the study groups also resulted in obvious age and sex differences between both groups. In the high-risk group 63% were male and the mean age was about 65 y, compared with the low-risk group where the mean age was about 41 y and 38% were male. Although precise age information was available, the large differences in age between the two risk groups prevented extensive correction for this variable. In preliminary analyses, including age as a continuous variable, it was determined that a simple dichotomy at 50 y retained most of the available information for this variable (**Table I**). After dichotomizing the age variable at 50 y, pooling hair colors into black/dark brown, light brown/fair, and red/auburn groups, and recategorizing skin color as olive/medium and fair, it was found that only sex exerted an effect on pigmentation phenotype. This was determined by using logistic regression to

Table I. Phenotypic characteristics and OR between high-risk and low-risk groups

Phenotype	Measures	High-risk group		Low-risk group		Crude odds ratios	Adjusted odds ratios[a]
		n	(%)	n	(%)		
Any BCC		43	(39)	0	(0)		
Any SCC		20	(18)	0	(0)		
SK	None	3	(2.5)	109	(100)		
	>0	108	(97)	0	(0)		
Facial elastosis	None	0	(0)	109	(100)		
	++	111	(100)	0	(0)		
Hair color	Black	6	(5.5)	13	(12)	Black/dark brown = 1	Black/dark brown = 1
	Dark brown	28	(26)	46	(44)		
	Auburn	5	(4.5)	4	(4)		
	Red/ginger	11	(10)	2	(2)	9.04 (1.86, 43.78)[b]	17.96 (2.76, 148.9)[b]
	Light brown	49	(45)	33	(31)	2.44 (1.28, 4.64)	Light brown/fair =
	Fair	10	(9)	7	(6)	2.35 (0.80, 6.87)	1.85 (0.74, 4.61)
		n = 109		n = 105			
Skin color	Olive	0	(0)	15	(14)	Olive/medium = 1	Olive/medium = 1
	Medium	31	(28)	49	(45)		
	Fair	80	(72)	45	(41)	2.81 (1.57, 5.02)	6.40 (2.56, 16.03)
Skin UV response	Only tans	6	(5)	12	(11)	1	1
	Burns then tans	72	(65)	77	(61)	1.87 (0.67, 5.25)	1.84 (0.69, 4.89)
	Only burns	33	(30)	20	(18)	3.30 (1.07, 10.18)	9.81 (1.94, 49.54)
Back freckling	None	10	(9.5)	33	(31)	1	1
	+	33	(32)	54	(51)	2.02 (0.88, 4.62)	3.58 (2.02, 6.37)
	++	39	(37.5)	17	(16)	7.57 (3.05, 18.78)	12.84 (5.69, 28.97)
	+++	22	(21)	2	(2)	36.3 (7.25, 181.82)	46.01 (16.99, 124.62)
		n = 104		n = 106			
Age at 1994	30–49	10		87			
	50–79	101		22			
Sex	Male	69	(62)	38	(35)		
	Female	42	(38)	71	(65)		
Total samples		111		109			

simultaneously control for age and sex in the association between pigmentation phenotype and risk group with the amalgamation of categories to provide sufficient numbers for analysis. Even after adjusting for age and sex, the associations between risk group and red hair and fair skin color, and skin UV sensitivity remain significant [**Table I**, adjusted odds ratios (OR)].

Association between MC1R genotype, pigmentation phenotype, and UV sensitivity Analysis of MC1R variants in combined high- and low-risk samples revealed significant associations with each phenotype that was scored (**Table II**). The "any" variant allele category took into account the sum of all nine variants assayed by allele-specific oligonucleotide, expressed as a total of none, one, or two variants. There were no individuals in this study with a total of greater than two variants. The red hair color ("RHC") variant allele category took into account only the sum of the three variants, which have previously been associated with RHC and melanoma risk: Arg151Cys, Arg160Trp, and Asp294His (Valverde *et al*, 1995; Box *et al*, 1997; Smith *et al*, 1998; Palmer *et al*, 2000).

Any MC1R variant allele was significantly associated with hair color, and the chi-square comparison showed this association to be largely the result of increased MC1R variants in red and lighter hair colors. This association was even more significant when only the three previously identified "RHC" variants were considered. When the remaining six variants were grouped, their association with hair color was not detectable, confirming that the majority of the association between MC1R and hair color is mediated through the three "RHC" variants. **Table II** also presents the OR for each color grouping *vs* the combined reference category of dark brown and black hair colors. The OR were calculated by pooling individuals with one or two variants, whereas those carrying a fully consensus genotype were used as the reference category (Box *et al*, 1997). The results illustrated an important difference between this and earlier studies in that there was a significant association between the light brown hair color and "RHC" MC1R variants, as well as the very strong association between "RHC" variants and red hair (Box *et al*, 1997; Smith *et al*, 1998; Palmer *et al*, 2000). Of the seven individuals in the study who were homozygotes or compound heterozygotes for these "RHC" variants, five were found in the red or auburn color groupings, one had fair and one had light brown hair color. Examination of individual variants showed the strongest association between Arg160Trp and red hair [OR = 8.50 95% CI (2.70, 26.78)]. Arg160Trp was not significantly over-represented in any other hair colors and accounted for 7% of haplotypes. The Arg151Cys variant, at 7% of haplotypes was significantly over-represented in both the red and light brown hair color groupings, respectively [OR = 6.60 95% CI (1.80, 24.24), OR = 3.94 95% CI (1.36, 11.38)]. The elevated frequency of Arg151Cys variants in the light brown hair color grouping will account for a large part of the association between "RHC" variants and the light brown hair color. Asp294His was more common in individuals with red hair [OR = 4.55 95% CI (0.60, 34.26)]; however, as this variant is relatively infrequent, representing 2.3% of haplotypes, this association was not statistically significant. Of the remaining variants, Val60Leu, Val92Met, and Arg163Gln were distributed largely independently of hair color, failed to show any association with red hair, and were present at a frequency of 11.7%, 9.3%, and 5.1% of haplotypes, respectively. Asp84Glu, Arg142His, and Ile155Thr were too infrequent to test successfully for association, representing only 0.9%, 0.9%, and 1.6% of haplotypes, respectively. Low sample numbers also prohibited calculation of significant disequilibrium estimates between individual variants.

The strong tendency towards an increased number of MC1R variants as hair colors lightened and became red was also seen as

Table II. Association of MC1R variant genotype with scored phenotypes

Phenotype	Any variant			RHC variants[b]		
	OR	95% CI	Significance[a]	OR	95% CI	Significance
Hair color						
Dark brown/black	1.00			1.00		
Red/ginger/auburn	3.82	1.05–13.85		9.88	3.49–27.88	
Light brown	1.23	0.66–2.29	$p < 0.004$	3.09	1.49–6.39	$p < 0.0001$
Fair	1.96	0.59–6.49		3.08	0.98–9.68	
Skin color						
Olive/medium	1.00		$p < 0.008$	1.00		$p = 0.006$
Fair	1.78	1.01–3.15	$P_T = 0.002$	2.41	1.30–4.48	$P_T = 0.0004$
Skin response to UV						
Tans only	1.00			1.00		
Burns then tans	2.48	0.92–6.66	$p = 0.057$	1.77	0.49–6.45	$p = 0.066$
Burns only	4.27	1.38–13.23	$P_T < 0.004$	4.12	1.07–16.00	$P_T = 0.004$
Back freckling						
None	1.00			1.00		
+	0.79	0.37–1.70		1.44	0.60–3.44	
++	1.61	0.67–3.84	$p = 0.04$	1.79	0.71–4.51	$p < 0.008$
+++	2.68	0.77–9.29	$P_T < 0.005$	3.20	1.08–9.49	$P_T = 0.008$

[a]All probability values calculated by χ^2 comparison of none, one, or two variants for the "any" or "RHC" categories *vs* unpooled phenotype divisions. OR were calculated using "consensus" and "one or two variants" categories, consensus genotype was defined by Box, *et al* (1997). P_T obtained from Mantel–Haenszel test for trend.
[b]Variants include R151C, R160W, D294H.

Table III. OR for MC1R variants in NMSC risk group

NMSC status	Total	Any variant			RHC variants		
		OR	95% CI	Significance[a]	OR	95% CI	Significance
Low-risk group	109	1			1		
High-risk group	111	3.12	1.72–5.66	$p < 0.0001$	3.15	1.7–5.82	$p = 0.0002$
Any BCC	43	1.76	0.82–3.82	$P_T = 0.32$	1.72	0.86–3.45	$P_T = 0.03$
Any SCC	20	2.98	0.85–10.54	$P_T = 0.20$	1.63	0.63–4.20	$P_T = 0.55$
Any BCC or SCC	52	2.25	1.08–4.70	$P_T = 0.046$	1.73	0.91–3.30	$P_T = 0.03$
Any SK	108	3.15	1.73–5.74	$p < 0.0001$	3.41	1.84–6.32	$p < 0.0001$

[a]All probability values were calculated by χ^2 comparison of none, one, or two variants *vs* each risk group category. OR were calculated using "consensus" and "1 or 2 variants" categories, P_T obtained from Mantel–Haenszel test for trend.

Table IV. Association of MC1R variant alleles with NMSC risk[a]

Variant allele	OR (95% CI) for risk group
V60L	1.4 (0.7, 2.7)
V92M	2.1 (1.0, 4.4)
R151C	2.6 (1.2, 6.3)
I155T	1.0 (0.2, 5.1)
R160W	3.4 (1.5, 8.8)
R163Q	1.7 (0.7, 4.7)
D294H	15.0 (2.6, 283)

[a]Logistic regression analysis includes all variants simultaneously; the consensus sequence is the reference category. D84E and R142H were not included due to low numbers.

skin colors tended to lighten and skin to diminish in tanning ability (**Table II**); however, the modest association between "RHC" variants and back freckling, and the significant trend for more variants in more heavily freckled individuals when considering all samples combined, disappeared when assessed in each risk group alone. When considering all variants combined, the association with skin color was significant ($p = 0.0078$) and was only slightly improved by separating out the three "RHC" variants ($p = 0.0064$). As expected there was a strong correlation between skin color and tendency to sunburn with fair-skinned people most likely to burn and never tan ($p < 0.0001$) and all MC1R variants tested together were marginally associated with the propensity to sunburn ($p = 0.06$), as were the "RHC" variants ($p = 0.02$). Those people who tended to develop back freckling also tended to sunburn (Mantel–Haenszel test for trend $p = 0.02$) and were more likely to have fair skin ($p = 0.01$).

Association between MC1R genotype and NMSC risk group A statistically significant over-representation of MC1R variant alleles was found in the high-risk group, an association that was largely due to the action of the three "RHC" variants, which were important in the association with hair color, i.e., Arg151Cys, Arg160Trp, and Asp294His, as seen in **Table III**. Furthermore, consideration of the remaining six variants together failed to show a significant association with the high-risk group, although there was a tendency for more variants in the high-risk group OR = 1.55 95% CI [0.91, 2.64]. **Table IV** presents the association of each individual variant with prevalence of NMSC, including the three "RHC" variants, which each showed OR that are significantly greater than 1, although Val92Met was marginally significant. The Asp84Glu and Arg142His variants were unable to be tested due to

Table V. Association of MC1R variants with NMSC risk after stratified on the basis of hair and skin color

Skin color	Hair color	Percent (no.) with an MC1R variant		Adjusted OR [c] (95% CI)
		High risk	Low risk	
Fair	Red/ginger/auburn	93 (15)	50 (4)	
	Light brown/fair	75 (44)	57 (21)	2.59 (1.12–6.11)
	Black/dark brown	80 (20)	12 (18)	
		(n = 79)	(n = 43)	
Olive/medium	Red/ginger/auburn	100 (1)	100 (2)	
	Light brown/fair	80 (15)	58 (19)	2.74 (1.04–7.90)
	Black/dark brown	71 (14)	49 (41)	
		(n = 30)	(n = 62)	
Total samples		109[a]	105[b]	

[a]Hair color data was not recorded for two individuals.
[b]Hair color data was not recorded for four individuals.
[c]Stratum-specific OR is adjusted for hair color, by the use of the Mantel–Haenszel procedure.

low numbers. The Asp294His allele gave the strongest association; of the 10 Asp294His variants identified in this study, only one was found in the low-risk group. An important observation was that all seven of the individuals with two ''RHC'' changes were found in the high-risk group.

Association of MC1R variants with the constituent subgroups that make up the high-risk group was also tested, and revealed a similar trend to that observed between MC1R variants and high-risk group. Association of the ''any'' variant category with the high-risk group was largely mediated through the ''RHC'' class of variants alone (**Table III)**. As there were only 43 cases with BCC and 20 cases with SCC, it was difficult to demonstrate statistical significance between these groups and MC1R variants; however, ''any'' and ''RHC'' variants were over-represented in individuals with BCC, with Arg160Trp alone significant in its association with BCC (OR = 2.54 95% CI (1.08, 5.95)]. When the association was tested with pooling of BCC and SCC cases the ''any'' variant category was also significant. The association of MC1R variant alleles with SK was more readily detectable, with both the ''any'' variant and ''RHC'' variant groupings significantly increased in individuals with SK (**Table III)**. Grouping the remaining variants not classed as ''RHC'' revealed a tendency for a greater frequency in the SK group (OR = 1.49 95% CI (0.88, 2.54)].

Finally, the data were stratified on hair and skin color to determine if the MC1R variants were acting through their effects on pigmentation. It can be seen that the association between MC1R variants and risk group remained significant after stratification on hair color in individuals with both light and dark skin color (**Table V**).

DISCUSSION

The results reported in this study are largely consistent with previous efforts describing the association between MC1R variant alleles, pigmentation phenotype, and melanoma (Valverde *et al*, 1995; Box *et al*, 1997; Smith *et al*, 1998; Palmer *et al*, 2000), but go further to say that gene variants at this locus are also important in determining skin susceptibility to BCC, SCC, and SK. Furthermore, this study has examined all nine of the common variants identified in the south-east Queensland population (Box *et al*, 1997) and the British Isles (Valverde *et al*, 1995; Smith *et al*, 1998). Of the earlier attempts to examine the relationship between MC1R variants and SCC and/or BCC, only an association with the Asp294His variant has been reported (Smith *et al*, 1998). Another report that also examined the Asp294His and Val92Met variants did not successfully detect an obvious relationship between these MC1R gene variants and skin cancer risk (Ichii-Jones *et al*, 1999).

As the number of Nambour community samples available to this study were relatively low, it was impossible to attribute significance to the OR that describe the relationship between MC1R variant alleles and risk of BCC and SCC when considered separately. After pooling all variant alleles and considering individuals with BCC or SCC together, however, a significant OR of 2.25 was seen for developing one of these forms of skin cancer in individuals carrying at least one variant allele compared with those who do not carry a variant (**Table III)**. As there were far more individuals who had developed at least one SK, and the presence of SK is widely considered a good surrogate indicator for later development of both BCC and SCC, presence of a strong association between SK and all variants pooled indicates that the association between BCC and SCC, given greater sample numbers, will be strong. Indeed, a small proportion of solar keratoses have been shown to give rise directly to SCC.

It is an important conclusion that, as is the case with melanoma, the association between MC1R variants and the propensity to develop solar lesions is mediated largely through three variants, Arg151Cys, Arg160Trp, and Asp294His, which are also associated with red hair, fair skin color, and tanning ability. Evidence has been presented that these three variants have a diminished capacity to activate cyclic adenosine monophosphate signaling (Frandberg *et al*, 1998; Schioth *et al*, 1999). This study also confirms the lack of association of three high frequency variants, Val60Leu, Val92Met, and Arg163Gln, with any hair or skin color, or with presence of a solar lesion. The remaining variants were unable to be associated with phenotype or risk group separately due to low frequency. It is interesting to note that the trend for increasing MC1R variants as the level of back freckling increased (**Table II**) was completely abolished when each risk group was considered independently, indicating that risk group sampling probably accounts for this relationship. Back freckling in older people is considered predominantly as an indicator of solar skin damage and the concurrent high levels of UV exposure, which are required to generate solar lesions, are often recorded by a high degree of back freckling. Although MC1R variants are associated with the red hair phenotype, one characteristic of which is freckling, there is a clear physiologic distinction between the ephelides of red hair and the UV-induced back freckles recorded in this study (Rhodes *et al*, 1991; Bastiens *et al*, 1999).

A heterozygote effect of MC1R genotype on pigmentation and melanoma risk has previously been demonstrated (Rees and Flanagan, 1999; Healy *et al*, 2000; Palmer *et al*, 2000). When considering the nature of a heterozygote effect in this study, those individuals with one variant had double the odds for presence of a solar lesion when compared with individuals with two consensus alleles, OR = 2.4 [95% CI (1.27, 4.54) low-risk reference category] confirming that simply possessing one variant copy of MC1R increases risk. In a similar manner to Healy *et al* (2000), heterozygotes were also more likely to show sun sensitivity compared with those with the consensus genotype, with respective OR for the ''burns only'' and ''burns, then tans'', categories of 2.27 [95% CI (1.14, 4.67)] and 3.62 [95% CI (0.94, 16.12)] using the ''tans only'' reference category. Heterozygote individuals were also significantly more likely to have fair skin color compared with those with a consensus MC1R genotype (data not shown). Those individuals with two variants have an OR of 5.5 [95% CI (2.48, 12.1) low-risk reference category] for presence of a solar

lesion; however, the confidence limits were too broad to distinguish between a linear multiplicative or simple additive manner accurately, by which MC1R alleles act to increase propensity for solar damage.

The final aim of this analysis was to determine whether any association with risk group remained after stratification of MC1R genotype on skin and hair color, thereby determining whether the heterozygote effect, as seen in the melanoma study (Palmer *et al*, 2000), was selectively evident in individuals with darker skin categories. There was not enough statistical power to attribute significance to differences between the fair and olive/medium skin color groups when examining the association of MC1R variants with risk group in the different strata of skin and hair colors; however, a similar trend to the results obtained for the melanoma study was observed when ''RHC'' variants alone were considered. The association between ''RHC'' variants and risk group tended to disappear after controlling for hair color in individuals with fair skin ($p = 0.13$); however, in those with olive/medium skin, the association marginally persisted ($p = 0.046$). When logistic regression was used to simultaneously model all skin and hair color groups as controls for the association of total and ''RHC'' variants alone, the association between variants and risk group persisted (any variant $p = 0.01$, RHC variants $p = 0.01$), indicating an overall residual association of MC1R variants and risk group over and above that seen due to the association between MC1R and skin and hair color.

This may be due in part to reporting bias where individuals who are asked to give their own assessment of skin color are more likely to report their skin as lighter or darker depending upon the presence or absence of a solar lesion (Green and Martin, 1990). Additionally, the two risk groups for which MC1R genotypes were available may accentuate the heterozygote effect discussed here and in earlier studies (Healy *et al*, 2000; Palmer *et al*, 2000). It may be the case that individuals with a single MC1R variant are more likely to be in the high-risk group due either to (i) a visibly minimal but functionally significant reduction in the sunscreen capacity of the melanins they produce, (ii) or due to an increased level of phototoxic or mutagenic pheomelanin or metabolites, which affect not just the melanocyte but also the surrounding skin cells that contain the melanin granules. Therefore, the presence of at least one variant remains informative in terms of predicting risk for developing a solar lesion beyond that information gained through observation of pigmentation phenotype.

As the profiles of risk mediated through MC1R genotype are for the most part consistent for very distinct tumor cell types (melanoma, BCC, and SCC), a commonality of predisposition is emphasized. MC1R genotype, in heterozygous and homozygous variant forms, probably influences risk largely through its effect on skin UV sensitivity and increased propensity to sustain UV damage, thereby resulting in increased prevalence of all forms of skin cancers.

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