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The Role of Melanocortin-1 Receptor Polymorphism in Skin Cancer Risk Phenotypes

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We have examined melanocortin-1 receptor (MC1R) variant allele frequencies in the general population and in a collection of adolescent dizygotic and monozygotic twins to determine statistical associations of pigmentation phenotypes with increased skin cancer risk. This included hair and skin color, freckling, mole count and sun exposed skin reflectance. Nine variants were studied and designated as either strong *R* (OR = 63; 95% CI 32–140) or weak *r* (OR = 5; 95% CI 3–11) red hair alleles. Penetrance of each MC1R variant allele was consistent with an allelic model where effects were multiplicative for red hair but additive for skin reflectance. To assess the interaction of the brown eye color gene BEY2/OCA2 on the phenotypic effects of variant MC1R alleles we imputed OCA2 genotype in the twin collection. A modifying effect of OCA2 on MC1R variant alleles was seen on constitutive skin color, freckling and mole count. In order to study the individual effects of these variants on pigmentation phenotype we have established a series of human primary melanocyte strains genotyped for the MC1R receptor. These include strains which are MC1R wild-type consensus, variant heterozygotes, and homozygotes for strong *R* alleles Arg151Cys and Arg160Trp. Ultrastructural analysis demonstrated that only consensus strains contained stage III and IV melanosomes in their terminal dendrites whereas Arg151Cys and Arg160Trp homozygous strains contained only immature stage I and II melanosomes. Such genetic association studies combined with the functional analysis of MC1R variant alleles in melanocytic cells should provide a link in understanding the association between pigmentary phototypes and skin cancer risk.

Key words: MC1R, OCA2, Freckle, Mole, Melanocyte

INTRODUCTION

Although there are complex mutagenic actions of ultraviolet (UV) radiation on the different skin cells that give rise to basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma it is clear that each is causally related to the degree or intensity of sun exposure. Epidemiological studies of these skin cancers have shown that SCC is strongly associated with an individual's total or occupational UV exposure while the incidence of BCC is less strongly related with sun exposure, and is only weakly associated with pigmentation characteristics. In contrast, recreational sun exposure, history of sunburn, pigmentation phenotype and number of benign nevi are more predictive factors of melanoma (1). The incidence rate of each tumor is greatest in fair-skinned sun-sensitive individuals, indicating the importance of the innate ability to respond to UV light through the increased synthesis of melanin and a skin tanning response. However, the absolute amount and differential light-absorbing properties of the melanin biopolymer within the epidermal melanocytes must be considered in response to sun exposure, leaving the idea of a protective tanning response in question. Melanin can be viewed as either photoprotective or photosensitizing, depending on the type and composition of the pigment itself (2). Of central importance in determining an individual's risk for skin

Abbreviations – BCC, basal cell carcinoma; BEY2, brown eye color-2; MC1R, melanocortin-1 receptor; MSH, α-melanocyte-stimulating hormone; OCA2, oculocutaneous albinism-2; OR, odds ratio; QF, Queensland foreskin; RHC, red hair color; SCC, squamous cell carcinoma; TYR, tyrosinase; TYRP1, tyrosinase related protein-1; UV, ultraviolet

cancer is the direct characterization of the chemical properties of the melanin pigment (3) and of the genes underlying the regulation of its synthesis (4, 5).

Hormonal stimulation of the melanocortin-1 receptor (MC1R) expressed on the cell membrane surface is central to the tanning response of human melanocytes following UV irradiation (6). Although the hormonal signaling pathways are yet to be fully defined, a synergistic action of α-melanocyte-stimulating hormone (α-MSH) together with other mitogens on protein phosphorylation has been reported (7–9). These pathways converge inducing changes in gene expression largely through the microphthalmia transcription factor (MITF) which appears to be critical for activation of the eumelanogenic pathway (10, 11). Combined treatment of melanocytes with UV and α-MSH also potentiates cell dendricity (12) and transfer of pigment to keratinocytes (13).

The MC1R locus is highly polymorphic in human populations with variant forms of the receptor underlying the diverse range of human pigmentation phenotypes and skin phototypes (5). Several of the MC1R variant alleles have been associated with the red hair and fair skin (denoted RHC; red hair color) phenotype, a condition that is caused by the synthesis of a high level of pheomelanin which can place individuals at a higher risk of skin cancer [reviewed in (14)]. To quantify the relative contribution of each independent MC1R variant allele to pigmentary phenotypes associated with high skin cancer risk, we have examined statistical associations of some common MC1R gene variants with red hair, skin color, degree of freckling and nevus counts in a large collection of adolescent twins, parents and siblings.

Cultured human melanocytes are now considered a powerful in vitro tool to study human pigmentation as, dependent upon culture conditions, they continue to express the original melanogenic phenotype characteristic of the skin type from which they were derived (15, 16). The great advantage of long-term or immortal clonal melanocyte cultures is that large amounts of a single, pure, cell type can be prepared for biochemical, cellular, molecular and genetic analyses (17, 18). Culture of human melanocytes of defined MC1R genotype may provide a leading experimental approach to defining the physiological consequences for each MC1R allele in response to ligand binding (19, 20). We have performed a systematic genotype screen for several common MC1R polymorphisms using melanocytes cultured from individual foreskins to ascertain clonal cell strains. Characterization of the response to α-MSH in these strains will help assess the functional consequences of the RHC variant alleles and the roles they play in determining melanocyte cell phenotypes associated with high skin cancer risk.

MC1R Variant RHC Allele Penetrance

Previous studies examining MC1R variant alleles in relation to hair color have all been consistent in finding the RHC phenotype to be a recessive trait, although the reported influence of some alleles has varied between studies (14). Homozygote or compound heterozygote variant MC1R genotype carriers are generally red-haired, but as this is not always the case it is likely that other loci are involved in the expressivity of the trait (21). In addition, red hair occurs in a significant proportion of heterozygote consensus MC1R allele carriers, some alleles displaying greater influence than others. It is unlikely that each of these variant alleles represent complete loss of receptor function or that they have a simple Mendelian recessive mode of inheritance; rather variant alleles likely represent a linear series of differential strength alleles.

The relationship between MC1R gene variants and red hair, skin reflectance, degree of freckling and nevus count were investigated in a large sample of adolescent twins, parents and sibs, constituting 2331 family members in 645 pedigrees (22). MC1R genotyping was performed for 1779 individuals within 460 of these pedigrees. Excluding one member of each genotyped MZ twin pair, there were 1569 individuals with complete MC1R genotype, hair color, eye color and sex recorded. The RHC-variant alleles Arg151Cys, Arg160Trp, Asp294His are common in the Queensland population and are responsible for most of the RHC in this community consistent with our earlier reports (21, 23–25), where at least one of these three alleles is found in 93% of individuals with red hair.

Penetrance of each variant allele for red hair and fair skin phenotypes was modeled in a logistic regression analysis (22). This indicated that the three RHC alleles Arg151Cys, Arg160Trp and Asp294His were highly associated with red hair and fair skin, showing odds ratios (OR) of 118 (95% CI 51–272), 50 (95% CI 22–116) and 94 (95% CI 34–263), respectively, compared with low strength alleles Val60Leu and Val92Met with OR 6 (95% CI 3–15) and 5 (95% CI 2–13) relative to the consensus allele for red hair. The Asp84Glu allele was relatively strongly associated with RHC, recording an OR of 62 (95% CI 18–224); the Arg163Gln gave only a weak two-fold increase in association with red hair. The regression model assumes that these alleles act multiplicatively on expression of the red hair trait. From these analyses it can be concluded that the Asp84Glu, Arg151Cys, Arg160Trp, Asp294His variants can be considered strong RHC alleles, which we designate '*R*' with combined OR of 63 (95% CI 32–140). The Val60Leu, Val92Met, Arg163Gln variants are relatively weak RHC alleles and are designated '*r*' with combined OR of 5 (95% CI 3–11).

The RHC penetrances of the six genotypes formed upon combining the consensus '+' allele together with *R* and *r* were also examined using the multiplicative regression model. There was high concordance between the observed and expected frequencies of red hair for each grouped genotype. Significant numbers of red heads were seen only for *R/r* and *R/R* genotypes, where observed vs. expected gave 10.8% vs. 11.8%, and 67.1% vs. 62.1%, respectively. The frequency of red hair in those with a heterozygous *R/+* genotype was only 1.5% vs. 2.5%, *r/r* with 0.9% vs. 1%, *r/+* with 1% vs. 0.2%, while there was no red head observed or expected with a +/+ consensus genotype.

Consensus MC1R +/+ genotype carriers also showed the darkest induced skin color with mean skin reflectances of 60.5% for the inner arm and 49.8% for the back of the hand. To address the quantitative relationship of variant MC1R

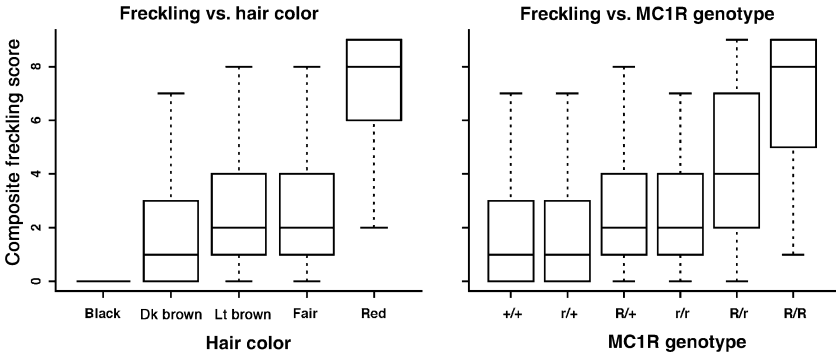

Fig. 1. Box plot of the mean, SD and range of the composite freckling score (*y*-axis) by hair color (*x*-axis, left panel), and by composite MC1R genotype (*x*-axis, right panel). The composite freckling score of an individual was the summation of the degree of freckling using a 4-point scale of nil (0) to severe freckling (3) on the three separate body sites including the back of the hand, the face and shoulder. This gives a range from a baseline of 0 to a total maximum of 9 (*y*-axis).

alleles with skin reflectance, the increase in the mean skin reflectance measurement per variant allele was calculated relative to these values for the +/+ genotype (22). In general, variant alleles acted in an additive manner to increase skin reflectance, for the inner arm *R* alleles increased the reflectance by +1.9% and demonstrated a greater effect than *r* alleles which increased it by only +0.9%. This difference was even more pronounced for exposed skin on the back of the hand, with increases of +1.5% for *R* compared with +0.3% for *r*.

MC1R Variant RHC Allele Associations with Freckling and Mole Count

Several of the genetic studies of MC1R variant alleles in relation to the RHC phenotype have suggested an association with number of freckles (23, 26, 27) or sun-induced lentigines (24). It has been reported that carriers of two MC1R variants have as much as a 11-fold increased risk of freckling and two-fold higher risk of solar lentigines (28). There was a very wide range in the number of nevi (3–422), seen in the twin collection and of freckles (nil to severe) with 76% of individuals having some freckling present on at least one of the three body sites examined.

Figure 1 shows the degree of freckling in the sample population plotted against hair color and by comparison with the composite MC1R genotypes based on the consensus +, strong *R* and weak *r* alleles. No individual designated as black-haired displayed freckling and this may be due to ethnic definition of this phenotype in our sample collection. There was a non-linear increase in freckling score when grouped by hair color ranging from lowest with dark brown, slightly higher and equivalent levels in light brown and fair individuals, with a dramatically higher level in those with red hair – 80% of red heads were in the severe category compared with only 17% of other hair colors (22). When clustered by MC1R genotype there was again a dramatic relationship with freckling that reflected that seen by phenotype. There appeared to be a dosage effect for freckling for the RHC alleles commensurate with their penetrance for red hair which shows the intimate interactions of these traits. The +/+ and *r*/+ samples had the lowest levels, followed by *R*/+ and *r*/*r* with almost equivalent levels, with high scores in *R*/*r* and extreme freckling in *R*/*R* carriers. The fraction attributable to carrying at least one variant MC1R allele increased linearly with the degree of freckling from 1 to 9 ranging from 23.4% for mild to 100% for severely freckled subjects (22).

There was also a non-linear correlation between total composite freckle score and mole count in the adolescent twin collection group as plotted in Fig. 2. Those having greater numbers of freckles or more freckling sites also scored an increased number of nevi until severe freckling became apparent, whereupon mole number decreased. Red-haired subjects had the greatest number of freckles and least number of moles. We also tested for association with composite MC1R genotype. Although no consensus homozygote +/+ had a severe freckling score, there was a positive correlation between total mole count and a composite freckling score. There was little effect of the *r* allele on this correlation, but the *R*/*R* genotype had the lowest number of moles and greatest number of freckles, consistent with the phenotypic association found in red heads. In the heterozygous state, the *R*/+ genotype displayed an initial positive association with moliness until severe freckling was reached, after which there was a significant decrease in the number of moles.

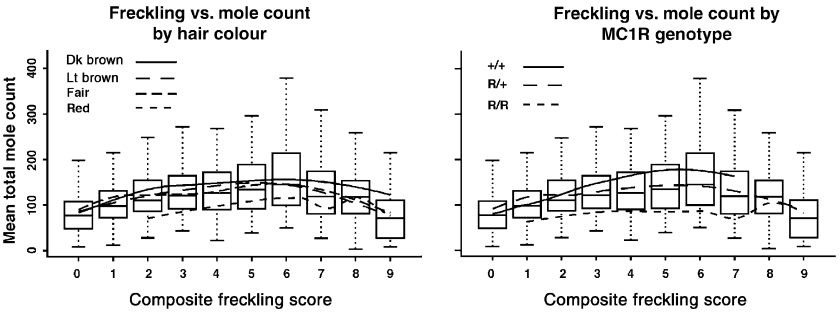

Fig. 2. Association between freckling, moliness by hair color (left panel) and by MC1R genotype (right panel). The composite freckling score (*x*-axis) was plotted against the mean of mole count (*y*-axis) for individuals with that score. The mean, SD and range are indicated by the horizontal line, vertical boxes and bars. The plot of the mean value of mole count per composite freckling score for each hair color and MC1R genotype is shown by the solid and broken lines as indicated in the legend.

Interaction of MC1R and BEY2/OCA2 Eye Color in Freckling and Mole Count

Other genes are likely to modify MC1R genetic effects in determination of pigmentation phenotype and it is notable that an epistatic interaction with the OCA2 gene, which is mutated in type II albinism and encodes the P-protein, has recently been suggested (29). Polymorphism of the OCA2 gene almost certainly underlies the previous assignment of the brown eye (BEY2) loci to chromosome 15q (30), with two OCA2 variant alleles recently shown to be associated with non-blue eye colors (31). To assess the interaction of the BEY2/OCA2 color gene on the phenotypic effects of variant MC1R alleles we performed a combined segregation-linkage analysis of eye and hair color with the D15S165 genotype marker which is 2 Mb centromeric of the OCA2 gene on chromosome 15q11.2–15q12. This confirmed the linkage and recessive inheritance of blue eye color with the OCA2 locus and provided a frequency of 21% for the dominant brown eye *B* allele in our sample population (22). Using the D15S165 marker and reported eye color we imputed individual genotypes in multiple replicates of the dataset, with blue-eyed individuals more than 97% likely to be assigned as recessive *b/b*.

We tested for the genetic interaction between strong MC1R variant *R* alleles associated with fair/pale skin and of dominant *B* and recessive *b* OCA2 alleles in the determination of skin color, degree of freckling and mole count. There was a significantly greater proportion of fair/pale skinned individuals of *b/b* genotype than of *B/B* or *B/b* genotype (*B/–*), and this difference in frequency significantly increased when also carrying a strong *R* allele ($P < 0.001$). All individuals of *b/b*, *R/R* genotype were in the fair/pale skin category but this decreased to 83.8% with fair/pale skin in individuals of *B/–*, *R/R* genotype ($P = 0.02$), the remainder having medium skin color. This proportionate lightening in all genotypic groups when carrying both recessive *b* and *R* alleles indicates additive action of MC1R and BEY2/OCA2 loci on constitutive skin color.

A modifying effect of BEY2/OCA2 on MC1R is also seen for freckling score and mole count as shown in Fig. 3, where having a *b/b* genotype increased freckling compared with combined *B/–* and *R/–* carriers. The *R/R* genotype also increased the combined freckling score in an additive fashion. There was a slight but non-significant increase in the mean nevi count for those of a *b/b* genotype compared with *B/–* and *R/–* combined genotypes. In contrast, there was a significant decrease in the number of moles in individuals of *b/b*, *R/R* genotype from 111 to 61. The MC1R modifier effect is purely recessive as no decrease in mole count is seen in the *R/–* heterozygotes.

Functional Analysis of MC1R Variant RHC Alleles in Primary Human Melanocytes

Cultured human melanocytes have been found to express high affinity receptors for the α-MSH ligand on their surface with approximately 700 binding sites per cell (32–34). Primary melanocyte cultures are an ideal system in which to study human pigmentary differences. Under appropriate growth conditions they can continue to express the original melanogenic phenotype characteristic of the skin type from which they were derived (35–38). We have performed a systematic genotype screen for several of the common MC1R polymorphisms using melanocytes cultured from individual foreskins to ascertain clonal cell strains (39). Over 1000 foreskin samples were processed. The haplotype frequencies for the RHC variant alleles in those strains that were genotyped were 8.3, 7.3 and 1.6% for Arg151Cys, Arg160Trp, and Asp294His. Notably, this is comparable with the haplotype frequency range of 11.0, 7.0 and 2.7%, respectively, for these variants seen in our previous screen of MC1R alleles present in the Southeast Queensland population (22, 25). It was our intention to isolate homozygous strains for each of these alleles to allow the specific functional characterization of individual forms of the receptor.

Over 300 newly established cultures of human foreskin melanocyte strains (QF) were successfully established and genotyped from this initial screen with many wildtype +/+ and heterozygous +/– strains, including five Arg151Cys–/– homozygotes and one Arg160Trp–/– homozygote identified. When MC1R genotype was considered, all consensus +/+ allele strains had very dark pellets, being scored as black or dark brown by eye. When strains with a variant genotype were grouped, homozygous Arg151Cys–/– and Arg160Trp–/– cell pellets were considerably less pigmented, whereas heterozy-

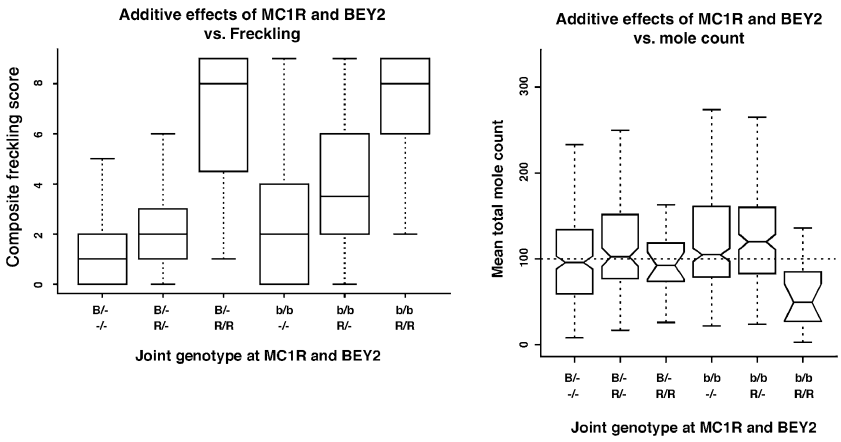

Fig. 3. Genetic interaction of MC1R and BEY2/OCA2 in freckling and mole count. Box plot of the mean, standard deviation and range of the composite freckling score (*y*-axis, left panel) and mean total mole count (*y*-axis, right panel) by joint MC1R and OCA2 genotype (*x*-axis). The *B* allele is dominant for brown eye color, *b* is recessive for blue eye color, – is any non-*b* or non-*R* allele.

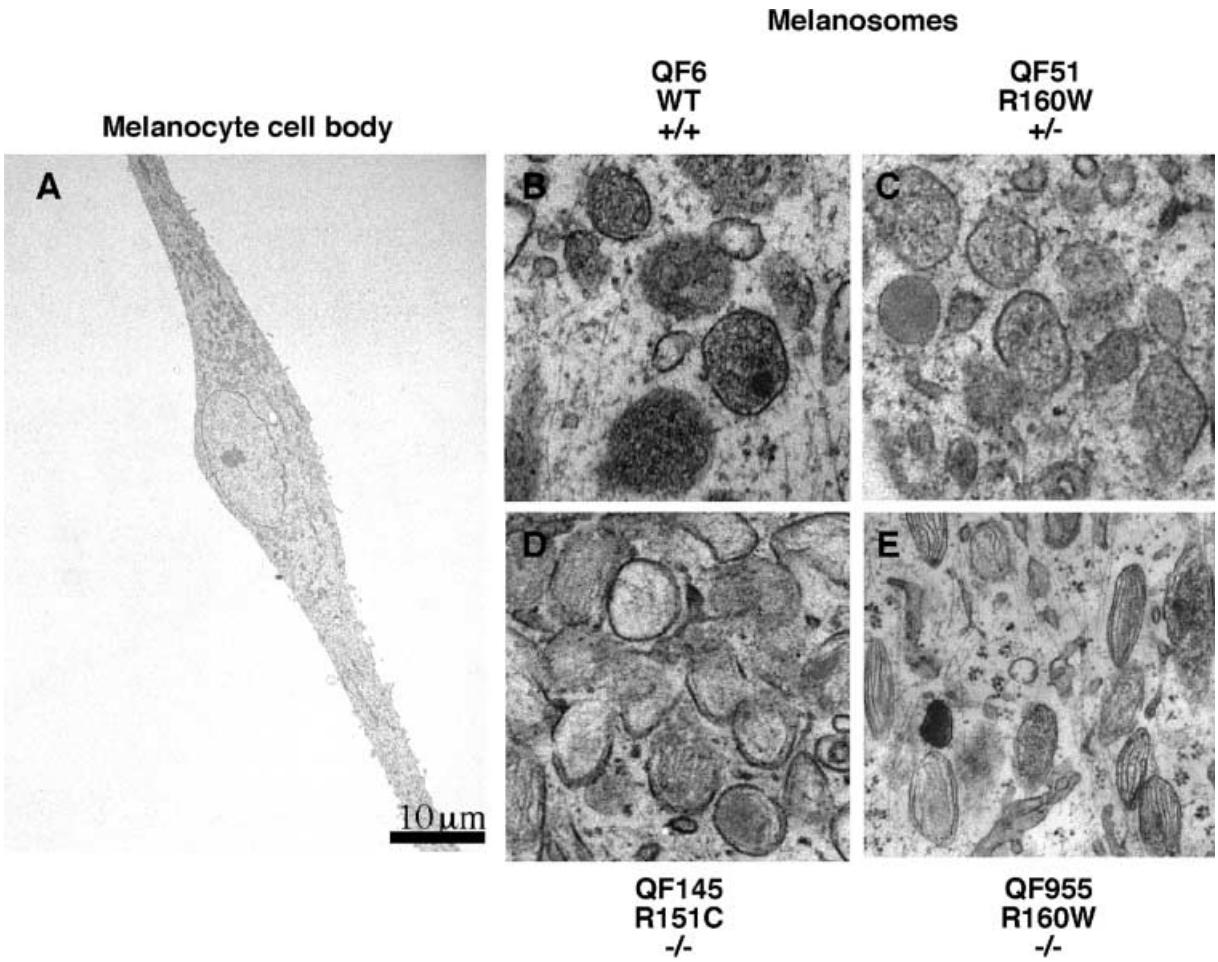

Fig. 4. Ultrastructure of melanosomes of cultured QF melanocytes. Transmission electron microscopy of melanocytes was performed on Epon-embedded sections. Panel A indicates the typical bi-dendritic morphology of melanocytes in culture seen at low resolution. Panels B–E are taken at the terminus of the dendrite showing the distinct ultrastructure of the melanosomes at high magnification with the QF strain and MC1R genotype as indicated.

gous Arg151Cys+/– and Arg160Trp+/– cell pellets varied from black to white but were generally of intermediate pigmentation (39).

To determine if this correlation of MC1R genotype with level of pigmentation was due to variation in the expression of melanogenic enzymes, several of the QF melanocyte strains of each genotype were examined using a range of monoclonal antibodies and levels measured by immunohistochemical staining (39). The tyrosinase enzyme TYR was present in each strain at relatively constant levels, whereas the TYRP1 and SILV proteins were expressed at high levels in some strains but were undetectable in others. However, no apparent correlation of any of the pigmentation markers with MC1R wild-type, Arg151Cys, Arg160Trp, heterozygous or homozygous genotypes could be deduced from these expression profiles. The melanogenic potential of these strains was tested using a DOPA staining protocol. Each QF melanocyte strain tested displayed a similar potential for melanin formation upon incubation with the DOPA precursor melanogenic substrate independent of MC1R genotype.

Ultrastructural Examination of MC1R Variant RHC Allele Melanocytes

Pigment in melanocytes is packaged within melanosomes which mature through a four stage process, beginning with immature stage I and II melanosomes which are unable to synthesize melanin maturing into stage III and IV melanosomes capable of melanin synthesis. Late stage melanosomes are transported to the ends of the dendrites in preparation for passaging to keratinocytes (40). We examined and compared the ultrastructure of several MC1R genotyped primary melanocyte cell strains using transmission electron microscopy (39). Cells from all strains were elongated in shape with dendritic processes as seen in low resolution scanning of the melanocyte cell body (Fig. 4, panel A). High resolution examination of the melanosomes contained within the dendritic terminal regions was performed using several QF strains.

Wild-type QF6+/+ melanocytes were heavily pigmented and displayed typical melanin-dense stage III–VI melanosomes, some of these were located at the terminal ends of the

dendrites while others were scattered through the cytoplasm alongside immature melanosomes (panel B). The heterozygous strain QF51 Arg160Trp+/– contained some mature but less opaque melanosomes in the dendritic tips (panel C). The lumens of these melanosomes had lost the internal structure evident in the immature melanosomes but did not show the same degree of melanin accumulation as wild type melanosomes. In contrast the two homozygous strains QF145 Arg151Cys–/– and QF955 Arg160Trp–/– contained only immature stage I and II melanosomes (panels D and E). In QF145 cells, the melanosomes acquired the size and shape of later stage structures but retained the internal striations of immature granules. In QF955 cells, there was a marked accumulation of elongated striated stage II melanosomes in the dendrites. Homozygous cells were thus found to have immature melanosomes but were devoid of mature melanosomes, the correlation of MC1R genotype with melanin density within melanocyte pellets is thus reflected in the appearance and maturation of the melanosomes as seen by electron microscopy (Fig. 4).

Taken together the cell pellet color and cellular ultrastructure of cultured primary melanocyte strains are consistent in showing an MC1R genotype–phenotype correlation with respect to melanin processing and storage. Tracing MC1R genotypic differences to the cellular level demonstrates that a lack of fully formed mature melanin granules and the accumulation of eumelanosome-like immature granules underlie the physical basis to the RHC phenotype.

SUMMARY

Population-based studies of MC1R variant alleles have demonstrated strong genetic associations with red hair and pale skin phenotype and an increased risk of all forms of skin cancer (14). We have attempted to quantify the penetrance of each of nine relatively common variant MC1R alleles for pigmentary traits including red hair, fair skin, freckling and nevus count in a representative sample of the white Australian population. There is a clear distinction in the strength of these alleles in relation to the penetrance for RHC, and modeling of each allele supported the designation of strong *R* and weak *r* RHC variants, where effects were multiplicative for the penetrance of red hair but additive for skin reflectance (22). Moreover, there is also a clear role of such designated RHC alleles in the appearance, density and number of body sites that display freckling on the skin, with 100% of those severely affected having variant MC1R genotypes.

There have been conflicting reports on the association between nevi and freckling in many studies that have been conducted on risk factors for skin cancer (41). A general correlation between mole and freckle number was apparent in our adolescent sample, those having more freckles also reporting an increased number of nevi. However, within the pale-skinned highly freckled group, red hair was associated with a decreased mole count. This phenotypic association was examined at the molecular level by plotting the strong *R* allele MC1R variant genotype against mole count and freckling score (Fig. 2). In addition to confirming genotypically that *R/R* homozygotes had more freckles and fewer moles, an *R/+* heterozygous effect was apparent, with lower mole counts seen as freckle score increased. Such a heterozygous carrier effect on freckling and nevi is consistent with that already reported for skin phototype (42), skin color and melanoma risk (23), which indicate that variant MC1R alleles do not behave in a strictly recessive manner.

A modifier effect of the BEY2/OCA2 gene on several of the human pigmentary traits associated with MC1R variant alleles was found during the analysis of RHC genotypes. These findings support the influence of the BEY2/OCA2 locus genetic interactions on skin color, freckling and nevus count. The significant reduction in nevus count in those of *b/b*, *R/R* genotype (Fig. 3) indicates that this is a true genetic recessive effect. The mechanism by which RHC variant alleles prevent mole formation is entirely unknown, although it may be postulated that there is a mutually exclusive relationship between freckling and mole formation.

Although such genetic analysis of MC1R variants in population-based studies have provided strong associations with pigmentary phenotypes that are linked with skin cancer risk, they do not yet enable predictive outcomes for an individual or fully explain the underlying mechanisms which make people with these genotypes more prone to skin cancer. The availability of a series of primary melanocyte strains of defined MC1R genotype will help provide insight into the cellular basis to these high risk pigmentary traits. MC1R variant alleles that are associated with increased skin cancer risk may sensitize melanocytes directly to DNA damage after UV exposure (43) and the signal transduction pathways and cellular responses involved may now be amenable to comparative studies in vitro. Our ultrastructural observation that melanocytes of RHC homozygous genotype produce immature melanosomes which are able to be transported to the terminal dendritic regions (39) may reflect such physiological responses are possible within the skin. If so, an individual of this genotype may pass immature melanosomes to the keratinocytes upon initiation of a tanning response and thereby give diminished photoprotection to keratinocytes. If this is the case the idea of a protective tanning response will remain illusory. Moreover, future studies may provide a possible mechanistic basis for the epidemiological differences of BCC, SCC and melanoma incidence that arise through the interaction of an individual's sun exposure (1) and their MC1R genotype.

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REFERENCES

1 Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B 2001;63:8–18
2 Hill HZ, Li W, Xin P, Mitchell DL. Melanin: a two edged sword? Pigment Cell Res 1997;10:158–161
3 Vincensi MR, d'Ischia M, Napolitano A, Procaccini EM, Riccio G, Monfrecola G, Santoianni P, Prota G. Phaeomelanin versus eumelanin as a chemical indicator of ultraviolet sensitivity in fair-skinned subjects at high risk for melanoma: a pilot study. Melanoma Res 1998;8:53–58
4 Sturm RA, Box NF, Ramsay M. Human pigmentation genetics: the difference is only skin deep. Bioessays 1998;20:712–721

5 Sturm RA, Teasdale RD, Box NF. Human pigmentation genes: identification, structure and consequences of polymorphic variation. Gene 2001;277:49–62
6 Suzuki I, Im S, Tada A, Scott C, Akcali C, Davis MB, Barsh G, Hearing V, Abdel-Malek Z. Participation of the melanocortin-1 receptor in the UV control of pigmentation. J Invest Dermatol Symp Proc 1999;4:29–34
7 Im S, Moro O, Peng F, Medrano EE, Cornelius J, Babcock G, Nordlund JJ, Abdel-Malek ZA. Activation of the cyclic AMP pathway by alpha-melanotropin mediates the response of human melanocytes to ultraviolet B radiation. Cancer Res 1998;58:47–54
8 Tada A, Suzuki I, Im S, Davis MB, Cornelius J, Babcock G, Nordlund JJ, Abdel-Malek ZA. Endothelin-1 is a paracrine growth factor that modulates melanogenesis of human melanocytes and participates in their responses to ultraviolet radiation. Cell Growth Differ 1998;9:575–584
9 Tada A, Pereira E, Beitner-Johnson D, Kavanagh R, Abdel-Malek ZA. Mitogen- and ultraviolet-B-induced signaling pathways in normal human melanocytes. J Invest Dermatol 2002;118:316–322
10 Bertolotto C, Abbe P, Hemesath TJ, Bille K, Fisher DE, Ortonne JP, Ballotti R. Microphthalmia gene product as a signal transducer in cAMP-induced differentiation of melanocytes. J Cell Biol 1998;142:827–835
11 Price ER, Horstmann MA, Wells AG, Weilbaecher KN, Takemoto CM, Landis MW, Fisher DE. Alpha-melanocyte-stimulating hormone signaling regulates expression of microphthalmia, a gene deficient in Waardenburg syndrome. J Biol Chem 1998;273:33042–33047
12 Scott GA, Cassidy L. Rac1 mediates dendrite formation in response to melanocyte stimulating hormone and ultraviolet light in a murine melanoma model. J Invest Dermatol 1998;111:243–250
13 Virador VM, Muller J, Wu X, Abdel-Malek ZA, Yu ZX, Ferrans VJ, Kobayashi N, Wakamatsu K, Ito S, Hammer JA, Hearing VJ. Influence of alpha-melanocyte-stimulating hormone and ultraviolet radiation on the transfer of melanosomes to keratinocytes. Faseb J 2002;16:105–107
14 Sturm RA. Skin colour and skin cancer – MC1R, the genetic link. Melanoma Res 2002;12:405–416
15 Bessou S, Surleve-Bazeille JE, Pain C, Donatien P, Taieb A. Ex vivo study of skin phototypes. J Invest Dermatol 1996;107:684–688
16 Smit NP, Kolb RM, Lentjes EG, Noz KC, van der Meulen H, Koerten HK, Vermeer BJ, Pavel S. Variations in melanin formation by cultured melanocytes from different skin types. Arch Dermatol Res 1998;290:342–349
17 Bennett DC, Medrano EE. Molecular regulation of melanocyte senescence. Pigment Cell Res 2002;15:242–250
18 Halaban R. The regulation of normal melanocyte proliferation. Pigment Cell Res 2000;13:4–14
19 Abdel-Malek Z, Scott MC, Suzuki I, Tada A, Im S, Lamoreux L, Ito S, Barsh G, Hearing VJ. The melanocortin-1 receptor is a key regulator of human cutaneous pigmentation. Pigment Cell Res 2000;13:156–162
20 Sturm RA, Duffy DL, Box NF, Newton RA, Shepherd AG, Chen W, Marks LH, Leonard JH, Martin NG. Genetic Association and Cellular Function of MC1R Variant Alleles in Human Pigmentation. Ann N Y Acad Sci, in press
21 Box NF, Wyeth JR, O'Gorman LE, Martin NG, Sturm RA. Characterization of melanocyte stimulating hormone variant alleles in twins with red hair. Human Mol Genet 1997;6:1891–1897
22 Duffy DL, Box NF, Chen W, Palmer JS, Montgomery GW, James MR, Hayward NK, Green A, Martin NG, Sturm RA. Interactive effects of MC1R and OCA2 on melanoma risk phenotypes, in press
23 Palmer JS, Duffy DL, Box NF, Aitken JF, O'Gorman LE, Green AC, Hayward NK, Martin NG, Sturm RA. Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype? Am J Hum Genet 2000;66:176–186
24 Box NF, Duffy DL, Irving RE, Russell A, Chen W, Griffyths LR, Parsons PG, Green AC, Sturm RA. Melanocortin-1 receptor genotype is a risk factor for basal and squamous cell carcinoma. J Invest Dermatol 2001a;116:224–229
25 Box NF, Duffy DL, Chen W, Stark M, Martin NG, Sturm RA, Hayward NK. MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. Am J Hum Genet 2001b;69:765–773
26 Smith R, Healy E, Siddiqui S, Flanagan N, Steijlen PM, Rosdahl I, Jacques JP, Rogers S, Turner R, Jackson IJ, Birch-Machin MA, Rees JL. Melanocortin 1 receptor variants in an Irish population. J Invest Dermatol 1998;111:119–122
27 Flanagan N, Healy E, Ray A, Philips S, Todd C, Jackson IJ, Birch-Machin MA, Rees JL. Pleiotropic effects of the melanocortin 1 receptor (MC1R) gene on human pigmentation. Hum Mol Genet 2000;9:2531–2537
28 Bastiaens M, ter Huurne J, Gruis N, Bergman W, Westendorp R, Vermeer BJ, Bavinck JN. The melanocortin-1-receptor gene is the major freckle gene. Hum Mol Genet 2001;10:1701–1708
29 Akey JM, Wang H, Xiong M, Wu H, Liu W, Shriver MD, Jin L. Interaction between the melanocortin-1 receptor and P genes contributes to inter-individual variation in skin pigmentation phenotypes in a Tibetan population. Hum Genet 2001;108:516–520
30 Eiberg H, Mohr J. Assignment of genes coding for brown eye colour (BEY2) and brown hair colour (HCL3) on chromosome 15q. Eur J Hum Genet 1996;4:237–241
31 Rebbeck TR, Kanetsky PA, Walker AH, Holmes R, Halpern AC, Schuchter LM, Elder DE, Guerry D. P gene as an inherited biomarker of human eye color. Cancer Epidemiol Biomarkers Prev 2002;11:782–784
32 Donatien PD, Hunt G, Pieron C, Lunec J, Taieb A, Thody AJ. The expression of functional MSH receptors on cultured human melanocytes. Arch Dermatol Res 1992;284:424–426
33 De Luca M, Siegrist W, Bondanza S, Mathor M, Cancedda R, Eberle AN. Alpha melanocyte stimulating hormone (alpha MSH) stimulates normal human melanocyte growth by binding to high-affinity receptors. J Cell Sci 1993;105:1079–1084
34 Thody AJ, Hunt G, Donatien PD, Todd C. Human melanocytes express functional melanocyte-stimulating hormone receptors. Ann N Y Acad Sci 1993;680:381–390
35 Iozumi K, Hoganson GE, Pennella R, Everett MA, Fuller BB. Role of tyrosinase as the determinant of pigmentation in cultured human melanocytes. J Invest Dermatol 1993;100:806–811
36 Abdel-Malek Z, Swope V, Collins C, Boissy R, Zhao H, Nordlund J. Contribution of melanogenic proteins to the heterogeneous pigmentation of human melanocytes. J Cell Sci 1993;106:1323–1331
37 Barker D, Dixon K, Medrano EE, Smalara D, Im S, Mitchell D, Babcock G, Abdel-Malek ZA. Comparison of the responses of human melanocytes with different melanin contents to ultraviolet B irradiation. Cancer Res 1995;55:4041–4046
38 Hunt G, Todd C, Thody AJ. Unresponsiveness of human epidermal melanocytes to melanocyte-stimulating hormone and its association with red hair. Mol Cell Endocrinol 1996;116:131–136
39 Leonard JH, Marks LH, Chen W, Cook AL, Boyle GM, Smit DJ, Brown DL, Stow JL, Parsons PG, Sturm RA. Screening of human primary melanocytes of defined melanocortin-1 receptor genotye: pigmentation marker, ultrastructual and UV-survival studies. Pigment Cell Res 2003;16:198–207
40 Marks MS, Seabra MC. The melanosome: membrane dynamics in black and white. Nat Rev Mol Cell Biol 2001;2:738–748
41 Gallagher RP, McLean DI. The epidemiology of acquired melanocytic nevi. A brief review. Dermatol Clin 1995;13:595–603
42 Healy E, Flannagan N, Ray A, Todd C, Jackson IJ, Matthews JN, Birch-Machin MA, Rees JL. Melanocortin-1-receptor gene and sun sensitivity in individuals without red hair. Lancet 2000;355:1072–1073
43 Scott MC, Wakamatsu K, Ito S, Kadekaro AL, Kobayashi N, Groden J, Kavanagh R, Takakuwa T, Virador V, Hearing VJ, Abdel-Malek ZA. Human melanocortin 1 receptor variants, receptor function and melanocyte response to UV radiation. J Cell Sci 2002;115:2349–2355