

to allow independent verification. Second, comparing crude rates with age-standardized rates is invalid. This is because melanoma rates have been increasing in older people but decreasing in younger people during a period in which more Australians are surviving to old age. This means that crude melanoma rates calculated for the year 1982 relate to an entirely different population structure versus those calculated for 2011. Without properly accounting for these changes, the differences between crude and standardized rates are uninterpretable, and no valid comparisons can be made.

We considered the issue of population dilution in our article, and we referenced Dr. Czarnecki's original article positing his hypothesis (Czarnecki, 2014). We also referenced the subsequent paper by Baade et al. (2015) that elegantly disproved it. In their article, Baade et al. modeled melanoma incidence in Australia under the full range of hypothetical scenarios that might explain Australia's population growth between 1982 and 2011—that is, from being 100% attributable to migration to 0% attributable to migration. Regardless of the assumed level of migration, the decline in age-

standardized melanoma incidence in Australia was apparent across all scenarios, from which the authors concluded that there is “strong evidence against the hypothesis that the observed decrease in melanoma incidence among young Australians since the mid-1990s can be explained solely by the increasing overseas migration and any resultant lowering of the ‘at risk’ population in Australia.” We agree with their conclusion.

In summary, we agree that population dilution is of interest and may explain some of the decline in the Australian melanoma incidence rates, but we disagree with the assertion that melanoma incidence is rising in young susceptible Australians. As argued by others (Baade et al., 2015), the timing of the changes in melanoma incidence, coupled with the divergent trends among younger and older Australians, are consistent with birth cohort and period effects that are best explained by primary prevention campaigns that commenced nationally in the 1980s.

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CONFLICT OF INTEREST

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Association of Melanocortin-1 Receptor Variants with Pigmentary Traits in Humans: A Pooled Analysis from the M-Skip Project



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TO THE EDITOR

Skin pigmentation is due to the accumulation of eumelanin, which is brown-black pigment and photoprotective, and pheomelanin, which is yellow-red pigment and may promote carcinogenesis (Valverde et al., 1995). The melanocortin-1 receptor (MC1R) gene regulates the amount and type of pigment production and is a major determinant of skin phototype (Garcia-

Borron et al., 2005; Valverde et al., 1995). Binding of α -melanocyte stimulating hormone to MC1R stimulates the enzymatic activity of adenylate cyclase enzyme, thereby elevating intracellular cyclic adenosine monophosphate (cAMP) levels. MC1R is highly polymorphic, especially in Caucasians: more than 200 coding region variants have been described to date (Garcia-Borron et al., 2014; Gerstenblith

et al., 2007; Perez Oliva et al., 2009). Six variants—D84E, R142H, R151C, I155T, R160W, and D294H—have been designated as “R” alleles because of their strong association with the “red hair color” phenotype characterized by red hair, fair skin, freckles, and sun sensitivity. The V60L, V92M, and R163Q variants are found to have a weaker association with the red hair color phenotype and have been designated as “r” alleles (Garcia-Borron et al., 2014; Raimondi et al., 2008).

Previous studies demonstrated that several alleles are associated with phenotypic characteristics and that MC1R variants are associated with both

Abbreviations: cAMP, cyclic adenosine monophosphate; MC1R, melanocortin-1 receptor; SOR, summary odds ratio; WT, wild-type

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Table 1. Summary odds ratios for the association between combined MC1R variants and phenotypic characteristics

Phenotypic characteristic	MC1R	Studies/control	SOR (95% CI)	I ² (%) ³	P-value ³
Hair color—fair versus dark ¹	Wild-type	13/1,371	1.00 (reference)		
	Any variant	13/2,758	1.91 (1.38–2.65)	59	<0.01
	1 variant	13/1,991	1.55 (1.12–2.15)	39	0.07
	2+ variants	13/767	3.32 (2.34–4.72)	62	<0.01
Hair color—red versus others	Wild-type	7/705	1.00 (reference)		
	Any variant	7/1,474	3.54 (1.91–6.55)	0	0.80
	1 variant	7/1,016	1.18 (0.57–2.44)	0	0.83
	2+ variants	7/458	10.17 (5.28–19.58)	0	0.77
Eye color—fair versus dark ²	Wild-type	14/1,530	1.00 (reference)		
	Any variant	14/2,832	1.12 (0.96–1.30)	12	0.33
	1 variant	14/2,079	1.11 (0.94–1.32)	10	0.35
	2+ variants	14/753	1.16 (0.93–1.45)	0	0.80
Skin type—I, II versus III, IV	Wild-type	14/1,540	1.00 (reference)		
	Any variant	14/3,046	2.26 (1.81–2.83)	49	0.02
	1 variant	14/2,211	1.95 (1.51–2.53)	41	0.06
	2+ variants	14/8,35	3.58 (2.68–4.78)	42	0.05
Freckles—yes versus no	Wild-type	9/1,067	1.00 (reference)		
	Any variant	9/2,257	2.52 (1.99–3.20)	33	0.16
	1 variant	9/1,528	2.00 (1.52–2.64)	36	0.13
	2+ variants	9/729	4.47 (3.25–6.15)	38	0.12

Significant ORs and P-values are in bold.

Abbreviations: CI, confidence intervals; MC1R, melanocortin-1 receptor; OR, odds ratio; SOR, summary odds ratio.

¹Fair hair colors were red, blond, dark blonde, light brown. Dark hair colors were brown, black, dark brown.

²Fair eye colors were blue, green, gray, hazel. Dark eye colors were brown, black.

³I² and Q test P-value are measures of between-study heterogeneity (see [Supplementary Methods](#) online).

associated with skin type I/II and freckles ([Supplementary Table S2](#) online). The three variants that seemed to play the most important role in skin type determination and the presence of freckles were D84E, R151C, and D294H. Red hair color was significantly associated with all MC1R variants except for V92M and R163Q.

We visualized the associations between hair color, eye color, skin type, freckles, and the three main studied geographical areas by multiple correspondence analysis ([Supplementary Figure S1a](#) or [b](#) online). A two-dimensional multiple correspondence analysis solution, with dimension 1 on the horizontal axis and dimension 2 on the vertical axis, was considered the most adequate because the first and second dimension presented Benzecri-adjusted inertias of 85.31% and 11.31%, respectively ([Supplementary Table S3](#) online), accounting for 96.62% of the total association. The extreme red hair color phenotype (red hair, skin type I, and freckles) was associated either with carrying at least two MC1R variants ([Supplementary Figure S1a](#)) or with the presence of major penetrant (“R”) alleles ([Supplementary Figure S1b](#)). We suggest that dimension 1 can be interpreted as a “pigmentation score” because it differentiates well between dark and fair phenotypic characteristics. The median pigmentation score increased with increasing number of MC1R variants, and for single MC1R variants it was higher ($P < 0.0001$) compared with WT subjects ([Supplementary Figure S2](#) online).

Seven of the nine MC1R variants analyzed in this study, V60L, D84E, R142H, R151C, I155T, R160W, and D294H, are clearly hypomorphic with significant reduction in cAMP signaling potential ([Beaumont et al., 2007; Herraiz et al., 2012; Kadarko et al., 2010; Scott et al., 2002](#)). Within this group of variants, the lowest SOR for red hair, skin type I/II, or freckles corresponds to V60L. Interestingly, this variant was also the one with the smallest functional impairment in terms of coupling to the cAMP pathway, when the seven variants analyzed here were compared under identical experimental conditions ([Herraiz et al., 2012](#)).

melanoma and nonmelanoma skin cancer ([Han et al., 2006; Pasquali et al., 2015; Scherer et al., 2008; Tagliabue et al., 2015](#)) with a stronger role for darker-pigmented populations, suggesting that nonpigmentary pathways link MC1R with skin cancer development. Because the role and strength of each MC1R variant in determining specific phenotypic characteristics and the red hair color phenotype remains unclear, we performed a pooled analysis of individual-level data from the M-SKIP project, described in full elsewhere ([Raimondi et al., 2012](#)). We selected from the M-SKIP database all 5,366 cancer-free controls with MC1R gene sequenced and information on at least one of the following phenotypic characteristics: hair color, eye color, skin type, and freckles, thus including 16 independent studies from 18 publications ([Supplementary Table S1](#) online).

We found greater summary odds ratios (SORs) for carriers of two MC1R variants compared with carriers of only one variant allele ([Table 1](#)).

Furthermore carriage of any MC1R variant, one variant and two or more variants, compared with not having such variants (i.e., wild-type [WT] subjects), was significantly associated with fair hair color, skin type I/II, and presence of freckles. Red hair color was significantly associated with carrying any MC1R variant (SOR; 95% confidence interval: 3.54; 1.91–6.55) and with carrying two or more variants (SOR; 95% confidence interval: 10.17; 5.28–19.58), but not with carrying one MC1R variant (SOR; 95% confidence interval: 1.18; 0.57–2.44). No significant association was observed for light eye color and MC1R. Sensitivity analyses indicated that the observed between-study heterogeneity may be attributable to single studies: when we excluded the studies that were outliers, we obtained similar pooled odds ratios as the original ones, but no longer with evidence of heterogeneity (results not shown). No evidence of publication bias was found by Egger’s test. All the investigated MC1R variants compared with WT subjects were positively

Results also showed that V92M and R163Q behave as “r” alleles, with a weak albeit significant association with cutaneous phenotypic traits. In heterologous systems, V92M has been reported to display either a slight functional impairment (Herraiz et al., 2012) or normal coupling to the cAMP pathway (Beaumont et al., 2007), whereas R163Q apparently signals as efficiently as WT. Therefore, it appears that the ability of V92M or R163Q to activate the cAMP pathway is similar, if not identical to WT. This suggests that other mechanisms account for their association with cutaneous phenotypic characteristics, for example, V92M or R163Q might impair functional coupling to signaling module(s) different from the cAMP cascade. MC1R promiscuously binds to a variety of intracellular partners with signaling potential and this ability might depend on WT conformation. However, little is known as to the effects of other variants on MC1R binding to its various protein partners, and the phenotypic consequences of such molecular interactions also remain largely unknown. Further research is needed to understand the scaffolding properties of MC1R, the functional consequences of the formation of signaling complexes orchestrated by the receptor, and the effects on these processes of the myriad of natural variants in the *MC1R* gene.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <http://dx.doi.org/10.1016/j.jid.2016.05.099>.

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Low Levels of Genetic Heterogeneity in Matched Lymph Node Metastases from Patients with Melanoma

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TO THE EDITOR

In our previous experience, a high consistency of *BRAF* and *NRAS* mutation patterns was observed between primary tumors and lymph node metastases in patients with advanced

melanoma (Colombino et al., 2012). Conversely, increasing rates of discrepancies in *BRAF*/*NRAS* mutation patterns were found between primary melanomas and metastases in other sites (brain or, mostly, skin) (Colombino

et al., 2012). When the distribution of *BRAF*/*NRAS* mutations was evaluated in a larger cohort, the high rate of consistency in sequence variations of these two genes was further confirmed between primary melanomas and lymph node metastases (142/156; 91%) (Colombino et al., 2013; unpublished data). However, intraindividual heterogeneity of *BRAF* mutations has been

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