Race, ethnicity, and melanocortin-1-receptor polymorphisms are associated with post-burn hypertrophic scarring: a prospective cohort study

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Abstract

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Objective: To assess the association between melanocortin 1 receptor (MC1R) single-nucleotide polymorphisms (SNPs) and severity of post-burn hypertrophic scarring (HTS).

Background: People of color seem predisposed to HTS, but this association has not been quantified, nor have genetic factors been identified. Recent evidence indicates that melanocortin signaling has an anti-inflammatory effect, and MC1R loss-of-function is associated with fibrogenesis.

Methods: Between 2007 and 2013 we prospectively enrolled adults admitted with deep burns and obtained blood for DNA isolation. Subjects were evaluated over time using

the Vancouver Scar Scale (VSS) and asked to rate their itching. Genotyping was performed for 8 MC1R SNPs. Testing for association with severe HTS (VSS>7) and itch severity (0-10) was based on multivariate regression with adjustment for known HTS risk factors.

Results: Of 425 subjects, (median burn size 6.8% body surface area), 77% were White and 88% were non-Hispanic. The prevalence of severe HTS (VSS>7) was 49%, and the mean itch score was 3.9. In multivariate analysis, Hispanic ethnicity (prevalence ratio [PR] 1.32) and Asian (PR 1.61), Black/African American (PR 1.95), and Native American (PR 1.84) race were independently associated with severe HTS. Two MC1R SNPs, R151C (P = 0.033) and R163Q (P = 0.005), were significantly associated with severe HTS. Hispanic ethnicity and Asian race were associated with increased pruritus, and MC1R T314T was associated with decreased pruritus.

Conclusions: Race and ethnicity are significantly associated with post-burn HTS and itch severity, and MC1R R151C and R163Q are the first SNPs to be associated with post-burn HTS.

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Introduction

Hypertrophic scarring (HTS) is a fibroproliferative response to cutaneous injury that occurs in over 70% of burns requiring hospital admission[1], resulting in scar raised above the skin level but within the boundaries of the original wound. In addition to disfigurement, HTS is associated with chronic neuropathic pain and pruritus, functional impairment, and psychological morbidity, which collectively contribute to decreased quality of life[2]. There are no effective methods to prevent or treat HTS[3], and development of new therapies has been limited by incomplete understanding of HTS pathophysiology[4]. Previously reported risk factors include female sex, young age, burn depth and extent, burn site (neck, upper limb), number of operations, delayed wound-healing, and use of meshed skin grafts[1,5]. In addition, it has been suggested that individuals of dark-skinned race are at increased risk of post-burn HTS[6-8], implying a genetic mechanism. However, it is not known to what degree race influences the development of HTS after burns, nor have the genetic determinants of HTS been identified.

Given the apparent association between skin pigmentation and HTS formation, genes involved in skin pigmentation may contribute to HTS. Melanocortin signaling is known to be a chief determinant of pigmentation, as binding of α-melanocyte-stimulating hormone (α-MSH) to the G-protein-coupled melanocortin 1 receptor (MC1R) causes melanocytes to produce dark eumelanin in favor of light pheomelanin, imparting dark color to the skin and hair[9]. The MC1R gene is highly polymorphic with over 80 known variant alleles, many of which impact function[10]. Some of these loss-of-function variants are associated

with red hair and fair skin[11] and increased risk of skin cancers, especially melanoma[12,13]. However, several MC1R variants are also known to be common among dark-skinned races that seem predisposed to HTS; in one study, the loss-of-function R163Q single-nucleotide polymorphism (SNP) had a minor allele frequency (MAF) of 70% among East/Southeast Asians and 100% in Native Americans[14].

A growing body of evidence suggests a role for MC1R signaling in wound healing. Melanocortin signaling induces an anti-inflammatory cytokine-expression profile in leukocytes[15], inhibits synthesis of extracellular matrix in dermal fibroblasts[16], and reduces skin fibrosis in a murine model[17], with MC1R knockout mice exhibiting increased skin fibrosis[18]. Our lab has shown that α-MSH and MC1R are up-regulated by epidermal keratinocytes and dermal fibroblasts in human burn wounds and hypertrophic scar[19]. These data suggest that MC1R signaling may have an anti-inflammatory, anti-fibrotic role in wound healing, and MC1R loss-of-function might lead to excessive inflammation and fibrosis, contributing to HTS. Thus, the primary purpose of our study was to examine the association between race/ethnicity and risk of developing severe HTS and to determine whether MC1R SNPs are associated with increased risk for severe HTS. In addition, since post-burn pruritus is thought to have a neuropathic mechanism[20] and melanocortin signaling has been implicated in neuropathic pain[21], we sought to determine whether MC1R SNPs are associated with post-burn pruritus in a secondary analysis.

Methods

Study design, population, and setting

After obtaining University of Washington Institutional Review Board approval and a Federal Certificate of Confidentiality from the National Institutes of Health, we conducted a prospective cohort study of patients admitted to the UW Regional Burn Center from 2007 through 2013. We enrolled adults (age ≥18) whose burns were at least deep partial-thickness or had delayed healing (≥2 weeks), placing them at increased risk of HTS. Each subject provided a blood sample for genotyping, and subject characteristics including age, sex, race, ethnicity, burn size, and number of operations were obtained from the medical record. Self-reported race and ethnicity were recorded as separate variables in accordance with National Institutes of Health guidelines[22]. After hospital discharge, subjects were seen 1-2 times in follow-up, with the first visit generally occurring within six months of injury and the second after six months post-injury. For inclusion in the analysis, subjects were required to have at least one visit at least 3 months post-injury. At each clinic visit, scars were assessed by a research nurse using the Vancouver Scar Scale (VSS)[23], and subjects rated their scar-associated itch on a scale from 0 to 10, with 0 indicating no itch.

Exposures and outcomes

Our primary exposures were race, ethnicity, and MC1R genotype. Our primary outcome was severe HTS, which we defined as total VSS score >7 at any follow-up, consistent

with our previous approach[8]. We chose severe HTS (with a relatively high VSS cut-off) rather than mere presence of HTS as our primary outcome because we anticipated a high prevalence of HTS due to subject enrollment based on wound depth and healing time. We examined the highest itch score (0-10) from any follow up as a secondary outcome.

Genotyping

Subjects provided 2 mL of venous whole blood in a Vacutainer K_2EDTA tube (Becton-Dickinson, Franklin Lakes, NJ). Genomic DNA was isolated using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA) followed by ethanol precipitation and quantification using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). Genotyping was performed on 20 ng DNA samples using pre-designed TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA) in 384-well plates with a Viia7 instrument (Applied Biosystems, Foster City, CA) per manufacturer guidelines. Samples were genotyped for 8 MC1R SNPs (Figure 1), including 7 of the most extensively characterized loss-of-function[24-27] missense variants: rs1805005 (V60L), rs2228479 (V92M), rs1805007 (R151C), rs1110400 (I155T), rs885479 (R163Q), rs3212366 (F196L), and rs1805009 (D294H). The effect of the synonymous variant rs2228478 (T314T) on MC1R function is unknown, but it was included because it is prevalent among those of African descent[10,14], a population thought to be at increased risk of HTS[6,7].

Statistical analysis

Continuous variables are summarized as mean (standard deviation [SD]) or, if skewed, median (interquartile range [IQR]). Categorical variables are summarized as number (percent). Testing for differences in scar outcomes between early and late follow-up visits was performed using a paired two-tailed *t*-test. As our primary outcome measure, we report the prevalence of severe HTS among subjects returning for their first or second post-injury follow-up visit. We chose prevalence rather than incidence because we did not have complete follow-up, precluding reliable estimation of the cumulative incidence of severe HTS in our cohort. Prevalence-ratio estimates describing the association between race or ethnicity and development of severe HTS were obtained using Poisson regression with robust standard errors. In multivariate analysis, age, sex, percent total body surface area (%TBSA) burned, and number of operations were included as covariates.

The minor allele frequency (MAF) for each MC1R SNP was calculated as follows:

$$\text{MAF} = \frac{\#\ \text{rare alleles}}{2 \times (\#\ \text{subjects})} \times 100\,.$$

Rare SNPs (MAF<5% in our overall study population) were excluded from association testing. Testing for Hardy-Weinberg equilibrium was performed using the chi-square test. To test for associations between individual MC1R SNPs and HTS severity, we fit a multivariate Poisson regression model including age, sex, percent total body surface area (%TBSA) burned, race, and ethnicity as well as the MC1R SNP genotype

variables, which were coded as 0, 1, or 2 (for the number of variant alleles present) and modeled as continuous, assuming an additive model of inheritance. Haplotype frequencies were estimated from our unphased genotype data using the expectation-maximization algorithm[28]. Haplotypes with frequency >5% were tested for association with HTS severity using multivariate log-binomial regression, with adjustment for HTS risk factors and race/ethnicity as above.

In a secondary, exploratory analysis of risk factors for post-burn pruritus, univariate analysis of itch was performed using one-way analysis of variance (ANOVA). We used linear regression with robust standard errors for multivariate modeling of itch score, the continuous dependent variable. Regression models with and without MC1R SNP variables were fit as described above for modeling risk of severe HTS, but with the addition of HTS severity as an adjustment covariate, as it has previously been associated with post-burn pruritus[29], and we were interested in the effect of race/ethnicity on itch beyond their effect on HTS severity. Haplotypes with frequency >5% were tested for association with itch score using multivariate linear regression with adjustment for race/ethnicity and known risk factors for post-burn pruritus including HTS severity. In linear regression analyses, statistical inference was based on partial F tests of regression-coefficient estimates.

In all Poisson and log-binomial regression analyses, statistical inference was based on Wald tests of regression-coefficient estimates, and exponentiated coefficients are

reported as prevalence ratios with their Wald-type 95% confidence intervals. Haplotype estimation and association testing were performed using the Haplo Stats package in R version 3.0.2; all other analyses were performed in Stata 13.0 (StataCorp, College Station, TX), with $P<0.05$ considered statistically significant.

Study power

Assuming a 50% prevalence of severe HTS, a SNP MAF of 0.10, and an additive genetic model, approximately 390 subjects would be required to achieve 80% power to detect prevalence ratios of 1.4 and 1.8 for heterozygotes and rare homozygotes, respectively, at a type I error rate of 0.05. Given that >400 subjects were included in the analysis, we conclude that our study was adequately powered. Sample size calculations were performed using the Genetic Power Calculator[30].

Results

Of 586 burned adults enrolled, 425 had provided a blood sample for genotyping and had been seen in follow-up at least three months post-injury by the time of our analysis. These were predominantly white non-Hispanic males, consistent with patient demographics of our burn center (Table 1). The majority (64%) required at least one operation, reflecting enrollment based on presence of deep burns. At a median follow-up of 7 months (range 3-20), the mean VSS score was 7.4 (SD 2.3), and 208 (49%) had severe HTS (highest VSS>7). The mean itch score was 3.9 (SD 3.0). Of 300 subjects who were evaluated twice, both prevalence of severe HTS and mean itch score were significantly higher at early (median 3.2 months) compared to later (median 7.5 months) follow-up visits: 46% vs. 30% had severe HTS, respectively (P<0.0001), and mean itch scores were 3.6 vs. 2.7, respectively (P<0.0001), consistent with the known tendency for scar[31] and itch[32] severity to diminish over time.

MC1R SNPs were common among our study subjects, with 68% of subjects carrying at least one copy of one variant allele, consistent with previous estimates in comparable populations[10]. Prevalence of individual SNPs varied considerably according to race and ethnicity: R163Q was much more common among Native Americans and Asians compared to Whites and among Hispanics compared to non-Hispanics, and T314T was highly prevalent among Blacks and Asians compared to Whites (Figure 2). All SNPs were in Hardy-Weinberg equilibrium (P>0.30 for all SNPs in the overall study population; data not shown). Three SNPs were rare (MAF<5%) in our study subjects, including F196L, which was not detected in any of the 425 subjects screened; these

were excluded from subsequent analysis, leaving 5 for association testing (V60L, V92M, R151C, R163Q, and T314T).

Race, ethnicity, and MC1R genotype are associated with scar severity

In unadjusted analysis, the prevalence of severe HTS varied significantly according to race (P<0.0001), with a higher prevalence among Asians (PR 1.45; 95% CI: 1.05-2.00), Blacks (PR 1.78; 95% CI 1.34-2.36), and Native Americans (PR 1.98; 95% CI: 1.52-2.57) compared to Whites. There was no significant difference in prevalence of severe HTS for Hispanics compared to non-Hispanics (PR 1.15; 95% CI: 0.88-1.51; P = 0.32). In a multivariate model adjusting for several known risk factors for HTS, Hispanic ethnicity and Asian, Black, and Native American race were each independently associated with risk of severe HTS (Table 2). Burn size and number of operations were also independently associated with HTS severity, corroborating previous reports[5,8].

When the multivariate model was expanded to include genotype data for five MC1R SNPs, R151C (PR_{adj} 1.30; 95% CI: 1.02-1.66) and R163Q (PR_{adj} 1.30; 95% CI: 1.08-1.55) were independently associated with severe HTS (Table 3). The R163Q variant was very common among Native Americans, Asians, and Hispanics and thus explained some of the excess risk for severe HTS in those groups, but neither R151C nor R163Q was common among Blacks/African Americans and thus did not account for their increased risk of severe HTS (Figure 2). To examine associations between combinations of MC1R SNPs and HTS severity, we performed haplotype analysis (Table 4). Most haplotypes containing multiple rare alleles were quite rare, with the

exception of V92M/T314T, with an estimated haplotype frequency of 8.6%. After excluding rare haplotypes (frequency<5%), the only haplotypes significantly associated with HTS severity were those containing the R163Q (*P* = 0.013) or R151C (*P* = 0.037) variant alleles alone (Table 4). Thus, haplotype analysis did not reveal any associations beyond those detected in individual SNP analysis (Table 3).

Race, ethnicity, and MC1R genotype are associated with post-burn pruritus

In univariate testing, itch severity varied significantly according to race (*P* = 0.048); mean itch score was 5.2 for Asians, 4.9 for Blacks, and 4.3 for Native Americans, compared to 3.7 for Whites. Itch scores were also higher among Hispanics (mean 4.6) compared to Non-Hispanics (mean 3.8), although this difference did not quite reach statistical significance (*P* = 0.06). In a multivariate model including clinical and demographic factors only, female sex, burn size, HTS severity, Hispanic ethnicity, and Asian race were independently associated with itch score (Table 5). When MC1R SNP variables were included in the model, the T314T variant was significantly associated with decreased itch score (*P* = 0.018), with each additional copy of T314T being associated with 1.28 point lower mean itch score (95% CI: 0.21-2.29; Table 6). Testing of haplotypes did not reveal any significant associations with itch score after adjustment for age, sex, burn size, number of operations, HTS severity, race, and ethnicity (*P*>0.10 for all haplotypes with frequency >5%; data not shown).

Discussion

Despite decades of research, our understanding of HTS pathophysiology is still far from complete[33], likely due in part to the paucity of epidemiologic studies of HTS risk factors[1]. Although there are several references to race or skin color as a risk factor for HTS in the burn literature[6-8], the association between race and post-burn HTS has not been rigorously analyzed. Gangemi and colleagues performed an extensive analysis of post-burn HTS, elegantly enumerating a number of patient- and scar-specific risk factors[5]. However, they did not examine the role of race or ethnicity. Here we have shown that Hispanic ethnicity and Asian, Black, and Native American race are independently associated with severe HTS after adjusting for factors known to be associated with HTS risk. With the exception of Native American race, previously reported in a subset of the presently analyzed cohort[8], these are novel findings. Although the precision of our prevalence-ratio estimates is limited by the relatively small number of non-White and Hispanic subjects in our study, our estimates provide a preliminary measure of the excess risk of severe HTS associated with these racial and ethnic groups and thus have immediate clinical utility in the counseling of burned patients and in guiding preventive measures. Moreover, they strongly imply the existence of predisposing genetic variants.

Our observation that MC1R SNPs R151C and R163Q are associated with excess risk for severe HTS is the first published association between genetic polymorphisms and post-burn HTS risk. These and other MC1R SNPs have been extensively studied, largely due to their role in skin pigmentation and melanoma risk. The cell-membrane-bound G-protein-coupled MC1R triggers increased cAMP expression upon binding its

ligand, α-MSH[9]. Compared to wild-type MC1R, the R151C variant displays dramatically decreased cell-surface localization and α-MSH binding[24] as well as decreased cAMP production in response to α-MSH[34]. The R163Q variant is associated with decreased affinity for α-MSH[26,27], decreased cell-surface localization[24], and decreased baseline cAMP production[27] compared to wild-type. These functional impairments are thought to contribute to the red-hair/fair-skin phenotype[35] and increased melanoma risk[36] by interfering with melanocortin signaling in melanocytes (albeit with incomplete penetrance, especially of the R163Q variant[11]).

However, MC1R is expressed by a number of other cell types including fibroblasts[15,19], which are thought to be key mediators of HTS[37]. In response to cutaneous injury, fibroblasts proliferate, differentiate into myofibroblasts, deposit extracellular matrix, and mediate scar contraction[38]. Melanocortin signaling has been shown to decrease fibroblast collagen synthesis[16] and proliferation[39], indicating an overall anti-fibrogenic effect. Accordingly, decreased MC1R expression in keloid-derived human fibroblasts confers loss of α-MSH-induced inhibition of collagen synthesis and myofibroblast transformation[40], and MC1R loss of function leads to increased skin fibrosis in a murine model[18]. The R151C and R163Q variants have specifically been associated with increased fibroblast proliferation at baseline and loss of α-MSH-induced decrease in proliferation[39]. In addition, the red Duroc pig, which forms thick, fibroproliferative scar closely resembling human HTS[41], is known to have presumably loss-of-function MC1R SNPs[42] as well as inherently fibrogenic dermal fibroblasts[43]. Hence, the R151C and

R163Q MC1R loss-of-function variants may lead to increased HTS severity through impaired melanocortin-mediated regulation of the fibroblast response to injury.

Although the R163Q SNP appears to account for some of the excess risk for severe HTS among Hispanics, Asians, and Native Americans, the R151C nor R163Q variants were rare (MAF 3.3%) and absent, respectively, in Blacks/African Americans, the group at highest risk for severe HTS (Table 2). Consistent with other studies[10,14], we found that the translationally silent T314T variant was the only highly prevalent MC1R SNP in individuals identifying as Black/African American (Figure 2). It is widely assumed that genes influencing skin pigmentation are under selection, and preservation of the consensus MC1R amino-acid sequence among Africans is thought to reflect evolutionary selection pressure against sensitivity to deleterious UV radiation[44,45]. Although to our knowledge the effect of the T314T variant has not been studied experimentally, it is thought to be associated with preserved MC1R function. Given our results and the selection pressure thought to affect pigmentation genes in individuals of African origin, we speculate that genetic variants responsible for increased HTS in this group may be less likely to be in pigmentation genes.

In a secondary analysis, we explored factors associated with post-burn pruritus, one of the most common and debilitating burn sequela[46]. Consistent with previous reports[29,47], we found that female sex, burn size, and HTS severity were significantly associated with post-burn pruritus. In addition, we report that Hispanic ethnicity and Asian race are independently associated with post-burn itch severity (Tables 5). Given that our analysis

was adjusted for HTS severity, these results not only suggest a genetic etiology for post-burn pruritus, but also indicate that the responsible genetic variants may be distinct from those predisposing to HTS. Although MC1R SNPs R151C and R163Q were significantly associated with severe HTS, they were not associated with more severe post-burn pruritus (Table 6). The only significant genetic association was with the synonymous T314T variant, which was linked to decreased severity of post-burn pruritus. As described above, it is likely that this synonymous variant is associated with preserved function of the MC1R protein. However, synonymous mutations may exert phenotypic effects by altering protein expression through a variety of mechanisms, including by affecting mRNA stability[48]. Thus, if the relationship were causal, our finding that the T314T variant is significantly associated with decreased itch severity could reflect over-expression of functional MC1R, resulting in an anti-neuroinflammatory effect. Given the exploratory nature of this secondary analysis, our data call for further studies to confirm our findings and to identify additional genetic variants associated with post-burn pruritus.

This study has several limitations. As a candidate-gene associated study in a genetically admixed population, it is subject to confounding by population substructure, which can lead to detection of spurious associations due to common ancestry[49]. To address this issue, we adjusted our analyses for self-identified race and ethnicity. Although self-identified race/ethnicity has been shown to correlate well with ancient genetic ancestry[50], the imprecision of these designations may result in some degree of residual confounding. Our results warrant validation in an independent cohort, ideally

with more robust adjustment for population substructure. Even in the absence of confounding by population structure, due to the nature of genetic association studies, the significant associations that we detected between the R151C and R163Q variants and HTS severity may not be due to a direct relationship between these SNPs and HTS, but could instead reflect an association with any genetic locus in linkage disequilibrium with the R151C or R163Q loci. Hence, experimental studies will be required to ascertain the biological mechanism underlying any detected association. Finally, we have studied only 8 of the over 80 known MC1R variants. Thus, we are unable to directly detect associations between HTS severity and MC1R polymorphisms that were not among those we genotyped. Mindful of this limitation, we focused our analysis on SNPs that are known to alter MC1R function and those that are among the most common. We were unable to obtain a genotyping assay for the R160W SNP, which is a well-studied loss-of-function[24,34] variant that is common in the U.S., Europe, and Australia[10]. Thus, further studies will be required to examine this and other MC1R SNPs of interest.

In summary, our findings suggest that Hispanic ethnicity and Asian, Black, and Native American race are independently associated with risk of developing severe HTS. We report MC1R SNPs R151C and R163Q as the first genetic variants to be associated with increased severity of post-burn HTS. These SNPs may cause dysfunctional inflammatory and fibrogenic responses that lead to increased scarring after burn injury. We also report increased severity of post-burn pruritus in association with Hispanic ethnicity and Asian race, and decreased post-burn pruritus in association with the

MC1R T314T variant. Our findings warrant replication in an independent clinical cohort, and additional studies are required to elucidate the biological mechanism linking MC1R SNPs to post-burn HTS as well as identify additional genetic variants conferring risk for post-burn HTS and pruritus.

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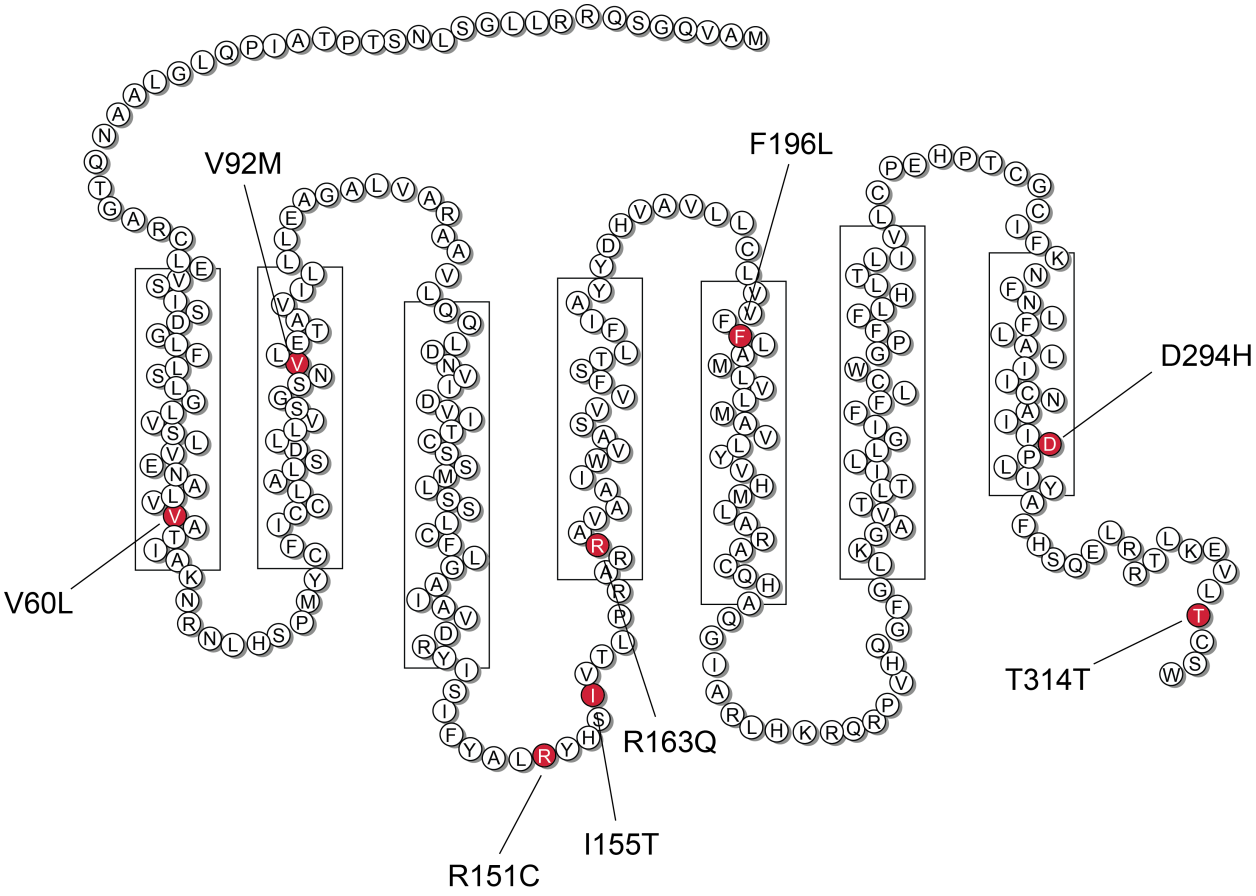

FIGURE 1. The eight SNPs considered in this study are named according to their corresponding amino-acid substitutions, denoted by single-letter amino-acid codes and residue number, as indicated in this cartoon of the MC1R amino-acid sequence. Boxes indicate the seven transmembrane domains. The diagram was adapted by permission from Macmillan Publishers Ltd: *Journal of Investigative Dermatology*, Ringholm et al.[26], copyright 2004.

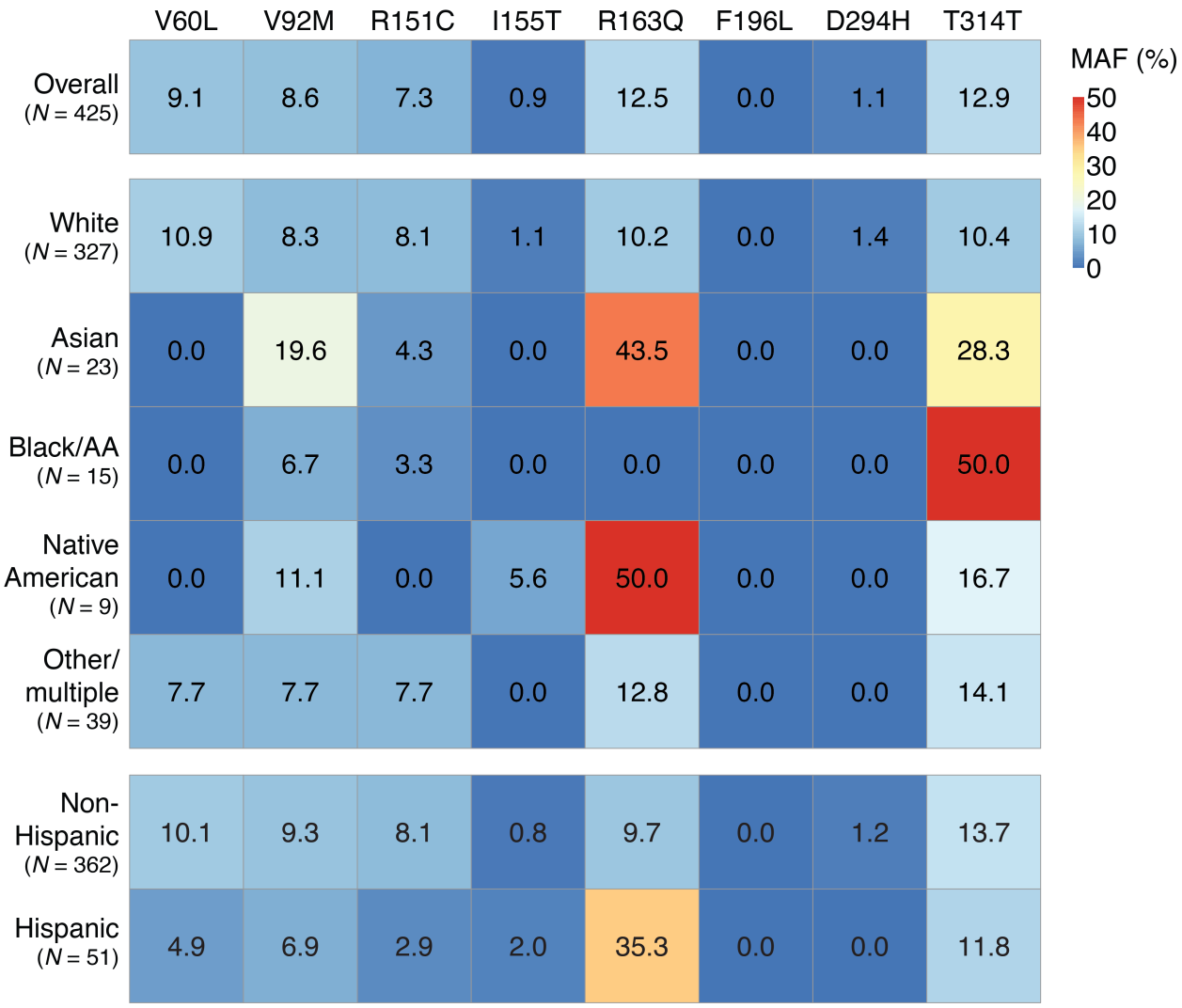

FIGURE 2. Overall and race- and ethnicity-specific frequencies of seven MC1R SNPs are illustrated in a heat map to highlight variations in SNP frequencies across racial and ethnic groups. The numbers of subjects in the racial and ethnic subgroups do not sum to 425 due to missing or unknown race/ethnicity for 12 subjects. MAF, minor allele frequency.

TABLE 1. Summary* of 425 subjects.

Age[#] (years)	40	(28-52)
Sex		
Male	298	(70%)
Female	127	(30%)
Ethnicity[§]		
Non-Hispanic	362	(88%)
Hispanic	51	(12%)
Race[§]		
White	327	(79%)
Asian	23	(6%)
Black/AA	15	(4%)
Native American	9	(2%)
Other/multiple	39	(9%)
Burn size[#] (%TBSA)	7	(3-15)
Number of operations		
0	152	(36%)
≥1	273	(64%)

*Data presented as number (%), except where indicated. [#]Reported as median (interquartile range). [§]Missing or reported as unknown for 12 subjects. AA, African American; %TBSA, percent total body surface area burned.

TABLE 2. Clinical and demographic factors independently associated with severe HTS (*N* = 412).

	PR_{adj}	95% CI	*P*
Age	0.97*	0.91-1.04	0.454
Female sex	0.97	0.79-1.21	0.808
Burn size	**1.10#**	**1.04-1.17**	**0.001**
≥1 Operation	**1.35**	**1.06-1.73**	**0.015**
Hispanic ethnicity	**1.32**	**1.01-1.73**	**0.045**
Race (Ref: White)			
Asian	**1.61**	**1.18-2.21**	**0.003**
Black/AA	**1.95**	**1.47-2.59**	**<0.001**
Native American	**1.84**	**1.43-2.37**	**<0.001**
Other/multiple	1.17	0.85-1.62	0.325

*For 10 additional years of age. #For an additional 10% total body surface area burned. **Bold** indicates statistical significance (*P*<0.05). PR_{adj}, adjusted prevalence ratio; AA, African American; Ref, referent category.

TABLE 3. MC1R SNPs independently associated with severe HTS (*N* = 412).

MC1R SNP	PR_{adj}*	95% CI	*P*
V60L	1.01	0.78-1.33	0.918
V92M	1.10	0.76-1.58	0.614
R151C	**1.30**	**1.02-1.66**	**0.033**
R163Q	**1.30**	**1.08-1.55**	**0.005**
T314T	1.11	0.83-1.49	0.476

*Adjusted prevalence-ratio associated with each additional copy of the rare allele, adjusted for age, sex, burn size, number of operations, race, and ethnicity. **Bold** indicates statistical significance ($P<0.05$).

TABLE 4. Haplotype frequencies and association[#] with severe HTS (*N* = 412).

	V60L	V92M	R151C	I155T	R163Q	D294H	T314T	Freq. (%)	PR_{adj}	95% CI	*P*
1								55.7		Ref	
2					■			**12.5**	**1.92**	**1.48-2.50**	**0.013**
3	■							9.8	1.49	1.13-1.96	0.730
4		■					■	8.6	1.09	0.85-1.39	0.146
5			■					**7.9**	**1.76**	**1.34-2.31**	**0.037**
6							■	3.4	*	*	*
7						■		1.1	*	*	*
8				■			■	0.9	*	*	*
9		■			■		■	0.1	*	*	*
10	■		■					<0.1	*	*	*
11			■	■			■	<0.1	*	*	*

[#]Based on log-binomial multivariate regression with adjustment for age, sex, number of operations, burn size, race, and ethnicity. *Rare haplotype (<5%) precluded association testing. In each haplotype, white cells indicate the common allele and black cells the rare allele. **Bold** indicates a statistically significant association ($P<0.05$). PR_{adj}, adjusted prevalence ratio.

TABLE 5. Clinical and demographic factors independently associated with severity of post-burn pruritus (*N* = 411).

	Coef.*	95% CI	*P*
Age	−0.07[#]	−0.25-0.12	0.474
Female sex	**0.94**	**0.33-1.54**	**0.003**
Burn size	**0.30[§]**	**0.09-0.52**	**0.006**
≥1 Operation	0.36	−0.23-0.96	0.232
Severe HTS	**1.47**	**0.89-2.04**	**<0.001**
Hispanic ethnicity	**1.09**	**0.23-1.95**	**0.013**
Race (Ref: White)			
Asian	**1.50**	**0.42-2.59**	**0.007**
Black/AA	1.09	−0.49-2.68	0.176
Native American	−0.34	−1.79-1.12	0.651
Other/multiple	0.78	−0.22-1.77	0.126

*Linear regression coefficient, representing the difference in mean itch score associated with each factor. [#]For 10 additional years of age. [§]For an additional 10% total body surface area burned. **Bold** indicates statistical significance (*P*<0.05). AA, African American; Ref, referent category.

TABLE 6. MC1R SNPs independently associated with severity of post-burn pruritus (*N* = 411).

MC1R SNP	Coef.*	95% CI	*P*
V60L	−0.13	−0.77-0.51	0.693
V92M	0.39	−0.79-1.56	0.520
R151C	0.13	−0.61-0.86	0.733
R163Q	0.32	−0.33-0.96	0.332
T314T	**−1.25**	**−2.29-−0.21**	**0.018**

*Linear regression coefficient, representing the difference in mean itch score associated with each additional copy of the rare allele, adjusted for age, sex, burn size, number of operations, race, ethnicity, and HTS severity. **Bold** indicates statistical significance (*P*<0.05).