Race and melanocortin 1 receptor polymorphism R163Q are associated with post-burn hypertrophic scarring: a prospective cohort study

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Abstract

The genetic determinants of post-burn hypertrophic scarring (HTS) are unknown, and melanocortin 1 receptor (MC1R) loss-of-function leads to fibrogenesis in experimental models. To examine the associations between self-identified race and MC1R single-nucleotide polymorphisms (SNPs) with severity of post-burn HTS, we conducted a prospective cohort study of burned adults admitted to our institution over 7 years. Subjects were evaluated using the Vancouver Scar Scale (VSS), asked to rate their itching, and genotyped 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 risk factors. Of 425 subjects analyzed, 77% identified as White. The prevalence of severe HTS (VSS>7) was 49%, and the mean itch score was 3.9. In multivariate analysis, Asian (prevalence ratio [PR] 1.54; 95% CI: 1.13-2.10), Black/African American (PR 1.86; 95% CI: 1.42-2.45), and Native American (PR 1.87; 95% CI: 1.48-2.35) race were independently associated with severe HTS. MC1R SNP R163Q was also significantly (P<0.001) associated with severe HTS. Asian race (linear regression coefficient 1.32; 95% CI: 0.23-2.40) but not MC1R SNP genotype was associated with increased itch score. We conclude that MC1R genotype may influence post-burn scarring.

Introduction

Hypertrophic scarring (HTS) is a fibroproliferative response to cutaneous injury that occurs in over 70% of burns requiring hospital admission (Lawrence et al., 2012), 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 (Bock et al., 2006). There are no effective methods to prevent or treat HTS (Friedstat and Hultman, 2014), and development of new therapies has been limited by incomplete understanding of HTS pathophysiology (van der Veer et al., 2009). Known 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 (Gangemi et al., 2008; Lawrence et al., 2012). In addition, individuals of dark-skinned race appear to be at increased risk of post-burn HTS (Bombaro et al., 2003; Deitch et al., 1983; Thompson et al., 2013). Since race reflects geographic ancestral origin and population genetic structure (Risch et al., 2002), this association suggests 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 (Lin and Fisher, 2007). The MC1R gene is highly polymorphic with over 80 known variant alleles, many of which alter function (Gerstenblith et al., 2007). Some of these loss-of-function variants are associated with red hair and fair skin (Flanagan et al., 2000) and increased risk of skin cancers, especially melanoma (Kennedy et al., 2001; Sturm, 2002). 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 an allele frequency of 70% among East/Southeast Asians and 100% in Native Americans (Rana et al., 1999).

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 (Brzoska et al., 2008), inhibits synthesis of extracellular matrix in dermal fibroblasts (Bohm et al., 2004), and reduces skin fibrosis in a murine model (Kokot et al., 2009), with MC1R knockout mice exhibiting increased skin fibrosis (Bohm and Stegemann, 2014). 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 (Muffley et al., 2011). 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. The primary purpose of our study was to examine the association between self-identified race 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 (Goutos, 2013) and melanocortin signaling has been implicated in neuropathic pain (Caruso et al., 2014), we sought to determine whether MC1R SNPs are associated with post-burn pruritus in a secondary analysis.
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. The racial distribution of subjects who were not genotyped or had incomplete clinical follow-up did not differ significantly from that of the 425 subjects included in the analysis, who were predominantly White 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 (Gauglitz et al., 2011) and itch (Goutos et al., 2009) severity to diminish over time. Average length of follow-up did not vary with race (data not shown).

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 (Gerstenblith et al., 2007). Prevalence of individual SNPs varied considerably according to race: R163Q was much more common among Native Americans and Asians compared to Whites, and T314T was highly prevalent among Blacks and Asians compared to Whites (Figure 1). All SNPs were in Hardy-Weinberg equilibrium (P>0.30 for all SNPs in the overall study population; data not shown). Three rare SNPs (MAF<5% in our overall study population) were excluded from subsequent analysis, leaving 5 for association testing: V60L, V92M, R151C, R163Q, and T314T.

Race 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 (prevalence ratio [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. In a multivariate model adjusting for several known risk factors for HTS, 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 (Gangemi et al., 2008; Thompson et al., 2013).

When the multivariate model was expanded to include genotype data for five MC1R SNPs, the R163Q variant (PRadj 1.35; 95% CI: 1.14-1.53) was independently associated with severe HTS after accounting for multiple testing (Table 3). This significant association persisted when the multivariate analysis was limited to White subjects only (Table 3), indicating that the association was not driven by the Asian and Native American subjects, who had a higher prevalence of both severe HTS (Table 2) and R163Q (Figure 1b). In a model including an interaction between R163Q genotype and race, there was no evidence of
effect modification by race ($P = 0.27$), although this analysis was likely under-powered due to small numbers in the non-white race categories.

To examine associations between combinations of MC1R SNPs and HTS severity, we performed haplotype analysis (Table S1). Only V92M and T314T showed a considerable degree of linkage disequilibrium (Figure S1), and V92M/T314T was the only relatively common (frequency 8.6%) haplotype containing multiple rare alleles. After excluding rare haplotypes (frequency<5%), the only haplotype significantly associated with HTS severity contained the R163Q ($P<0.001$) allele alone; this result was consistent between analyses of the overall study population and of White subjects only (Table S1). Thus, haplotype analysis did not reveal any associations beyond those detected in individual SNP analysis.

**Race is 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. In a multivariate model including clinical and demographic factors only, female sex, burn size, HTS severity, and Asian race were independently associated with itch score (Table 4). When MC1R SNP variables were included in the model, there was a near-significant association between the T314T variant and decreased itch score ($P = 0.029$), with each additional copy of T314T being associated with 1.18 point lower mean itch score (95% CI: 0.12-2.23) in the overall study population; however, this association did not reach significance after accounting for multiple testing (defined as $P<0.01$), nor was it significant in the stratified analysis limited to Whites only (Table 5). Haplotype analysis did not reveal any significant associations with itch score after adjustment for age, sex, burn size, number of operations, HTS severity, and race ($P>0.01$ for all haplotypes with frequency >5%; data not shown).

**Discussion**

Despite decades of research, our understanding of HTS pathophysiology is still far from complete (Tredget et al., 2014), likely due in part to the paucity of epidemiologic studies of HTS (Lawrence et al., 2012). Here we have shown that Asian, Black, and Native American race are independently associated with severe HTS. Although the relatively small number of non-White subjects limits the precision of our prevalence-ratio estimates, these estimates provide a preliminary measure of the excess risk of severe HTS associated with these racial 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.

In addition, we report that MC1R SNP R163Q is associated with excess risk for severe HTS. Although this result will require replication prior to clinical translation, it represents a first step toward personalized, genome-based management of burn scars, in line with the President's recently announced Precision Medicine Initiative (Collins and Varmus, 2015). In addition, investigating the potential mechanism linking this SNP to HTS severity may advance our understanding of HTS pathophysiology. The cell-membrane-bound G-protein-coupled MC1R triggers increased cAMP expression upon binding its ligand, α-MSH (Lin...
and Fisher, 2007). Compared to wild-type MC1R, the R163Q variant is associated with decreased affinity for \(\alpha\)-MSH (Doyle et al., 2012; Ringholm et al., 2004), decreased cell-surface localization (Beaumont et al., 2005), and decreased baseline cAMP production (Doyle et al., 2012) compared to wild-type. These functional impairments are thought to contribute to the red-hair/fair-skin phenotype (Valverde et al., 1995) and increased melanoma risk (Rodriguez and Setaluri, 2014) by interfering with melanocortin signaling in melanocytes (albeit with incomplete penetrance (Flanagan et al., 2000)).

However, MC1R is expressed by a number of other cell types including fibroblasts (Brzoska et al., 2008; Muffley et al., 2011), which are thought to be key mediators of HTS (Sarrazy et al., 2011). In response to cutaneous injury, fibroblasts proliferate, differentiate into myofibroblasts, deposit extracellular matrix, and mediate scar contraction (Tomasek et al., 2002). Melanocortin signaling has been shown to decrease fibroblast collagen synthesis (Bohm et al., 2004) and proliferation (Stanisz et al., 2011), indicating an overall anti-fibrogenic effect. Accordingly, decreased MC1R expression in keloid-derived human fibroblasts confers loss of \(\alpha\)-MSH-induced inhibition of collagen synthesis and myofibroblast transformation (Luo et al., 2013), and MC1R loss of function leads to increased skin fibrosis in a murine model (Bohm and Stegemann, 2014). The R163Q variant specifically has been associated with increased fibroblast proliferation at baseline and loss of \(\alpha\)-MSH-induced decrease in proliferation (Stanisz et al., 2011). In addition, the red Duroc pig, which forms thick, fibroproliferative scar closely resembling human HTS (Engrav et al., 2011), is known to have presumably loss-of-function MC1R SNPs (Dun et al., 2007) as well as inherently fibrogenic dermal fibroblasts (de Hemptinne et al., 2008). Hence, the R163Q MC1R loss-of-function variant may lead to increased HTS severity through impaired melanocortin-mediated regulation of the fibroblast response to injury. If confirmed, this mechanism could provide a biological rationale for topical application of \(\alpha\)-MSH to burns and other cutaneous wounds to prevent or treat HTS.

Although the R163Q SNP appears to account for some of the excess risk for severe HTS among Asians and Native Americans, it was not present in Blacks/African Americans, the group at highest risk for severe HTS (Table 2). Consistent with other studies (Gerstenblith et al., 2007; Rana et al., 1999), we found that the translationally silent T314T variant was the only highly prevalent MC1R SNP in individuals identifying as Black/African American (Figure 1b). 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 (Harding et al., 2000; John et al., 2003). 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 (Bell and Gabriel, 2009). Consistent with previous reports (Carrougher et al., 2013; Van Loey et al., 2008), we found that female sex, burn size, and HTS severity were significantly associated with post-burn pruritus. In
addition, we report that Asian race is independently associated with post-burn itch severity (Table 4). 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. Indeed, although MC1R R163Q was significantly associated with severe HTS, it was not associated with more severe post-burn pruritus, nor were any of the other MC1R SNPs we tested (Table 5). Our data call for further studies to confirm our findings and to identify 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 (Cordell and Clayton, 2005). We addressed this issue in two ways: we adjusted for self-identified race and we performed stratified analyses of White subjects only. Although self-identified race has been shown to correlate well with ancient genetic ancestry (Tang et al., 2005), the imprecision of racial designations may result in some degree of residual confounding. Since Whites have different degrees of genetic admixture, neither the adjusted nor the stratified analyses can account for substructure present in this group. Thus, 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 R163Q variant and HTS severity may not be due to a direct relationship between this SNP and HTS, but could instead reflect an association with any genetic locus in linkage disequilibrium with the R163Q locus. Hence, experimental studies will be required to ascertain the biological mechanism underlying the detected association. Finally, we have studied only 8 of the over 80 known MC1R variants, focusing our analysis on relatively common SNPs that are known to alter MC1R function. Further studies will be required to examine other MC1R SNPs of interest.

In summary, our findings suggest that Asian, Black, and Native American race are independently associated with risk of developing severe HTS, and Asian race is associated with increased severity of post-burn pruritus. In addition, the MC1R SNP R163Q is associated with increased severity of post-burn HTS. This SNP may cause a dysfunctional inflammatory and fibrogenic response that lead to increased scarring after burn injury. Our findings warrant replication in an independent clinical cohort, and additional studies are required to elucidate the biological mechanism linking MC1R R163Q to post-burn HTS as well as identify additional genetic variants conferring risk for post-burn HTS and pruritus.

Materials and 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. The study was conducted according to Declaration of Helsinki protocols, and all participants provided written, informed consent. 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 was recorded separately from ethnicity in accordance with National Institutes of Health guidelines (NIH, 2001). 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) (Baryza and Baryza, 1995), 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 self-identified race and MC1R genotype. We did not examine ethnicity as an exposure of interest since it is a social/cultural rather than geographic designation (NIH, 2001; Risch et al., 2002) and the objective of our study was to consider genetic influences on HTS. Our primary outcome was severe HTS, which we defined as total VSS score >7 at any follow-up, consistent with our previous approach (Thompson et al., 2013). 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**

Genomic DNA was isolated from venous whole blood using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA) followed by 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 1a), including 7 of the most extensively characterized loss-of-function (Beaumont et al., 2005; Doyle et al., 2012; Newton et al., 2005; Ringholm et al., 2004) 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 (Gerstenblith et al., 2007; Rana et al., 1999), a population thought to be at increased risk of HTS (Bombaro et al., 2003; Deitch et al., 1983).

**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). We tested for differences in scar outcomes between early and late follow-up visits 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.

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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 racial groups 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. We chose Poisson rather than logistic regression anticipating that severe HTS would be common (Thompson et al., 2013), in which case estimated odds ratios would fail to approximate prevalence ratios.

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 with robust standard errors including age, sex, percent total body surface area (%TBSA) burned, and race (to adjust for confounding by population structure) 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. We did not adjust our regression analyses for ethnicity (Hispanic vs. non-Hispanic) because Hispanics are a highly admixed population, with individuals having varying proportions of European, Native American, and African ancestry. Accordingly, ethnic designation has been shown to be uninformative about population genetic structure beyond race (Stephens et al., 2001). To test for effect modification, we fit a second regression model including a multiplicative interaction between SNP genotype and race in addition to all the variables included in the original model. Haplotypes were inferred from our unphased genotype data using the expectation-maximization algorithm (Long et al., 1995). Haplotypes with frequency >5% were tested for association with HTS severity using multivariate Poisson regression with cluster-robust standard errors, with adjustment for HTS risk factors and race as above. To further address potential confounding by population structure, all genetic association analyses were repeated using data from the White subjects only.

In a secondary, exploratory analysis of risk factors for post-burn pruritus, univariate analysis of itch was performed using one-way analysis of variance. 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 a covariate, as it has previously been associated with post-burn pruritus (Carrougher et al., 2013), and we were interested in the effect of race on itch beyond its effect on HTS severity. Haplotypes with frequency >5% were tested for association with itch score using multivariate linear regression with adjustment for race and known risk factors for post-burn pruritus.
pruritus including HTS severity. In linear regression analyses, statistical inference was based on partial F tests of regression-coefficient ($\beta$) estimates.

In Poisson 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 linkage disequilibrium analyses were performed using the Haplo Stats and LDheatmap packages, respectively, in R version 3.0.2; all other analyses were performed in Stata 13.0 (StataCorp, College Station, TX). Statistical significance was based on a type-I error rate of 0.05. In regression models containing multiple SNPs or haplotypes, the $P$-value significance threshold was adjusted by Bonferroni correction to account for multiple testing.

**Study power**

Assuming a 50% prevalence of severe HTS, a SNP MAF of 0.10, and an additive genetic model, approximately 372 subjects would be required to achieve 80% power to detect prevalence ratios of 1.5 and 2.0 for heterozygotes and rare homozygotes, respectively, at a type I error rate of 0.01. 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 (Purcell et al., 2003).

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Location and prevalence of the MC1R variants studied. (a) 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 modified from Ringholm et al. (2004) with permission. (b) Overall and race-specific frequencies of seven MC1R SNPs are illustrated in a heat map to highlight variations in SNP frequencies across racial groups. The numbers of subjects in the racial subgroups do not sum to 425 due to missing or unknown race for 12 subjects. MAF, minor allele frequency.
Table 1

Summary\(^1\) of 425 subjects.

|                  |       |
|------------------|-------|
| Age\(^2\) (years)| 40    |
| Sex              |       |
| Male             | 298   |
| Female           | 127   |
| Ethnicity\(^3\)  |       |
| Non-Hispanic     | 362   |
| Hispanic         | 51    |
| Race\(^3\)       |       |
| White            | 327   |
| Asian            | 23    |
| Black/AA         | 15    |
| Native American  | 9     |
| Other/multiple   | 39    |
| Burn size\(^2\) (%TBSA) | 7  |
| Number of operations |       |
| 0                | 152   |
| ≥1               | 273   |

\(^1\) Data presented as number (%), except where indicated.

\(^2\) Reported as median (interquartile range).

\(^3\) 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 = 413).

| Factor            | PR<sub>adj</sub> | 95% CI      | P    |
|-------------------|-----------------|-------------|------|
| Age               | 0.97<sup>1</sup> | 0.91-1.03   | 0.338|
| Female sex        | 0.96            | 0.78-1.19   | 0.711|
| Burn size         | 1.10<sup>2</sup> | 1.03-1.16   | 0.003|
| ≥1 Operation      | 1.33            | 1.05-1.70   | 0.020|
| Race (Ref: White) |                 |             |      |
| Asian             | 1.54            | 1.13-2.10   | 0.006|
| Black/AA          | 1.86            | 1.42-2.45   | <0.001|
| Native American   | 1.87            | 1.48-2.35   | <0.001|
| Other/multiple    | 1.18            | 0.87-1.60   | 0.298|

<sup>1</sup> For 10 additional years of age.
<sup>2</sup> For an additional 10% total body surface area burned.

Bold indicates statistical significance (P<0.05).

PR<sub>adj</sub>: adjusted prevalence ratio; AA, African American; Ref, referent category.
Association between MC1R SNPs and risk of severe HTS.

| MC1R SNP | \( PR_{\text{all}}^1 \) | 95% CI  | \( P \) | \( PR_{\text{adj}}^2 \) | 95% CI  | \( P \) |
|----------|-----------------|---------|------|-----------------|---------|------|
| V60L     | 1.00            | 0.76-1.31 | 0.995 | 1.04            | 0.79-1.38 | 0.778 |
| V92M     | 1.08            | 0.75-1.55 | 0.681 | 0.78            | 0.47-1.30 | 0.340 |
| R151C    | 1.29            | 1.01-1.64 | 0.042 | 1.25            | 0.95-1.66 | 0.115 |
| R163Q    | 1.35            | 1.14-1.53 | <0.001 | 1.46            | 1.20-1.78 | <0.001 |
| T314T    | 1.12            | 0.84-1.50 | 0.440 | 1.45            | 0.94-2.24 | 0.095 |

1 Adjusted prevalence-ratio associated with each additional copy of the rare allele, estimated using data from all study subjects, and adjusted for age, sex, burn size, number of operations, and race.

2 Adjusted prevalence-ratio associated with each additional copy of the rare allele, estimated using data from White subjects only, and adjusted for age, sex, burn size, and number of operations.

Bold indicates statistical significance after accounting for multiple testing in each model (\( P<0.01 \)).
Table 4
Clinical and demographic factors independently associated with severity of post-burn pruritus (N = 412).

|                  | β  | 95% CI      | P    |
|------------------|----|-------------|------|
| Age              | −0.09<sup>2</sup> | −0.27-0.09 | 0.337|
| Female sex       | 0.90 | 0.29-1.51   | 0.004|
| Burn size        | 0.28<sup>3</sup> | 0.06-0.50  | 0.012|
| ≥2 Operation     | 0.29 | −0.30-0.89  | 0.330|
| Severe HTS       | 1.54 | 0.97-2.12   | <0.001|
| Race (Ref: White) |     |             |      |
| Asian            | 1.32 | 0.23-2.40   | 0.017|
| Black/AA         | 0.90 | −0.67-2.47  | 0.261|
| Native American  | −0.27 | −1.69-1.16 | 0.711|
| Other/multiple   | 0.75 | −0.23-1.74  | 0.132|

<sup>1</sup>Linear regression coefficient, representing the difference in mean itch score associated with each factor.

<sup>2</sup>For 10 additional years of age.

<sup>3</sup>For an additional 10% total body surface area burned.

**Bold** indicates statistical significance (P<0.05).

AA, African American; Ref, referent category.
Association between MC1R SNPs and severity of post-burn pruritus.

| MC1R SNP | $\beta^1$  | 95% CI    | P    | $\beta^2$  | 95% CI    | P    |
|----------|-------------|-----------|------|-------------|-----------|------|
| V60L     | -0.18       | -0.82-0.46| 0.583| -0.13       | -0.81-0.56| 0.718|
| V92M     | 0.28        | -0.91-1.48| 0.645| 0.22        | -1.67-2.10| 0.822|
| R151C    | 0.09        | -0.65-0.83| 0.810| 0.26        | -0.52-1.04| 0.515|
| R163Q    | 0.55        | -0.07-1.18| 0.084| 0.57        | -0.17-1.32| 0.130|
| T314T    | -1.18       | -2.23-0.12| 0.029| -1.36       | -3.13-0.41| 0.131|

$^1$ Linear regression coefficient estimated using data from all study subjects, representing the difference in mean itch score associated with each additional copy of the rare allele, and adjusted for age, sex, burn size, number of operations, race, and HTS severity.

$^2$ Linear regression coefficient estimated using data from White subjects, representing the difference in mean itch score associated with each additional copy of the rare allele, and adjusted for age, sex, burn size, number of operations, and HTS severity.