Published in final edited form as:

J Surg Res. 2018 November ; 231: 448–452. doi:10.1016/j.jss.2018.07.018.

MC1R gene polymorphisms are associated with dysfunctional immune responses and wound infection after burn injury

Damien W. Carter, MD^{a,*}, Ravi F. Sood, MD, MS^b, Max E. Seaton, MD^c, Lara A. Muffley, BS^b, Shari Honari, RN, BSN^b, Ann M. Hocking, PhD^b, Saman A. Arbabi, MD, MPH^b, and Nicole S. Gibran, MD, FACS^b

^aDepartment of Surgery, Maine Medical Center, Tufts University School of Medicine, Portland, Maine ^bDepartment of Surgery, University of Washington, Washington ^cDepartment of Surgery, University of Maryland, College Park, Maryland

Abstract

Background: Systemic inflammatory response syndrome (SIRS) is associated with organ failure and infectious complications after major burn injury. Recent evidence has linked melanocortin signaling to anti-inflammatory and wound-repair functions, with mutations in the melanocortin 1 receptor (MC1R) gene leading to increased inflammatory responses. Our group has previously demonstrated that MC1R gene polymorphisms are associated with postburn hypertrophic scarring. Thus, we hypothesized that MC1R single nucleotide polymorphisms (SNPs) would be associated with increased burn-induced SIRS and increased infectious complications.

Methods: We performed a retrospective cohort study of adults (>18 y of age) admitted to our burn center with >20% total body surface area (TBSA) partial/full thickness burns between 2006 and 2013. We screened for five MC1R SNPs (V60L, V92M, R151C, R163Q, T314T) by polymerase chain reaction from genomic DNA isolated from blood samples. We performed a detailed review of each patient chart to identify age, sex, race, ethnicity, %TBSA burned, burn wound infections (BWIs), and 72-hr intravenous fluid volume, the latter a surrogate for a dysfunctional inflammatory response to injury. Association testing was based on multivariable regression.

Results: Of 106 subjects enrolled, 82 had complete data for analysis. Of these, 64 (78%) were male, with a median age of 39 and median burn size of 30% TBSA. A total of 36 (44%) subjects developed BWIs. The median total administered IV crystalloid in first 72h was 24.6 L. In multivariate analysis, the R151C variant allele was a significant independent risk factor for BWI (adjusted prevalence ratio 2.03; 95% CI: 1.21–3.39; $P = 0.007$), and the V60L variant allele was independently associated with increased resuscitation fluid volume ($P = 0.021$).

Conclusions: This is the first study to demonstrate a significant association between genetic polymorphisms and a nonfatal burn-induced SIRS complication. Our findings suggest that MC1R

*Corresponding author: Department of Surgery, Maine Medical Center Burn Service, Tufts University School of Medicine, Portland, ME 04102. Tel.: +1 2076617078, fax: +1 2077740459., dcarter@mmc.org, penecillamine@gmail.com (D.W. Carter).

Disclosure

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in this article.

polymorphisms contribute to dysfunctional responses to burn injury that may predict infectious and inflammatory complications.

Introduction

The American Burn Association (http://www.ameriburn.org/resources_factsheet.php) estimates that in the US over 1,000,000 burns occur each year with 486,000 patients with thermal injuries seeking medical care and 40,000 hospitalized. Approximately 4000 burn patients ultimately succumb to their injury.¹ Three of 4 deaths are attributable to inhalation injury and/or a dysfunctional immune response that leads to inflammatory or infectious complications.² Severe burn injury is often coupled with a profound systemic inflammatory response syndrome (SIRS). Although burn-induced SIRS is an anticipated response to injury, an overstimulated inflammatory system may lead to organ failure and infectious complications.³ Similarly, certain genetically determined aspects of the immune response may be protective, resulting in reduced infectious complications and organ dysfunction.

Melanocortin peptides such as adrenocorticotrophic hormone and melanocyte-stimulating hormone (MSH) are secreted by the anterior pituitary gland in response to injury.⁴ Recently, melanocortin signaling has been found to be an important component of the inflammatory response in burn wounds.⁴ Specifically, α -MSH—a ligand that interacts with melanocortin-1 receptor (MC1R) is ubiquitous in the inflammatory milieu of burn-injured skin.⁴ Convincing evidence has linked melanocortin signaling to anti-inflammatory and wound-repair functions.⁵ Our group has previously demonstrated that MC1R gene polymorphisms are associated with postburn hypertrophic scar severity.⁶ Though melanocortin signaling after burn injury has been shown to have a primarily anti-inflammatory function, certain polymorphisms in the MC1R gene may lead to dysfunctional responses after a severe burn. Specifically, certain mutations in the MC1R gene have been shown to increase the duration of the inflammatory state in animal models.^{7,8}

Based on the growing body of evidence demonstrating an important role for melanocortin signaling in the inflammatory response after burn injury, we hypothesized that MC1R single nucleotide polymorphisms (SNPs) would be associated with increased burn-induced SIRS and infectious complications.

Methods

Study design and data collection

Following approval from the University of Washington Institutional Review Board, we conducted a retrospective candidate-gene association study in subjects already enrolled in a prospective cohort study of hypertrophic scarring (HTS). We had prospectively enrolled adults (age ≥ 18) admitted to the UW Medicine Regional Burn Center between 2007 and 2013 with deep burns, placing them at increased risk of HTS. At the time of enrollment, each subject provided a whole blood sample for genotyping and age, sex, race, ethnicity, and burn size were prospectively obtained from the electronic medical record with additional IRB approval. The outcomes of interest in the present study (development of burn wound infection (BWI) and volume of fluid resuscitation within the first 72 h postinjury) were

ascertained retrospectively by electronic health record review for subjects with a burn size 20% total body surface area (TBSA). BWI was confirmed by the presence of a wound culture result with $> 10^5$ cfu/high power field and/ or $>30\%$ graft loss in a particular site as confirmed by an attending surgeon note in the patient chart.

SNP genotyping

Our primary exposures of interest were MC1R SNP genotypes, which had been determined previously in a study done by our group concerning hypertrophic scar formation.⁶ Subjects provided 2 mL of venous whole blood, and genomic DNA was isolated using a QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands) and quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). Samples were genotyped for eight MC1R SNPs using 20 ng DNA and predesigned TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA) in 384-well plates with a Viia7 instrument (Applied Biosystems, Foster City, CA) per manufacturer guidelines. Of the MC1R SNPs studied, seven were non synonymous⁹ missense variants: rs1805005 (V60L), rs2228479 (V92M), rs1805007 (R151C), rs1110400 (I155T), rs885479 (R163Q), rs3212366 (F196L), and rs1805009 (D294H). One was a synonymous variant rs2228478 (T314T) whose effect on MC1R function is unknown (Figure 1).

Statistical analysis

Subject characteristics are summarized as median (interquartile range [IQR]) for continuous variables and number (percent) for categorical variables (Table 1). Rare SNPs (minor allele frequencies [MAF] $<5\%$) were excluded from association testing. Testing for Hardy-Weinberg equilibrium was performed using the chi-square test. To test for an association between MC1R SNPs and BWI, for each SNP we fit a multivariate Poisson regression model with an indicator of burn wound infection as the dependent variable and age, sex, TBSA burned, race, and ethnicity as adjustment covariates. MC1R SNP genotype variables were coded as 0, 1, or 2 (for the number of variant alleles present) and modeled as continuous, assuming an additive model of inheritance. In Poisson regression analyses, statistical inference was based on Wald tests of regression coefficient estimates, and exponentiated coefficients are reported as risk ratios with their 95% confidence intervals.

In our second analysis, we used multivariate linear regression with robust standard errors to model resuscitation fluid volume, the continuous dependent variable. Regression models were otherwise fit as described above for modeling of BWI, with statistical inference based on partial F tests of regression coefficient estimates. All analyses were performed in Stata 13.0 (StataCorp, College Station, TX). Statistical significance was based on Bonferroni correction for multiple testing with a family-wise error rate of 0.05.

Results

Demographic characteristics

A total of 106 subjects met inclusion criteria. Eighty-two had complete clinical data for analysis. Of these (Table 1), 64 (78%) were male, with a median age of 39 (SD = 13.7, range: 18–74) and median %TBSA burn of 30% (SD = 12.6, range: 20%–65%). A total of

36 (44%) of subjects developed BWI. The median total IV crystalloid (IVF) volume administered 72 h following injury was 24.6L. The total IVF volume at 72 h was used as a surrogate for severity of systemic inflammatory response. SNP MAFs were V60L: 9.1%, V92M: 9.6%, R151C: 7.3%, I155T: 0.9%, R163Q: 12.5%, F196L: 0.0% (No subjects had any copies of this SNP), D294H: 1.1%, T314T: 12.9%. All SNPs were in Hardy-Weinberg equilibrium ($P > 0.30$ for all SNPs). We analyzed only those SNPs with MAF $> 5\%$.

As anticipated, chart review for significant comorbidities yielded minimal comorbid disease in this population (Table 2). In addition, there were very few elderly subjects in our cohort. A notable finding was approximately 15% of subjects had mental diseases (schizophrenia, bipolar disorder, anxiety d/o and severe depression). This finding is consistent with other demographic studies of burn patients.¹⁰ There were 22 complications other than BWI noted in the cohort: seven patients with acute respiratory distress syndrome and/or pneumonia, sepsis with confirmed bacteremia in seven subjects, 2 subjects with *C. difficile* infection, and six other (two “Integra infections”, two lower extremity deep venous thrombosis, and two sacral decubitus ulcers). These complications were not analyzed in the cohort due to their low overall incidence.

R151C polymorphism is a significant independent risk factor for BWI—After adjustment for age, sex, race/ethnicity, and burn size, the R151C polymorphism was a significant independent risk factor for BWI (adjusted prevalence ratio 2.03; 95% CI: 1.21–3.39; $P = 0.007$), using a P value threshold of 0.01 (0.05/5 SNPs tested) to define significance (Table 3).

The V60L polymorphism is independently associated with increased resuscitation fluid volume—As shown in Table 3, using a linear regression model, the V60L polymorphism was found to be associated with increased resuscitation fluid volume administration ($P = 0.021$), although this association did not quite reach statistical significance after correcting for multiple testing (significance threshold $P < 0.01$). IVF volume was used as a surrogate for inflammatory response to injury as there was minimal organ failure (by Denver and SOFA scores) in the cohort (Table 4).

Discussion

A dysfunctional inflammatory response to burn injury has been shown in previous work by others to lead to SIRS-related inflammatory complications—such as acute respiratory distress syndrome and acute kidney injury—as well as infectious complications.^{3,11} Recent evidence has implicated an important role for melanocortin signaling in chronic inflammation in the burn wound that is associated with HTS.⁶ Others have demonstrated that the melanocortin signaling pathway is responsible for a number of acute anti-inflammatory functions.^{5,6} Previous work done by our group on the same cohort of patients described above demonstrated a strong association between certain MC1R polymorphisms and severity of HTS.⁶ This association in burn scarring leads us to the question of whether or not certain genetic polymorphisms also have a deleterious effect on the compensatory anti-inflammatory response system (CARS).

In this article, we have demonstrated that there is a significant association between the R151C MC1R variant allele and clinically observed BWI. Likewise, we appear to have demonstrated a link between exuberant inflammatory response to burn injury and the V60L variant allele for the melanocortin 1 receptor.

Briefly, the melanocortin signaling system begins in the central nervous system. The melanocortin peptides are derivative products of proopiomelanocortin (POMC). The POMC gene is primarily expressed by the pituitary, arcuate nucleus of the hypothalamus, and nucleus of the solitary tract in the brain stem.³ This prohormone gives rise to β -endorphins, as well as the MSH variants.¹²

α -MSH interacting with the MC1R is a well-described signaling pathway in this system. MC1R is expressed by cutaneous melanocytes, where it has a key role in determining skin and hair pigmentation.¹³ However, other cell types in the skin also express MC1R, including keratinocytes, fibroblasts, endothelial cells, and antigen-presenting cells.^{5,14} In this respect, MC1R is expressed by leukocytes and macrophages where it mediates the anti-inflammatory and immunomodulatory properties of melanocortins.¹⁵ This anti-inflammatory signaling pathway has been noted to occur within areas of burn injured skin.⁶

Although this is a preliminary study in a small cohort of patients, we believe the “signal” cannot be ignored. The genetics of inflammatory signaling are increasingly important in our understanding of how to potentially alter dysfunctional responses to achieve improved outcomes. Specifically, this type of inquiry may lead to improved targeting of immunomodulatory therapies to a cohort of patients most likely to benefit from treatment.¹⁶ In continuing to unwrap the genetic intricacies of these proinflammatory and anti-inflammatory pathways, we may be able to further improve outcomes in the small number of severely burn-injured patients who do not respond to our current standards of resuscitation therapy.

This is a preliminary study using a small cohort of patients and thus has some limitations. First, our small number of patients limits the precision of our analysis. Nonetheless, the fact that we identified borderline significant associations with two polymorphisms are encouraging and suggest that further study in this area is warranted. Second, there was a small number of ethnic and racial minorities in our cohort. This can lead to spurious false-positive associations due to confounding by population substructure.^{17,18} We attempted to address this by adjusting our analyses for race/ethnicity; but given the small numbers of racial/ethnic minorities, some degree of residual confounding likely affects our analysis. Third, the use of resuscitation intravenous fluid volumes at 72 h has never been reported as a surrogate for systemic inflammation. We had initially intended to use a standardized multiorgan failure score to measure dysfunctional immune response in our cohort but found that very few patients developed significant organ failure. We believe this is a testament to advances in modern burn care. Also, because most patients were enrolled in the study after discharge from the hospital, this was likely a “healthier” group of large burns compared with patients who died during hospitalization. However, as expected, IVF volumes did increase with age and burn size and we did find an association between one MC1R SNP and resuscitation volume using a linear regression model. Fourth, definition of BWI is imprecise

and often subjective particularly in assessment of graft loss. Where possible, we confirmed BWI by quantitative cultures. However, several patients who had what appeared to be wound infections clinically were never subjected to a quantitative culture. In these cases, we gleaned from the charts whether or not a BWI was diagnosed by the attending physician. The subjectivity of this analysis is a clear weakness of this study.

This is the first study to demonstrate a significant association between genetic polymorphisms and a burn-induced SIRS complication. Our findings suggest that MC1R polymorphisms may lead to dysfunctional responses to burn injury that may predict infectious and inflammatory complications. Understanding the mechanisms linking these genetic polymorphisms to increased inflammation and infection may lead to the development of novel therapies to improve clinical outcomes after severe burn injury.

REFERENCES

1. Blaisdell LL, Chace R, Hallagan LD, Clark DE. A half-century of burn epidemiology and burn care in a rural state. *J Burn Care Res* 2012;33:347–353. [PubMed: 22002206]
2. Herndon DN, Blakeley PE. Teamwork for total burn care: achievements, directions, and hopes. In: Herndon DN, ed. *Total Burn Care* Philadelphia, PA: Saunders; 2007:9–13; 3.
3. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. *Clin Microbiol Rev* 2006;19:403–434. [PubMed: 16614255]
4. Carter D, Warsen A, Mandell K, Cuschieri J, Maier R, Arbabi S. Delayed topical p38 MAPK inhibition attenuates full thickness burn wound inflammatory signaling. *J Burn Care Res* 2014;35:e83–e92. [PubMed: 23666384]
5. Muffley LA, Zhu KQ, et al. Spatial and temporal localization of the melanocortin 1 receptor and its ligand α -melanocyte-stimulating hormone during cutaneous wound repair. *J Histochem Cytochem* 2011;59:278–288. [PubMed: 21378282]
6. Auriemma M, Brzoska T, Klenner L, et al. α -MSH-stimulated tolerogenic dendritic cells induce functional regulatory T cells and ameliorate ongoing skin inflammation. *J Invest Dermatol* 2012;132:1814–1824.
7. Sood RF, Hocking AM, et al. Race and melanocortin 1 receptor polymorphism R163Q are associated with post-burn hypertrophic scarring: a prospective cohort study. *J Invest Dermatol* 2015;135:2394–2401. [PubMed: 26030184]
8. Doyle JR, Fortin JP, et al. Selected melanocortin 1 receptor single nucleotide polymorphisms differentially alter multiple signaling pathways. *J Pharmacol Exp Ther* 2012;342:318–326. [PubMed: 22547573]
9. Herraiz C, Journé F, Abdel-Malek Z, Ghanem G, Jiménez-Cervantes C. Signaling from the human melanocortin 1 receptor to ERK1 and ERK2 mitogen-activated protein kinases involves transactivation of cKIT. *Mol Endocrinol* 2011;25:138–156. [PubMed: 21084381]
10. Chang MH, Ned RM, Hong Y, et al. Racial/ethnic variation in the association of lipid-related genetic variants with blood lipids in the US adult population. *Circulatory Cardiovasc Genet* 2011;4:523–533.
11. Palmu R, Suominen K, Vuola J, Isometsu E. Mental Disorders among acute burn patients. *Burns* 2010;36:1072–1079. [PubMed: 20483541]
12. Kang S, Tanaka T, Kishimoto T. Therapeutic uses of anti-interleukin-6 receptor antibody. *Interleukin Immunol* 2015;27:21–29.
13. Matejec R, Kayser F, Schmal F, et al. Effects of corticotropin releasing hormone on proopiomelanocortin derivatives and monocytic HLA-DR expression in patients with septic shock. *Peptides* 2013;47:133–141. [PubMed: 23891702]
14. Saternus R, Pilz S, Gräber S, et al. A closer look at evolution: variants (SNPs) of genes involved in skin pigmentation, including EXOC2, TYR, TYRP1, and DCT, are associated with 25(OH)D serum concentration. *Endocrinology* 2015;156:39–47. [PubMed: 25396269]

15. García-Borrón JC, Olivares C. Melanocortin 1 receptor and skin pathophysiology: beyond colour, much more than meets the eye. *Exp Dermatol* 2014;23:387–388. [PubMed: 24372738]
16. Cooper A, Robinson SJ, Pickard C, Jackson CL, Friedmann PS, Healy E. Alpha-melanocyte-stimulating hormone suppresses antigen-induced lymphocyte proliferation in humans independently of melanocortin 1 receptor gene status. *J Immunol* 2005;175:4806–4813. [PubMed: 16177130]
17. Zhang AQ, Zeng L, Gu W, et al. Clinical relevance of single nucleotide polymorphisms within the entire NLRP3 gene in patients with major blunt trauma. *Crit Care* 2011;15:R280. [PubMed: 22112657]
18. Sharma S, Murphy A, Howrylak J, et al. The impact of self-identified race on epidemiologic studies of gene expression. *Genet Epidemiol* 2011;35:93–101. [PubMed: 21254216]

MC1R Molecule and corresponding SNP amino acid composition

Fig 1 –.
MC1R Molecule and corresponding SNP amino acid composition. (Color version of figure is available online.)

Table 1 –

Study population.

N	82
Age, y (SD) range	39.6 (13.7) 18–74
Sex, <i>n</i> (%)	
Male	64 (78.0)
Female	18 (21.9)
Race/Ethnicity, <i>n</i> (%)	
White/Caucasian	64 (78.0)
Hispanic/Latino	6 (7.3)
Asian	3 (3.7)
AA or black	2 (2.4)
Other/Multiracial	8 (9.8)
Burn wound infection	36 (43.9)
Infectious complication(s)	45 (54.9)
Pulmonary complication	7 (8.5)
No complication	39 (47.6)

AA = African American.

Table 2 –

Comorbidities.

System	18–35 y	36–55 y	56–74 y
N	22	49	11
Hypertension, <i>n</i> (%)	1 (4.5)	5 (10.2)	2 (18.2)
Diabetes, <i>n</i> (%)	2 (9.1)	1 (2.0)	0 (0.0)
Pulmonary, <i>n</i> (%)	2 (9.1)	5 (10.2)	2 (18.2)
Cardiovascular, <i>n</i> (%)	2 (9.1)	3 (6.1)	1 (9.1)
Psychiatric	1 (4.5)	10 (20.4) *	1 (9.1) *
Neurological	2 (9.1) *	4 (8.2)	1 (9.1)

Pulmonary: asthma/chronic obstructive pulmonary disease/ obstructive sleep apnea; Cardiovascular: coronary artery disease/ myocardial infarction/ cerebrovascular accident/venous thrombo-embolism; Psychiatric: depression, bipolar, schizophrenia, schizo-affective, conversion, PTSD; Neurological: seizures, paraplegia, cerebral palsy, Alzheimer's, and multiple sclerosis.

* Patients may have more than one comorbidity.

Table 3 –

Bum wound infection and MC1R SNP data.

MC1R SNP's	RR	95% CI	P
V60L	0.82	0.46–1.45	0.488
V92M	1.06	0.46–2.40	0.896
R151C	2.03	1.21–3.39	0.007*
R163Q	1.06	0.72–1.58	0.757
T314T	1.38	0.69–2.76	0.366

Poisson regression model with burn wound infection as outcome and SNP as exposure.

Adjustment covariates: age, sex, burn size, race, ethnicity.

Bold signifies the SNP association with Burn wound infection.

* P-value 0.05 or 95% confidence interval does not cross 1.

Table 4 –

72h volume of resuscitation and Mc1R.

MC1R SNP's	Coefficient	95% CI	P
V60L	6052	942 to 11,163	0.021 *
V92M	321	−6821 to 7463	0.929
R151C	−370	−6920 to 6179	0.911
R163Q	−2638	−9934 to 4659	0.473
T314T	688	−5105 to 6480	0.814

Linear regression model with 72 h IVF as outcome and SNP as exposure. Adjustment covariates: age, sex, burn size, race, ethnicity, Bold signifies the SNP association with Burn wound infection.

* P-value 0.05 or 95% confidence interval does not cross 1.