HHS Public Access

Author manuscript

J Immunotoxicol. Author manuscript; available in PMC 2017 February 02.

Published in final edited form as:

J Immunotoxicol. 2016 September ; 13(5): 738–744. doi:10.3109/1547691X.2016.1173135.

Association of MHC region SNPs with irritant susceptibility in healthcare workers

Berran Yucesoy[a], **Yerkebulan Talzhanov**[b], **M. Michael Barmada**[b], **Victor J. Johnson**[c], **Michael L. Kashon**[a], **Elma Baron**[d], **Nevin W. Wilson**[e], **Bonnie Frye**[a], **Wei Wang**[a], **Kara Fluharty**[a], **Rola Gharib**[f], **Jean Meade**[g], **Dori Germolec**[h], **Michael I. Luster**[i], and **Susan Nedorost**[d]

[a]Health Effects Laboratory Division, CDC/NIOSH, Morgantown, WV, USA

[b]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

[c]BRT-Burleson Research Technologies, Morrisville, NC, USA

[d]University Hospitals Case Medical Center, Case Western Reserve University, Cleveland, OH, USA

[e]Department of Pediatrics, School of Medicine, University of Nevada, Reno, NV, USA

[f]Department of Dermatology, School of Medicine, West Virginia University, Morgantown, WV, USA

[g]Office of Director, CDC/NIOSH, Morgantown, WV, USA

[h]Toxicology Branch, DNTP/NIEHS, Research Triangle Park, NC, USA

[i]School of Public Health, West Virginia University, Morgantown, WV, USA

Abstract

Irritant contact dermatitis is the most common work-related skin disease, especially affecting workers in “wet-work” occupations. This study was conducted to investigate the association between single nucleotide polymorphisms (SNPs) within the major histocompatibility complex (MHC) and skin irritant response in a group of healthcare workers. 585 volunteer healthcare workers were genotyped for MHC SNPs and patch tested with three different irritants: sodium lauryl sulfate (SLS), sodium hydroxide (NaOH) and benzalkonium chloride (BKC). Genotyping was performed using Illumina Goldengate MHC panels. A number of SNPs within the MHC Class I (OR2B3, TRIM31, TRIM10, TRIM40 and IER3), Class II (HLA-DPA1, HLA-DPB1) and Class III (C2) genes were associated (p <0.001) with skin response to tested irritants in different genetic models. Linkage disequilibrium patterns and functional annotations identified two SNPs in the *TRIM40* (*rs1573298)* and *HLA-DPB1* (*rs9277554)* genes, with a potential impact on gene regulation. In addition, SNPs in *PSMB9* (rs10046277 and *ITPR3* (rs499384) were associated with

CONTACT: Dr. Berran Yucesoy, berranyucesoy@gmail.com, formerly of the Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown WV, USA.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.
The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

hand dermatitis. The results are of interest as they demonstrate that genetic variations in inflammation-related genes within the MHC can influence chemical-induced skin irritation and may explain the connection between inflamed skin and propensity to subsequent allergic contact sensitization.

Keywords

Genetics; healthcare workers; irritant contact dermatitis; MHC

Introduction

Irritant contact dermatitis (ICD) is an innate immune reaction elicited most frequently by wet/dry cycles, but also exacerbated by direct contact with the skin from detergents, oils, strong acids and bases and volatile chemicals. It is the most common occupational skin disease due to wet work in the setting of low ambient indoor humidity and responsible for ~80% of all cases of occupational contact dermatitis (Meding & Swanbeck 1987; Lushniak 1995). A high prevalence has been documented in specific industries such as healthcare, metal-working, hairdressing, agriculture and food preparation (Tupker 2003; Jungbauer et al. 2004; NIOSH 2012). A 1-year prevalence of hand dermatitis was reported to be between 9.7% and 11.8% for the general population, whereas a higher prevalence (17–30%) was reported for healthcare workers (Smit et al. 1993; Meding & Jarvholm 2002; Luk et al. 2011; Ibler et al. 2012).

ICD creates danger signal cytokines that predispose the same individual to allergic contact dermatitis (ACD) (Matzinger 2002). Like ACD, a wide range of inter-individual variation exists in the development and expression of ICD, independent of the atopic status (Basketter et al. 1998; Magina et al. 2003). Mechanisms underlying these differences in susceptibility are not fully understood although extrinsic (e.g. climate) as well as intrinsic factors (e.g. reparative capacity of the skin, genetics) are thought to influence development and severity (Tupker 2003). A strong role for genetics was first demonstrated in family studies which indicated that differences in susceptibility can be inherited (Bryld et al. 2000). Given the role of inflammation in the disease process, genetic polymorphisms in several cytokine genes, including tumor necrosis factor-α (*TNFα*), interleukin-1α (*IL-1α*), *IL-1β, IL-8, IL-10*, have been examined. Among those, only the *TNFα* rs1800629 promoter SNP was found to be associated with susceptibility to experimentally induced ICD (Allen et al. 2000; de Jongh et al. 2008; Davis et al. 2010; Landeck et al. 2012). However, it should be noted that the genetic susceptibility to ICD is largely unexplored.

The MHC spans nearly 4 Mb and encodes more than 180 highly polymorphic genes, many of which influence immune regulation and susceptibility to complex diseases. HLA class I molecules are important in the regulation of inflammatory responses, whereas HLA class II molecules play a role in the activation of the T cells recognizing the HLA–peptide complex. Although the HLA complex is one of the most extensively studied regions in the human genome, it has not yet been investigated with regard to ICD development. In addition to genes in the HLA complex, several functionally important genes are located in this region,

including the *TNFα, TNFβ* and *TAP* (transporter associated with antigen processing) genes. We previously showed that a low irritancy threshold to sodium lauryl sulfate (SLS), but not to sodium hydroxide (NaOH) or benzalkonium chloride (BKC) – combined with frequent hand washing and winter season – correlated with irritant hand dermatitis in the subset of patients from one study site in this study cohort of healthcare workers exposed to water and detergent hazards (Callahan et al. 2013). Previous literature also suggested that the immune response varied with different irritants (Willis et al. 1993).

Based on the role of MHC genes in the regulation of immune and inflammatory responses, this study sought to investigate the role of genetic variation within this region in skin irritation response to various types of irritants.

Materials and methods

Study population

The study population consisted of 585 healthcare workers (nurses, physicians and technicians) from the two participating University Hospitals (Case Medical Center and West Virginia University Hospitals). Volunteers with no history of psoriasis and or inflammatory skin disease requiring medical attention and capable of giving informed consent were recruited for the study. A history of or current mild irritant hand dermatitis or intermittent chapped hands were not an exclusion criterion. Subjects who were pregnant, using immunosuppressive, immunomodulatory or anti-inflammatory medications, receiving ultraviolet therapy or tanning salon usage were excluded. The volunteers' current skin condition was classified at each study visit by a dermatologist based on objective skin symptoms as mild, moderate or severe hand dermatitis. Moderate or severe dermatitis is characterized by erythema, papules, vesicles, fissures, exhibiting a clear eczematous picture. Mild dermatitis is exhibited as erythema, slight chapping and scaling of the skin. Information on participants' health status (e.g. asthma, dermatitis/eczema, seasonal allergies and family history of dermatitis) and skin exposure history (e.g. the number of daily hand washing and use of soap or hand cleanser) were collected by questionnaire. Among participants, 22.9% had hand dermatitis at any study visit; however, none of them was severe. Blood samples were collected for genetic analysis. All study procedures were approved by the Institutional Review Boards of the participating institutions.

Genotyping

Whole blood samples were collected for genetic analysis and genomic DNA was extracted using the QIAamp blood kit (QIAGEN Inc., Chatsworth, CA). Genotyping was performed according to the standard protocol provided by Illumina using the MHC Panel Set and Golden Gate protocol (Illumina Inc., San Diego, CA). Three MHC oligonucleotide pools, MHC Mapping Panel, MHC Exon-Centric Panel and MHC Panel combining both mapping and exon-centric panels, were used. Each panel covered 1228, 1293 and 2400 SNPs, respectively. Independent loci covered in the MHC panels are spaced at an average of 2.08 kb (range: 0.005–71.05 kb). Genotyping was performed in a 16-well format using universal BeadChips (Illumina Inc., San Diego, CA). A total of 250 ng to 1 μg DNA was used for each assay depending on the source. Genotypes were auto called and manually revised using

GenomeStudio software (Illumina Inc., San Diego, CA). The genotype confidence score of the assay was set to 0.25 in GenomeStudio Genotyping module.

Irritants, patch testing and transepidermal water loss

SLS (99% pure), NaOH and BKC (all from Sigma, St. Louis, MO) were employed as model irritants. Aqueous solutions of SLS at concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10 and 20%, NaOH at 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0%, and BKC at 0.1, 0.5, 1.0 and 2.5% were applied in 0.2 ml volumes to 5 mm Finn Chambers (Allerderm, Petaluma, CA) and affixed to the intact non-inflamed skin of the back with Scanpor tape. Distilled water served as a negative control. Twenty percent SLS, the minimum level classified as irritant (R38) by European Commission criteria, served as a positive control. Subjects wore the taped patches for 24 h and reactions were graded by visual assessment of the patch sites using a three-point grading scale of increasing irritation ("0" no reaction; "+" weakly positive reaction characterized by mild erythema across most of the treatment site; "++" strong positive reaction characterized by spreading erythema with edema) (Basketter et al. 1997).

The transepidermal water loss (TEWL), an indicator of the skin barrier integrity, was measured using an evaporimeter (VapoMeter SWL4, Delfin Technologies, Kuopio, Finland). Three readings (grams per hour·meter squared, g/m^2h) at each site were taken from the upper inner arm, from the back and from the side of forefinger and the means were calculated.

Study design

The study was conducted in a cross-sectional study design in two phases. Phase I was designed to determine an effective concentration range for each irritant (using concentration ranges given above) that would be used for the second phase. Forty healthcare workers were assessed in this phase. Individual differences in skin response were noted starting at concentrations of 2.5% SLS, 1% NaOH and 0.5% BKC. Based on this, the concentration range for Phase II was set as: 2.5, 5.0 and 20% SLS; 1, 2.5 and 5.0% NaOH; and 0.5, 1.0 and 2.5% BKC.

Statistical analyses

SNP-specific deviations from the Hardy–Weinberg Equilibrium were tested using chi-squared goodness-of-fit tests. Responsiveness to three concentrations of each irritant was coded as low, medium (moderate) and high. These variables were turned into binary variables by calling no and low responders as controls and moderate and high responders as cases and included in the logistic regression analysis. Alleles that were not called in a sample were coded as missing in the analysis. A threshold of 2% was used for missing rates per individual and per SNP. For each dataset, SNPs were called and filtered separately and then merged using PLINK version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink) (Purcell et al. 2007). The final dataset contained 2131 SNPs for 585 subjects. The total genotype rate for the merged dataset was 0.75.

Statistical analysis was performed using PLINK. As underlying genetic models are unknown *a priori*, several different models were tested and overall significance of test results

confirmed by exploring functional elements in linkage disequilibrium with our interesting findings. As such, we used a conservative discovery-based threshold for *p* values corresponding to a = 0.001, without any multiple testing correction, as this study is meant to be exploratory and hypothesis generating. Association between each SNP and irritant response was analyzed using three genetic models, including: a dominant model (comparing homozygous wild-type vs. variant allele-carrying genotypes), a recessive model (comparing wild-type allele-carrying vs. homozygous variant genotypes) and an additive model (cumulative effect of each additional variant allele). Logistic regression model, with adjustments for potential cofounders was used to test for differences between irritancy thresholds according to genotypes. Potential confounders were separately selected for each irritant from a larger set of measured variables using group comparison of the means between cases and controls. Any variable that had a significant difference in the means was then used in stepwise regression model to eliminate any potential confounder that did not have any influence on the outcome variable. Based on this, skin response to SLS was adjusted for gender, population (represents different recruitment sites), season (coded binary as cold vs. warm) and indoor humidity when patch test was interpreted. Skin response to NaOH was adjusted for gender, population and indoor humidity, whereas response to BKC was adjusted only for gender and population. Although age did not appear to be a significant confounder in stepwise regression model, we repeated analysis with additional adjustment for age and compared the results with those obtained by the final model.

To test the association of SNPs with the development of ICD, subjects were assigned to the case or control group based on the development of hand dermatitis during the study period. As ICD from wet work, as in our cohort of healthcare workers, usually occurs during cold months (October to March), only subjects examined during these months were included in this analysis (Callahan et al. 2013). The measured variables were individually tested for association with hand dermatitis and stepwise regression model was used to eliminate any confounder that did not have any influence on outcome. Based on this, the results were adjusted for hand washing frequency and TEWL measurement on the forefinger.

Linkage disequilibrium (LD) and haplotype blocks were assessed using default parameters in Haploview (Broad Institute, Boston, MA) (Barrett et al. 2005). Pairwise LD was calculated only for SNPs within 200 kb. RegulomeDB (http://regulome.stanford.edu) was used to annotate any SNP with known and predicted regulatory elements (Johnson et al. 2008). SNAP (Broad Institute, Boston, MA) tools were used to update annotations of interesting SNPs according to dbSNP135 and to find proxy SNPs within 500 kb based on LD and physical distance (Boyle et al. 2012).

Results

Characteristics of the study subjects

The demographics variables of the participants that were included in the analysis are described in Table 1. The main study population consisted of 585 subjects from among a larger sample of 654 healthcare workers. A total of 69 samples were excluded from the analyses due to ineligibility or incomplete information. The mean age of the population was 37 years and 478 of them were female. While 15.2% of the study population had a family

history of dermatitis or eczema, 22.9% of them had hand dermatitis at any time during the study. History of hand dermatitis requiring medical attention was an exclusion criterion for study entry to mitigate the risk of enrolling a study subject with allergic contact hand dermatitis; none of the subjects had or developed severe hand dermatitis during the study.

Association between SNP, irritancy threshold and ICD

The MHC panel allowed examination of 2131 SNPs in 158 genes within the MHC region. All genotype frequencies were in Hardy–Weinberg Equilibrium. After adjusting for confounders, a number of SNPs in Olfactory Receptor, Family 2, Subfamily B, Member 3 (*OR2B3*), Complement Component 2 (*C2*), Tripartite Motif (TRIM) (*TRIM40, TRIM31, TRIM10*), Immediate Early Response 3 (*IER3*) and, Major Histocompatibility Complex, Class II, DP Beta (*HLA-DPA1* and *HLA-DPB1*) genes were associated with skin irritation response. We reported any SNP that reached a discovery threshold level $p < 0.001$. Table 2 summarizes the associations found in three genetic models; p values that reached the same significance level after additional adjustment for age are marked in bold. The *OR2B3* (rs2050231) and *C2* (rs9332739) SNPs showed an association with responses to 5% and 20% SLS, respectively. The *IER3* (rs8512) SNP was associated with response to 2.5% NaOH, whereas *TRIM40* (rs1573298), *TRIM10* (rs1557608) and *TRIM31* (rs1264701) SNPs were associated with response to 5% NaOH under different genetic models. The *HLA-DPB1* (rs9277554, rs3117228 and rs3130188) SNPs were associated with responses to 1% BKC, whereas the *HLA-DPA1* (rs406477) SNPs were associated with responses to both 1% and 2.5% BKC. None of the other polymorphisms that were examined showed any interesting association with the irritancy response.

After adjusting for confounders, SNP (rs10046277 and rs499384) in *PSMB9* (proteasome subunit beta type 9) and *ITPR3* (Inositol 1,4,5-Trisphosphate Receptor, Type 3) genes, respectively were associated with hand dermatitis ($p < 0.001$) (Table 3). While the TEWL measurements from the back and upper inner arm did not show any association with skin irritant response, TEWL/forefinger was associated with hand dermatitis and included in the final model as a covariate. Additional adjustment for age did not change the p values presented in Table 3.

Association between irritancy response and haplotypes

A number of interesting associations were identified between inferred haplotypes and response to irritants. Table 4 shows haplotype frequencies and associations that passed the discovery threshold. Variation in response to irritants was significantly associated with seven haplotypes. Haplotypes constructed by the SNPs that were mapped to the *AIF1, BAT2, HLA-DQA2* and *DAXX* genes were related to the response to 5% and 20% SLS. Haplotype correlated with response to 5% NaOH was composed of SNPs that mapped to the *HLA-G* gene. While haplotypes constructed by SNPs within the *RPS18, B3GALT4, C6orf11, HKE2, RGL2* and *TAPBP* genes were associated with responses to 0.5% BKC, haplotypes composed of SNPs mapped to the *HLA-DQB2, HLA-DOB, HLA-DOA* and *HLA-DPA1* genes were associated with a response to 1% BKC. Only one of these haplotypes (GGGA) contained a SNP (rs406477) that was identified in the logistic regression analysis. Both haplotype and single SNP were associated with response to BKC.

Regulatory information for “interesting” associations

The 10 unique SNPs identified from the initial analyses were imported to the SNP Annotation and Proxy Search (SNAP) tool (Johnson et al. 2008) to find additional SNPs in complete linkage disequilibrium (using an $r^2 = 1$). This led to the identification of additional correlated SNPs using data from the International HapMap Project (International HapMap Consortium et al. 2010). The total set of SNPs was then used as input to the RegulomeDB (Boyle et al. 2012) web resource that integrates data from the ENCODE projects and other data sources regarding various types of functional assays including DNaseI-seq, ChIP-seq, RNAseq and eQTL analyses. SNPs with RegulomeDB scores between 1 and 3 (inclusive, where scoring refers to available data types supporting a functional role for the variant) and related genes are listed in Table 5. Functional annotations showed that SNP *rs9277554,* associated with skin response to BKC, affects expression of its own gene (*HLA-DPB1).* Linkage disequilibrium pattern revealed that *TRIM40 rs1573298* SNP, associated with skin response to NaOH, was strongly correlated with other functional SNPs. These SNPs were found to regulate the expression of *HCG4, HLA-A, HLA-G, HLA-H, KIT, NDUFS1* and *TFG* genes. Regarding association with ICD, functional annotations showed that ITPR3/ rs499384 SNP (RegulomeDB score =2b) affected binding of GATA1 (GATA binding protein 1). RegulomeDB gave “no data” score for the rs10046277 SNP.

Discussion

The present study demonstrates that genetic variations within the MHC region can influence chemical-induced skin irritation. Keratinocytes play the major role in the immunological response to irritants by releasing cytokines and upregulating MHC Class II (HLA-DR) and cell adhesion molecules, upon skin barrier damage. The MHC region represents plausible candidate for studying the genetic basis of skin irritant response as it harbors multiple genes involved in immune regulation within the skin during an inflammatory reaction.

The genetic basis of irritant susceptibility has been largely understudied. In an earlier study, *HLA-A, HLA-B, HLA-DRA* and *HLA-DRB* genetic patterns were determined in metal worker trainees with and without ICD and no association was found between HLA alleles and ICD (Iliev et al. 2001). This study analyzed three frequently used skin irritants [SLS (an anionic surfactant), NaOH (a strong base) and BKC (cationic surfactant)]. SLS and NaOH are non-sensitizers whereas BKC can cause allergic sensitization. Increased susceptibility to one irritant was not always indicative of an increased susceptibility to other irritants that could be related to the dose and different penetration capabilities or the nature of the chemicals. Alternatively, there may be genetic differences in irritant susceptibility.

In our analysis, these model irritants produced different association patterns. Among three irritants, only BKC might have a potential for inducing contact sensitization. Although some have considered allergic patch test responses misclassified irritant responses (Basketter et al. 2004), we do recognize that sensitization to BKC occurs. Allergic sensitization usually leads to more severe skin symptoms than irritant dermatitis. Severe hand dermatitis was an exclusion criteria in subject recruitment; none of the volunteers had or developed severe hand dermatitis during the study period. In addition, the incidence of BKC is low in patients evaluated for allergic contact dermatitis from the same geographic region as these volunteer

subjects (Dao et al. 2012). In this population without severe dermatitis, it is unlikely that many, if any, of the current irritant patch test results were also the result of an allergic response. However, as most sensitizers are also irritants, this would not alter our results even if some of our subjects had both irritant and allergic responses.

This study also found that the *HLA-DPA1 (rs406477)* and *DPB1 (rs9277554, rs3117228, rs3130188)* SNPs were associated with response to moderate and high levels of BKC in both the additive and dominant models. Allergic contact dermatitis requires "danger signals" from innate immune response that can occur via non-HLA mechanisms such as IER3. An irritant chemical can cause inflammation that then predisposes individuals to sensitization from other chemical substances. These findings suggested that HLA-DP variations might influence sensitization from substances that are both irritant and allergenic. As it is possible that associated SNPs are associated with nearby functional SNPs due to extensive patterns of LD, we previously identified highly correlated SNPs within 500 kb and assessed their regulatory potential (Montgomery et al. 2010). Functional annotation of SNPs showed that three SNPs in the *HLA-DPB1* genes were correlated and regulated the expression level of their own gene. Thus, it is biologically plausible that gene variants within the HLA-DPB gene can influence immunoregulatory mechanisms in the skin during irritant-induced inflammation, and in turn, contribute to the variability in response to chemical irritants in susceptible subjects.

Three SNPs in the *TRIM40* (rs1573298), *TRIM10* (rs1557608) and *TRIM31* (rs1264701) genes were associated with response to high concentration (5%) NaOH in dominant and additive models. TRIM 10, 31 and 40 are members of the TRIM multigene family that encodes as many as 100 genes in humans. Although the majority of TRIM genes remain largely uncharacterized, several of them have been implicated in innate immunity and antiviral defense (Ozato et al. 2008; Kawai & Akira 2011; McNab et al. 2011).

The *IER3* rs8512 SNP was found to be associated with response to 2.5% NaOH in a recessive genetic model. *IER3* is a member of MHC Class I genes whose role in immune response (e.g. elimination of pathogens, restoration of epithelial barrier functions) and inflammation was elucidated by Arlt and Schafer (2011). IER3 was shown to be expressed in the skin (Feldmann et al. 2001). A role of IER3 deficiency in enhanced inflammatory responses was shown for *Leishmania* infections in IER3 knockout mice (Akilov et al. 2009); the lack of IER3 resulted in increased susceptibility to leishmaniosis and skin inflammatory response. IER3 has also been associated with psoriasis, an inflammatory skin disease worsened by irritation (Koebnerization) (Zhu et al. 2011). The association of IER3 with NaOH, the most alkaline irritant examined, may reflect genetic susceptibility to degradation of skin barrier by the elevated pH under the patch test with subsequent innate immune response.

The roles of *OR2B3, C2, IER3 and TRIM40* genes have not been characterized in the context of skin irritation response and we were unable to find information pertaining to the possible functional role for the SNP within these genes. However, as irritant response is considered to involve innate immunity and inflammation, it is plausible to expect that genetic variability within TRIM and IER3 genes might influence skin irritation response.

Most SNPs within the haplotype blocks mapped to genes with unidentified immune functions (e.g. *AIF1, BAT2, DAXX, HLA-G, RPS18, B3GALT4, C6orf11, HKE2, RGL2 and TAPBP)*. Only one haplotype (GGGA) included a SNP (*rs406477*) that was identified in the logistic regression analysis (Table 3). The rest of the SNP identified in logistic regression analysis were not included in associated haplotypes because either haplotypes that include these SNPs did not reach our discovery threshold (p <0.001) or some of these SNPs were not associated with any haplotypes.

Functional annotations showed that the HLA-DPB1 rs9277554 variant affects expression of its own gene, supporting a regulatory role for this SNP. On the other hand, TRIM40 rs1573298 SNP correlated with other functional SNPs that regulate the expression of several genes (*HCG4, HLA-A, HLA-G, HLA-H, KIT, NDUFS1* and *TFG*) involved in the regulation of inflammatory reaction.

The *PSMB9* rs10046277 and *ITPR3* rs499384 SNPs were associated with hand dermatitis after adjusting for confounders. The *PSMB9* gene is involved in the degradation of proteins into peptides, for subsequent antigen presentation by MHC Class I molecules (Ghannam et al. 2014). No information was found regarding the possible functional role of the rs10046277 SNP. *ITPR3* is known to play an important role in the regulation of the hair cycle in the skin (Sato-Miyaoka et al. 2012). The *ITPR3* rs499384 SNP effects binding of GATA1, a transcription factor needed for cell development and maturation. However, the exact role of *PSMB9* and *ITPR3* genes/SNPs in the ICD process remains unknown.

The p value results in this study were not corrected for multiple comparisons because our analysis was based on the well-defined role of the MHC in immune responses and we were interested in generating leads for further study rather than being the definitive study of genetic associations in this region. Herein, we reported all tests that reached a discovery threshold of p <0.001 and highlighted the functional relevance of associated SNP to determine which might be interesting results to follow-up on.

To our knowledge, this is the first report implicating specific MHC SNP in irritant susceptibility in a high-risk occupational population. Although the exact mechanism underlying enhanced susceptibility remains to be determined, MHC SNPs seem to contribute to chemical irritancy threshold. That is, different MHC variants are associated with different chemical irritants. Strong allergic sensitizers are also irritants and this property is thought to contribute to their potency. The contribution of the MHC to irritant response may tie to subsequent allergic sensitization by the same or different antigens. These results offer new avenues for future studies of genes contributing to an increased irritant response. Confirmatory studies are warranted to validate the results reported herein and identify causative alleles behind these associations using high-resolution mapping.

Acknowledgments

The authors wish to thank the volunteers who participated in this study. The authors also acknowledge the efforts of Debbie Velickoff from the WVU Pediatrics Department. The authors also thank Drs. Linda Sargent, Scott Auerbach and Ruth Lunn for their critical reviews.

Funding information

This study was supported in part by an inter-agency agreement between NIOSH and NIEHS (AES12007001-1-0-6) as a collaborative National Toxicology Program research activity.

References

Akilov OE, Ustyugova IV, Zhi L, Hasan T, Wu MX. Enhanced susceptibility to *Leishmania* infection in resistant mice in the absence of immediate early response gene *X-1*. J Immunol. 2009; 183:7994–8003. [PubMed: 19923449]

Allen MH, Wakelin SH, Holloway D, Lisby S, Baadsgaard O, Barker JN, McFadden JP. Association of TNFA gene polymorphism at position −308 with susceptibility to irritant contact dermatitis. Immunogenetics. 2000; 51:201–205. [PubMed: 10752629]

Arlt A, Schafer H. Role of the immediate early response 3 (*IER3*) gene in cellular stress response, inflammation, and tumorigenesis. Eur J Cell Biol. 2011; 90:545–552. [PubMed: 21112119]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

Basketter D, Reynolds F, Rowson M, Talbot C, Whittle E. Visual assessment of human skin irritation: A sensitive and reproducible tool. Contact Dermatitis. 1997; 37:218–220. [PubMed: 9412749]

Basketter DA, Marriott M, Gilmour NJ, White IR. Strong irritants masquerading as skin allergens: The case of benzalkonium chloride. Contact Dermatitis. 2004; 50:213–217. [PubMed: 15186375]

Basketter DA, Miettinen J, Lahti A. Acute irritant reactivity to sodium lauryl sulfate in atopics and non-atopics. Contact Dermatitis. 1998; 38:253–257. [PubMed: 9667441]

Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, et al. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 2012; 22:1790–1797. [PubMed: 22955989]

Bryld LE, Agner T, Kyvik KO, Brondsted L, Hindsberger C, Menne T. Hand eczema in twins: A questionnaire investigation. Br J Dermatol. 2000; 142:298–305. [PubMed: 10730764]

Callahan A, Baron E, Fekedulegn D, Kashon M, Yucesoy B, Johnson VJ, Domingo DS, Kirkland B, Luster MI, Nedorost S. Winter season, frequent hand washing, and irritant patch test reactions to detergents are associated with hand dermatitis in healthcare workers. Dermatitis. 2013; 24:170–175. [PubMed: 23857011]

Dao H, Fricker C, Nedorost ST. Sensitization prevalence for benzalkonium chloride and benzethonium chloride. Dermatitis. 2012; 23:162–166. [PubMed: 22828255]

Davis JA, Visscher MO, Wickett RR, Hoath SB. Influence of TNFα polymorphism-308 and atopy on irritant contact dermatitis in healthcare workers. Contact Dermatitis. 2010; 63:320–332. [PubMed: 20731689]

de Jongh CM, John SM, Bruynzeel DP, Calkoen F, van Dijk FJ, Khrenova L, Rustemeyer T, Verberk MM, Kezic S. Cytokine gene polymorphisms and susceptibility to chronic irritant contact dermatitis. Contact Dermatitis. 2008; 58:269–277. [PubMed: 18416756]

Dimas AS, Deutsch S, Stranger BE, Montgomery SB, Borel C, Attar-Cohen H, Ingle C, Beazley C, Gutierrez Arcelus M, Sekowska M, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. Science. 2009; 325:1246–1250. [PubMed: 19644074]

Feldmann KA, Pittelkow MR, Roche PC, Kumar R, Grande JP. Expression of an immediate early gene, *IEX-1*, in human tissues. Histochem Cell Biol. 2001; 115:489–497. [PubMed: 11455449]

Ghannam K, Martinez-Gamboa L, Spengler L, Krause S, Smiljanovic B, Bonin M, Bhattarai S, Grutzkau A, Burmester GR, Haupl T, et al. Up-regulation of immunoproteasome subunits in myositis indicates active inflammation with involvement of antigen-presenting cells, CD8 T-cells and IFNγ. PLoS One. 2014; 9:e104048. [PubMed: 25098831]

Ibler KS, Jemec GB, Flyvholm MA, Diepgen TL, Jensen A, Agner T. Hand eczema: prevalence and risk factors of hand eczema in a population of 2274 healthcare workers. Contact Dermatitis. 2012; 67:200–207. [PubMed: 22624885]

Iliev D, Elsner P, Tuor C, Hinnen U. Are certain genetic patterns more common in persons with irritant contact dermatitis? A study in metalworker trainees. Am J Contact Dermatitis. 2001; 12:232–233. [PubMed: 11783427]

Altshuler DM, Gibbs RA, Peltonen L. International HapMap Consortium. Integrating common and rare genetic variation in diverse human populations. Nature. 2010; 467:52–58. [PubMed: 20811451]

Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: A web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics. 2008; 24:2938–2939. [PubMed: 18974171]

Jungbauer FH, Lensen GJ, Groothoff JW, Coenraads PJ. Exposure of the hands to wet work in nurses. Contact Dermatitis. 2004; 50:225–229. [PubMed: 15186378]

Kawai T, Akira S. Regulation of innate immune signaling pathways by the tripartite motif (TRIM) family proteins. EMBO Mol Med. 2011; 3:513–527. [PubMed: 21826793]

Landeck L, Visser M, Kezic S, John SM. Impact of TNFα polymorphisms on irritant contact dermatitis. Contact Dermatitis. 2012; 66:221–227. [PubMed: 22404198]

Luk NM, Lee HC, Luk CK, Cheung YY, Chang MC, Chao VK, Ng SC, Tang LS. Hand eczema among Hong Kong nurses: A self-report questionnaire survey conducted in a regional hospital. Contact Dermatitis. 2011; 65:329–335. [PubMed: 21831240]

Lushniak BD. The epidemiology of occupational contact dermatitis. Dermatol Clin. 1995; 13:671–680. [PubMed: 7554514]

Magina S, Barros MA, Ferreira JA, Mesquita-Guimaraes J. Atopy, nickel sensitivity, occupation, and clinical patterns in different types of hand dermatitis. Am J Contact Dermatitis. 2003; 14:63–68. [PubMed: 14749022]

Matzinger P. The danger model: A renewed sense of self. Science. 2002; 296:301–305. [PubMed: 11951032]

McNab FW, Rajsbaum R, Stoye JP, O'Garra A. Tripartite-motif proteins and innate immune regulation. Curr Opin Immunol. 2011; 23:46–56. [PubMed: 21131187]

Meding B, Jarvholm B. Hand eczema in Swedish adults - changes in prevalence between 1983 and 1996. J Invest Dermatol. 2002; 118:719–723. [PubMed: 11918722]

Meding B, Swanbeck G. Prevalence of hand eczema in an industrial city. Br J Dermatol. 1987; 116:627–634. [PubMed: 2954578]

Montgomery SB, Sammeth M, Gutierrez-Arcelus M, Lach RP, Ingle C, Nisbett J, Guigo R, Dermitzakis ET. Transcriptome genetics using second generation sequencing in a Caucasian population. Nature. 2010; 464:773–777. [PubMed: 20220756]

NIOSH. [Accessed December 2015] 2012. Available from: http://www.cdc.gov/niosh/topics/skin/

Ozato K, Shin DM, Chang TH, Morse HC. TRIM family proteins and their emerging roles in innate immunity. Nat Rev Immunol. 2008; 8:849–860. [PubMed: 18836477]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]

Sato-Miyaoka M, Hisatsune C, Ebisui E, Ogawa N, Takahashi-Iwanaga H, Mikoshiba K. Regulation of hair shedding by the type 3 IP3 receptor. J Invest Dermatol. 2012; 132:2137–2147. [PubMed: 22572820]

Smit HA, Burdorf A, Coenraads PJ. Prevalence of hand dermatitis in different occupations. Int J Epidemiol. 1993; 22:288–293. [PubMed: 8505186]

Tupker RA. Prediction of irritancy in the human skin irritancy model and occupational setting. Contact Dermatitis. 2003; 49:61–69. [PubMed: 14641352]

Willis CM, Stephens CJ, Wilkinson JD. Differential patterns of epidermal leukocyte infiltration in patch test reactions to structurally unrelated chemical irritants. J Invest Dermatol. 1993; 101:364–370. [PubMed: 8370975]

Zeller T, Wild P, Szymczak S, Rotival M, Schillert A, Castagne R, Maouche S, Germain M, Lackner K, Rossmann H, et al. Genetics and beyond –the transcriptome of human monocytes and disease susceptibility. PLoS One. 2010; 5:e10693. [PubMed: 20502693]

Zhu KJ, Lv YM, Yin XY, Wang ZX, Sun LD, He SM, Cheng H, Hu DY, Zhang Z, Li Y, et al. Psoriasis regression analysis of MHC loci identifies shared genetic variants with vitiligo. PLoS One. 2011; 6:e23089. [PubMed: 22125590]

Table 1

Demographics of study groups.

Demographics	***N* = 585**
Age (years; median, range)	37, 18 to 70
Gender (F/M/missing)	478/88/19
Ethnicity (non-Hispanic whites/others/missing)	523/37/25
Season (warm vs. cold)[a]	356/229
Population (WVU vs. CWU)[b]	500/85
Family history of dermatitis or eczema (%)	15.2
Hand Dermatitis (%) during any research visit	22.9
TEWL	
Arm (median, range)	9.78, −5.8 to 65.53
Back (median, range)	9.53, 7.67 to 173.5
Forefinger (median, range)	21.48, −6.53 to 139.33

[a]Season variable was coded according to time of patch testing: warm – April through September; cold – October through March.

[b]Population variable represents the location of subject recruitment (WVU: West Virginia University; CWU: Case Western Reserve University).

Table 2

Logistic regression analysis for skin irritant response.

									p Value		
Irritant	**Gene**	**Position**	**Location**	**SNP**	**Genotype**	***N***	**Mean**	**(95% CI)**	**ADD**	**DOM**	**REC**
SLS_M	OR2B3	−11695	>10 kb coding	rs2050231	GG	58	1.50	1.36–1.64			
					CG	142	1.45	1.36–1.54			
					CC	108	1.63	1.53–1.72		**0.0008882**	
SLS_H	C2	[104/34]	coding	rs9332739	GG	500	1.87	1.83–1.90			
					GC	36	1.65	1.48–1.82			
					CC	1	2.00	–		0.0008002	
NAOH_H	TRIM40	−569	flanking_3UTR	rs1573298	GG	183	1.40	1.32–1.47			
					GC	240	1.12	1.08–1.17			
					CC	116	1.05	1.01–1.10	**3.03E-05**	**3.97E-05**	
NAOH_H	TRIM10	−1144	flanking_3UTR	rs1557608	CC	38	1.15	1.02–1.27			
					AC	210	1.14	1.09–1.19			
					AA	289	1.23	1.18–1.28	**0.000587**		
NAOH_H	TRIM31	−4318	flanking_3UTR	rs1264701	CC	328	1.15	1.11–1.19			
					CA	194	1.26	1.19–1.32			
					AA	15	1.20	0.97–1.43		0.0007839	
NAOH_M	IER3	[380/355]	3UTR	rs8512	GG	377	1.09	1.06–1.12			
					GA	147	1.04	1.00–1.07			
					AA	13	1.33	1.02–1.65			**0.0001745**
BKC_M	HLA-DPA1	−27150	>10 kb coding	**rs406477**	GG	10	1.00	–			
					AG	99	1.16	1.09–1.24			
					AA	199	1.31	1.24–1.38	**0.0003624**	**0.0006043**	
BKC_M	HLA-DPB1	−562	flanking_3UTR	rs9277554	GG	241	1.25	1.20–1.31			
					GA	243	1.15	1.11–1.20			
					AA	53	1.08	1.00–1.16	**0.000316**	**0.0005235**	
BKC_M	HLA-DPB1	−1459	flanking_3UTR	rs3117228	CC	242	1.25	1.19–1.30			
					CA	238	1.16	1.11–1.20			
					AA	57	1.1	1.01–1.18	**0.0007473**		

									p Value		
Irritant	**Gene**	**Position**	**Location**	**SNP**	**Genotype**	***N***	**Mean**	**(95% CI)**	**ADD**	**DOM**	**REC**
BKC_M	HLA-DPB1	−2200	flanking_3UTR	rs3130188	GG	58	1.09	1.01–1.18			
					AG	237	1.16	1.11–1.21			
					AA	242	1.25	1.19–1.30	**0.0007149**		
BKC_H	HLA-DPA1	−27150	>10 kb coding	**rs406477**	GG	10	1.00	–			
					AG	99	1.28	1.18–1.38			
					AA	199	1.43	1.35–1.51	**0.0004029**		

SLS_M: 5%; SLS_H: 20%; NaOH_M: 2.5%; NaOH_H: 5%; BKC_M: 1.0%; BKC_H: 2.5%.

Results are adjusted for potential confounders specific to each irritant; Bold *p* values represent significance after additional adjustment for age.

ADD: additive genetic model; DOM: dominant genetic model; REC: recessive genetic model.

Bold markers are significant in haplotype analysis.

Table 3

Association of MHC variants with ICD.

								p Values (adjusted)		
Gene	**Position**	**Location**	**SNP**	**Genotype**	**N**	**Mean**	**(95% CI)**	**ADD**	**DOM**	**REC**
PSMB9	−27723	>10 kb coding	rs10046277	GG	195	0.58	0.51–0.65			
				GA	37	0.3	0.14–0.45	0.0006785		
				AA	0				0.0006785	
ITPR3	−7254	flanking_5UTR	rs499384	GG	181	0.49	0.42–0.57			
				GA	44	0.8	0.67–0.92			
				AA	7	0.71	0.26–1.17		0.0006775	

Results are adjusted for hand washing frequency and TEWL measurement on the forefinger; additional adjustment for age resulted in same *p* values.

ADD: additive genetic model; DOM: dominant genetic model; REC: recessive genetic model.

Table 4

Haplotype-based test results.

Variable	BP1	BP2	SNP1	SNP2	Haplotype	F	OR	STAT	*p* Values	Genes
SLS_L	31582025	31587938	rs3132451	rs3763295	GTGGA	0.0297	0.0358	8.78	0.0007999	AIF1 | BAT2
SLS_M	32695082	32706334	rs5029394	rs7773694	AAG	0.011	0.0919	8.61	1.00E-04	HLA-DQA2
SLS_M	33303989	33313427	rs3130266	rs3130013	GCAAC	0.0257	0.247	9.19	0.0009999	DAXX
NAOH_H	29821896	29831008	rs2734980	rs1611732	GGGCA	0.0103	155	12.1	3.00E-04	HLA-G
BKC_L	33242825	33280629	rs458434	rs3106190	CGGAACAAGGGC	0.0131	14.4	9.2	0.0006999	RPS18|B3GALT4|C6orf11|HKE2|RGL2|TAPBP
BKC_M	32744145	32758394	rs2857205	rs7758736	GGGCAG	0.316	0.531	10.2	0.0005999	HLA-DQB2|HLA-DOB
BKC_M	33001488	33016728	rs380468	rs375912	GGGA	0.186	0.414	11	0.0007999	HLA-DOA|HLA-DPA1

BP1: physical position of left-most (5′) SNP (base-pair); BP2: physical position of right-most (3′) SNP (base-pair).

SNP1: left-most (5′) SNP; SNP2: left-most (3′) SNP.

F: Frequency; OR: Estimated odds ratio; STAT: Test statistic (*t* from Wald test); *p* Values: Empirical *p* values from permutation procedures (10,000 permutations).

Haplotype containing a SNP (rs406477) that is individually associated with skin irritant response is shown in bold.

Results are adjusted for confounders specific to each irritant.

Table 5

Regulatory potential of associated/correlated SNPs and affected genes.

CHR	SNP	Proxy	BP	Distance	eQTL	References
6	rs1573298	rs9261518	30116984	825	HCG4|HLA-A|HLA-G|HLA-H|KIT|NDUFS1|TFG	(Zeller et al. 2010)
6	rs1573298	rs9261519	30117100	941	HCG4|HLA-A|HLA-G|HLA-H|KIT|NDUFS1|TFG	
6	rs1573298	rs2844775	30179421	63262	HCG4|HLA-A|HLA-G|HLA-H|KIT|NDUFS1|TFG	
6	rs1573298	rs1042338	30152788	36629	HCG4|HLA-A|HLA-G|HLA-H|KIT|NDUFS1|TFG	
6	rs9277554	rs9277554	33055537	0	HLA-DPB1	(Dimas et al. 2009)
6	rs3117228	rs9277554	33055537	897	HLA-DPB1	
6	rs3130188	rs9277554	33055537	1638	HLA-DPB1	

Proxy: correlated SNP.

Distance: physical distance between SNP of interest and its proxy.

eQTL: list of genes affected by interesting and proxy SNPs.