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Genetic basis of irritant susceptibility in health care workers

Berran Yucesoy, PhD, **Yerkebulan Talzhanov, PhD**, **M. Michael Barmada, PhD**, **Victor J. Johnson, PhD**, **Michael L. Kashon, PhD**, **Elma Baron, MD**, **Nevin W. Wilson, MD**, **Bonnie Frye, BS**, **Wei Wang, MS**, **Kara Fluharty, BS**, **Rola Gharib, MD**, **Jean Meade, MD, PhD**, **Dori Germolec, PhD**, **Michael I. Luster, PhD**, and **Susan Nedorost, MD**

Health Effects Laboratory Division (Drs. Yucesoy, Kashon, Frye, Wang, Fluharty, Meade), CDC/NIOSH, Morgantown, WV; Department of Human Genetics (Drs. Talzhanov and Barmada), University of Pittsburgh, Pittsburgh, PA; BRT-Burleson Research Technologies (Dr. Johnson), Morrisville, NC; University Hospitals Case Medical Center (Drs. Baron and Nedorost), Case Western Reserve University, Cleveland, OH; Department of Pediatrics, School of Medicine (Dr. Wilson), University of Nevada, Reno, NV; Department of Dermatology, School of Medicine (Dr. Gharib), West Virginia University, Morgantown, WV; Toxicology Branch, DNTP/NIEHS (Dr. Germolec), Research Triangle Park, NC; School of Public Health (Dr. Luster), West Virginia University, Morgantown, WV

Abstract

Objective—To investigate the association of single nucleotide polymorphisms (SNPs) within genes involved in inflammation, skin barrier integrity, signaling/pattern recognition and antioxidant defense with irritant susceptibility in a group of health care workers.

Materials and Methods—The 536 volunteer subjects were genotyped for selected SNPs and patch tested with three model irritants: sodium lauryl sulfate (SLS), sodium hydroxide (NaOH) and benzalkonium chloride (BKC). Genotyping was performed on genomic DNA using Illumina Goldengate custom panels.

Results—The *ACACB* (rs2268387, rs16934132, rs2284685), *NTRK2* (rs10868231), *NTRK3* (rs1347424), *IL22* (rs1179251), *PLAU* (rs2227564), *EGFR* (rs6593202) and *FGF2* (rs308439) SNPs showed association with skin response to tested irritants in different genetic models (all at $p<0.001$). Functional annotations identified two SNPs in *PLAU* (rs2227564) and *ACACB* (rs2284685) genes with a potential impact on gene regulation. In addition, *EGF* (rs10029654), *EGFR* (rs12718939), *CXCL12* (rs197452), and *VCAM1* (rs3917018) genes showed association with hand dermatitis ($p<0.005$)

Conclusions—The results demonstrate that genetic variations in genes related to inflammation and skin homeostasis can influence responses to irritants and may explain inter-individual variation in the development of subsequent contact dermatitis.

*Address correspondence to: Berran Yucesoy, PhD, Formerly of the Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown WV, berranyucesoy@gmail.com.

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Keywords

irritant contact dermatitis; genetics; health care workers

INTRODUCTION

Irritant contact dermatitis (ICD) is an inflammatory response of the skin to a variety of triggers in the absence of sensitization and accounts for 50–80% of all occupational dermatitis cases. [1,2,3] ICD is commonly seen in people who have repetitive contact with weak irritants (i.e. rapid evaporation of water especially in the presence of detergents and soaps) and mainly effects hands. [4] At risk industries include (but are not limited to) those that involve “wet work” such as healthcare, printing, metal machining, food preparation, painting, and beauty services. A number of internal (e.g., atopy, age, sex, body region and genetic predisposition) and external (e.g., physical and chemical properties of the irritant, concentration, exposure duration) factors influence susceptibility to ICD. External factors trigger a number of events including skin barrier disruption, activation of epidermal keratinocytes, cellular damage, and release of inflammatory mediators such as cytokines, chemokines and adhesion molecules. [5]

There is wide biological variation in skin response to irritants, and susceptibility factors involved in the regulation of epidermal homeostasis are still largely unexplored. Given the role of cytokines in inflammatory processes, genetic polymorphisms in several cytokine genes, including tumor necrosis alpha (TNFα), interleukin-1 alpha (IL-1α), IL-1β, IL-8, IL-10, have been investigated. Among those, TNFα -308 and IL1A-889 SNPs were found to be associated with susceptibility to ICD. [6-10] Polymorphisms in the filaggrin (FLG) gene (R501X and 2282del4) have also been shown to be predisposing factors for atopic dermatitis and chronic ICD. [11-13] However, conflicting results have been reported and no clear association was established between clinically diagnosed or experimentally-induced irritant dermatitis and FLG variations. [14-18]

In the present study, we aimed to comprehensively investigate the association of genetic variations in selected candidate genes involved in inflammation, skin barrier integrity, signaling/pattern recognition, and antioxidant defense with irritant susceptibility. Since acute inflammatory response in epidermis depends on the chemical structure of the applied irritant. [19] three structurally different irritants, sodium lauryl sulfate (SLS), NaOH (sodium hydroxide) and benzalkonium chloride (BKC), were used as model irritants. To date, most published studies have investigated a small number of genes and/or SNPs and focused on clinical ICD. In this regard, assessing variability in skin response to experimental irritants under controlled conditions may provide insights into the mechanism underlying ICD risk.

METHODS

Study population

The study population consisted of 536 health care 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, or 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, family history of dermatitis) and skin exposure history (e.g., the number of daily hand washings and use of soap or hand cleanser) were collected by questionnaire. Blood samples were collected for genetic analysis. Study procedures were approved by the Institutional Review Boards of participating institutions. The demographics variables of the participants that were included in the analysis are given in Table 1.

Genotyping and SNP selection

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 Illumina Golden Gate protocol (Illumina Inc., San Diego, CA). A total of 250 ng to 1μg DNA was used for each assay depending on the source. Genotypes were auto called using GenomeStudio software (Illumina, Inc., San Diego, CA). An oligonucleotide pool assay (OPA) was designed by selecting candidate genes that are known and/or suspected to be involved in the ICD process. In SNP selection, 10 kb upstream and 10 kb downstream regions were included in accordance with design score validations and SNPs closer than 60 bp to another SNP were excluded to accommodate the assay. The SNPs included in the OPA had a MAF of >5% in HapMap/CEU population. Selected genes and their chromosomal locations are presented in Supplementary Table 1.

Model irritants, patch testing, and transepidermal water loss (TEWL)—Sodium lauryl sulfate (SLS) (99% pure), sodium hydroxide (NaOH) and benzalkonium chloride (BKC) (Sigma-Aldrich Co. LLC., San 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 concentrations of 0.1%, 0.25%, 0.5%, 1.0%, 2.5% and 5.0% and BKC at concentrations of 0.1, 0.5, 1.0, 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. Since patch tests for irritant contact dermatitis are not standardized, a range of concentrations was used in the first phase of our study to determine the concentrations which resulted in inter-individual variation of response. Distilled water served as a negative control. 20% SLS, the minimum level classified as irritant (R38) by European Commission criteria, served as a positive control. Subjects wore the taped patches for 24 hours and reactions were graded by visual assessment of the patch sites using a 3 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). [20]

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 (g/m2h) at each site were taken from the upper inner arm, from the back, and from the side of forefinger and the means were calculated. The inner arm and back sites were assessed as these would be expected to show high TEWL in patients with genetic barrier defect e.g. atopic dermatitis, whereas the forefinger would be expected to have higher TEWL both from genetic barrier defects and from environmental factors such as wet-dry cycles due to hand washing. These wet-dry cycles are more pronounced in low humidity conditions when indoor heating is used in cold weather.

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 health care workers were assessed in this phase. Individual differences in skin response were observed 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% of SLS; 1%, 2.5% and 5.0% of NaOH; and 0.5%, 1.0% and 2.5% of 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 for no response, medium (moderate) for a weakly positive macular reaction, and high for a papular or vesicular reaction. These variables were turned into binary variables by calling low responders as controls and moderate and high responders as cases and included in the logistic regression analysis. The genotype confidence score of the assay was set to 0.25 in GenomeStudio Genotyping module. 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. [21] The final dataset contained 1074 SNPs for 536 subjects. The total genotype rate for the dataset was 0.99.

Statistical analysis was performed using PLINK. Since 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, that included a dominant model (comparing homozygous wild-type vs. variant allele-carrying genotypes), 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 confounder that did not have any influence on the outcome variable. Based on this, skin responses to SLS were adjusted for gender, population (represents different recruitment sites), season (coded binary as cold vs warm) and indoor humidity when the patch test was interpreted. Skin responses to NaOH were adjusted for gender, population and indoor humidity, while responses to BKC were adjusted only for gender and population. Based on a stepwise regression model, age did not appear to significantly affect skin responses. However, we repeated analysis with additional adjustment for age and compared the results with those obtained by the final model.

In order 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. Since ICD from wet work, as in our cohort of health care workers usually occurs during cold months (October-March), only subjects examined during these months were included in this analysis. [22] This prevented erroneously including subjects who might develop wintertime ICD in the control group. 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 the outcome. Based on this, the results were adjusted for hand washing frequency and TEWL measurement on the forefinger. The results were not corrected for multiple comparisons since our analysis was based on the defined biological role of selected genes. Instead, we reported all tests that reached a ($p<0.001$ for skin irritant response and $p<0.005$ for ICD) level of significance and highlighted the functional relevance of significantly associated SNPs.

Linkage disequilibrium (LD) and haplotype blocks were assessed using default parameters in Haploview. [23] Pairwise LD was calculated only for SNPs within 200kb. RegulomeDB was used to annotate SNPs with known and predicted regulatory elements. [24] SNAP tools were used to update annotations of interesting SNPs according to dbSNP135 and to find proxy SNPs within 500kb based on LD and physical distance. [25]

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 654 health care workers. 118 subjects were excluded from the analyses due to ineligibility or incomplete information, leaving 536 subjects for the final analysis. The mean age of the population was 36 with the majority (451) being female. While 15.3% of the study population had a family history of dermatitis or eczema, 22.4% had hand dermatitis at any time during the study.

Association between SNPs, irritancy threshold and ICD

A number of samples were excluded from the analyses due to incomplete genotype information. The custom panel allowed examination of 1074 SNPs in 188 genes. All genotype frequencies were in Hardy Weinberg Equilibrium. After adjusting for confounders, a number of SNPs in genes that code for *ACACB* (Acetyl-CoA Carboxylase Beta), *NTRK2*

(Neurotrophic Tyrosine Kinase, Receptor, Type 2), *NTRK3* (Neurotrophic Tyrosine Kinase, Receptor, Type 3), *IL22* (interleukin 22), *PLAU* (Plasminogen Activator, Urokinase), *EGFR* (Epidermal Growth Factor Receptor), and *FGF2* (Fibroblast Growth Factor 2) were significantly 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 that reached a significant level ($p<0.001$). Additional adjustment for age did not change the *p* values. The *ACACB (rs2268387 and rs16934132)* SNPs were associated with response to 2.5% SLS, whereas *NTRK2 (rs10868231)* SNP was associated with response to 5% SLS. Another *ACACB (rs2284485)* SNP was associated with the response to the highest dose (20%) of SLS. The *IL22 (rs1179251)* SNP was associated with response to 1% NaOH, while the *PLAU (rs2227564) and EGFR (rs6593202) SNPs* were also associated with response to 2.5% and 5% of NaOH respectively. Finally the *FGF2 (rs308439)* and *NTRK3 (rs1347424)* SNPs showed association with response to 0.5% and 1% BKC respectively. None of the other polymorphisms that were examined showed any significant association with the irritancy response.

A regression analysis showed that SNPs in *EGF* (rs10029654), *EGFR* (rs12718939), *CXCL12* (rs197452), and *VCAM1* (rs3917018) genes were significantly associated with hand dermatitis ($p<0.005$) after adjusting for potential confounders (Table 3). *P* values that reached the same significance level after additional adjustment for age are marked in bold.

Association between haplotypes and skin irritant response

Variation in response to irritants was significantly associated with three haplotypes (TA, AGA, GGG). Table 4 shows these haplotypes and their frequencies. TA haplotype constructed by SNPs that were mapped to the NTRK2 gene was associated with response to 2.5% NaOH. Both AGA and GGG haplotypes were correlated with response to 5% NaOH and constructed by SNPs that were mapped to the EGFR gene. These haplotypes contained the rs6593202 SNP that was also identified in the logistic regression analysis.

Regulatory information for significant associations

The significant SNPs identified from initial analyses were used as input to the SNP Annotation and Proxy Search (SNAP) tool [25] to find additional SNPs in complete linkage disequilibrium (using an r2 of 1). A combination of the original and correlated SNPs were then used as input to the RegulomeDB [24] web resource, which 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) are listed in Table 5. *PLAU (rs2227564)* and *ACACB (rs2284685)* SNPs were found to regulate the expression of *ECD* and *KCTD10* genes, respectively.

Regarding association with hand dermatitis, functional annotations showed that *VCAM1*/rs3917018 SNP (RegulomeDB score = 3a) affects binding of POLR2A (Polymerase (RNA) II (DNA Directed) Polypeptide A) and falls within CUX2 (Cut-Like Homeobox 2), FOXO1 (Forkhead Box O), FOXO4, and FOXO6 binding motifs. While rs12718939 and rs197452

SNPs showed minimal binding evidence (RegulomeDB scores 5 and 6), the rs10029654 returned with “no data” score.

DISCUSSION

We previously reported an association between individual irritation responsiveness and the risk for hand dermatitis in our study population. Subjects with a positive patch test to 2.5% SLS were more likely to have occurrence of hand dermatitis (IRR=1.78, 95% CI: 0.92, 3.45). [22] In the present study, we investigated the genetic basis of irritant susceptibility in a **larger** healthcare workers population and identified nine significant SNPs in seven candidate genes. The ACACB SNPs (rs2268387, rs16934132 and rs2284685) showed association with response to low and high levels of SLS in different genetic models. The enzyme encoded by the ACACB gene is involved in the synthesis of fatty acids. Its role in protecting skin barrier was shown in a murine model where increased mRNA expression was observed after barrier disruption. [26] Buraczewska et al. reported an association between high expression of ACACB and low transepidermal water loss (TEWL) in untreated skin. [27] Another study investigating skin barrier restoration after exposure to SLS showed ACACB to be one of the important genes in lipid synthesis and skin barrier repair. [28] Although the regulation of ACACB in keratinocytes is not completely understood, altered expression of lipid processing regulators, such as peroxisome proliferator-activated receptors (PPARa and PPARc), in SLS-exposed skin and the correlation between TEWL and ACACB argues for the relevance of the gene in skin barrier formation and homeostasis. [29,30] It is possible that altered levels of ACACB lead to increased penetration of irritants through the skin, and subsequent development of inflammation. Based on this, it is biologically plausible that genetic variations in the ACACB gene may influence its regulation and consequently result in inter individual variation in response to irritant exposure.

The IL-22 rs1179251 SNP was associated with skin response to 1% NaOH. Recent studies showed the importance of IL-22 in skin barrier impairment. IL-22 was found to increase epidermal barrier dysfunction by down-regulating filaggrin and the enzymes involved in profilaggrin to filaggrin processing. [31] IL-22 has also been reported to promote epidermal hyperplasia, inhibit the differentiation of keratinocytes and promote their migration and plays a role in regulating antimicrobial peptides (AMPs) and matrix metalloproteinase (MMP) in epidermis. [32-37] High levels of IL-22 expression have been observed in chronic atopic dermatitis and its levels were positively associated with severity of disease. [38] Interestingly, atopic dermatitis is known to be worsened by alkaline pH, and NaOH is a very alkaline irritant. IL-22 was also shown to mediate IL-23-induced epidermal hyperproliferation and dermal inflammation in psoriasis. [39,40] Although not studied in the context of skin irritancy, genetic variations of the IL22 gene have been associated with psoriasis and its severity. [41-43] Our results show, for the first time, that genetic variability within IL-22 may also contribute to skin irritant response.

The PLAU rs2227564 and EGFR rs6593202 SNPs were associated with skin responses to medium and high levels of NaOH, respectively. The Plau [urokinase-type plasminogen activator (U-PA)] has been shown to be expressed in skin keratinocytes and known to be involved in degradation of the extracellular matrix.[44] Although there is no information

related to role of PLAU in skin irritation responses, a recent study reported that extended increased expression of extracellular matrix-related genes (e.g., Col3A1, TGFβ3) including PLAU was associated with hypertrophic scar formation.[45]

EGFR signaling plays a role in keratinocyte proliferation and differentiation and ultimately influences the wound healing process. [46-48] The EGFR signaling pathway was found to be crucial for skin development and homeostasis. [49] Increased EGFR expression was found in chronic inflammatory skin diseases including psoriasis and atopic dermatitis, possibly by regulating cytokine and chemokine secretion by keratinocytes. [49-51] Polymorphisms in the EGFR gene have been investigated in relation to skin rash observed after cancer treatment with EGFR-inhibitors, and there are studies assessing their contribution to the innate immune response which mediates the skin responses induced by irritants. [52] This finding extends our knowledge of the role of EGFR gene variations in skin homeostasis.

The FGF2 rs308439 SNP was associated with response to medium and high levels of BKC. FGF2 is known to be a potent angiogenic factor and endothelial cell mitogen and has been implicated in diverse biological processes including wound healing. It was shown that FGF2 enhances the recruitment of monocytes, T cells, and PMNs in response to various inflammatory stimuli and increases expression of adhesion molecules on cytokine-activated epithelial cells. [53] The presence of angiogenic factors including FGF2 has been associated with more intense accumulation of leukocytes and exacerbation of injury during inflammation. [54,55] SNPs in NTRK2 (rs10868231) and NTRK3 (rs1347424) genes were associated with responses to medium levels of SLS and BKC. However, the roles of NTRK2 and NTRK3 genes have not been characterized in the skin. Although there is no information about the role of significant genes and their variations in irritancy response, their biological role in skin homeostasis and previous reports on their role in inflammatory processes support our findings.

Haplotype analysis showed that SNPs within the haplotype blocks mapped to NTRK2 and EGFR genes. Two haplotypes (AGA and GGG) included a SNP *(rs6593202)* that was identified by logistic regression analysis (Table 2). These findings further support the involvement of EGFR and NTRK2 in skin irritant responses. Functional annotations showed that PLAU rs2227564 and ACACB rs2284685 SNPs regulate ECD (Ecdysoneless) and KCTD10 (potassium channel tetramerisation domain containing 10) genes, respectively. Although KCTD10 may be associated with DNA synthesis and cell cycle control [56,57] the biological role of these genes has not been fully characterized and there was no information pertaining to the possible role of these genes in skin irritation response. As such, the results should be considered preliminary but should allow for more direct studies into the associations that were identified.

Examination of our subjects for development of ICD on the hands revealed that SNPs in EGF (rs10029654), EGFR (rs12718939), CXCL12 (rs197452), and VCAM1 (rs3917018) genes were associated with ICD after adjusting for potential confounders. Among these SNPs, only the *VCAM1*/ rs3917018 showed functional evidence. *VCAM1* is an adhesion molecule that plays a role in skin inflammatory responses. [58] RegulomeDB showed that the rs3917018 affects the binding of POLR2A and overlaps with CUX2 and FOXO (1,4, and 6)

binding motifs. They all play a role in the regulation of transcription. FOXO transcription family is also known to be involved in wound healing. [59] The association of this SNP with ICD could be related to its overlap with these binding motifs. EGF, EGFR, and CXCL12 are known to play important roles in epithelial homeostasis and wound healing. [47,60,61] EGFR has been shown to be essential for toll-like receptor signaling which is a key player in innate immunity. [52,62,63] EGF family members have also been shown to be involved in cutaneous immune/inflammatory responses. [64] In the present study, EGFR variants showed association with both skin irritant response and ICD. Since ICD is mediated by the innate immune system, it is plausible to expect that EGFR variants may play a role in the ICD process. However, since no functional data is available for SNPs in these genes, it is difficult to determine their exact involvement in ICD pathogenesis.

Limitations of our study include the exclusion of subjects with a history of inflammatory skin disease requiring medical attention which likely decreased the prevalence of atopic dermatitis patients in our cohort. Also, chronic exposure to irritants may result in 'hardening' which may have reduced acuity of dermatitis and caused failure to detect some cases on examination. In addition, the healthy worker effect may have caused some genetically predisposed patients to change careers and reduced their representation in our cohort. Also, we were unable to study filaggrin (FLG) mutations that were found to be associated with atopic dermatitis in this analysis. Only two FLG SNPs, rs11582620 and rs2184953, were included in the final panel due to low designability rank scores of most commonly studied FLG SNPs [i.e. rs61816761 (R501X), rs41370446 (2282del4)]. This prevents comparison to prior literature reporting associations between atopic dermatitis and FLG variants rs61816761, rs41370446, rs138726443 (R2447X), and rs150597413 (S3247X). [11,12,17] The SNPs included in our study have not been studied in Caucasians. However, our results are in line with two other studies reporting no association between these SNPs and atopic dermatitis in Japanese population. [65,66]

The p-value results in this study were not corrected for multiple comparisons since our analysis was based on the well-defined role of the selected genes in skin irritation response and we were interested in generating hypotheses for further study rather than being the definitive study of genetic associations of these genes. We reported all test results that reached a discovery threshold of $p<0.001$ and highlighted the functional relevance of associated SNPs to determine which might be interesting results to follow up on.

To our knowledge, this is the first report implicating ACACB, NTRK2, NTRK3, IL-22, PLAU, EGFR, FGF2 gene variants in irritant susceptibility in a high risk occupational population. Although the exact mechanism underlying enhanced susceptibility remains to be determined, these SNPs seem to modify skin response to irritants and contribute significantly to chemical irritancy threshold. Large prospective studies are warranted to confirm these findings and identify causative alleles using high-density genome scans. Genetic markers for predisposition to specific irritants may be useful to choose individualized hand hygiene methods. Genetic mechanisms underlying ICD may also be helpful to guide development of new therapies. Because ICD creates 'danger signals' that predispose to allergic contact dermatitis, prevention of ICD would be expected to reduce the development of the more debilitating occupational allergic contact hand dermatitis as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographics of the study population

Demographics	**N=536**
Age (median, range)	36, 18-70
Gender (F/M/missing)	451/67/18
Ethnicity (non-hispanic whites/others/missing)	483/29/24
Season (warm vs. cold) *	331/205
Population (WVU vs. CWU) **	459/77
Family history of dermatitis or eczema (%)	15.3
Hand Dermatitis (%) during any research visit	22.4
TEWL	
Arm (median, range)	9.7, −5.8 − 65.53
Back (median, range)	9.5, 7.67 − 173.5
Forefinger (median, range)	21.3, −6.53 − 96.8

* Season variable was coded according to time of patch testing: warm - April through September; cold - October through March

** Population variable represents the location of subject recruitment (WVU-West Virginia University; CWU-Case Western Reserve University)

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Table 2

Logistic regression analysis for skin irritant response

Irritant	Gene	Position	Location	SNP	Chr	Genotype	N	Mean	(95% CI)	P		
										ADD	DOM	REC
SLS_L	*ACACB*	−820	intron	rs2268387	12	GG	95	1.60	1.50-1.70			
						AG	259	1.35	1.29-1.41			
						AA	182	1.34	1.27-1.42	0.0002617		3.20E-05
SLS_L	*ACACB*	−4907	intron	rs16934132	12	GG	85	1.35	1.24-1.45			
						AG	267	1.34	1.29-1.40			
						AA	184	1.48	1.41-1.56		0.0003871	
SLS_M	*NTRK2*	−1558	intron	rs10868231	9	GG	52	1.51	1.37-1.65			
						AG	205	1.49	1.42-1.55			
						AA	279	1.64	1.58-1.69		0.0007386	
SLS_H	*ACACB*	−1007	intron	rs2284685	12	GG	103	1.84	1.77-1.91			
						CG	257	1.89	1.85-1.93			
						CC	176	1.76	1.70-1.82		0.0009052	
NAOH_L	*IL22*	−242	intron	rs1179251	12	GG	8	1.38	0.94-1.81			
						CG	92	1.10	1.04-1.17			
						CC	436	1.02	1.00-1.03	0.0002163	0.0007886	
NAOH_M	*PLAU*	[53/38]	coding	rs2227564	10	GG	329	1.12	1.09-1.16			
						GA	183	1.03	1.00-1.05			
						AA	24	1.08	0.96-1.20		0.0005885	
NAOH_H	*EGFR*	−37643	intron	**rs6593202**	7	GG	394	1.20	1.16-1.24			
						GA	130	1.26	1.18-1.33			
						AA	12	1.5	1.17-1.83	0.0006236		
BKC_L	*FGF2*	−23897	intron	rs308439	4	CC	1	1.00	-			
						AC	51	1.37	1.24-1.51			
						AA	484	1.14	1.11-1.17		0.0008263	

Irritant	Gene	Position	Location	SNP	Chr	Genotype	N	Mean	(95% CI)	P		
										ADD	DOM	REC
BKC_M	*NTRK3*	−10816	intron	rs1347424	15	GG	206	1.18	1.12-1.23			
						GA	264	1.18	1.14-1.23			
						AA	66	1.31	1.19-1.42			0.0006351

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 confounders specific to each irritant; Additional adjustment for age resulted in same p values

ADD: Additive genetic model; DOM: Dominant genetic model; REC: Recessive genetic model

Bold marker is significant in haplotype analysis

Table 3

Association of gene variants with ICD

Gene	Position	Location	SNP	Genotype	N	Mean	(95% CI)	P (adjusted)		
								ADD	DOM	REC
EGF	−175	intron	rs10029654	GG	114	0.64	0.55-0.73			
				GA	115	0.52	0.43-0.61			
				AA	23	0.26	0.07-0.46	**0.001326**		**0.003475**
EGFR	−18262	intron	rs12718939	GG	107	0.49	0.39-0.58			
				GA	125	0.56	0.47-0.65			
				AA	20	0.85	0.68-1.02	0.001159		0.003111
CXCL12	−1125	intron	rs197452	GG	184	0.6	0.53-0.67			
				GA	63	0.43	0.3-0.55			
				AA	5	0.2	−0.36-0.76	**0.002309**	**0.003884**	
VCAM1	−1457	intron	rs3917018	GG	129	0.66	0.58-0.74			
				GA	92	0.41	0.31-0.52			
				AA	31	0.52	0.33-0.7		**0.003906**	

Results are adjusted for hand washing frequency and TEWL measurement on the forefinger; Bold p values represent significance after additional adjustment for age; ADD: Additive genetic model; DOM: Dominant genetic model; REC: Recessive genetic model

Table 4

Haplotype based test results

Variable	Chr	BP1	BP2	SNP1	SNP2	Haplotype	F	OR	STAT	P	Genes
NAOH_M	9	87449460	87450766	rs7870666	rs1867283	TA	0.482	3.73	10.1	0.0009999	*NTRK2*
NAOH_H	7	55105320	55124701	rs12718939	rs6593202	**AGA**	0.138	2.49	11	0.0008999	*EGFR*
NAOH_H	7	55105320	55124701	rs12718939	rs6593202	**GGG**	0.565	0.454	11.3	0.0008999	*EGFR*

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: Empirical p-values from permutation procedures (10,000 permutations)

Haplotypes containing a SNP (rs6593202) 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	BP	eQTL	Reference
chr10	rs2227564	75673100	*ECD*	[67]
chr12	rs2284685	109686781	*KCTD10*	[68]

BP: Physical position of the SNP

eQTL: List of genes affected by significant SNPs