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# Impact of Cytokine and Cytokine Receptor Gene Polymorphisms on Cellular Immunity after Smallpox Vaccination

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## Abstract

We explored associations between SNPs in cytokine/cytokine receptor genes and cellular immunity in subjects following primary smallpox vaccination. We also analyzed the genotype-phenotype associations discovered in the Caucasian subjects among a cohort of African-Americans. In Caucasians we found 277 associations ($p<0.05$) between gene SNPs and inter-individual variations in IFN-α, IL-12p40, IL-1β, IL-2, and TNF-α secretion levels. A collection of SNPs in the *IL1RN, IL2RB, IL4R, IL6, IL10RB, IL12A*, and *IL12RB2* genes had consistent associations among both Caucasians and African-Americans. A regulatory SNP (rs452204) in the *IL1RN* gene was significantly associated with higher levels of IL-2 secretion in an allele dose-dependent manner in both race groups ($p=0.05$ for Caucasians and $p=0.002$ for African-Americans). *IL12RB2* polymorphism rs3790567 was associated with a dose-related decrease in IL-1β secretion ($p=0.009$ for Caucasians and $p=0.01$ for African-Americans). Our results demonstrate that variations in smallpox vaccine-induced cytokine responses are modulated by genetic polymorphisms in cytokine and cytokine receptor genes.

### Keywords

Immunogenetics; SNPs; Smallpox Vaccine; Cytokine; Cytokine Receptor; Cellular Immunity; Caucasians; African-Americans

## 1. Introduction

Smallpox (vaccinia virus) immunization induces a spectrum of inter-individual variations in protective immune responses (Henderson et al., 2008). Human Leukocyte Antigen (*HLA*), cytokine/cytokine receptor, vitamin D (*RXRA*) receptor and other genes are known to play a

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significant part in host immune response variation to smallpox immunization (Ovsyannikova et al., 2011; Stanley, Jr. et al., 2007; Davis et al., 2010; Haralambieva et al., 2011a). Studies have also demonstrated genetic predisposition for an adverse event (AE) after smallpox vaccine (Stanley, Jr. et al., 2007; Reif et al., 2008; Crowe, Jr., 2007). Genetic variations that influence immune responses to smallpox vaccine may also differ across ethnic groups. A recent study of humoral immunity to smallpox vaccine in healthy adults found differences in single-nucleotide polymorphisms (SNPs) distributions among Caucasians and African-Americans (Haralambieva et al., 2011a). This study also found common SNPs in *IL18* and *IL18R1* genes significantly associated with inter-individual variations in smallpox vaccine-induced antibody levels in Caucasians and African-Americans (Haralambieva et al., 2011a). However, not much is known about the effects of polymorphisms in cytokine/cytokine receptor genes on markers of vaccinia-induced cellular immunity.

Cytokines play an essential role in controlling immune responses to pathogens, including vaccinia virus (Biron, 1994; McKinney et al., 2006). The host vaccinia-virus interaction triggers an anti-viral immune response in the host, which leads to activation of cytokine and signaling pathways. Because of the significance of cytokines in driving innate and adaptive immune responses, gene polymorphisms that influence regulation of cytokine production or cytokine activity may influence smallpox vaccine-specific immune responses. Unlike vaccine-induced antibody levels changes in cytokine levels have not been correlated with protection, and the clinical/biological relevance of specific cytokine levels is unclear. Nonetheless, vaccinia virus-elicited cytokines have been linked to host response and immunity. For example, adverse events, such as fever, lymphadenopathy, and localized or generalized rash have been reported in association with higher levels of cytokines (IFN-γ, TNF-α, IL-2, IL-5, and IL-10) following smallpox vaccine (Rock et al., 2004). Polymorphisms in the *IL1* and *IL18* genes have been connected to an increased incidence of fever and variations in humoral immunity after immunization with smallpox vaccine (Stanley, Jr. et al., 2007). Thus, understanding genetic determinants that influence inter-individual variations in cytokine production after smallpox vaccination may offer additional insights into factors that influence immunity and AEs in response to the smallpox vaccine.

We conducted a population-based study of smallpox vaccine antibody response and genotyped a large group of healthy subjects after a single dose of smallpox vaccine (Haralambieva et al., 2011a; Ovsyannikova et al., 2011). In this study, we explored relationships between SNPs in candidate cytokine and cytokine receptor genes and cell-mediated immunity as measured by cytokines *in vitro* in subjects following primary smallpox vaccination. We also examined the genotype-phenotype associations discovered in our Caucasian subjects among an internal cohort of African-Americans.

## 2. Materials and methods

### 2.1. Study Subjects

Details of this study's recruitment are described in our previous publications (Kennedy et al., 2009; Ovsyannikova et al., 2011; Haralambieva et al., 2011a). The description of our study subjects is nearly identical to what has been previously published (Ovsyannikova et al., 2012; Kennedy et al., 2009; Ovsyannikova et al., 2011; Haralambieva et al., 2011a). Briefly, we enrolled 1,076 healthy subjects (age 18 to 40 years) in Rochester, MN and San Diego, CA. All study subjects received a single dose of Dryvax between 2002 and 2006 and had a documented vaccine "take," or development of the pustule, at the vaccination site following immunization. We collected demographic and vaccination information including age at enrollment, gender, race, ethnicity, and time since previous vaccination. The Institutional Review Boards of both the Mayo Clinic and Naval Health Research Center approved the study, and we obtained written informed consent from each subject.

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### 2.2. Cytokine measurements

We purified peripheral blood mononuclear cells (PBMCs), as previously described (Ryan et al., 2009; Umlauf et al., 2011). Our description of cytokine measurements is similar to those we published previously (Ryan et al., 2009; Umlauf et al., 2011). We optimized viral stimulation conditions, as previously described (Ryan et al., 2009; Ovsyannikova et al., 2011) and they are: IFN-β, IL-2, IL-18: MOI = 5 (24 hours); IL-12p40, IL-12p70, TNF-α, IL-1β: MOI = 0.5 (24 hours); IFN-α: MOI = 0.05 (4 days); IL-4, IL-10: MOI = 0.05 (7 days). We used ELISA kits for IL-1β, IL-2, IL-4, IL-10, IL-12p40, IL-12p70, and TNF-α (BD Pharmingen, San Diego, CA), IFN-α (PBL Biomedical Laboratories, Piscataway, NJ), IFN-β (PBL Biomedical Laboratories, New Brunswick, NJ), and IL-18 (MBL International, Woburn, MA) to detect cytokines from culture supernatants. The levels of sensitivity for the IL-1β, IL-2, IL-4, IL-10, IL-12p40, IL-12p70, TNF-α, IFN-α, IFN-β, and IL-18 assays were 3.9 pg/ml, 7.8 pg/ml, 7.8 pg/ml, 7.8 pg/ml, 31.3 pg/ml, 7.8 pg/ml, 7.8 pg/ml, 12.5 pg/ml, 250 pg/ml, and 25.6 pg/ml, respectively.

### 2.3. Genotyping methods

The genotyping methods we used for this study have been previously described and our description of the methods is identical of those we previously published (Haralambieva et al., 2011a). TagSNPs within and 10kb upstream and downstream of the 32 candidate cytokine and cytokine receptor genes were selected using the LD tagSNP selection approach from the Hapmap Phase II (http://www.hapmap.org), Seattle SNPs (http://pga.mbt.washington.edu/), NIEHS SNPs (http://egp.gs.washington.edu/), and NCBI (http://www.ncbi.nlm.nih.gov/projects/SNP/). The selection criteria included: SNPs with validation data, successful predictive genotyping scores for Illumina GoldenGate assays, a minor allele frequency (MAF) 0.05, and a pairwise linkage disequilibrium (LD) threshold of $r^2$ 0.90.

The selected 785 candidate SNPs were genotyped using two custom-designed 384-plex Illumina GoldenGate™ assays (Illumina Inc., San Diego, CA), TaqMan custom assays (Applied Biosystems, Foster City, CA) and pyrosequencing. All SNPs selected for the custom oligo-pooled assays had Illumina design scores > 0.4 and all DNA samples (n=250 ng each) were genotyped following the manufacturer's instructions. Genotype calls were made using the genotyping module of BeadStudio 2 software. The Illumina genotyping success rate was 89%, and the study success rate was 98% (Haralambieva et al., 2011a).

### 2.4. SNP genotyping

SNP genotyping for this study has been previously described (Haralambieva et al., 2011a). The description of the SNP genotyping methods is nearly identical to those we previously published (Haralambieva et al., 2011b). In brief, we genotyped 1,076 subjects for 785 known SNPs in the Th1 (*IL2, IFNG, IL12A, IL12B*), Th2 (*IL4*) and innate/proinflammatory (*IL6, IL10, IL18, IL1B, TNFA, IFNA, IFNB*) cytokine genes and the corresponding Th1 (*IL2RA, IL2RB, IL2RG, IFNGR1, IFNGR2, IL12RB, IL12RB2*), Th2 (*IL4R*) and innate/inflammatory (*IL6R, IL6ST, IL10RA, IL10RB, IL18R1, IL1R1, IL1R2, IL1RN, IFNAR1, IFNAR2, TNFRSF1A, TNFRSF1B*) cytokine receptors genes. Subject exclusions were made on the basis of inadequate/poor quality of DNA (n=5) or low call rates (n=15, call rates <0.95). SNPs were excluded based on complete genotyping failure, low call rates (n=25, call rates <0.95) or MAF<0.05 (n=50, MAF=0). A total of 701 SNPs met all genotyping quality assessments and were used for analysis in a set of 1,056 subjects, which included 580 Caucasians and 217 African-Americans.

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## 2.5. Statistical methods

**2.5.1. Racial classification—**The description of both our statistical analyses and methods is identical or nearly identical to those we previously published (Ovsyannikova et al., 2012; Haralambieva et al., 2011a). Self-declared race and ethnicity were collected for all individuals. We supplemented this demographic information with genetic data using a subset of 25,000 autosomal SNPs with call rates above 99% from the Illumina Infinium HumanHap650Y bead chip array for African-Americans, and the HumanHap550 array for Caucasians, run in conjunction with the Golden Gate candidate SNP assay. SNPs were chosen by chromosome and positioned in such a way as to adequately cover the entire genome, with a minimum distance of 100 kb between each SNP. Using these SNPs, we employed a principal components (PC)-based approach similar to that used by the Eigenstrat software package to assess population structure. The resulting first four eigenvectors, with additional guidance from the self-declared race and ethnicity data, revealed four unique clusters of subjects: 580 in Group 1, assumed to be primarily of European descent; 217 in Group 2, assumed to be primarily of African descent; 54 in Group 3, assumed to be primarily of Asian descent; and 229 in Group 4, assumed to be primarily of Hispanic ethnicity. A total of 219 subjects did not fit into one of these four clusters. The resulting Caucasian and African-American racial groups were used for all subsequent comparisons described herein.

**2.5.2. Statistical analyses—**Statistical analyses and the description of our methods are identical to those used in our candidate gene association studies of smallpox vaccine immunity (Haralambieva et al., 2011a; Ovsyannikova et al., 2011). Tests for deviation from the assumption of Hardy-Weinberg equilibrium (HWE) were performed after accounting for the racial clusters defined above using an approach similar to that described by Schaid, *et al.* (Schaid et al., 2006).

Data were descriptively summarized using frequencies and percentages for categorical variables, and medians and inter-quartile ranges (IQRs) for continuous variables. Assessment of cytokine secretion resulted in six recorded values: three prior to stimulation with vaccinia virus and three post-stimulation. For descriptive purposes, a single response measurement per individual was obtained for each outcome by subtracting the median of the three unstimulated values from the median of the three stimulated values.

Associations of cytokine secretion with SNP genotypes were formally evaluated using linear regression models. In contrast to the descriptive analyses, formal evaluations used repeated measures analyses to simultaneously model the multiple observations per subject. We accounted for within-subject correlations using generalized estimating equations (GEEs), fitting an unstructured within-person variance-covariance matrix for each model. Associations comparing stimulation-induced differences in cytokine secretion with SNP genotypes were evaluated by including the SNP variable in the regression model, together with a variable representing stimulation status. The resulting SNP-by-stimulation status interaction was then tested for statistical significance. These repeated measures models are similar to paired t-tests, in that they compare differences between the two stimulation states within each individual across racial groups. SNPs were modeled assuming an ordinal genotypic effect based on the number of copies of the minor allele. Two sets of analyses were carried out. First, associations of all 701 SNPs with each cytokine secretion response variable were examined within the Caucasian (discovery) subset. All such associations with resulting p-value $< 0.05$ were re-examined in the African-American (replication) subset. SNPs with $p<0.05$ for a given cytokine in each racial subset, and with associations in the same direction for each subset, were considered statistically significant. All analyses adjusted for the following set of demographic and clinical variables: gender; age at blood

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draw (quartiles); time from smallpox immunization to blood draw (quartiles); time from blood draw to assay (quartiles); shipping temperature of the sample (frozen or ambient); and time of year when the sample was shipped (warm weather months April–September vs. cold weather months October–March).

Inverse cumulative normal (probit) data transformations were used to correct for data skewness in all linear regression models. All statistical tests were two-sided and all analyses were carried out using the SAS software system (SAS Institute, Inc., Cary, NC).

# 3. Results

## 3.1. Subjects' Demographics and Cytokine Responses

The overall demographic and immune response variables of our study subjects have been previously published (Haralambieva et al., 2011a; Ovsyannikova et al., 2011; Umlauf et al., 2011; Kennedy et al., 2009). In brief, our study cohort consisted of 580 Caucasian (discovery cohort) and 217 African-American subjects. There were 422 Caucasian males and 158 Caucasian females in this study (Table 1). The African-American cohort consisted of 174 males and 43 females.

The cytokine secretion profile was consistent with a broad-based immune response characterized by secretion of innate/proinflammatory and Th1 cytokines in response to vaccinia virus stimulation. In this study, we utilized inactivated vaccinia virus in order to minimize viral immunomodulation. We observed high levels of IL-1β and TNF-α that are well recognized proinflammatory cytokines known to cause fever in humans. Table 2 outlines the secretion of IFN-α, IL-12p40, IL-1β, IL-2, and TNF-α cytokines from subjects in each racial group. We were unable to detect any vaccinia-induced secretion of IL-4, IL-10, IL-12p70, IL-18, and IFN-β above the measurable limit of detection of the ELISA assay.

## 3.2. Associations between SNPs in Cytokine and Cytokine Receptor Genes and Vaccinia-Specific IFN-α, IL-12p40, IL-1β, IL-2, and TNF-α Secretion in Caucasians

In the Caucasian cohort (discovery cohort, n=580), we found 277 significant associations (at the level $p<0.05$) between polymorphisms in cytokine/cytokine receptor genes and variations in IFN-α (n=34 SNPs) (Supplemental Table 1), IL-12p40 (n=44 SNPs) (Supplemental Table 2), IL-1β (n=79 SNPs) (Supplemental Table 3), IL-2 (n=60 SNPs) (Supplemental Table 4), and TNF-α (n=60 SNP) (Supplemental Table 5) secretion levels. A non-synonymous *IFNAR2* polymorphism (rs4986956/Phe8Ser, p=0.007) was significantly associated with a five-fold allele dose-related decrease in IFN-α secretion (Supplemental Table 1). Two genetic variants in the promoter and intronic regions of *IL12A* gene (rs640039, p=0.0002 and rs2243131, p=0.001) demonstrated associations with variations in IFN-α secretion (three-fold and two-fold, respectively).

Two coding synonymous *IL6* (rs2069849/Phe201Phe, p=0.009) and non-synonymous *IL4R* (rs3024677/Val579Ile, p=0.01) polymorphisms were associated with vaccinia-specific IL-12p40 secretion (Supplemental Table 2). Further, the minor allele G of a promoter SNP (rs640039) from the *IL12A* gene was associated with an allele dose-dependent increase (1.5-fold) in IL-12p40 secretion (p=0.006).

In addition, two synonymous genetic variants in the *IL18* (rs549908/Ser35Ser, p=0.002) and *IL6* (rs2069849/Phe201Phe, p=0.005) genes demonstrated associations with variations in IL-1β secretion (Supplemental Table 3). Of note, a synonymous *IL6* polymorphism rs2069849 exhibited highly significant associations with the production of vaccinia-specific IL-12p40 (p=0.009) and IL-1β (p=0.005). Caucasians demonstrated significant associations

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between two promoter SNPs (rs3093668, p=0.0005 and rs3093726, p=0.0006) in the *TNF* gene and secreted IL-1β.

Sixty significant SNP associations (p<0.05) with vaccinia-specific IL-2 secretion were also found in Caucasians. Three of them were coding SNPs in the *IL4R* gene: non-synonymous rs1805015/Ser503Pro (p=0.004), non-synonymous rs1805011/Glu400Ala (p=0.009), and synonymous rs2234898/Leu414Leu (p=0.01) (Supplemental Table 4). We also found significant associations (p<0.009) between multiple SNPs in the *IL10* gene and IL-2 secretion.

Lastly, the minor allele A of an intronic SNP (rs360722, p=0.0005) within the *IL18* gene was associated with an allele dose-dependent decrease (2-fold) in TNF-α secretion (Supplemental Table 5).

### 3.3. Associations between SNPs in Cytokine and Cytokine Receptor Genes and Vaccinia-Specific Cytokine Secretion in African-Americans

All SNPs with p<0.05 in the Caucasian analyses were examined in the African-American racial subset. In our African-American cohort, we found several SNP associations (p<0.05) with vaccinia-specific IFN-α, IL-12p40, IL-1β, IL-2, and TNF-α secretion levels (Table 3) that were consistent with the Caucasian cohort findings. In particular, an intronic SNP rs3024672 in the *IL4R* gene was significantly associated with lower levels of IFN-α secretion in an allele dose-dependent manner in both race groups (72 vs. 24 pg/ml, p=0.01 for Caucasians and 58 vs. 33 pg/ml, p=0.003 for African-Americans).

Two intronic SNPs rs2243131 (p=0.03 for Caucasians and p=0.02 for African-Americans) and rs2069844 (p=0.002 for Caucasians and p=0.02 for African-Americans) in the *IL12A* and *IL6* genes, respectively, were significantly associated with IL-12p40 production in an allele dose-dependent manner in both race groups (Table 3).

Five previously discovered *IL12RB2* polymorphism associations in Caucasians were also observed with vaccinia-specific IL-1β secretion in African-Americans. Intronic SNPs (rs1874791, rs3790565, rs3790566, rs946685, and rs3790567; pair-wise $r^2$ 0.92) in the *IL12RB2* gene were all significantly associated with IL-1β secretion in both race groups in an allele dose-dependent manner (range of p-values from 0.01 to 0.002).

One *IL1RN* (rs452204) and two *IL2RB* (rs3218266 and rs2284034; $r^2$=0.21) polymorphisms identified in Caucasians were associated with vaccinia-specific IL-2 secretion and were similarly associated in African-Americans. Genetic variant rs2069763 was a coding synonymous SNP (exon 1, Leu38Leu) that was associated with an allele dose-related increase in IL-2 production in Caucasians (15.7 vs. 25.6 pg/ml, p=0.04), but with an allele dose-related decrease in IL-2 production in African-Americans (17.9 vs. 9.9 pg/ml, p=0.04) (data not shown).

Finally, SNP rs946685 in the *IL12RB2* gene was significantly associated with TNF-α secretion in an allele dose-dependent manner in both race groups (188 vs. 128 pg/ml, p=0.02 for Caucasians and 184 vs. 155 pg/ml, p=0.02 for African-Americans).

## 4. Discussion

We found that the variations in cell-mediated (cytokine) immune responses to smallpox vaccine are significantly influenced by genetic variants in cytokine and cytokine receptor genes. In total, we discovered 277 significant associations (p<0.05) between polymorphisms in cytokine/cytokine receptor genes (n=32) and variations in vaccinia-specific IFN-α, IL-12p40, IL-1β, IL-2, and TNF-α secretion in Caucasians (Supplemental Tables 1–5).

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Multiple non-coding and coding polymorphisms in these genes were putatively associated with variations in cytokine responses, with many of these SNPs associated in an allele dose-related manner. A number of SNP associations (n=13) belonging to the *IL1RN, IL2RB, IL4R, IL6, IL10RB, IL12A*, and *IL12RB2* genes demonstrated concordance between Caucasians and African-Americans. Furthermore, SNP associations in the *IL4R, IL6, IL12A*, and *IL12RB2* genes demonstrated allele dose-related responses that were consistent in the two racial groups. As an example, we identified a SNP in the *IL1RN* (rs452204) gene whose minor allele is associated with a considerable decrease (1.5-fold for Caucasians and three-fold for African-Americans) in vaccinia-specific IL-2 secretion levels in a dose-related manner. Similarly, the intronic *IL12RB2* polymorphism (rs3790567) previously reported to be associated with giant cell-arteritis (Rodriguez-Rodriguez et al., 2011) demonstrated significant associations with decreased IL-1β secretion in a dose-related manner (1.5-fold for Caucasians and 1.7-fold for African-Americans) after vaccinia virus stimulation. It is important to define the functional consequences of these replicated genetic variants that are associated with inter-individual variations in cytokine responses to smallpox vaccine, and such studies are ongoing in our laboratory.

Polymorphisms in coding and non-coding domains of genes coding for cytokine/cytokine receptors can influence various effects of cytokine biology. Among these effects are altered gene and/or protein expression and function, alternate splice variant usage, altered transcriptional factor activity, methylation and miRNA function (van Deventer, 2000; Haukim et al., 2002; Bidwell et al., 2001; Bidwell et al., 1999). Cytokine receptor polymorphisms can alter gene expression and affect receptor protein function (van Deventer, 2000;Hackstein et al., 2001;van de Vosse et al., 2003). Functional genetic variants can be found for all cytokines, as well as for their respective receptors. For example, a specific haplotype in the *IL4* gene has been demonstrated to be associated with reduced predisposition to the development of fever in smallpox vaccine-naïve individuals (Stanley, Jr. et al., 2007). One potential mechanism behind the association between the *IL4* gene haplotype, which includes the known functional SNP rs2243250, and a decreased risk for fever might be the influence of a Th2 type-modulated increase in IL-4 production and concomitant suppression of IFN-γ production and Th1 responses (Stanley, Jr. et al., 2007; Nelms et al., 1999).

Race-specific analyses demonstrated that a specific polymorphism in the *IL2* gene (coding synonymous rs2069763, Leu38Leu) is associated with vaccinia-specific IL-2 production in an allele dose-dependent manner in both racial groups (Caucasians and African-Americans). This specific polymorphism may have a functionally significant effect on the gene product (i.e., IL-2 protein secretion level). The Th1 type cytokine, IL-2, is a critical immunoregulatory cytokine required for T cell proliferation and plays an important role in adaptive immunity (Malek, 2002). Of interest, a positive correlation ($p$=0.0009) between IL-2 secretion and vaccinia-specific neutralizing antibody titer was found within this cohort of study subjects (Ryan et al., 2009; Umlauf et al., 2011). Also, previously cited *IL18R1* SNPs rs2080289 and rs2241116 were associated with both an increased neutralizing antibody titer ($p$=0.001) and IL-2 ($p$=0.029), and TNF-α ($p$=0.023) secretion level, respectively, in an allele dose–dependent manner in Caucasians (Supplemental Tables 4 and 5) (Haralambieva et al., 2011a). Notably, the *IL2* genetic polymorphism rs2069763 has also been associated with high measles vaccine-induced antibody and high lymphoproliferative responses (Dhiman et al., 2007). This SNP has also been linked to human papillomavirus (HPV)-related cervical and vulvar cancers (Hussain et al., 2008). These observations suggest an important role for this genetic variant in the modulation of immune responses to multiple viral pathogens.

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Significant variation in immune responses to smallpox vaccine was observed in our study subjects. We found evidence for vigorous production of innate/proinflammatory and Th1 cytokines (IFN-α, IL-12p40, IL-1β, and TNF-α) that indicates the importance of the early innate and inflammatory immune pathways after smallpox vaccination in the generation of protective immunity. These inter-individual variations in cytokine immune responses after smallpox immunization are likely to result in part from genetic variability between individuals. Studies have demonstrated that IL-1β, IL-18, TNF-α, and the interferons are inhibited by specific vaccinia strain proteins (Dunlop et al., 2003; Moss and Shisler, 2001), and removal of these inhibitory proteins can reduce the virulence of the resulting vaccinia strains (Reading and Smith, 2003; Mossman et al., 1996). These data indicate that alterations in individual cytokine levels, or in the overall balance of cytokines, significantly influence immune response to smallpox vaccine and disease susceptibility.

We found evidence for increased production of IL-1β and TNF-α, which are well recognized proinflammatory cytokines that cause fever in humans (Dinarello, 2004). This increased cytokine production may be due to actions modulated by a transcription factor-kB (NF-kB) that is required for transcription of several cytokines, including IL-1 and TNF-α (Jin and Wang, 2003). NF-kB plays a role in regulating cellular responses to viral antigens and infection. Conversely, the binding of NF-kB and other transcription factors (Jak/STAT/IRF) to regulatory regions can be interrupted by promoter polymorphism(s) (Jin and Wang, 2003). A recent study examining associations between AEs following smallpox vaccination and SNPs in 19 candidate genes found that at the single-gene level, variations within the *IL1* and *IL18* genes were associated with adverse responses, specifically fever or vaccine-induced febrile responses after smallpox vaccination (Stanley, Jr. et al., 2007). Our analysis in Caucasians demonstrated a number of significant associations, at the level $p<0.05$, between SNPs in the *IL1R* (n=15 associations), *IL18* (n=6 associations), and *IL18R1* (n=28 associations) genes and vaccinia-specific IL-1β secretion levels (Supplemental Table 3). It is important to replicate these candidate gene SNPs discovered in our work and conduct follow-up studies of genetic variants to gain insights into the functions of replicated polymorphisms and the mechanisms by which they may contribute to smallpox vaccine immunity. Even if these individual SNPs do not provide causative insights into smallpox vaccine immune response, they may lead to further studies providing mechanistic insights (Debouck, 2009).

The strengths of our population-based study include a large sample of healthy Caucasian and African-American individuals with well-documented demographic and immunization data. Genotyping across 32 cytokine and cytokine receptor genes allowed us to examine associations of both relatively common and uncommon alleles with vaccinia-specific cytokine responses and with demographic variables of interest.

The limitations of our study include the genotyping of candidate cytokine and cytokine receptor gene families, and no other candidate immune response genes. Therefore, these individual cytokine/cytokine receptor SNPs may influence only a small proportion of the heritability of the phenotype expression (i.e., cellular immunity). Further, our analysis in African-Americans was conducted using a smaller group of subjects and thus has restricted power. Statistical analysis only included Caucasians and African-Americans, narrowing the conclusions regarding genetic associations in other racial or ethnic groups.

Our analyses were conducted in two independent study groups that had different racial backgrounds: African- and Caucasian-American. Because of the genetic differences between these two groups, we must acknowledge that it is possible that genetic control of immune responses may differ between them. However, if there are indeed functional variants that have a direct effect on the development of immune responses to smallpox vaccine, and these

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functional variants are present in both genetic populations, the presence of a consistent genetic association within each of the two populations provides much stronger evidence in favor of that association.

Additionally, we acknowledge the importance of multiple testing issues in this study. We examined 701 SNPs across five different cytokine secretion measures in the Caucasian cohort, resulting in 3,505 tests of association. Thus, some of the associations reported herein could be false positive associations. Under the complete null hypothesis of no SNP associations and under the assumption of independent tests of hypothesis (an assumption not completely met due to linkage disequilibrium present in some SNP clusters), we would expect approximately 175 statistically significant tests by chance alone. It is noteworthy that we identified 277 SNP associations with $p<0.05$, suggesting that a large number of our SNP associations are likely to be true positive results. Similarly, of the 277 SNP associations with $p<0.05$ in the Caucasian cohort, we would expect approximately seven to successfully replicate in the African-American cohort (assuming two-sided $p<0.05$ and association in the same direction as Caucasian estimate, effectively resulting in a one-sided test with $p<0.025$). We successfully replicated 13 SNP associations in the African-American cohort, higher than that expected by chance alone.

In conclusion, our results suggest that genetic diversity at cytokine and cytokine receptor loci may contribute to variations in cytokine responses following smallpox vaccination. Vaccine-induced adaptive immune responses in our study subjects were characterized by high levels of innate/proinflammatory and Th1 TNF-α, IL-1β, IL-12p40, and IFN-α cytokines. It is plausible that high production of these cytokines originates from genetic polymorphisms in the cytokine and cytokine receptors and/or activation of downstream signaling molecules and pathways. Several SNP associations in Caucasian subjects with variations in vaccinia-induced secreted IFN-α, IL-12p40, IL-1β, IL-2, and TNF-α cytokines were replicated in African-Americans. These replicated associations were found within the key cytokine and cytokine receptor *IL1RN, IL2RB, IL4R, IL6, IL10RB, IL12A*, and *IL12RB2* genes, indicating these genetic factors have an important role in cellular immunity. This study enhances our understanding of genetic determinants involved in inter-individual variation of cellular immune responses following smallpox vaccination, and may be applicable to other viral diseases as well. Our findings will inform further studies intended to confirm the results reported herein, and may provide novel insights into functional mechanisms behind these genetic associations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **SNPs** | Single-nucleotide polymorphisms |
| **HLA** | Human Leukocyte Antigen |

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| | |
|---|---|
| **IL** | Interleukin |
| **HWE** | Hardy-Weinberg equilibrium |
| **IQR** | Inter-quartile range |

## References

1. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun. 1999; 1:3–19. [PubMed: 11197303]
2. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases: Supplement 1. Genes Immun. 2001; 2:61–70. [PubMed: 11393658]
3. Biron CA. Cytokines in the generation of immune responses to, and resolution of, virus infection. Curr. Opin. Immunol. 1994; 6:530–538.
4. Crowe JE Jr. Genetic predisposition for adverse events after vaccination. J Infect Dis. 2007; 196:176–177. [PubMed: 17570102]
5. Davis NA, Crowe JE Jr, Pajewski NM, McKinney BA. Surfing a genetic association interaction network to identify modulators of antibody response to smallpox vaccine. Genes Immun. 2010; 8:630–636. [PubMed: 20613780]
6. Debouck C. Integrating genomics across drug discovery and development. Toxicol. Lett. 2009; 186:9–12.
7. Dhiman N, Ovsyannikova IG, Cunningham JM, Vierkant RA, Kennedy RB, Pankratz VS, Poland GA, Jacobson RM. Associations between measles vaccine immunity and single nucleotide polymorphisms in cytokine and cytokine receptor genes. J Infect Dis. 2007; 195:21–29. [PubMed: 17152005]
8. Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. J Endotoxin. Res. 2004; 10:201–222.
9. Dunlop LR, Oehlberg KA, Reid JJ, Avci D, Rosengard AM. Variola virus immune evasion proteins. Microbes. Infect. 2003; 5:1049–1056.
10. Hackstein H, Hecker M, Kruse S, Bohnert A, Ober C, Deichmann KA, Bein G. A novel polymorphism in the 5′ promoter region of the human interleukin-4 receptor alpha-chain gene is associated with decreased soluble interleukin-4 receptor protein levels. Immunogenetics. 2001; 53:264–269. [PubMed: 11491529]
11. Haralambieva IH, Ovsyannikova IG, Dhiman N, Kennedy RB, O'Byrne M, Pankrantz VS, Jacobson RM, Poland GA. Common SNPs/haplotypes in IL18R1 and IL18 genes are associated with variations in humoral immunity to smallpox vaccination in Caucasians and African-Americans. J. Infect. Dis. 2011a; 204:433–441.
12. Haralambieva IH, Ovsyannikova IG, Kennedy RB, Vierkant RA, Pankratz SV, Jacobson RM, Poland GA. Associations between single nucleotide polymorphisms and haplotypes in cytokine and cytokine receptor genes and immunity to measles vaccination. Vaccine. 2011b; 29:7883–7895. [PubMed: 21875636]
13. Haukim N, Bidwell JL, Smith AJ, Keen LJ, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases: Supplement 2. Genes Immun. 2002; 3:313–330. [PubMed: 12209358]
14. Henderson, DA.; Borio, LL.; Grabenstein, JD. Smallpox and vaccinia. In: Plotkin, SA.; Orenstein, WA.; Offit, PA., editors. Vaccines. 2008. p. 773-804.
15. Hussain SK, Madeleine MM, Johnson LG, Du Q, Malkki M, Wilkerson HW, Farin FM, Carter JJ, Galloway DA, Daling JR, Petersdorf EW, Schwartz SM. Cervical and vulvar cancer risk in relation to the joint effects of cigarette smoking and genetic variation in interleukin 2. Cancer Epidemiol. Biomarkers Prev. 2008; 17:1790–1799.

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16. Jin P, Wang E. Polymorphism in clinical immunology - From HLA typing to immunogenetic profiling. J Transl Med. 2003; 1:8. [PubMed: 14624696]
17. Kennedy RB, Ovsyannikova IG, Pankratz VS, Vierkant RA, Jacobson RM, Ryan MA, Poland GA. Gender effects on humoral immune responses to smallpox vaccine. Vaccine. 2009; 27:3319–3323. [PubMed: 19200827]
18. Malek TR. T helper cells, IL-2 and the generation of cytotoxic T-cell responses. Trends Immunol. 2002; 23:465–467. [PubMed: 12297409]
19. McKinney BA, Reif DM, Rock MT, Edwards KM, Kingsmore SF, Moore JH, Crowe JE Jr. Cytokine expression patterns associated with systemic adverse events following smallpox immunization. J Infect Dis. 2006; 194:444–453. [PubMed: 16845627]
20. Moss B, Shisler JL. Immunology 101 at poxvirus U: immune evasion genes. Semin. Immunol. 2001; 13:59–66.
21. Mossman K, Nation P, Macen J, Garbutt M, Lucas A, McFadden G. Myxoma virus M-T7, a secreted homolog of the interferon-gamma receptor, is a critical virulence factor for the development of myxomatosis in European rabbits. Virology. 1996; 215:17–30. [PubMed: 8553583]
22. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. Annu Rev Immunol. 1999; 17:701–738. [PubMed: 10358772]
23. Ovsyannikova IG, Kennedy RB, O'Byrne M, Jacobson RM, Pankratz VS, Poland GA. Genome-wide association study of antibody response to smallpox vaccine. Vaccine. 2012; 30:4182–4189. [PubMed: 22542470]
24. Ovsyannikova IG, Vierkant RA, Pankratz VS, Jacobson RM, Poland GA. Human leukocyte antigen genotypes in the genetic control of adaptive immune responses to smallpox vaccine. J Infect Dis. 2011; 203:1546–1555. [PubMed: 21592983]
25. Reading PC, Smith GL. Vaccinia virus interleukin-18-binding protein promotes virulence by reducing gamma interferon production and natural killer and T-cell activity. J Virol. 2003; 77:9960–9968. [PubMed: 12941906]
26. Reif DM, McKinney BA, Motsinger AA, Chanock SJ, Edwards KM, Rock MT, Moore JH, Crowe JE Jr. Genetic Basis for Adverse Events after Smallpox Vaccination. J Infect Dis. 2008; 198:16–22. [PubMed: 18454680]
27. Rock MT, Yoder SM, Talbot TR, Edwards KM, Crowe JE Jr. Adverse events after smallpox immunizations are associated with alterations in systemic cytokine levels. 2004; 189:1401–1410.
28. Rodriguez-Rodriguez L, Carmona FD, Castaneda S, Miranda-Filloy JA, Morado IC, Narvaez J, Mari-Alfonso B, Gomez-Vaquero C, Amigo-Diaz E, Rios-Fernandez R, Blanco R, Llorca J, Fernandez-Gutierrez B, Martin J, Gonzalez-Gay MA. Role of rs1343151 IL23R and rs3790567 IL12RB2 polymorphisms in biopsy-proven giant cell arteritis. J Rheumatol. 2011; 38:889–892. [PubMed: 21285166]
29. Ryan JE, Dhiman N, Ovsyannikova IG, Vierkant RA, Pankratz VS, Poland GA. Response surface methodology to determine optimal cytokine responses in human peripheral blood mononuclear cells after smallpox vaccination. J Immunol Methods. 2009; 341:97–105. [PubMed: 19038260]
30. Schaid DJ, Batzler AJ, Jenkins GD, Hildebrandt MA. Exact tests of Hardy-Weinberg equilibrium and homogeneity of disequilibrium across strata. Am. J Hum. Genet. 2006; 79:1071–1080.
31. Stanley SL Jr, Frey SE, Taillon-Miller P, Guo J, Miller RD, Koboldt DC, Elashoff M, Christensen R, Saccone NL, Belshe RB. The immunogenetics of smallpox vaccination. J Infect Dis. 2007; 196:212–219. [PubMed: 17570108]
32. Umlauf BJ, Ovsyannikova IG, Haralambieva IH, Kennedy RB, Vierkant RA, Pankrantz VS, Jacobson RM, Poland GA. Correlations between vaccinia-specific immune responses within a cohort of armed forces members. Viral Immunol. 2011; 24:415–420. [PubMed: 21958369]
33. van de Vosse E, Lichtenauer-Kaligis EGR, van Dissel JT, Ottenhoff THM. Genetic variations in the interleukin-12/interleukin-23 receptor (beta1) chain, and implications for IL-12 and IL-23 receptor structure and function. Immunogenetics. 2003; 54:817–829. [PubMed: 12671732]
34. van Deventer SJH. Cytokine and cytokine receptor polymorphisms in infectious disease. Intensive Care Med. 2000; 26(Suppl 1):S98–S102. [PubMed: 10786965]

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## Highlights

- Genetic variations influencing vaccine immunity differ across ethnic groups
- Gene SNP associations with smallpox vaccine cellular immunity were examined
- Thirteen SNPs had consistent associations among Caucasians and African-Americans
- Genetic diversity may contribute to immune response variations post vaccination

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**Table 1**

Demographic characteristics of the Caucasian and African-American study cohorts.

| Variable | African-American (N=217) | | Caucasian (N=580) | |
|---|---|---|---|---|
| | **N** | **%** | **N** | **%** |
| Gender | | | | |
| Male | 174 | 80 | 422 | 73 |
| Female | 43 | 20 | 158 | 27 |
| Age quartile | | | | |
| 19–21 years | 49 | 23 | 133 | 23 |
| 22–23 years | 39 | 18 | 156 | 27 |
| 24–27 yeas | 54 | 25 | 167 | 29 |
| 28–40 years | 75 | 34 | 124 | 21 |
| Time from immunization to blood draw | | | | |
| 1–8 months | 53 | 24 | 149 | 26 |
| 9–14 months | 46 | 21 | 147 | 25 |
| 15–33 months | 44 | 20 | 161 | 28 |
| 34–48 months | 74 | 35 | 123 | 21 |

N, number of subjects.

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## Table 2

Vaccinia-specific cytokine immune responses in the Caucasian and African-American study cohorts.

| Cytokine | | N | Mean (pg/ml) | Std | Median (pg/ml) | Q1 | Q3 |
|---|---|---|---|---|---|---|---|
| IFN-α | African-American | 209 | 76.0 | 86.0 | 49.3 | 12.0 | 120.5 |
| | Caucasian | 560 | 94.9 | 97.4 | 67.5 | 22.6 | 143.0 |
| IL-12p40 | African-American | 189 | 93.6 | 118.9 | 65.6 | 30.1 | 146.6 |
| | Caucasian | 478 | 89.6 | 111.2 | 62.3 | 27.6 | 117.6 |
| IL-1β | African-American | 192 | 101.4 | 146.7 | 46.8 | 24.2 | 104.6 |
| | Caucasian | 496 | 112.6 | 202.1 | 55.1 | 27.6 | 146.5 |
| IL-2 | African-American | 185 | 19.9 | 33.0 | 15.0 | 1.2 | 32.9 |
| | Caucasian | 465 | 27.5 | 37.3 | 18.6 | 5.2 | 38.3 |
| TNF-α | African-American | 192 | 197.8 | 201.8 | 162.8 | 77.1 | 310.5 |
| | Caucasian | 496 | 224.6 | 230.5 | 170.7 | 93.1 | 342.8 |

For each subject the response reflects secreted cytokine levels in pg/ml (median of vaccinia-specific stimulated response measured in triplicate, minus median of unstimulated response in triplicate).

N, number of subjects; Std, standard deviation. Q1 and Q3 reflect the 25% and 75% inter-quartile ranges. The levels of vaccinia-specific IL-4, IL-10, IL-12p70, IL-18, and IFN-β were below the level of detection limit of ELISA assays.

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## Table 3

Replicated single-nucleotide polymorphisms associated with vaccinia-specific cytokine secretion.

| | | | African-American | | | Caucasian | | |
|---|---|---|---|---|---|---|---|---|
| **Gene/Cytokine** | **SNP ID** | **Genotype[a]** | **N** | **Median, pg/ml (IQR)** | **P-value[b]** | **N** | **Median, pg/ml (IQR)** | **P-value[b]** |
| IFN-α | | | | | | | | |
| IL4R | rs3024672 | GG | 73 | 58.5 (22.6,115.2) | 0.003 | 503 | 71.9 (27.4,147.2) | 0.012 |
| | | GA | 104 | 49.2 (11.6,121.9) | | 46 | 24.4 (4.5,95.8) | |
| | | AA | 28 | 33.5 (1.6,82.8) | | 0 | -- | |
| IL-12p40 | | | | | | | | |
| IL12A | rs2243131 | AA | 116 | 60.7 (26.3,124.7) | 0.022 | 322 | 59.7 (24.3,110.0) | 0.030 |
| | | AC | 65 | 71.1 (35.5,156.0) | | 124 | 65.8 (32.6,121.3) | |
| | | CC | 5 | 249.2 (146.6,252.2) | | 16 | 79.2 (44.3,131.3) | |
| IL6 | rs2069844 | CC | 116 | 68.6 (34.6,163.7) | 0.023 | 438 | 62.9 (29,118.5) | 0.002 |
| | | CA | 66 | 57.8 (30.1,115.0) | | 30 | 35.0 (18.3,77.9) | |
| | | AA | 3 | 11.7 (2.2,245.7) | | 0 | -- | |
| IL-1β | | | | | | | | |
| IL12RB2 | rs1874791 | GG | 24 | 71.4 (52.2,131.6) | 0.015 | 332 | 58.0 (29.8,149.4) | 0.005 |
| | | GA | 96 | 44.5 (25.6,125.7) | | 140 | 52.5 (26.6,144.4) | |
| | | AA | 69 | 43.0 (18.0,84.9) | | 14 | 33.4 (18.3,60.7) | |
| IL12RB2 | rs3790565 | AA | 24 | 71.4 (52.2,131.6) | 0.015 | 331 | 57.8 (29.7,150.1) | 0.005 |
| | | AG | 96 | 44.5 (25.6,125.7) | | 141 | 52.5 (27.1,139.1) | |
| | | GG | 69 | 43.0 (18.0,84.9) | | 14 | 33.4 (18.3,60.7) | |
| IL12RB2 | rs3790566 | GG | 21 | 64.3 (37.6,89.6) | 0.015 | 284 | 60.9 (30.8,153.8) | 0.019 |
| | | GA | 87 | 55.5 (25.6,145.0) | | 179 | 48.7 (25.6,134.4) | |
| | | AA | 78 | 39.9 (20.4,84.9) | | 23 | 44.6 (21.6,116.0) | |
| IL12RB2 | rs946685 | GG | 25 | 67.5 (50.9,130.5) | 0.003 | 336 | 58.4 (29.8,150.9) | 0.003 |
| | | GA | 95 | 46.0 (25.5,131.7) | | 136 | 51.3 (26.6,130.6) | |
| | | AA | 66 | 39.9 (18.0,84.4) | | 14 | 33.4 (18.3,60.7) | |
| IL12RB2 | rs3790567 | GG | 21 | 64.3 (37.6,89.6) | 0.013 | 286 | 60.9 (30.4,155.6) | 0.009 |
| | | GA | 87 | 55.5 (25.6,145.0) | | 178 | 47.9 (25.6,134.4) | |

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| | | | African-American | | | Caucasian | | |
|---|---|---|---|---|---|---|---|---|
| Gene/Cytokine | SNP ID | Genotype[a] | N | Median, pg/ml (IQR) | P-value[b] | N | Median, pg/ml (IQR) | P-value[b] |
| | | AA | 80 | 38.9 (19.2,84.6) | | 22 | 41.4 (21.6,108.5) | |
| IL-2 | | | | | | | | |
| IL1RN | rs452204 | GG | 48 | 7.5 (−2.8,24.3) | 0.002 | 164 | 19.2 (5.7,38.9) | 0.048 |
| | | GA | 96 | 18.0 (1.4,32.3) | | 220 | 15.7 (4.6,34.4) | |
| | | AA | 38 | 23.2 (0.9,49.5) | | 72 | 29.9 (8.6,57.8) | |
| IL10RB | rs2834170 | GG | 88 | 19.0 (1.5,34.4) | 0.042 | 456 | 18.7 (5.4,38.1) | 0.030 |
| | | GA | 80 | 14.3 (1.2,35.0) | | 1 | −20 (−20,−20) | |
| | | AA | 14 | 6.4 (−4.0,11.9) | | 0 | -- | |
| IL2RB | rs3218266 | GG | 117 | 15.0 (0.3,30.6) | 0.033 | 197 | 16.2 (3.5,36.5) | 0.007 |
| | | GC | 49 | 21.5 (3.5,37.0) | | 203 | 19.3 (5.9,37.4) | |
| | | CC | 12 | 19.6 (6.0,41.9) | | 55 | 20.7 (9.5,43.8) | |
| IL2RB | rs2284034 | GG | 111 | 10.8 (−1.3,27.5) | 0.038 | 203 | 15.1 (3.3,36.3) | 0.009 |
| | | GA | 59 | 20.6 (3.8,41.8) | | 193 | 19.5 (6.3,37.4) | |
| | | AA | 10 | 19.6 (5.4,42.0) | | 52 | 20.3 (10.0,40.2) | |
| TNF-α | | | | | | | | |
| IL12RB2 | rs946685 | GG | 25 | 183.6 (97.8,298.9) | 0.024 | 336 | 187.9 (95.3,347.2) | 0.023 |
| | | GA | 95 | 161.5 (77.3,319.4) | | 136 | 153.4 (89.0,333.2) | |
| | | AA | 66 | 154.6 (66.2,280.8) | | 14 | 127.9 (82.9,189.7) | |

A-Adenine, C-Cytosine, G-Guanine

-- No subject with that genotype

[a]Values are presented as homozygous major allele/heterozygous/homozygous minor allele.

[b]Repeated measures linear regression modeling approach

Analyses adjusted for gender; age at blood draw (quartiles); time from smallpox immunization to blood draw (quartiles); time from blood draw to assay (quartiles); shipping temperature of the sample (frozen or ambient); and time of year when the sample was shipped (warm weather months April–September vs. cold weather months October–March).

Only SNPs with statistically significant findings (P<0.05) in both racial groups, and with associations in the same direction in each group, are presented.