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Genome-Wide Analysis of Polymorphisms Associated with Cytokine Responses in Smallpox Vaccine Recipients

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Abstract

The role that genetics plays in response to infection or disease is becoming increasingly clear as we learn more about immunogenetics and host-pathogen interactions. Here we report a genome-wide analysis of the effects of host genetic variation on cytokine responses to vaccinia virus stimulation in smallpox vaccine recipients. Our data show that vaccinia stimulation of immune individuals results in secretion of inflammatory and Th1 cytokines. We identified multiple SNPs significantly associated with variations in cytokine secretion. These SNPs are found in genes with known immune function, as well as in genes encoding for proteins involved in signal transduction, cytoskeleton, membrane channels and ion transport, as well as others with no previously identified connection to immune responses. The large number of significant SNP associations implies that cytokine secretion in response to vaccinia virus is a complex process controlled by multiple genes and gene families. Follow-up studies to replicate these findings and then pursue mechanistic studies will provide a greater understanding of how genetic variation influences vaccine responses.

Keywords

smallpox vaccine; vaccinia; GWAS; genome-wide association; SNP; immune response; cytokines

Introduction

Smallpox, a deadly disease caused by variola virus, plagued mankind until a decades-long effort by the World Health Organization led to its eradication in 1980 (Fenner 1988). Edward Jenner pioneered the use of cross-protective poxviruses as safe and effective vaccines (Jenner 1798). This work led to the eventual development of the vaccinia-based vaccines used successfully in the eradication effort. These live viral vaccines induced high

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Ethical Standards

All experiments described here comply with the current, applicable U.S. laws.

Conflict of Interests

The authors do not have any conflicts of interest to report.

levels of humoral and cell-mediated immunity in vaccinated subjects (Combadiere et al. 2004; Crotty et al. 2003; Frey et al. 2002; Hammarlund et al. 2003; Kennedy et al. 2009b), but also caused adverse events, some of which were life-threatening (Fulginiti 2003; Fulginiti et al. 2003), and resulted in the cessation of routine vaccination shortly after endemic smallpox was eradicated. A large percentage of the current U.S. population have conditions that contraindicate receipt of the vaccine (immunosuppression due to: cancer, organ transplants, HIV infection, heart conditions, and skin diseases such as eczema or psoriasis)(Fenner 1989). Altered cytokine responses to vaccinia inoculation in individuals with atopic dermatis are believed to be responsible for the higher incidence of eczema vaccinatum (Grigoryev et al. 2010; Howell et al. 2006; Scott et al. 2007). Furthermore, Th2 responses are correlated with impaired viral clearance,(Freyschmidt et al. 2007) while animal models where expression of key cytokines are intentionally over- or under-expressed clearly indicate that cytokine production in response to poxvirus infection or inoculation can greatly influence the course of the viral infection (Foong et al. 2009; Kohyama et al. 2007; Sharma et al. 1996; Tian et al. 2009; van Den Broek et al. 2000). Genetic variations can have a profound influence on disease susceptibility, progression, and resolution, as well as on vaccine-induced immune responses. Here we present data on genome-wide SNPs associated with variations in cytokine responses in a well-characterized cohort of over 1,000 healthy adult recipients of the smallpox vaccine. Identifying the genetic elements controlling cytokine secretion in response to viral infection or vaccination will assist in creation of next-generation vaccines that elicit immune responses with the optimal Th1/Th2 balance as well as other cytokines (Th17, inflammatory cytokines) necessary for robust immune protection.

Materials and Methods

Subject Recruitment

Study subjects were recruited from military personnel who were recent recipients of a single dose of the Dryvax® smallpox vaccine and from civilian healthcare workers who participated in the civilian smallpox immunization program at the Mayo Clinic in Rochester, MN. Military personnel were recruited from the Naval Health Research Center (NHRC) in San Diego, CA (Kennedy et al. 2009a). All participants were in good general health, had received one, and only one, dose of the smallpox vaccine within the last four years and had a documented vaccine “take”, indicating successful immunization. Approval from the Institutional Review Boards of both the Mayo Clinic and NHRC was obtained, as was written informed consent from each subject prior to all study procedures.

Isolation of peripheral blood mononuclear cells (PBMC)

Each subject underwent a single blood draw of approximately 100 mL, with blood collected in heparinized tubes and shipped overnight at room temperature. PBMCs from each subject were isolated within 24 hours of blood draw using Accuspin (Sigma, St. Louis, MO) tubes containing HISTOPAQUE®-1077 (Sigma) according to established procedures. Isolated PBMCs (1×10^7 cells/mL) were resuspended in culture medium supplemented with 10% dimethyl sulfoxide (Protide Pharmaceuticals, St. Paul, MN) and 20% fetal bovine serum (FBS; Hyclone, Logan, UT), frozen for 18hrs in controlled-rate freezing containers, and in liquid nitrogen for long-term storage (Ryan et al. 2009).

SNP typing and QC

The Gentra Puregene Blood kit (Gentra Systems Inc., Minneapolis, MN) was used to extract DNA from biospecimens prior to quantification by Picogreen (Molecular Probes, Carlsbad, CA). Genome-wide SNP typing was performed using the Infinium HumanHap550 or HumanHap650Y BeadChip arrays for the Caucasian and African-American subjects, respectively. After whole genome amplification, fragmentation and hybridization, samples were imaged on an Illumina BeadArray reader. The genotyping module of BeadStudio 2 was used to make the clustering and genotyping calls, which were then transferred to a SAS database for later analyses. Quality control checks included: genotyping reproducibility using paired samples, removal of monomorphic SNPs, call rate cutoffs of < 95% for both individual SNPs and subjects, and a check for Hardy-Weinberg Equilibrium (HWE). As subjects from two races were genotyped, tests for deviation from HWE were performed in a race-stratified fashion similar to the exact test of Schaid, et al (Schaid et al. 2006).

ELISA Assays

Frozen PBMC aliquots were recovered as previously described (Ovsyannikova et al. 2005; Ryan et al. 2009). Briefly, cells were thawed and resuspended in culture medium supplemented with 50IU/ml of IL-2 (Proleukin®, Chiron, Emeryville, CA) overnight. After washing, cells were resuspended at a concentration of 2×10^6 cells/mL for use in the cytokine secretion assays.

Vaccinia virus (NYCBOH) was grown and titered according to established procedures (Earl et al. 2001; Kennedy et al. 2009a). A single viral stock was prepared for all assays and was inactivated using psoralen and UV light in order to minimize viral modulation of immune responses (Ryan et al. 2009).

2×10^5 PBMCs were plated in each well of 96-well round bottom plates. Experimental conditions included: a single well containing PHA (5ug/ml) as a positive control, triplicate wells containing culture medium (unstimulated wells), and triplicate wells with vaccinia virus (stimulated wells). Cytokine-specific vaccinia stimulation was optimized based on multiplicity of infection (MOI) and length of time in culture as previously described (Ryan et al. 2009) and is as follows: IFNβ, IL-2, IL-18: MOI=5, 24 hours; IL-12p40, IL-12p70, TNFα, IL-1β: MOI 0.5, 24 hours; IFNα: MOI=0.05, 4 days; IL-4, IL-10: MOI=0.05, 7 days; IL-6: MOI=5, 8 days.

Cytokine levels in culture supernatant were detected using commercial ELISA-based kits for IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p40, IL-12 p70, and TNF-α (all from BD Pharmingen), IFN-α (PBL Biomedical Laboratories, Piscataway, NJ), IFN-β (PBL Biomedical Laboratories) and IL-18 (MBL International, Woburn, MA). Optical density readings at 450 nm were converted to cytokine concentrations in pg/ml using the reference standards included in each assay.

Population stratification

As study participants were of more than one race, we selected 22,863 SNPs with >99% call rates that were spaced at approximately 100kb intervals and used these SNPs in the principal

components (PC) implemented in the Eigenstrat software package to assess population structure (Price et al. 2006). Using the same genotype data, we employed a clustering procedure similar to what is implemented in the Structure software program (Pritchard et al. 2000) to assign Caucasian or African-American racial group membership to subjects who had not self-reported a conclusive racial declaration. After defining genetic racial groups, we extracted the first four Eigenstrat axes of variation (Price et al. 2006) within each group and used these as covariates in the race-specific analyses in order to account for residual population stratification.

Statistical analyses

These analytical methods are identical to those employed in our other genome-wide association study of smallpox vaccine response. Assessment of cytokines resulted in six recorded values: those from the three unstimulated and the three vaccinia stimulated wells. The median of the unstimulated wells was subtracted from the median value of the stimulated wells to create a summary measure of each cytokine secretion level for each individual.

Associations between the levels of each cytokine level and the SNP genotypes were assessed separately within the two racial groups using linear regression models. SNPs were modeled assuming an additive genotypic effect. Formal evaluations of significance utilized repeated measures analyses that used all of the multiple observations per subject while accounting for within-subject correlations using generalized estimating equations (GEEs). As there were measurements of cytokine levels in both the stimulated and unstimulated state for each individual, the primary test for genetic association between a SNP and the outcome of interest was a test for interaction between the ordinal SNP variable and a variable identifying whether the result arose from a stimulated or an unstimulated state. The tests of the significance of these interactions are similar to paired t-tests: both compare differences in the two stimulation states within an individual among genotype-defined groups of individuals. All 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); time of year when the sample was shipped (warm-weather months April-September vs. cold-weather months October-March); and the first four Eigenstrat axes of variation. An inverse cumulative normal (probit) transformation was used for the cytokine variables in order to correct for data skewness. We used q-q plots to compare the observed and expected distribution of p-values for a given outcome across all SNPs. Genomic control lambda values were calculated to assess, and correct for, any potential residual inflation of significance in the race-specific results. All statistical tests were two-sided, and were performed using the R software package (unless otherwise indicated) (Team 2008). Due to the existence of several significant SNPs for which few subjects had two copies of the minor allele, additional models were run to further assess the effect of the minor alleles. These sensitivity analyses involved grouping the homozygous minor subjects with the heterozygous subjects, resulting in a dominant rather than an ordinal model. The models were run with the same adjusting factors as the ordinal model.

Results

SNP Typing Results

Subjects were genotyped using the Illumina Infinium HumanHap550 (Caucasians) or the HumanHap 650Y (African-American) BeadChip arrays. The HumanHap550 is a subset of SNPs from the HumanHap650Y; the following summary is for all SNPs, with the understanding that some SNPs were only run for the African-American subjects. Overall genotype concordance was high (97.9% including missing genotypes, 99.9% after excluding missing genotypes). SNPs with call rates below 95% were excluded (7.3%), as were 71 subjects with call rates less than 95%. Additional exclusions involved removing SNPs with minor allele frequencies below 1% and those that appeared to be out of HWE ($p<10^{-8}$). Of these 1,000 subjects, 580 formed the Caucasian racial cluster and 217 formed the African-American cluster. During analysis, SNPs with fewer than 10 observed minor alleles for a given outcome and racial cohort (Caucasian or African-American) were excluded from that outcome/race specific analysis.

Immune Outcomes

Cytokine responses for each subject were quantitated by ELISA assays and are outlined in Table 1. PBMCs from vaccine recipients exhibited a predominantly Th1 type response to viral stimulation characterized by robust secretion of IL-2, IL-12p40, TNFα, and IFNγ as well as high levels of proinflammatory cytokines IFNα, IL-1β, and IL-6 (Umlauf et al. 2011). In contrast, we saw low levels IL-10 and negligible amounts of IL-4, IL-12p70, IL-18, and IFNβ. Although we did find statistically significant associations for IL-4, IL-12p70, IL-18, and IFNβ the small differences in cytokine secretion are unlikely to be biologically meaningful, therefore results from these cytokines are not reported.

Genome Wide Analysis Results

Our results indicated that recall responses in smallpox vaccine recipients are primarily Th1, and that viral stimulation also induces a strong proinflammatory response in PBMCs. We found a number of significant genetic associations with variations in Th1-type cytokine production. After correction for the small degree of inflation of significance present in the p-value distributions, a number of SNPs for the phenotypes of interest were identified as meeting initial thresholds of significance. The QQ-plots and Manhattan plots indicating associations with IL-2 secretion for both our Caucasian and African-American cohorts are illustrated in Figure 1. SNP associations with Th1 cytokine secretion (IL-2, TNFα, IL-12p40) that reached a high level of significance ($p<5\times10^{-7}$) are listed in Tables 2–3 for Caucasians and African Americans respectively. Tables 5- 7 outline the significant associations found with the pro- inflammatory cytokines IL-1β, IFNα, and IL-6 respectively. Table 8 documents the genetic associations for IL-10, the only Th2 cytokine that was consistently secreted upon viral stimulation of PBMCs from vaccinated subjects. For each of the tables we have listed the function of the protein product (where known) underneath the gene name.

Discussion

Both animal models and large scale vaccination studies have demonstrated that poxviruses elicit a strong Th1 response. Our results indicate that this Th1 response is maintained in the vaccinia virus-specific memory T cell population as well. We have previously reported on genome-wide associations with markers of cellular immunity, namely IFNγ ELISPOT and quantitation of secreted IFNγ: in this report we extend our findings to additional measures including Th1, Th2, and inflammatory cytokines. Interestingly, we found considerable variations in cytokine responses to viral stimulation of PBMCs from vaccinated individuals (Table 1).

The relative absence of IFNβ or IL-18 secretion by PBMCs in response to vaccinia infection may be due to the immunomodulatory proteins encoded by poxviruses such as those described below. We inactivated the virus stock used in our experiments with psoralen and UV irradiation to crosslink viral DNA, resulting in >6 log reduction in infectivity. It is likely that this treatment inhibited viral production of immunomodulatory proteins (and we have previously reported on the detection of IFNg using live vs inactivated vaccinia virus)(Ryan et al. 2009), however we cannot rule out the possibility that NYCBOH-encoded proteins (A52R, A53R, B13R, B16R, B19R, C12L) affected secretion patterns of the cytokines assayed. A53R encodes CrmC, a soluble TNFR protein capable of sequestering TNFα and inhibiting TNF signaling. The NYCBOH genome also contains two additional genes for soluble TNFR proteins (C22L, B28R), although they contain mutations and are likely nonfunctional (Goebel et al. 1990). Some strains of vaccinia (Lister, USSR, Evans) also contain the K3R gene which encodes CrmE, yet another soluble TNFR.(Alcami et al. 1999; Reading et al. 2002) The B19R glycoprotein is a soluble receptor for IFN α/β, inhibiting IFNα/β from binding to cellular receptors and dampening antiviral responses (Colamonici et al. 1995; Symons et al. 1995). The E3L protein binds to dsRNA and inhibits PKR signaling, (Chang et al. 1992), IRF-dependent type I IFN synthesis, and TNFα production (Myskiw et al. 2009; Smith et al. 2001). Vaccinia viruses, including the NYCBOH strain, also encode C12L which is an IL-18 binding protein that inhibit IL-18 receptor activation, and may have hampered antibody-based detection of IL-18 in culture supernatant (Reading and Smith 2003; Smith et al. 2000; Symons et al. 2002). NYCBOH also encodes both B16R (an IL-1β inhibitor) and C10L (an IL-1R antagonist) which both block IL-1β signaling. We found high levels of TNFα, IFNα, IFNγ, IL-1β in spite of the virally encoded immunomodulatory proteins affecting each of these cytokines, providing support for our hypothesis that viral proteins are not interfering with cytokine secretion.

Although the median concentration of secreted IL-18 was low (0.7pg/ml in Caucasians and 1.3pg/ml in African Americans) we have previously reported a number SNPs in both IL18 and IL18R that are significantly associated with vaccinia neutralizing antibody titer after smallpox immunization (Haralambieva et al. 2011). Our analyses here have focused on the entire cohort, and it is quite possible that subsets of our cohort (for example: those making larger quantities of IL-18) behave differently from the group as a whole, and thus more focused studies may provide novel insights into the genetic control of smallpox vaccine responses.

One of the strengths of genome-wide association studies is the ability to identify novel genes/processes involved in control of diseases or biologic responses. Our results indicate that a large number of SNPs were associated with variations in cytokine response to vaccinia virus in subjects who had received the smallpox vaccine. One such SNP is rs16948200 in the nerve growth factor receptor gene NGFR. African-American individuals homozygous for the major allele (G) secrete over 12-fold more IL-2 than heterozygotes, while those homozygous for the minor allele (A) secrete essentially no IL-2. NGFR shares a similar structure to other members of the TNFR superfamily including the involvement of the downstream NF-kB and apoptotic pathways (Lotz et al. 1996). Nerve growth factor is produced by a variety of lymphoid cells and can influence proliferation, survival, differentiation and effector function immune cells (Garaci et al. 1999; Lambiase et al. 1997; Otten et al. 1989; Torcia et al. 1996). Our results indicate a possible connection between NGFR and IL-2, which may account for the proliferative and survival effects of NGF on immune cells. The intronic SNP rs4251424, in the interleukin-1 receptor-associated kinase 4 (*IRAK4*) gene, was associated with differential secretion of TNFα. In fact, African-Americans homozygous for the major allele (G) secreted nearly twice as much TNFα as heterozygous individuals. IRAK4 initiates a cascade of phosphorylation and signaling events in response to viral stimulation through TLRs (TLR7,8, and 9), resulting in cytokine production (Yang et al. 2005). IRAK4 has also been implicated in T cell activation and function (Suzuki et al. 2006). Thus, a SNP influencing IRAK4 expression or function may lead to distinct differences in T cell production of TNFα.

Rs11242417, an intronic SNP in the glial cell line-derived neurotrophic factor receptor alpha (*GFRA3*) gene, is associated with variations in IL-12p40 secretion in our Caucasian cohort. The GFRA3 gene encodes for a cellular receptor involved in neuronal development (Nishino et al. 1999) and although this same SNP is associated with schizophrenia (Souza et al. 2010) its' role in immune function is not known. In ourAfrican-America cohort, we identified rs859267 in *ADORA2B* as being associated with variations in IL-12p40 in an allele dose-dependent manner. Possession of the minor allele (C) leads to a 30% reduction in IL-12p40 for heterozygotes and a 70% reduction in IL-12p40 secretion in homozygotes (Table 4). *ADORA2B* encodes for the adenosine A2B G protein coupled receptor. Blockade of ADORA2B signaling enhances macrophage phagocytosis, cytokine production, and chemokine synthesis (Belikoff et al. 2011). Adenosine also inhibits IL-12 and TNFα release by dendritic cells through activation of the adenosine A2 receptor (Ben Addi et al. 2008; Panther et al. 2003), providing supporting evidence for our results showing that genetic polymorphisms in *ADORA2B* may affect IL-12 production by PBMCs.

IFNα production was also significantly associated with a number of SNPs in both of the racial groups we studied. In Caucasian subjects, possession of a single minor allele (A) at rs542631 in the *LTBP1* gene led to a 260% increase in IFNα secretion upon vaccinia stimulation. LTBP1 is involved in the trafficking and activation of latent TGFB complexes (Keski-Oja et al. 2004). Our data also show that another SNP near the same gene, rs6728024, is associated with variations in IL-6 secretion in African-American subjects, resulting in an almost three-fold, minor allele dose-dependent increase of IL-6. These associations are not surprising given the myriad effects TGF-β can have on cytokine

production by immune cells, including the induction of proinflammatory cytokines (TNFα, IL-1, IL-6) by monocytes (Bogdan and Nathan 1993). We also found a number of SNPs significantly associated with IFNα secretion in our African-American subjects, however, many of these SNPs (rs9493873 near *SGK1*, rs3095748 in *DAPK1*, rs2043599 near *NLRP8* and *NLRP13*, rs10517025 in *ATP8A1*, and rs17221323 in *DIP2C*) failed to reach genome-wide significance during our sensitivity analyses, indicating that these associations were likely driven by extremely high or low IFNα secretion measured in only a few subjects homozygous for the minor allele. Thus, these associations should be interpreted with caution.

Of particular interest with IL-6 is our finding that individuals heterozygous (GA) at rs2255327 in *BLK*, the B lymphoid tyrosine kinase, had levels of IL-6 two-fold higher than those homozygous for the major allele (GG). In addition to B cells, BLK is also expressed in granulocytes, monocytes, and macrophages (Okutani et al. 2006). BLK plays a critical role in B cell receptor signaling and subsequent development. Related Src family kinases activate cytokine production (IL-1, IL-6, TNFα) in macrophages in response to pathogen recognition by TLRs (Lowell 2004). SNPs in BLK are associated with autoimmune and inflammatory disorders such as: systemic lupus erythematosum (Harley et al. 2008; Ito et al. 2009; Yang et al. 2009), Sjogren's syndrome (Nordmark et al. 2011), systemic sclerosis (Gourh et al. 2010), and antiphospholipid antibody syndrome (Yin et al. 2009). Our results indicate that genetic variation in BLK may affect downstream IL-6 production.

We found a number of SNPs located near (rs16850885) or within (rs16850864, rs572987) the *MTHFD2L* gene associated with variations in IL-1β secretion in our Caucasian subjects. *MTHFD2L* codes for a methylenetetrahydrofolate dehydrogenase 2-like protein involved in carbon metabolism and conversion of folate to formate in the mitochondria (Bolusani et al. 2011). As shown in Figure 2, these SNPs are all in tight LD with one another. Another nearby SNP, rs7694828, is in tight LD with each of these three identified SNPs. Rs7694828 is located in a binding site of the transcriptional regulator CTCF (Kim et al. 2007). CTCF functions as an insulator, preventing the influence of cis-acting enhancers on gene activation (Bell et al. 1999). Yet another SNP (rs10014791) is also in high LD (r^2=1) with the three identified MTHFD2L SNPs and resides in a binding site for v-MAF. Thus, the identified SNPs may not directly affect the associated immune outcome, but rather tag nearby SNPs whose functional effects lead to variations in the downstream immune outcome. Most of the SNPs reported here are non-coding SNPs. This is due, in large part, to the composition of the HumanHap 550/650 chips: the vast majority of the SNPs on these platforms are in non-coding regions of the genome. Non-coding SNPs in splice sites, promoters, and regulatory regions can have as profound an influence on gene expression/function as coding SNPs, furthermore the SNPs we have identified may not be the causal SNPs, but rather tag coding/regulatory SNPs that actually impact gene/protein expression or function. We view this initial GWAS as a screening tool to identify genetic regions of interest for the future replication and fine-mapping efforts that we believe will be critical to elucidating the mechanisms behind the reported genetic associations.

Our study utilized individuals with documented evidence of vaccinia immunization, simultaneously analyzed multiple cytokines involved in both innate and adaptive responses

to poxviruses, and allowed us to assess associations within genetically defined racial subgroups. However, our study was limited in that: 1) we had a relatively small number of African-American subjects, 2) we examined a large, but not comprehensive, set of cytokines, and 3) we do not yet have an external validation data set. With these limitations in mind, we have nevertheless identified a number of novel genes containing SNPs associated with vaccinia-specific cytokine responses to smallpox vaccination. These SNPs are found in genes with known immune function, as well as in genes with no previously identified connection to immune responses, and in intergenic regions of the genome. We examined each significant ($p< 5 \times 10^{-7}$) race-specific association in the other racial group in a preliminary manner, using a cut-off of $p < 0.01$ in the second racial group. While we found a number of SNPs associated with similar trends in immune outcome, we did not find any SNP associations that reached this significance threshold. This initial replication assessment was likely hampered by racial effects and low sample sizes. Thus, important next steps are to expand the selection of cytokines examined (such as IL-17), fine-mapping to identify causal variants (both coding and regulatory), replication of the identified associations and fine-mapped variants in an independent cohort. Our findings may pinpoint novel means of immune regulation, and these newly identified SNPs may be excellent candidates for functional studies aimed at discovering the mechanisms behind such regulation.

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References

Alcami A, Khanna A, Paul NL, Smith GL. Vaccinia virus strains Lister, USSR and Evans express soluble and cell-surface tumour necrosis factor receptors. J Gen Virol. 1999; 80 (Pt 4):949–59. [PubMed: 10211965]

Belikoff BG, Hatfield S, Georgiev P, Ohta A, Lukashev D, Buras JA, Remick DG, Sitkovsky M. A2B adenosine receptor blockade enhances macrophage-mediated bacterial phagocytosis and improves polymicrobial sepsis survival in mice. J Immunol. 2011; 186:2444–53. [PubMed: 21242513]

Bell AC, West AG, Felsenfeld G. The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. Cell. 1999; 98:387–96. [PubMed: 10458613]

Ben Addi A, Lefort A, Hua X, Libert F, Communi D, Ledent C, Macours P, Tilley SL, Boeynaems JM, Robaye B. Modulation of murine dendritic cell function by adenine nucleotides and adenosine: involvement of the A(2B) receptor. Eur J Immunol. 2008; 38:1610–20. [PubMed: 18465770]

Bogdan C, Nathan C. Modulation of macrophage function by transforming growth factor beta, interleukin-4, and interleukin-10. Ann N Y Acad Sci. 1993; 685:713–39. [PubMed: 8363277]

Bolusani S, Young BA, Cole NA, Tibbetts AS, Momb J, Bryant JD, Solmonson A, Appling DR. Mammalian MTHFD2L encodes a mitochondrial methylenetetrahydrofolate dehydrogenase isozyme expressed in adult tissues. J Biol Chem. 2011; 286:5166–74. [PubMed: 21163947]

Chang HW, Watson JC, Jacobs BL. The E3L gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. Proc Natl Acad Sci U S A. 1992; 89:4825–9. [PubMed: 1350676]

Colamonici OR, Domanski P, Sweitzer SM, Larner A, Buller RM. Vaccinia virus B18R gene encodes a type I interferon-binding protein that blocks interferon alpha transmembrane signaling. J Biol Chem. 1995; 270:15974–8. [PubMed: 7608155]

Combadiere B, Boissonnas A, Carcelain G, Lefranc E, Samri A, Bricaire F, Debre P, Autran B. Distinct time effects of vaccination on long-term proliferative and IFN-gamma-producing T cell memory to smallpox in humans. J Exp Med. 2004; 199:1585–93. [PubMed: 15184506]

Crotty S, Felgner P, Davies H, Glidewell J, Villarreal L, Ahmed R. Cutting edge: long-term B cell memory in humans after smallpox vaccination. J Immunol. 2003; 171:4969–73. [PubMed: 14607890]

Earl PL, Moss B, Wyatt LS, Carroll MW. Generation of recombinant vaccinia viruses. Curr Protoc Protein Sci. 2001; Chapter 5(Unit 5):13. [PubMed: 18429179]

Fenner, F. Smallpox and its eradication. World Health Organization; Geneva: 1988.

Fenner F. Risks and benefits of vaccinia vaccine use in the worldwide smallpox eradication campaign. Res Virol. 1989; 140:465–6. discussion 487–91. [PubMed: 2685955]

Foong YY, Jans DA, Rolph MS, Gahan ME, Mahalingam S. Interleukin-15 mediates potent antiviral responses via an interferon-dependent mechanism. Virology. 2009; 393:228–37. [PubMed: 19729181]

Frey SE, Newman FK, Cruz J, Shelton WB, Tennant JM, Polach T, Rothman AL, Kennedy JS, Wolff M, Belshe RB, Ennis FA. Dose-related effects of smallpox vaccine. N Engl J Med. 2002; 346:1275–80. [PubMed: 11923489]

Freyschmidt EJ, Mathias CB, MacArthur DH, Laouar A, Narasimhaswamy M, Weih F, Oettgen HC. Skin inflammation in RelB(−/−) mice leads to defective immunity and impaired clearance of vaccinia virus. J Allergy Clin Immunol. 2007; 119:671–9. [PubMed: 17336617]

Fulginiti VA. Risks of smallpox vaccination. Jama. 2003; 290:1452. author reply 1452. [PubMed: 13129976]

Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part II. Adverse events. Clin Infect Dis. 2003; 37:251–71. [PubMed: 12856218]

Garaci E, Caroleo MC, Aloe L, Aquaro S, Piacentini M, Costa N, Amendola A, Micera A, Calio R, Perno CF, Levi-Montalcini R. Nerve growth factor is an autocrine factor essential for the survival of macrophages infected with HIV. Proc Natl Acad Sci U S A. 1999; 96:14013–8. [PubMed: 10570190]

Goebel SJ, Johnson GP, Perkus ME, Davis SW, Winslow JP, Paoletti E. The complete DNA sequence of vaccinia virus. Virology. 1990; 179:247–66. 517–63. [PubMed: 2219722]

Gourh P, Agarwal SK, Martin E, Divecha D, Rueda B, Bunting H, Assassi S, Paz G, Shete S, McNearney T, Draeger H, Reveille JD, Radstake TR, Simeon CP, Rodriguez L, Vicente E, Gonzalez-Gay MA, Mayes MD, Tan FK, Martin J, Arnett FC. Association of the C8orf13-BLK region with systemic sclerosis in North-American and European populations. J Autoimmun. 2010; 34:155–62. [PubMed: 19796918]

Grigoryev DN, Howell MD, Watkins TN, Chen YC, Cheadle C, Boguniewicz M, Barnes KC, Leung DY. Vaccinia virus-specific molecular signature in atopic dermatitis skin. J Allergy Clin Immunol. 2010; 125:153–159. e28. [PubMed: 20109744]

Hammarlund E, Lewis MW, Hansen SG, Strelow LI, Nelson JA, Sexton GJ, Hanifin JM, Slifka MK. Duration of antiviral immunity after smallpox vaccination. Nat Med. 2003; 9:1131–7. [PubMed: 12925846]

Haralambieva IH, Ovsyannikova IG, Dhiman N, Kennedy RB, O'Byrne M, Pankratz 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. 2011; 204:433–41. [PubMed: 21742843]

Harley JB, Alarcon-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP, Moser KL, Tsao BP, Vyse TJ, Langefeld CD, Nath SK, Guthridge JM, Cobb BL, Mirel DB, Marion MC, Williams AH, Divers J, Wang W, Frank SG, Namjou B, Gabriel SB, Lee AT, Gregersen PK, Behrens TW, Taylor KE, Fernando M, Zidovetzki R, Gaffney PM, Edberg JC, Rioux JD, Ojwang JO, James JA, Merrill JT, Gilkeson GS, Seldin MF, Yin H, Baechler EC, Li QZ, Wakeland EK, Bruner GR, Kaufman KM, Kelly JA. Genome-wide association scan in women with systemic lupus

erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. Nat Genet. 2008; 40:204–10. [PubMed: 18204446]

Howell MD, Gallo RL, Boguniewicz M, Jones JF, Wong C, Streib JE, Leung DY. Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus. Immunity. 2006; 24:341–8. [PubMed: 16546102]

Ito I, Kawasaki A, Ito S, Hayashi T, Goto D, Matsumoto I, Tsutsumi A, Hom G, Graham RR, Takasaki Y, Hashimoto H, Ohashi J, Behrens TW, Sumida T, Tsuchiya N. Replication of the association between the C8orf13-BLK region and systemic lupus erythematosus in a Japanese population. Arthritis Rheum. 2009; 60:553–8. [PubMed: 19180478]

Jenner, E. An inquiry into the causes and effects of the variolae vaccinae, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cow pox. Law; London: 1798.

Kennedy R, Pankratz VS, Swanson E, Watson D, Golding H, Poland GA. Statistical approach to estimate vaccinia- specific neutralizing antibody titers using a high throughput assay. Clin Vaccine Immunol. 2009a

Kennedy RB, Ovsyannikova IG, Jacobson RM, Poland GA. The immunology of smallpox vaccines. Curr Opin Immunol. 2009b; 21:314–20. [PubMed: 19524427]

Keski-Oja J, Koli K, von Melchner H. TGF-beta activation by traction? Trends Cell Biol. 2004; 14:657–9. [PubMed: 15564041]

Kim TH, Abdullaev ZK, Smith AD, Ching KA, Loukinov DI, Green RD, Zhang MQ, Lobanenkov VV, Ren B. Analysis of the vertebrate insulator protein CTCF-binding sites in the human genome. Cell. 2007; 128:1231–45. [PubMed: 17382889]

Kohyama S, Ohno S, Isoda A, Moriya O, Belladonna ML, Hayashi H, Iwakura Y, Yoshimoto T, Akatsuka T, Matsui M. IL-23 enhances host defense against vaccinia virus infection via a mechanism partly involving IL-17. J Immunol. 2007; 179:3917–25. [PubMed: 17785829]

Lambiase A, Bracci-Laudiero L, Bonini S, Bonini S, Starace G, D'Elios MM, De Carli M, Aloe L. Human CD4+ T cell clones produce and release nerve growth factor and express high-affinity nerve growth factor receptors. J Allergy Clin Immunol. 1997; 100:408–14. [PubMed: 9314355]

Lotz M, Setareh M, von Kempis J, Schwarz H. The nerve growth factor/tumor necrosis factor receptor family. J Leukoc Biol. 1996; 60:1–7. [PubMed: 8699112]

Lowell CA. Src-family kinases: rheostats of immune cell signaling. Mol Immunol. 2004; 41:631–43. [PubMed: 15220000]

Myskiw C, Arsenio J, van Bruggen R, Deschambault Y, Cao J. Vaccinia virus E3 suppresses expression of diverse cytokines through inhibition of the PKR, NF-kappaB, and IRF3 pathways. J Virol. 2009; 83:6757–68. [PubMed: 19369349]

Nishino J, Mochida K, Ohfuji Y, Shimazaki T, Meno C, Ohishi S, Matsuda Y, Fujii H, Saijoh Y, Hamada H. GFR alpha3, a component of the artemin receptor, is required for migration and survival of the superior cervical ganglion. Neuron. 1999; 23:725–36. [PubMed: 10482239]

Nordmark G, Kristjansdottir G, Theander E, Appel S, Eriksson P, Vasaitis L, Kvarnstrom M, Delaleu N, Lundmark P, Lundmark A, Sjowall C, Brun JG, Jonsson MV, Harboe E, Goransson LG, Johnsen SJ, Soderkvist P, Eloranta ML, Alm G, Baecklund E, Wahren-Herlenius M, Omdal R, Ronnblom L, Jonsson R, Syvanen AC. Association of EBF1, FAM167A(C8orf13)-BLK and TNFSF4 gene variants with primary Sjogren's syndrome. Genes Immun. 2011; 12:100–9. [PubMed: 20861858]

Okutani D, Lodyga M, Han B, Liu M. Src protein tyrosine kinase family and acute inflammatory responses. Am J Physiol Lung Cell Mol Physiol. 2006; 291:L129–41. [PubMed: 16581827]

Otten U, Ehrhard P, Peck R. Nerve growth factor induces growth and differentiation of human B lymphocytes. Proc Natl Acad Sci U S A. 1989; 86:10059–63. [PubMed: 2557615]

Ovsyannikova IG, Jacobson RM, Ryan JE, Vierkant RA, Pankratz VS, Jacobsen SJ, Poland GA. HLA class II alleles and measles virus-specific cytokine immune response following two doses of measles vaccine. Immunogenetics. 2005; 56:798–807. [PubMed: 15712014]

Panther E, Corinti S, Idzko M, Herouy Y, Napp M, la Sala A, Girolomoni G, Norgauer J. Adenosine affects expression of membrane molecules, cytokine and chemokine release, and the T-cell stimulatory capacity of human dendritic cells. Blood. 2003; 101:3985–90. [PubMed: 12446452]

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–9. [PubMed: 16862161]

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945–59. [PubMed: 10835412]

Reading PC, Khanna A, Smith GL. Vaccinia virus CrmE encodes a soluble and cell surface tumor necrosis factor receptor that contributes to virus virulence. Virology. 2002; 292:285–98. [PubMed: 11878931]

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; 77:9960–8. [PubMed: 12941906]

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]

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; 79:1071–80. [PubMed: 17186465]

Scott JE, ElKhal A, Freyschmidt EJ, MacArthur DH, McDonald D, Howell MD, Leung DY, Laouar A, Manjunath N, Bianchi T, Boes M, Oettgen HC, Geha RS. Impaired immune response to vaccinia virus inoculated at the site of cutaneous allergic inflammation. J Allergy Clin Immunol. 2007; 120:1382–8. [PubMed: 17889291]

Sharma DP, Ramsay AJ, Maguire DJ, Rolph MS, Ramshaw IA. Interleukin-4 mediates down regulation of antiviral cytokine expression and cytotoxic T-lymphocyte responses and exacerbates vaccinia virus infection in vivo. J Virol. 1996; 70:7103–7. [PubMed: 8794356]

Smith EJ, Marie I, Prakash A, Garcia-Sastre A, Levy DE. IRF3 and IRF7 phosphorylation in virus-infected cells does not require double-stranded RNA-dependent protein kinase R or Ikappa B kinase but is blocked by Vaccinia virus E3L protein. J Biol Chem. 2001; 276:8951–7. [PubMed: 11124948]

Smith VP, Bryant NA, Alcami A. Ectromelia, vaccinia and cowpox viruses encode secreted interleukin-18-binding proteins. J Gen Virol. 2000; 81:1223–30. [PubMed: 10769064]

Souza RP, Romano-Silva MA, Lieberman JA, Meltzer HY, MacNeil LT, Culotti JG, Kennedy JL, Wong AH. Genetic association of the GDNF alpha-receptor genes with schizophrenia and clozapine response. J Psychiatr Res. 2010; 44:700–6. [PubMed: 20116071]

Suzuki N, Suzuki S, Millar DG, Unno M, Hara H, Calzascia T, Yamasaki S, Yokosuka T, Chen NJ, Elford AR, Suzuki J, Takeuchi A, Mirtsos C, Bouchard D, Ohashi PS, Yeh WC, Saito T. A critical role for the innate immune signaling molecule IRAK-4 in T cell activation. Science. 2006; 311:1927–32. [PubMed: 16574867]

Symons JA, Adams E, Tscharke DC, Reading PC, Waldmann H, Smith GL. The vaccinia virus C12L protein inhibits mouse IL-18 and promotes virus virulence in the murine intranasal model. J Gen Virol. 2002; 83:2833–44. [PubMed: 12388820]

Symons JA, Alcami A, Smith GL. Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. Cell. 1995; 81:551–60. [PubMed: 7758109]

Team RDC. R: A language and environment for statistical computing. 2008.

Tian T, Liu L, Freyschmidt EJ, Murphy GF, Kupper TS, Fuhlbrigge RC. Overexpression of IL-1alpha in skin differentially modulates the immune response to scarification with vaccinia virus. J Invest Dermatol. 2009; 129:70–8. [PubMed: 18615110]

Torcia M, Bracci-Laudiero L, Lucibello M, Nencioni L, Labardi D, Rubartelli A, Cozzolino F, Aloe L, Garaci E. Nerve growth factor is an autocrine survival factor for memory B lymphocytes. Cell. 1996; 85:345–56. [PubMed: 8616890]

Umlauf BJ, Ovsyannikova IG, Haralambieva IH, Kennedy RB, Vierkant RA, Pankratz VS, Jacobson RM, Poland GA. Correlations between vaccinia-specific immune responses within a cohort of armed forces members. Viral Immunol. 2011; 24:415–20. [PubMed: 21958369]

van Den Broek M, Bachmann MF, Kohler G, Barner M, Escher R, Zinkernagel R, Kopf M. IL-4 and IL-10 antagonize IL-12-mediated protection against acute vaccinia virus infection with a limited

role of IFN-gamma and nitric oxide synthetase 2. J Immunol. 2000; 164:371–8. [PubMed: 10605032]

Yang K, Puel A, Zhang S, Eidenschenk C, Ku CL, Casrouge A, Picard C, von Bernuth H, Senechal B, Plancoulaine S, Al-Hajjar S, Al-Ghonaium A, Marodi L, Davidson D, Speert D, Roifman C, Garty BZ, Ozinsky A, Barrat FJ, Coffman RL, Miller RL, Li X, Lebon P, Rodriguez-Gallego C, Chapel H, Geissmann F, Jouanguy E, Casanova JL. Human TLR-7-, -8-, and -9-mediated induction of IFN-alpha/beta and -lambda Is IRAK-4 dependent and redundant for protective immunity to viruses. Immunity. 2005; 23:465–78. [PubMed: 16286015]

Yang W, Ng P, Zhao M, Hirankarn N, Lau CS, Mok CC, Chan TM, Wong RW, Lee KW, Mok MY, Wong SN, Avihingsanon Y, Lee TL, Ho MH, Lee PP, Wong WH, Lau YL. Population differences in SLE susceptibility genes: STAT4 and BLK, but not PXK, are associated with systemic lupus erythematosus in Hong Kong Chinese. Genes Immun. 2009; 10:219–26. [PubMed: 19225526]

Yin H, Borghi MO, Delgado-Vega AM, Tincani A, Meroni PL, Alarcon-Riquelme ME. Association of STAT4 and BLK, but not BANK1 or IRF5, with primary antiphospholipid syndrome. Arthritis Rheum. 2009; 60:2468–71. [PubMed: 19644876]

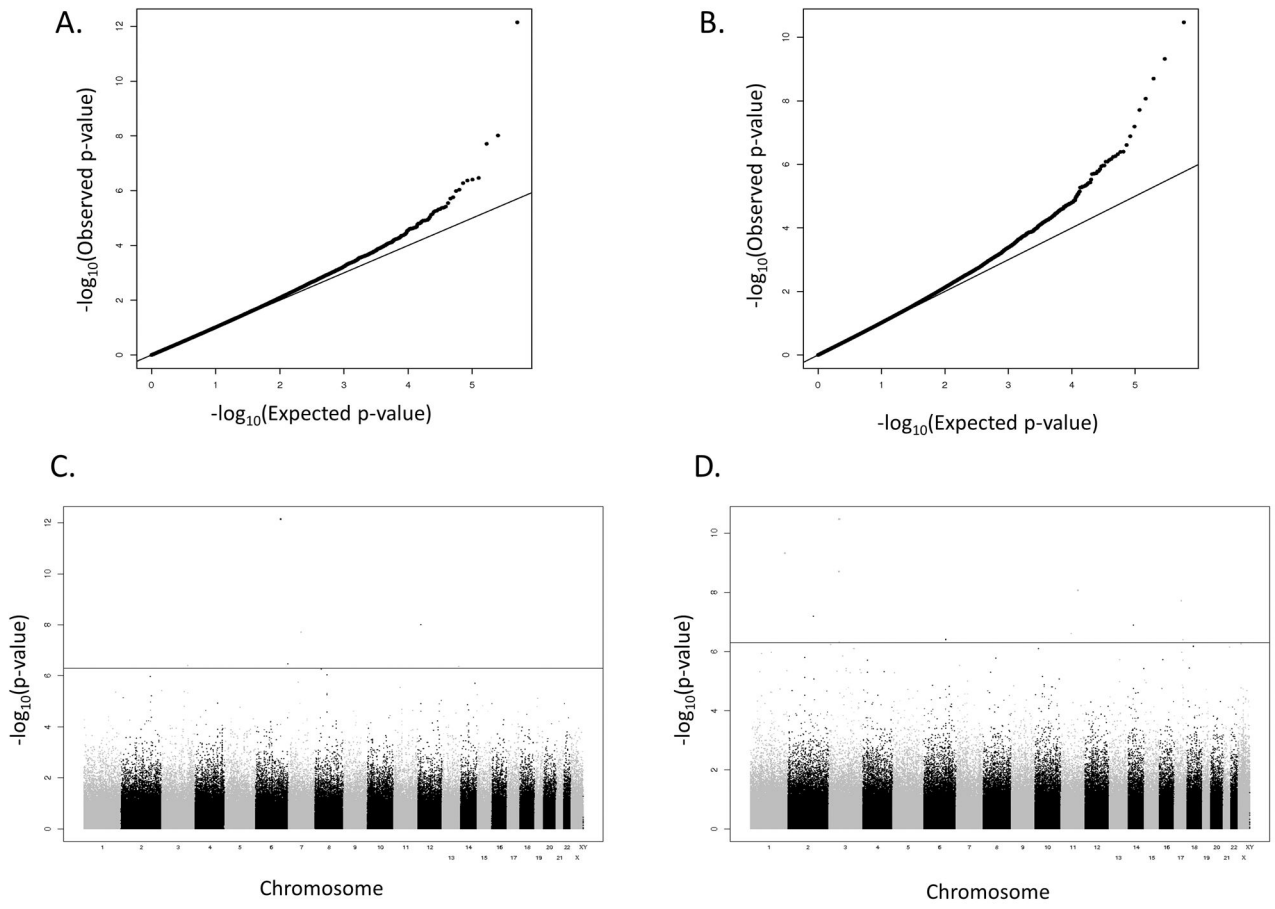

Figure 1. Quantile-Quantile and Manhattan plots of the expected (x-axis) and observed (y-axis) -log10 p-values for genotype associations with IL-2 secretion

a) the Caucasian cohort and b) the African-American cohort. The x-axis displays the –log10 of the p-value for each SNP association and the y-axis displays the chromosomes in alternating black and gray. p-values were adjusted for gender, age quartile at enrollment, time from immunization to blood draw, season and temperature sample was sent, time from blood draw to assay in quartiles, and the first 4 eigenvectors from the principal component analysis. c and d) Manhattan plot summary of GWAS results for IL-2 secretion for the Caucasian cohort (panel c) and the African-American cohort (panel d).

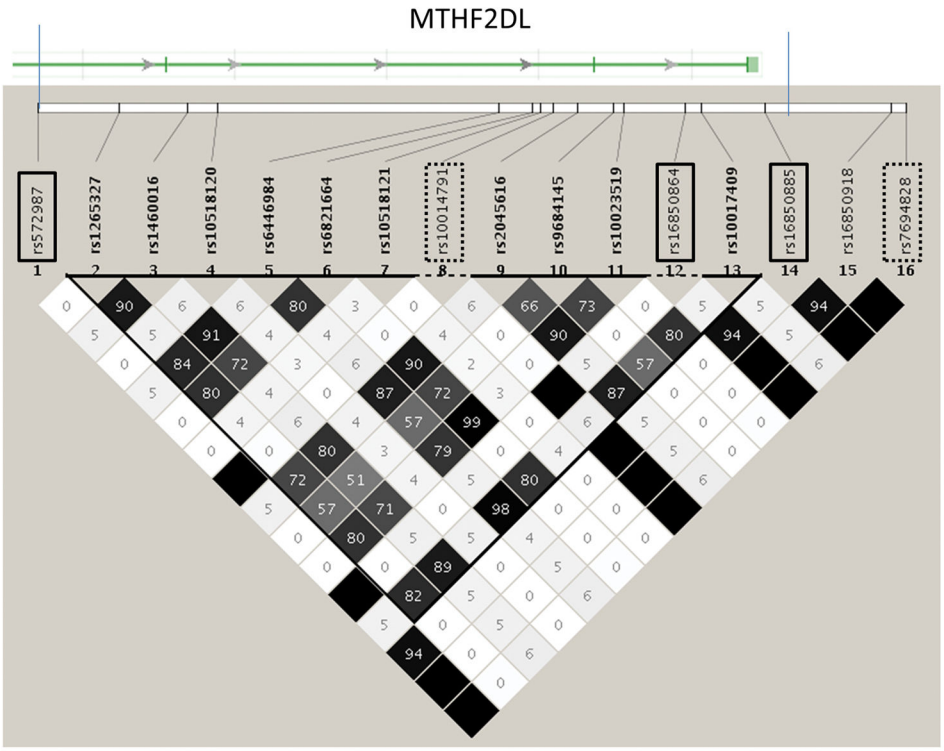

Figure 2. Genetic region containing a portion of the MTHFD2L gene and 3 SNPs significantly associated with variations in total IL-1β secretion in the Caucasian subjects

r^2 relationships between each pair of SNPs is indicated by the shading and number within each diamond on the LD plot. LD blocks (Gabriel definition) are represented by the bold triangle. SNPs showing significant associations in this study are highlighted in solid boxes, additional SNPs of interest are highlighted in hatched boxes. The vertical bar on top of the LD plot shows the introns/exons of the MTHFD2L gene aligned with the SNPs from the LD plot.

Table 1

Cytokine Response to Vaccinia Stimulation in Smallpox Vaccinees.

Caucasian Subjects						African-American Subjects				
Cytokine	**N**	**Mean**	**Std Dev**	**Median**	**IQR**	**N**	**Mean**	**Std Dev**	**Median**	**IQR**
IL-1β	450	113.6	209.0	55.6	28.1–148.3	185	100.7	145.1	46.9	24.9–102.2
IL-2	420	28.3	38.0	18.7	5.0–39.6	178	20.1	33.4	15.0	1.2–33.6
IL-4	490	2.1	15.6	0.6	−1.5–3.5	197	1.9	21.5	0.5	−3.0–3.0
IL-6	407	1253.8	1173.1	996.0	434.7–1914.7	177	1431.8	1143.2	1256.5	595.6–2173.0
IL-10	492	11.7	36.3	2.8	−0.3–11.2	198	9.1	27.0	2.0	−1.3–13.0
IL-12p40	435	87.9	109.4	62.9	27.4–115.2	182	93.8	120.1	65.6	30.1–146.6
IL-12p70	434	3.9	8.4	2.9	0.6–5.5	182	2.0	19.1	2.7	0.0–5.8
IL-18	420	1.1	20.2	0.7	−1.9–2.8	178	2.4	22.0	1.3	−0.8–3.9
IFNα	512	92.5	94.3	66.0	20.8–141.7	199	76.7	86.7	49.5	12.0–120.8
IFN β	429	2.9	15.1	1.7	−2.9–7.3	178	1.6	14.0	1.3	−4.8–7.1
TNFα	450	221.0	234.1	168.6	91.3–336.7	185	198.3	202.3	162.9	78.8–314.4

Immune outcome measurements in each racial cohort following stimulation with vaccinia virus.

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Table 2

SNPs showing significant association with secreted IL-2.

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
SNP associations in the Caucasian cohort									
rs9389316	6	GAPDHL19	5′upstream	59695	17	CC	391	19.7 (5.7,40.2)	7.12E-13
		GAPDH-like pseudogene				CA	17	4.3 (−1.7,12.1)	
						AA	0	(,)	
rs2268118	12	GRIN2B	intron	0	45	CC	363	20.7 (6.2,40.8)	9.70E-09
		ionotropic glutamate receptor				CA	37	14 (2.6,29.6)	
						AA	4	0.2 (−3.2,1.1)	
rs1403155*	7	AUTS2	5′upstream	1013906	13	GG	395	19.1 (5.5,39.6)	1.95E-08
		Autism susceptibility gene 2				GA	11	3.1 (−0.3,14.3)	
						AA	1	−1.4 (−1.4, −1.4)	
rs1473500	6	FRMD1	5′upstream	31177	26	GG	382	18.9 (5.8,39.3)	3.44E-07
		Protein interacting with angiotension II receptor				GA	26	7.8 (1.6,36.2)	
						AA	0	(,)	
rs10513432*	3	P2RY1	5′upstream	17371	31	AA	378	19.8 (5.8,40.5)	3.94E-07
		G-protein coupled purinergic receptor				AG	29	9.2 (2.5,15.1)	
						GG	1	−17.5 (−17.5, −17.5)	
rs1372791	13	LOC341604	5′upstream	292856	11	GG	397	19.3 (5.2,39.7)	4.26E-07
						GA	11	6.3 (1.8,14.4)	
						AA	0	(,)	
SNP associations in the African-American cohort									
rs13088281	3	RFT1	intron	0	15	CC	161	13.2 (0.6,30.4)	3.36E-11
		Oligosaccharide translocase				CA	15	41.8 (25.2,63.8)	
						AA	0	(,)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
rs908327*	1	TOMM20	3′downstream	180060	16	AA	160	17.9 (1.6,35.9)	4.74E-10
		Mitochondrial membrane translocase				AC	14	−5.2 (−10.9,9.8)	
						CC	1	−119.8 (−119.8, −119.8)	
rs17331151	3	ITIH3	3′downstream	1509	13	GG	163	14.1 (0.7,30.5)	1.98E-09
		ITIH4	3′downstream	2472		GA	13	49.5 (30.8,60.9)	
		Serine protease inhibitors				AA	0	(,)	
rs11223581*	11	CNTN5	intron	0	12	AA	163	14.2 (0.7,32.6)	8.51E-09
		Neuronal cell adhesion molecule			AG	10	26.4 (21.4,51.5)		
						GG	1	59.5 (59.5,59.5)	
rs16948200*	17	NGFR	5′upstream	6355	30	GG	144	19.2 (2.7,36.5)	1.93E-08
		Nerve growth factor receptor			GA	26	1.5 (−3.8,15.4)		
						AA	2	−4.8 (−7.2, −2.4)	
rs10432496	2	LOC100129594	5′upstream	29486	13	GG	163	17.1 (1.6,34.3)	6.41E-08
						GA	13	0.8 (−8.2,2.3)	
						AA	0	(,)	
rs11845208	14	ATP5GP2	5′upstream	377106	18	AA	157	18.8 (2.8,35.8)	1.30E-07
		ATP synthase				AG	18	−3.2 (−11.5,2.1)	
						GG	0	(,)	
rs4963243	11	DAGLA	intron	0	10	GG	166	15.7 (1.4,35.2)	2.45E-07
		Diacylglycerol lipase			GA	10	2.8 (−2.2,14.4)		
						AA	0	(,)	
rs1392089	6	LOC728727	intron	0	10	GG	165	17 (1.6,35.4)	3.97E-07
						GA	10	−7.5 (−25.1, −1.1)	
						AA	0	(,)	
rs7224438	17	BCAS3	intron	0	127	AA	71	23.7 (6.9,45.7)	4.03E-07
		Breast cancer amplified sequence 3			AG	79	9.6 (0.5,29.1)		
						GG	24	1.2 (−7.1,21.3)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
rs3796352	3	TMEM110	intron	0	13	GG	163	14.2 (0.9,30.9)	4.75E-07
		Transmembrane protein 110			GA	13	41.8 (19.5,60.7)		
						AA	0	(,)	

[a] rs SNP identification number,

[b] Chromosomal location of the indicated SNP,

[c] Gene or genetic region containing the indicated SNP,

[d] Location of the SNP relative to the gene or distance to nearest gene,

[e] Minor Allele Frequency

[f] Number of subjects with a given genotype,

[g] Median outcome measurement for each genotype group, expressed as the difference in the median unstimulated and stimulated values. The interquartile range is shown in parentheses,

[h] p-values were adjusted for gender, age quartile at enrollment, time from immunization to blood draw, season and temperature sample was sent, time from blood draw to assay in quartiles, and the first 4 eigenvectors from the principal component analysis.

* sensitivity analysis p-values are as follows: rs1403155 =3.56E-08, rs10513432 = 2.85E-06, rs908327 = 1.58E-07, rs11223581 = 4.92E-06, rs16948200 = 6.98E-08.

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Table 3

SNPs showing significant association with secreted TNFα

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
SNP associations in the Caucasian cohort									
rs738968*	22	LOC441996	3′downstream	95977	74	AA	364	181.1 (93.6,344.8)	8.43E-08
						AC	70	160.4 (84.1,310.9)	
						CC	2	108.5 (103.6,113.3)	
rs8141914*	22	LOC441996	3′downstream	105844	74	GG	363	179.6 (93.2,345)	9.78E-08
						GA	70	160.4 (84.1,310.9)	
						AA	2	108.5 (103.6,113.3)	
rs16994335*	22	LOC441996	3′downstream	52775	77	GG	361	182.6 (93.9,344.5)	1.30E-07
						GA	73	158.7 (82.6,319.2)	
						AA	2	108.5 (103.6,113.3)	
rs11889798*	2	C2orf83	intron	0	64	GG	375	188 (97.3,346.6)	3.87E-07
		LOC729968	3′downstream	295	64	GA	58	123.3 (58.8,274.6)	
						AA	3	101.6 (83.1,111.4)	
rs13006863*	2	SLC4A5	intron	0	257	GG	209	212.3 (106.9,374.9)	4.83E-07
						GA	189	147.8 (89,289.1)	
						AA	34	148.9 (100.9,292.5)	
SNP associations in the African-American cohort									
rs4251424	12	IRAK4	intron	0	11	GG	170	175.8 (94.3,323.4)	1.66E-08
		PUS7L	5′upstream	1230		GA	11	89.7 (39.2,114.1)	
		IL-1R associated kinase 4				AA	0	(,)	
rs13414205	2	CAMKMT	intron	0	37	GG	149	153 (72.2,298.1)	7.06E-08
		Calmodulin lysine N-methyltransferase				GA	29	224.7 (161.5,343.9)	
						AA	4	616.8 (443.4,702.6)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
rs758386	3	SLC6A20	synonymous	0	10	GG	170	171.6 (82,322.6)	2.41E-07
		Sodium bicarbonate cotransporter protein				GA	10	125.5 (48.4,213.5)	
						AA	0	(,)	

[a] rs SNP identification number,

[b] Chromosomal location of the indicated SNP,

[c] Gene or genetic region containing the indicated SNP,

[d] Location of the SNP relative to the gene or distance to nearest gene,

[e] Minor Allele Frequency

[f] Number of subjects with a given genotype,

[g] Median outcome measurement for each genotype group, expressed as the difference in the median unstimulated and stimulated values. The interquartile range is shown in parentheses,

[h] p-values were adjusted for gender, age quartile at enrollment, time from immunization to blood draw, season and temperature sample was sent, time from blood draw to assay in quartiles, and the first 4 eigenvectors from the principal component analysis.

[*] sensitivity analysis p-values are as follows: rs738968 = 2.37E-07, rs8141914 = 2.74E-07, rs16994335 = 3.59E-07, rs11889798 = 9-2.77E-06, rs13006863 = 4.13E-06,

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Table 4

SNPs showing significant association with secreted IL-12p40.

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
SNP associations in the Caucasian cohort									
rs7771911	6	LOC100131805	3′downstream	333903	11	CC	400	65.6 (27.9,116.7)	1.89E-12
						CA	11	39.9 (19.4,44.1)	
						AA	0	(,)	
rs17142462	7	KCND2	5′upstream	307090	10	GG	402	64 (27.6,116.1)	3.32E-09
						GA	10	36.7 (5.6,69.2)	
						AA	0	(,)	
rs7105056	11	BCO2	intron	0	14	GG	408	65 (28.7,116)	9.71E-09
						GA	14	28.2 (10.2,35.9)	
						AA	0	(,)	
rs7658486*	4	ARHGAP10	intron	0	43	GG	375	66.4 (29.3,121.5)	1.47E-08
						GA	39	39.1 (19.3,67.4)	
						AA	2	56 (41.6,70.4)	
rs11242417	5	GFRA3	intron	0	142	AA	289	71.5 (31.5,133)	5.56E-08
						AC	118	47.2 (18.8,88.6)	
						CC	12	41 (20.8,66.9)	
rs1584468	5	PRR16	intron	0	19	GG	403	66.4 (29,117.5)	8.64E-08
						GA	19	26.3 (11,43.5)	
						AA	0	(,)	
rs11034653	11	LOC100132631	3′downstream	507609	10	GG	412	62.7 (27.2,114.5)	1.72E-07
						GA	10	105.4 (89.6,142.3)	
						AA	0	(,)	
rs6484985	11	LOC100132631	3′downstream	527429	10	AA	412	62.7 (27.2,114.5)	1.72E-07
						AG	10	105.4 (89.6,142.3)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
						GG	0	(,)	
rs3736638*	7	COL1A2	intron	0	10	CC	411	63.2 (27.2,115.6)	2.81E-07
						CA	8	74.2 (58.8,102.2)	
						AA	1	121.4 (121.4,121.4)	
SNP associations in the African-American cohort									
rs859267*	17	ADORA2B	5′upstream	22210	46	AA	135	78 (39.8,156.2)	2.52E-07
						AC	40	53.7 (21.7,71.6)	
						CC	3	21.7 (5.5,67.7)	
rs6943090*	7	FKBP6	intron	0	44	GG	137	74.9 (39.8,155.5)	3.17E-07
		TRIM50	5′upstream	4563		GA	40	43.2 (18.8,113.2)	
						AA	2	-9.8 (-10.2,-9.3)	

[a] rs SNP identification number,

[b] Chromosomal location of the indicated SNP,

[c] Gene or genetic region containing the indicated SNP,

[d] Location of the SNP relative to the gene or distance to nearest gene,

[e] Minor Allele Frequency

[f] Number of subjects with a given genotype,

[g] Median outcome measurement for each genotype group, expressed as the difference in the median unstimulated and stimulated values. The interquartile range is shown in parentheses,

[h] p-values were adjusted for gender, age quartile at enrollment, time from immunization to blood draw, season and temperature sample was sent, time from blood draw to assay in quartiles, and the first 4 eigenvectors from the principal component analysis.

* sensitivity analysis p-values are as follows: rs7658486 = 5.84E-08, rs3736638 = 9.01E-05, rs859267 = 2.82E-07, rs6943090 = 3.93E-06.

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Table 5

SNPs showing significant association with secreted IL-1β.

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
SNP associations in the Caucasian cohort									
rs10998624*	10	SUPV3L1	5′upstream	192	12	GG	425	58.2 (29.7,156.6)	1.06E-09
		VPS26A	3′downstream	7184		GA	10	32.9 (14.5,57.9)	
						AA	1	19 (19,19)	
rs16850864	4	MTHFD2L	intron	0	10	GG	426	58 (29.7,151.2)	7.30E-09
						GA	10	23.6 (7,70.6)	
						AA	0	(,)	
rs16850885	4	EPGN	5′upstream	4064	10	GG	426	58 (29.7,151.2)	7.30E-09
		MTHFD2L	3′downstream	1324		GA	10	23.6 (7,70.6)	
						AA	0	(,)	
rs16850918	4	EPGN	3′downstream	5454	10	AA	426	58 (29.7,151.2)	7.30E-09
						AG	10	23.6 (7,70.6)	
						GG	0	(,)	
rs572987	4	MTHFD2L	intron	0	10	AA	426	58 (29.7,151.2)	7.30E-09
						AG	10	23.6 (7,70.6)	
						GG	0	(,)	
rs9582259	13	SLC15A1	intron	0	44	AA	391	60.7 (31.3,153.6)	2.99E-08
						AC	44	30.2 (16,79)	
						CC	0	(,)	
rs9835973	3	RAB6B	intron	0	152	GG	301	52.6 (25.5,125.5)	7.45E-08
						GA	118	70.5 (35.3,172)	
						AA	17	75.5 (46.1,141.8)	
rs902464	4	DCHS2	5′upstream	3673	10	GG	426	58.4 (29.9,151.2)	3.04E-07
						GA	10	22.8 (16.9,48.9)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
						AA	0	(,)	
rs9883650	3	MDS1	intron	0	71	AA	368	61.1 (31.8,158.7)	3.91E-07
						AC	61	34.5 (17.1,89.4)	
						CC	5	32.4 (21.7,48.9)	
rs16853574	3	MDS1	intron	0	62	AA	376	60.8 (31.5,158.7)	4.76E-07
						AG	52	33.2 (17.3,89.9)	
						GG	5	32.4 (21.7,48.9)	
SNP associations in the African-American cohort									
rs12247397*	10	LOC389936	5′upstream	85153	21.0	AA	160	53.9 (25.6,109.6)	3.33E-09
						AG	19	37.6 (24.7,75.7)	
						GG	1	44.7 (44.7,44.7)	
rs17000918*	22	FLJ44385	5′upstream	134939	15.0	CC	167	55.8 (28.5,117.8)	3.93E-09
						CA	11	29.7 (21.9,35.2)	
						AA	2	45 (30.3,59.7)	
rs11564024*	7	LOC392008	intron	0	31.0	AA	154	62.4 (30.8,125.8)	2.82E-08
						AC	25	23.7 (8.7,44.1)	
						CC	3	22.2 (14.8,62.2)	
rs17168526*	7	COL28A1	synonymous	0	21.0	AA	162	51.4 (28.8,118)	6.40E-08
						AG	17	62.3 (14.8,90.1)	
						GG	2	10.6 (5.3,15.9)	
rs12542677*	8	XKR4	intron	0	11.0	GG	172	52.7 (25.6,114.7)	2.32E-07
						GA	9	36.4 (24.7,74.2)	
						AA	1	43 (43,43)	
rs4827947*	X	LOC100128151	3′downstream	186536	21.0	GG	162	61.5 (28.4,124.5)	3.18E-07
						GA	19	33.3 (19.7,39.9)	
						AA	1	22.2 (22.2,22.2)	

[a] rs SNP identification number,

[b] Chromosomal location of the indicated SNP,

[c] Gene or genetic region containing the indicated SNP,

[d] Location of the SNP relative to the gene or distance to nearest gene,

[e] Minor Allele Frequency

[f] Number of subjects with a given genotype,

[g] Median outcome measurement for each genotype group, expressed as the difference in the median unstimulated and stimulated values. The interquartile range is shown in parentheses,

[h] p-values were adjusted for gender, age quartile at enrollment, time from immunization to blood draw, season and temperature sample was sent, time from blood draw to assay in quartiles, and the first 4 eigenvectors from the principal component analysis.

[*] sensitivity analysis p-values are as follows: rs10998624 = 1.58E-08, rs12247397 = 4.7E-10, rs17000918 = 2.33E-07, rs11564024 = 3.81E-08, rs17168526 = 4.75E-06, rs12542677 = 3.35E-08, rs4827947 = 1.43E-06.

Table 6

SNPs showing significant association with secreted IFNα.

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
SNP associations in the Caucasian cohort									
rs13181561	5	ECSM2	5′upstream	8585	248	AA	272	88.4 (35.8,174)	2.71E-14
		TMEM173	3′downstream	4644		AG	192	51.5 (12.4,112.1)	
						GG	28	5.6 (1.3,17.2)	
rs6573333	14	MNAT1	intron	0	14	CC	482	67.6 (22.5,142.8)	3.09E-10
						CA	14	37.9 (8.5,56)	
						AA	0	(,)	
rs6576443	15	ATP10A	3′downstream	29201	341	GG	212	77.7 (39.1,149.9)	6.20E-09
						GA	229	57.7 (16.8,140.2)	
						AA	56	31.9 (5.4,84.9)	
rs6573332	14	MNAT1	intron	0	16	GG	482	67.6 (22.5,142.8)	1.26E-08
						GA	16	38.4 (11.2,57.9)	
						AA	0	(,)	
rs7150492	14	MNAT1	intron	0	16	GG	481	67.7 (22.5,143)	1.33E-08
						GA	16	38.4 (11.2,57.9)	
						AA	0	(,)	
rs2925296	15	ATP10A	3′downstream	36278	356	GG	203	75.7 (39.3,149.1)	1.72E-08
						GA	234	59.8 (16.5,138.7)	
						AA	61	34.1 (7.6,101.5)	
rs10195263*	2	LOC344332	5′upstream	307939	20	AA	479	67.5 (22.8,142.6)	3.14E-08
						AG	18	23.7 (2.9,65.8)	
						GG	1	5.9 (5.9,5.9)	
rs542631	2	LTBP1	intron	0	10	CC	487	64.5 (20.6,139.2)	1.11E-07
						CA	10	168.5 (96.4,200.8)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
						AA	0	(,)	
rs261532	5	UBE2D2	intron	0	304	CC	245	83.7 (32.1,167)	2.50E-07
						CA	202	55.7 (18.3,114.9)	
						AA	51	31.8 (3.6,84.3)	
rs8012779*	14	MNAT1	intron	0	22	GG	477	69.6 (22.1,143)	4.03E-07
		TRMT5	3′downstream	6543		GA	20	49 (27.7,63.1)	
						AA	1	7.1 (7.1,7.1)	
SNP associations in the African-American cohort									
rs4078978*	2	WDR92	intron	0	12.0	GG	186	56 (16.7,122.3)	2.24E-18
						GA	8	1.6 (−0.2,7.7)	
						AA	2	−2.5 (−4.3, −0.7)	
rs381365	X	LOC100128265	3′downstream	47772	12.0	CC	184	56 (17.4,122.8)	2.30E-12
						CA	12	3.4 (2.3,21.9)	
						AA	0	(,)	
rs2048161	4	ZNF827	intron	0	15.0	GG	181	61.9 (18.4,124.1)	5.78E-12
						GA	15	8.8 (−0.7,17.3)	
						AA	0	(,)	
rs17252936*	X	MAMLD1	intron	0	13.0	AA	184	50.6 (14.3,122.3)	5.51E-10
						AG	11	30.6 (9.5,63.1)	
						GG	1	0 (0,0)	
rs11171846	12	TIMELESS	intron	0	10.0	GG	186	56 (15.5,122.3)	6.14E-10
						GA	10	14.2 (3.2,22)	
						AA	0	(,)	
rs12044963	1	KCND3	intron	0	12.0	CC	184	53 (15.7,122.8)	9.43E-10
						CA	12	12.9 (6.8,34.3)	
						AA	0	(,)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
rs6778194*	3	LOC152118	5′upstream	17628	12.0	AA	185	50.6 (15.4,122.2)	2.43E-09
						AC	8	23.1 (6.8,70)	
						CC	2	9.7 (−0.2,19.6)	
rs2272205*	2	COL4A4	intron	0	20.0	AA	178	59.6 (14.9,122.3)	2.84E-09
						AG	16	23.9 (10.5,49.8)	
						GG	2	10 (5,15)	
rs9408928	9	RAB14	intron	0	13.0	AA	183	53.6 (15.6,123.2)	4.07E-09
						AG	13	4.1 (2.7,24.4)	
						GG	0	(,)	
rs1540283	X	PHEX	intron	0	15.0	AA	181	60.6 (17.7,124.1)	9.40E-09
						AG	15	11 (−0.4,30.5)	
						GG	0	(,)	
rs2269466	X	PHEX	intron	0	15.0	AA	181	60.6 (17.7,124.1)	9.40E-09
						AG	15	11 (−0.4,30.5)	
						GG	0	(,)	
rs17007761	2	LOC728241	3′downstream	263187	17.0	CC	179	45.5 (11.1,111.1)	9.86E-09
						CA	17	114.8 (65.8,180.1)	
						AA	0	(,)	
rs4713226*	6	OR2H1	3′downstream	2315	14.0	GG	184	51.6 (15.2,122.3)	2.45E-08
						GA	10	17.1 (9,51.1)	
						AA	2	7 (0.4,13.5)	
rs9408926	9	CEP110	synonymous	0	12.0	GG	184	53 (15.2,122.8)	3.96E-08
						GA	12	11.6 (2.2,29.6)	
						AA	0	(,)	
rs210359	14	BMP4	3′downstream	248982	10.0	CC	186	51.6 (14.5,122.3)	7.61E-08
						CA	10	27.6 (4.8,48.5)	
						AA	0	(,)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
rs13067593*	3	LPP	intron	0	35.0	AA	162	63.3 (23.9,125.6)	7.88E-08
						AG	29	18.4 (1.1,58.5)	
						GG	3	4.1 (−3,12.3)	
rs5925760	X	PTCHD1	intron	0	10.0	GG	184	51.6 (13.5,122.8)	1.22E-07
						GA	10	28.3 (14.4,46.4)	
						AA	0	(,)	
rs7060947	X	ODZ1	intron	0	24.0	AA	169	63 (19.5,125.6)	1.31E-07
						AG	24	15.9 (2.2,38.2)	
						GG	0	(,)	
rs17221323*	10	DIP2C	intron	0	14.0	AA	183	52.5 (15.6,123.2)	1.39E-07
						AG	12	16.9 (2.9,50.8)	
						GG	1	0.9 (0.9,0.9)	
rs8127571	21	LOC100129027	3′downstream	84485	10.0	GG	186	53 (14.9,122.3)	1.86E-07
						GA	10	20.3 (4.4,37.7)	
						AA	0	(,)	
rs4839431*	1	CYMP	rna_exon	0	32.0	GG	163	53.6 (16.1,124.9)	2.11E-07
						GA	28	29.2 (3.2,62.1)	
						AA	2	−0.5 (−1.3,0.3)	
rs9807334	18	ELAC1	3′downstream	9671	10.0	GG	186	51.6 (14.9,122.3)	2.16E-07
						GA	10	14.7 (2.3,25.3)	
						AA	0	(,)	
rs10517025*	4	ATP8A1	intron	0	22.0	GG	176	47.6 (10.9,120.6)	2.17E-07
						GA	18	74.6 (52.6,127)	
						AA	2	156.7 (100.9,212.5)	
rs17714988	13	hCG_1820717	5′upstream	4677	10.0	AA	186	56 (14.9,122.3)	3.29E-07
						AG	10	15.7 (3.2,34.5)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
						GG	0	(,)	
rs9493873*	6	SGK1	5′upstream	80509	28.0	AA	168	62.6 (20.4,127.5)	4.18E-07
						AG	24	14.6 (2.5,48.5)	
						GG	2	4.8 (4.2,5.4)	
rs3095748*	9	DAPK1	intron	0	13.0	AA	184	50.6 (15.2,122.3)	4.64E-07
						AG	11	20.1 (2.1,71.3)	
						GG	1	0.4 (0.4,0.4)	
rs2043599*	19	NLRP13	5′upstream	7347	26.0	AA	171	58.5 (18.1,125.6)	4.77E-07
		NLRP8	5′upstream	8149		AG	24	18.9 (3.2,74.6)	
						GG	1	0.8 (0.8,0.8)	
rs10517038	4	ATP8A1	intron	0	18.0	GG	178	48.3 (11.1,119.3)	4.89E-07
						GA	18	74.6 (50.3,136.6)	
						AA	0	(,)	
rs3811769	4	ATP8A1	intron	0	18.0	AA	178	48.3 (11.1,119.3)	4.89E-07
						AG	18	74.6 (50.3,136.6)	
						GG	0	(,)	

[a] rs SNP identification number,

[b] Chromosomal location of the indicated SNP,

[c] Gene or genetic region containing the indicated SNP,

[d] Location of the SNP relative to the gene or distance to nearest gene,

[e] Minor Allele Frequency,

[f] Number of subjects with a given genotype,

[g] Median outcome measurement for each genotype group, expressed as the difference in the median unstimulated and stimulated values. The interquartile range is shown in parentheses,

[h] p-values were adjusted for gender, age quartile at enrollment, time from immunization to blood draw, season and temperature sample was sent, time from blood draw to assay in quartiles, and the first 4 eigenvectors from the principal component analysis.

* sensitivity analysis p-values are as follows: r rs10195263 = 5.51E-10, rs8012779 = 3.09E-06, rs4078978 = 3.9E-30, rs17252936 = 3.98E-07, rs6778194 = 9.5E-07, rs2272205 = 3.75E-07, rs4713226 = 4.62E-06, rs13067593 = 2.42E-07, rs17221323 = 3.18E-06, rs4839431 = 6.78E-06, rs10517025 = 3.33E-06, rs9493873 = 5.03E-06, rs3095748 = 9.4E-06, rs2043599 = 1.74E-06.

Table 7

SNPs showing significant association with secreted IL-6

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
SNP associations in the Caucasian cohort									
None									
SNP associations in the African-American cohort									
rs8096445	18	MEX3C	intron	0	15	GG	157	1175.9 (595.6,2063.9)	9.23E-09
						GA	15	2308.4 (1429,3259.6)	
						AA	0	(,)	
rs17444059	5	PDE4D	intron	0	12	AA	163	1337.4 (677.6,2276.4)	2.03E-08
						AG	12	507.8 (320.3,918.3)	
						GG	0	(,)	
rs6728021*	2	LTBP1	5′upstream	50888	20	AA	157	1129.4 (595.5,2032.7)	4.48E-08
						AG	16	2052 (1514,2798.5)	
						GG	2	3014.5 (2879.6,3149.4)	
rs1516489	3	LOC100129725	3′downstream	148218	10	AA	165	1337.4 (680.8,2274.6)	7.72E-08
						AC	10	274.3 (77.7,871.1)	
						CC	0	(,)	
rs17290760*	9	NDUFB6	intron	0	15	AA	161	1321.6 (674.3,2274.6)	1.17E-07
		TOPORS	5′upstream	3779		AG	13	933.7 (424,1552.7)	
						GG	1	329.6 (329.6,329.6)	
rs17299841*	6	C6orf190	3′downstream	7542	11	AA	164	1336.2 (679.2,2251)	1.59E-07
						AC	9	570 (366.5,891.2)	
						CC	1	329.6 (329.6,329.6)	
rs2501276*	1	CDC42	5′upstream	5496	14	GG	162	1234.1 (595.5,2056.1)	1.85E-07
						GA	12	2314 (1190.5,2983.4)	

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
						AA	1	2991.9 (2991.9,2991.9)	
rs2255327	8	BLK	intron	0	11	GG	162	1202.5 (595.5,2068.6)	2.90E-07
						GA	11	2390.3 (1463.8,3051.1)	
						AA	0	(,)	
rs2973662*	5	ODZ2	intron	0	16	GG	159	1139 (593.6,2048.3)	4.51E-07
						GA	14	2314.6 (1374.1,3053.6)	
						AA	1	3594.4 (3594.4,3594.4)	

[a] rs SNP identification number,

[b] Chromosomal location of the indicated SNP,

[c] Gene or genetic region containing the indicated SNP,

[d] Location of the SNP relative to the gene or distance to nearest gene,

[e] Minor Allele Frequency,

[f] Number of subjects with a given genotype,

[g] Median outcome measurement for each genotype group, expressed as the difference in the median unstimulated and stimulated values. The interquartile range is shown in parentheses,

[h] p-values were adjusted for gender, age quartile at enrollment, time from immunization to blood draw, season and temperature sample was sent, time from blood draw to assay in quartiles, and the first 4 eigenvectors from the principal component analysis.

* sensitivity analysis p-values are as follows: rs6728021 = 1.47E-06, rs17290760 = 1.13E-05, rs17299841 = 5.28E-05, rs2501276 = 2.6E-05, rs2973662 = 6.35E-6.

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Table 8

SNPs showing significant association with secreted IL-10

SNP ID[a]	Chromosome[b]	Gene[c]	Location[d]	Distance from Gene	MAF[e]	Genotype	N[f]	Median (IQR)[g]	p-value[h]
SNP associations in the Caucasian cohort									
rs16970881*	16	DNAH3	intron	0	39	GG	441	2.9 (−0.3,11.4)	1.27E-07
						GA	35	0.6 (−0.9,4)	
						AA	2	−0.7 (−1, −0.5)	
SNP associations in the African-American cohort									
rs13111850*	4	SPOCK3	5′upstream	108508	12.0	AA	177	2 (−1.1,12.9)	3.17E-11
						AG	8	0 (−2.2,5.1)	
						GG	2	−2.5 (−2.7, −2.2)	
rs13231718*	7	DYNC1I1	intron	0	10.0	AA	186	1.7 (−1.5,12.2)	5.38E-09
						AC	8	4 (2.1,20)	
						CC	1	10.6 (10.6,10.6)	
rs17231212	7	LOC100129730	5′upstream	27454	11.0	AA	184	2.3 (−0.8,13.4)	1.81E-08
						AG	11	−2.2 (−5, −0.1)	
						GG	0	(,)	
rs10055544*	5	LOC100131236	5′upstream	347030	11.0	GG	181	2 (−0.9,12.3)	1.36E-07
						GA	9	−2.8 (−4.5, −0.8)	
						AA	1	−8.1 (−8.1, −8.1)	
rs6679454	1	DAB1	intron	0	108	AA	100	3.8 (0.5,21.9)	2.89E-07
						AG	80	0.5 (−3.9,3.2)	
						GG	14	−1.9 (−4.7,11.1)	

[a] rs SNP identification number,

[b] Chromosomal location of the indicated SNP,

[c] Gene or genetic region containing the indicated SNP,

[d] Location of the SNP relative to the gene or distance to nearest gene,

[e] Minor Allele Frequency,

[f] Number of subjects with a given genotype,

[g] Median outcome measurement for each genotype group, expressed as the difference in the median unstimulated and stimulated values. The interquartile range is shown in parentheses,

[h] p-values were adjusted for gender, age quartile at enrollment, time from immunization to blood draw, season and temperature sample was sent, time from blood draw to assay in quartiles, and the first 4 eigenvectors from the principal component analysis.

[*] sensitivity analysis p-values are as follows: rs16970881 = 7.3E-07, rs13111850 = 9.06E-08, rs13231718 = 1.13E-11, rs10055544 = 8.51E-06.