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Multigenic Control of Measles Vaccine Immunity Mediated by Polymorphisms in Measles Receptor, Innate Pathway, and Cytokine Genes

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Abstract

Measles infection and vaccine response are complex biological processes that involve both viral and host genetic factors. We have previously investigated the influence of genetic polymorphisms on vaccine immune response, including measles vaccines, and have shown that polymorphisms in HLA, cytokine, cytokine receptor, and innate immune response genes are associated with variation in vaccine response but do not account for all of the inter-individual variance seen in vaccinated populations. In the current study we report the findings of a multigenic analysis of measles vaccine immunity, indicating a role for the measles virus receptor CD46, innate pattern-recognition receptors (DDX58, TLR2, 4, 5, 7 and 8) and intracellular signaling intermediates (MAP3K7, NFKBIA), and key antiviral molecules (VISA, OAS2, MX1, PKR) as well as cytokines (IFNA1, IL4, IL6, IL8, IL12B) and cytokine receptor genes (IL2RB, IL6R, IL8RA) in the genetic control of both humoral and cellular immune responses. This multivariate approach provided additional insights into the genetic control of measles vaccine responses over and above the information gained by our previous univariate SNP association analyses.

Keywords

measles vaccine; immunogenetics; vaccine response; multigenic SNP association; interferon response; cytokines; Toll-like receptors

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Disclosures

Dr. Poland chairs a safety evaluation committee for novel non-measles vaccines undergoing clinical studies by Merck Research Laboratories. Dr. Jacobson serves on a Safety Review Committee for a post-licensure study of Gardasil for Kaiser-Permanente.

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Introduction

Measles is an extremely infectious viral infection resulting in hundreds of thousands of deaths worldwide every year. The introduction of effective measles vaccines have drastically reduced both disease cases and mortality[1]. Nevertheless, 2008 saw the largest number of measles cases in Europe and the US in a decade, and the disease has once again been declared endemic in the UK[2]. The WHO has reported outbreaks and rising case numbers throughout Europe in the past few years, with over 30,000 cases reported in 2010[3]. In the US, the largest number of measles cases since 1996 had already occurred by May of 2011[4].

Measles infection and vaccine response are complex biological processes that involve both viral and host genetic factors. Our laboratory has investigated the role of genetic polymorphisms in vaccine response, including measles vaccines, and demonstrated that polymorphisms in HLA, cytokine, cytokine receptor, and innate immune response genes are associated with inter-individual variations in vaccine response but do not account for all of the variance seen in vaccinated populations[5–17]. Given the multifactorial nature of diseases and the complexity of biological responses, it is highly likely that multigenic interactions affect vaccine response and immune outcomes, a process that is better explained by the immune response network theory than by a dominant allele mechanism[18]. Increasingly sophisticated approaches are utilized to unravel multigenic associations with disease and treatment outcomes[19, 20] and serve as valuable complements to single SNP analyses in furthering our understanding of the complex genetic control of vaccine response. Accordingly, we took advantage of a wealth of existing genetic and immunologic data on healthy young recipients of two doses of MMR-II to examine potential multigenic interactions associated with robust or ineffective vaccine responses.

Materials and Methods

Subjects

Our study population consists of 821 subjects recruited from 2 previously reported, independent, age-stratified, random cohorts of children and young adults (11 – 22 years of age) in Rochester, MN[14, 17]. There was no known wild-type measles virus exposure in the community since the birth of the oldest subject. Each participant had medically documented receipt of exactly two doses of measles-mumps-rubella (MMR-II, Merck) vaccine. The parents of each participant provided written informed consent, while written assent was obtained from age-appropriate children. The Mayo Clinic Institutional Review Board (IRB) approved all study procedures. A single blood draw was obtained from each subject and DNA, serum, and PBMC samples were obtained as previously described[21].

TagSNP selection

One hundred twenty-six genetic loci encoding cytokines and cytokine receptors, Toll-like receptors and signaling molecules, antiviral proteins, measles virus receptors, vitamin A receptors, and other innate immunity-related proteins were identified as genes of interest from our data, literature searches and public databases[7–9, 14, 17, 22, 23]. All SNPs within each gene as well as those 5 kb upstream and downstream for each gene were included in our LD tagSNP selection algorithm[24]. The ldSelect program was used to bin SNPs with a minor allele frequency (MAF) ≥ 0.05 and a pairwise linkage disequilibrium (LD) threshold of $r^2 \geq 0.90$ among HapMap CEPH unrelated samples as well as successful Illumina predictive genotyping scores. The resulting selection of tagSNPs was further refined with SNPPicker[25]. Additionally, a list of putative functional “obligate” SNPs (including coding (nonsynonymous, synonymous) SNPs and SNPs in either the 5′ or 3′ untranslated regions

with a MAF ≥ 0.05) was added to the final list for a total of 2,304 SNPs. We followed the nomenclature defined by den Dunnen and Antonarakis[26].

Genotyping methods

Genomic DNA was extracted using Puregene kits (Gentra Systems Inc., Minneapolis, MN) and quantified by Picogreen (Molecular Probes) as described[14, 17]. We genotyped 2,304 SNPs from 126 candidate genes. SNPs were divided into two Illumina GoldenGate SNP panels (Illumina Inc., San Diego, CA) of 1,536 and 768 SNPs. Design scores for all were >0.4 , and all genotyping was performed as per manufacturer's protocols[6]. Genotype calls were made using Bead Studio 2 software. Quality control relied on control genomic DNA samples (CEPH family trio and two other genomic DNAs) and both replicate and inheritance datasets to assess and refine clustering. After removal of the SNPs that failed quality-assurance measures (HWE, amplification failure, poor clustering, or multiple replicate errors), we obtained genotyping data on 1,912 SNPs. The study sample genotyping success rate (including replicate samples) was 98%. After excluding samples due to insufficient DNA or inadequate DNA quality and removing genotyping failures as well as subjects with low call rates, this multigenic analysis focused on the 568 remaining Caucasians subjects.

Neutralizing Antibody Assay

We assessed measles-specific neutralizing antibody levels using a fluorescent plaque reduction neutralization assay as previously described[27]. Briefly, 4-fold serum dilutions were incubated with GFP-expressing measles virus (MV) for 1 hour before the addition to Vero cells for 43 hours. Fluorescent syncytia were imaged and counted and Karber's formula was used to determine the 50% end-point titer (Neutralizing Dose, ND₅₀). The inclusion of a WHO measles antibody standard enabled the transformation of ND₅₀ values into mIU/ml[27].

ELISPOT Assay

IFN γ cytokine response was quantified by an ELISPOT assay (R&D Systems, Minneapolis, MN) as previously described[28]. PHA (5 μ g/ml) served as a positive control, unstimulated samples contained PBMCs and culture medium only, while "stimulated" samples also contained measles virus at a multiplicity of infection (MOI) of 0.5. Plates were incubated for 42 hours and spots were developed and counted using optimized counting parameters (detection sensitivity, thresholds for minimum and maximum spot size, background hue, spot separation, removal of debris and/or overdeveloped regions) were created and maintained while counting all ELISPOT plates.

Statistical analyses

The analyses focused on attempting to identify multigenic contributions of SNPs identified from candidate immune response genes. Subject characteristics were first summarized and compared to observed measures of immunity to measles (i.e., neutralizing antibodies and IFN γ ELISPOT) as has been previously reported[14, 17]. The median and interquartile ranges of each measurement were summarized overall for each category of the descriptive variables. The observed measurements were compared across the demographic categories with analysis of variance (ANOVA) techniques. Neutralizing antibody levels were log-transformed before analysis, and differences in IFN γ ELISPOT counts between stimulated and unstimulated cells were analyzed on an inverse normal scale to meet linear models assumptions. Median values across the replicated measurements were used as the outcome variables, and all analyses were adjusted for the following set of covariates potentially

associated with measurements of response to measles vaccination: age at enrollment, gender, age at first measles vaccination, age at second measles vaccination, and cohort status.

A series of analyses was carried out to identify combinations of SNPs that might simultaneously influence measures of measles immune response. Our initial assessment was to individually examine the additional information gained from individual SNPs using a stepwise model-selection approach for the identification of groups of SNPs for each immune response measure. In this stepwise process, single ordinal SNP variables were added/removed one at a time, using p-values for entrance or removal of 0.01, until only SNPs contributing to the phenotypic association in question remained in the regression model. We applied randomization tests (repeated random re-assignment of phenotypes to SNP genotypes) to adjust for the fact that we identified associated SNPs in the same dataset in which the tests were performed[29]. These randomized data sets then provide estimates of the magnitude of effects that would be observed if no associations are present, and the observed model is then compared to this empirical distribution of results to obtain empirical p-values that are not sensitive to the model-selection approach. LD blocks were defined as described by Gabriel et al.[30]. All statistical tests were two-sided. SAS software (SAS Institute Inc., Cary, NC) was used for all analyses unless otherwise indicated.

Results

From the 2,304 SNPs selected for the study, we excluded 392 due to monomorphic expression in our population, genotyping failures, call rates < 95%, or a minor allele frequency (MAF) < 1%, leaving 1,912 SNPs for analysis. While the original cohort was 19.7% non-white, the studies described here focus on the Caucasian subset due to statistical power issues and the relatively small number of other racial groups (ranging in size from n=2 to n=89). After excluding individuals who did not meet inclusion criteria and for whom we had poor call rates (< 95%), 598 Caucasian subjects remained eligible for multigenic analysis. Our study population had a median age of 15 years old and was 56.5% male[31]. Additional demographic and immunologic information is presented in Table 1. We noticed that cohort status did affect both Ab titer and IFN γ ELISPOT results. While the two cohorts were broadly similar in terms of race, age, gender, ethnicity, they were not identical. Furthermore, the two cohorts were recruited 2 years apart and assays were conducted at different times. Any of these differences may account for the observed affect on immune outcomes. In our analyses we have adjusted for each of these covariates.

Humoral Immunity

While our univariate analyses in this study population identified many individual SNPs associated with variations in measles-specific immune outcomes[13, 15–17], these analyses may have missed interactions (e.g., one gene compensating for the loss of another gene's function) or multigenic effects (e.g., one alleles' effect only noticeable when additional genetic variants in other genes are also present). Our cohort had a median neutralizing antibody level of 858.5 IU/mL with an interquartile range of 423.0 – 1,762.0. We conducted a stepwise selection procedure that returned a list of 22 SNPs that were similarly associated with neutralizing antibody titer (p=0.046, Table 2). Of these 22 SNPs, 16 were also significantly associated with this immune outcome when examined in the univariate analysis[13, 15–17], and are shown in bold in Table 2. Interestingly, expanding the SNP association analyses to account for multigenic effects allowed us to detect additional significant associations with neutralizing antibody and SNPs in the RXRA, TLR4, IKBKE, RARB, and IL6 genes.

Cell-mediated Immunity

Our marker of cell-mediated immunity was the number of IFN γ -secreting cells (as assessed by IFN γ ELISPOT assay), rather than total cytokine secretion levels. Cytokine secretion patterns in this cohort in response to measles stimulation have been published previously[13, 15–17]. In this multigenic study we restricted our analyses to IFN γ ELISPOT results as our primary marker of cell-mediated immunity. Our cohort had a median IFN γ ELISPOT response of 36.0 spot forming units per 200,000 cells with an interquartile range of 15.0 – 71.0. A parallel set of multivariate analyses also indicated that 30 SNPs collectively contributed to variations in measles IFN γ ELISPOT outcome when considered simultaneously ($p=0.021$), Table 3. In contrast to our findings with neutralizing antibody levels, fewer than half of these 30 SNPs were also significant when analyzed individually. While several of these new SNPs were in genes containing significant univariate SNP associations, others were in genes not previously identified. Hence the multivariate analysis demonstrates that SNPs in the ACAA1, IL2RB, IL8RA, OAS2, and SLAMF1 genes also influence cellular immune responses to measles vaccine. Interestingly, two multivariate SNPs—rs4073 (5' UTR of IL8) and rs228937 (3' UTR of IL2RB)—were significantly associated with secreted IFN γ levels (rather than IFN γ ELISPOT) in the univariate analyses[13].

At the SNP level, only rs2243248 (in the IL4 gene) was present in both lists, although at the gene level several genes contributed to variations in both humoral and cellular immune outcomes. These genes included the measles viral receptor (CD46) and the vitamin A and vitamin D receptors (RARB and RXRA) as well as both cytokine and cytokine receptors (IL4, IL2RB, and IL8RA). Further examination of the LD structure of these regions indicated that for DDX58 a multivariate SNP (rs669260) and a univariate SNP (rs11795343), both associated with variations in neutralizing antibody titers, are in an LD block (Figure 1), while a number of SNPs in the IL6R gene (Figure 2) are in close LD, as are SNPs in the IL2RB gene (Figure 3).

Discussion

Candidate gene and genome-wide association studies initially focused on individual genetic variants found to be associated with the outcome(s) of interest, but are increasingly moving to a multivariate focus including haplotypes, pathways and networks. This is in recognition of the fact that many biological responses, including immunity to a pathogen or vaccine, consist of a series of tightly controlled, sequentially activated, and inter-linked response pathways – in the context of pathogen exposure or vaccination we have termed this ordered sequence of events the “immune response network” [5, 18]. According to this theory, these interactive pathways are controlled by genetic loci, and result in predictable immune outcomes. We have previously shown that many SNPs in these genes are univariately associated with variations in immune responses to measles immunization[7–9, 13–17, 22, 23]. Here we performed a SNP selection procedure that identified a number of SNPs that simultaneously affected either humoral or cellular immune responses to measles vaccine. This approach allows one to obtain additional insights into the genetic control of measles vaccine responses over and above the information gained by univariate SNP association analyses. In this study we focused on the interconnected nature of the following key areas: 1) cellular receptors for the measles virus (SLAM and CD46) that control virus binding and, consequently, initial infection[32–34]. 2) innate pathways (TLRs and intracellular signaling components) that detect pathogens and mediate the initial anti-viral responses as well as prime the more powerful and antigen-specific adaptive responses. 3) cytokines that serve to control both the adaptive immune responses that are ultimately responsible for viral neutralization and clearance. Our hypothesis was that the genetic polymorphisms present in

these key genes may be responsible, in part, for the observed variations in immune response to measles vaccine.

Viral proteins and/or nucleic acids are detected by host-pattern recognition receptors such as the TLR and RIG families, which activate various intracellular signaling cascades, and lead to the expression and function of antiviral resistance proteins. Our multigenic analysis indicates that genetic variation in the TLR4, TLR5, TLR7 and RIG1 (DDX58) genes are associated with alterations in neutralizing antibody titer while a SNP in TLR8 contributes to IFN γ ELISPOT response. Our results also indicate that genetic variation within key antiviral (PKR/EIF2AK2, MX1, VISA, and OAS2) and signaling (NFKB1A, IKBKE) pathways also contributed to genetic control of cellular and humoral immunity following measles vaccination as SNPs in these genes were included in our sets of genetic elements contributing to variations in IFN γ ELISPOT or measles neutralizing antibody responses. Polymorphisms in these genes may lead to variation in the ability of host cells to recognize viral infection, initiate antiviral resistance pathways, and prime subsequent adaptive immune responses.

Cytokines play important roles in controlling both innate and adaptive immune activity. According to our multigenic analysis, a small number of SNPs in cytokine (IFNA1, IL4, and IL6) and cytokine receptor genes (IL2RB and IL8RA) are part of the SNP set defining neutralizing antibody titers following measles vaccination. IFNA1 expression, stimulated by TLR or RIG recognition of virus, leads to the activation of downstream STAT/JAK signaling with myriad subsequent effects including the production of B cell factors BLYS and APRIL promoting B cell survival, differentiation, and Ig isotype switching[35]. IFNA1 can also synergize with IL6 to induce B cell differentiation into plasma cells promoting Ig isotype switching[35–37]. Interestingly, half of the genes identified by our multigenic analysis as important for cellular responses were cytokines or cytokine receptors, emphasizing the important role that these mediators play in cell-mediated immunity. These genes included: IL4, IL8, and IL12B, as well as IFNAR2, CSF2RB, IL2RB, IL6R, and IL8RA. Consistent with the effects of type I IFN on cellular immunity[36], we identified a number of genetic associations involved in components of IFN β secretion, a pathway that is actively down-regulated by measles virus V and C proteins, suggesting that IFN β responses play an important role in the development of measles-specific immunity[38–40].

Vitamin A regulates immune function, and its deficiency can increase susceptibility to infectious agents [41, 42], while vitamin A supplementation can improve antibody responses to measles vaccine[43–45]. We found that polymorphisms in the vitamin A receptors contributed to differential immune outcome to measles vaccine. This may be due to direct antiviral activities mediated by the binding of retinoic acid to its receptors (such as the expression of interferon response genes)[46], or through indirect actions on adaptive immune responses since signaling through retinoic acid is important in the development of B cells and the subsequent production of antibody[47], as well as in the control of both Fas induced and activation induced T cell death[48]. Similarly, vitamin D has known immunomodulatory roles[49, 50], but animal and human studies have reported conflicting results as to whether or not vitamin D has any effect on vaccine response[51–55]. Interestingly, a recent paper has shown that vitamin A inhibits the replication of MV through induction of RIG-I[56]. Our results parallel this finding by showing that polymorphisms in vitamin A receptors and in DDX58 coordinately influence immune responses to measles vaccine virus.

Our multigenic SNP analysis of associations with measles vaccination outcome identified several genetic regions of interest. Figure 1 shows the LD structure of DDX58, the gene encoding the RNA helicase RIGI. The LD block at the right of the figure includes rs669260,

an intronic SNP contributing to variations in measles-specific neutralizing antibody levels, and rs11795343, another intronic SNP where the G allele is associated with a dose-dependent increase in antibody level. Interestingly, these two SNPs share an LD block with the nonsynonymous SNP, rs10813831. Rs10813831 encodes an Arg to Cys change in the caspase recruitment domain resulting in different functional activity of RIG-I[57], raising the possibility that this functional SNP may be responsible for the associations described here. Another such region is the IL6R gene (Figure 2). Three SNPs (rs4379670, rs2229238, and rs4845371) were all significantly associated with IFN γ response in our multivariate analysis, but only one of these SNPs (rs4845371) was significant in the univariate analysis. Thus additional loci within the IL6R gene may influence IFN γ ELISPOT responses, but these influences would be missed unless combined, multivariate effects are considered. Two of these SNPs (rs2229238 and rs4379670) associated with variations in IFN γ ELISPOT outcome are located in an LD block near the 3' region of the gene. These SNPs are in close LD with rs2228145, a non-synonymous SNP, resulting in an aspartic acid to alanine substitution. This substitution is located at the proteolytic cleavage site responsible for the generation of soluble IL6R, and possession of the C allele is associated with an allele-dose dependent increase in both IL6 and sIL6R[58, 59]. Yet another region of interest is the IL2RB gene where we found 6 different SNPs implicated in control of immune responses to measles vaccine (Figure 3). Future fine-mapping of these regions followed by functional studies are may provide further insights into their role in the recognition of negative-strand RNA viruses and the development of immune responses against them.

These multigenic results reflect SNP associations that exist after controlling for the presence of other SNP effects. In several cases, univariate SNP associations lacked an ordinal trend, but, after controlling for multi-SNP effects, these same SNPs were associated with allele-dose dependent responses. This is perhaps due to the fact that the dose-related association between one SNP and an outcome may be distorted by the presence of other SNP effects when the other SNPs are in linkage disequilibrium with it. One example of this is rs2069824, where possession of the minor allele was associated with significant increases in neutralizing antibody titer in our multivariate model. Not only did our multivariate model identify some of the more prominent SNPs from single locus association studies, but they also provided evidence in favor of other SNP associations for variants that did not appear to associate strongly with the immune phenotypes when considered in isolation. One of these SNPs that was identified only after multigenic assessment was rs2069835 in IL6. This SNP displayed higher levels of humoral immunity among the heterozygotes than among either of the homozygote groups when assessed univariately. However, after accounting for other SNPs in our multigenic model, it showed a clear allele-dose response. This is likely due to the interplay of the two IL6 SNPs that were identified via the model selection procedure. Other SNPs however, lack a clear ordinal trend even after controlling for all other candidate SNPs. One possible explanation for this is that we have yet to account for all of the different SNPs and genes influencing measles-specific immune outcomes, another argument for conducting genome-wide association studies. Regardless, the multi-SNP results help to explain a greater proportion of the variability in the immune outcomes assessed.

The study results reported here have a few limitations. As with all SNP association studies, replication of our results in an independent cohort is important to correct for any false-positive associations. Additionally, our cohort provided limited power to detect significant epistatic associations. While our results support the hypothesis that measles vaccine immunity is controlled by multiple genes, our genotyping was limited to a small, select group of candidate genes. It is highly likely that there are additional elements involved in the genetic control of measles immunity, and these elements would only be identified through an expanded selection of candidate genes, or perhaps more appropriately, a genome-wide SNP association study that we are now planning. Follow up studies with larger numbers of

subjects will assist in elucidating multigenic effects, particularly those where multiple SNPs might interact and thereby compensate for one another. Additionally, a relatively limited number of multi-SNP approaches were employed. There are additional analytical approaches one can use but none of them have been widely accepted by the scientific community.

The ability to identify both single locus variants that affect immune outcome and gene-gene interactions, or even networks/pathways of interacting loci-controlling immune phenotype are increasingly common for cancer[60–62], neurologic and autoimmune disorders[63–65], and immune responses to viral vaccines including: rubella[31], smallpox[66], and now measles. Thus we are moving from single SNP association studies to multigenic analyses and eventually towards a more comprehensive, systems-level understanding of genetic influences on vaccine-induced immune responses[67]. A thorough understanding of how immunity is controlled will therefore establish a foundation for improving existing vaccines, identifying risk factors for adverse events, and developing effective next-generation vaccines that: activate appropriate innate and adaptive immune pathways leading to immunity, target CTL responses to well-defined and conserved epitopes, elicit suitable T helper (Th1/Th2/Th17) activity, stimulate robust antibody responses that do not merely bind to the pathogen but actively neutralize, opsonize, aggregate, fix complement, and/or activate antibody-dependent cell-mediated cytotoxicity, all while avoiding immunopathology. Thus, the logical next steps for these studies are: to replicate the significant findings, perform fine-mapping studies to identify likely causal variants, probe the mechanistic effects of the identified genetic variations, explore the downstream immunologic consequences of each variant, and to determine how to best use the resulting knowledge to appropriately manipulate immune responses to provide long-lasting protective immunity to measles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. de Vries RD, Stittelaar KJ, Osterhaus AD, de Swart RL. Measles vaccination: new strategies and formulations. *Expert review of vaccines*. 2008 Oct; 7(8):1215–23. [PubMed: 18844595]
2. Plemper RK, Snyder JP. Measles control--can measles virus inhibitors make a difference? *Curr Opin Investig Drugs*. 2009 Aug; 10(8):811–20.
3. Vaccines: The case of measles. *Nature*. 2011 May 26; 473(7348):434–5. [PubMed: 21614052]
4. CDC. Measles: United States, January--May 20, 2011. *Mmwr*. 2011 May 27; 60(20):666–8. [PubMed: 21617634]
5. Poland GA, Ovsyannikova IG, Jacobson RM. Vaccine immunogenetics: bedside to bench to population. *Vaccine*. 2008 Nov 18; 26(49):6183–8. [PubMed: 18598732]
6. Dhiman N, Ovsyannikova IG, Vierkant RA, Pankratz VS, Jacobson RM, Poland GA. Associations between cytokine/cytokine receptor single nucleotide polymorphisms and humoral immunity to measles, mumps and rubella in a Somali population. *Tissue antigens*. 2008 Sep; 72(3):211–20. [PubMed: 18715339]

7. Dhiman N, Ovsyannikova IG, Vierkant RA, Ryan JE, Pankratz VS, Jacobson RM, et al. Associations between SNPs in toll-like receptors and related intracellular signaling molecules and immune responses to measles vaccine: preliminary results. *Vaccine*. 2008 Mar 25; 26(14):1731–6. [PubMed: 18325643]
8. Dhiman N, Poland GA, Cunningham JM, Jacobson RM, Ovsyannikova IG, Vierkant RA, et al. Variations in measles vaccine-specific humoral immunity by polymorphisms in SLAM and CD46 measles virus receptors. *The Journal of allergy and clinical immunology*. 2007 Sep; 120(3):666–72. [PubMed: 17560639]
9. Ovsyannikova IG, Jacobson RM, Vierkant RA, Pankratz VS, Poland GA. HLA supertypes and immune responses to measles-mumps-rubella viral vaccine: findings and implications for vaccine design. *Vaccine*. 2007 Apr 20; 25(16):3090–100. [PubMed: 17280755]
10. Ovsyannikova IG, Ryan JE, Jacobson RM, Vierkant RA, Pankratz VS, Poland GA. Human leukocyte antigen and interleukin 2, 10 and 12p40 cytokine responses to measles: is there evidence of the HLA effect? *Cytokine*. 2006 Nov; 36(3–4):173–9. [PubMed: 17234427]
11. Dhiman N, Ovsyannikova IG, Cunningham JM, Vierkant RA, Kennedy RB, Pankratz VS, et al. Associations between measles vaccine immunity and single-nucleotide polymorphisms in cytokine and cytokine receptor genes. *The Journal of infectious diseases*. 2007 Jan 1; 195(1):21–9. [PubMed: 17152005]
12. Dhiman N, Ovsyannikova IG, Ryan JE, Jacobson RM, Vierkant RA, Pankratz VS, et al. Correlations among measles virus-specific antibody, lymphoproliferation and Th1/Th2 cytokine responses following measles-mumps-rubella-II (MMR-II) vaccination. *Clinical and experimental immunology*. 2005 Dec; 142(3):498–504. [PubMed: 16297162]
13. Haralambieva IH, Ovsyannikova IG, Kennedy RB, Vierkant RA, Pankratz VS, Jacobson RM, et al. Associations between single nucleotide polymorphisms and haplotypes in cytokine and cytokine receptor genes and immunity to measles vaccination. *Vaccine*. 2011 Oct 19; 29(45):7883–95. [PubMed: 21875636]
14. Haralambieva IH, Ovsyannikova IG, O'Byrne M, Pankratz VS, Jacobson RM, Poland GA. A large observational study to concurrently assess persistence of measles specific B-cell and T-cell immunity in individuals following two doses of MMR vaccine. *Vaccine*. 2011 Jun 15; 29(27):4485–91. [PubMed: 21539880]
15. Haralambieva IH, Ovsyannikova IG, Umlauf BJ, Vierkant RA, Shane Pankratz V, Jacobson RM, et al. Genetic polymorphisms in host antiviral genes: Associations with humoral and cellular immunity to measles vaccine. *Vaccine*. 2011 Nov 8; 29(48):8988–97. [PubMed: 21939710]
16. Ovsyannikova IG, Haralambieva IH, Vierkant RA, O'Byrne MM, Jacobson RM, Poland GA. Effects of vitamin A and D receptor gene polymorphisms/haplotypes on immune responses to measles vaccine. *Pharmacogenetics and genomics*. 2011
17. Ovsyannikova IG, Haralambieva IH, Vierkant RA, Pankratz VS, Jacobson RM, Poland GA. The role of polymorphisms in Toll-like receptors and their associated intracellular signaling genes in measles vaccine immunity. *Human genetics*. 2011 Mar 19.
18. Poland GA, Ovsyannikova IG, Jacobson RM, Smith DI. Heterogeneity in vaccine immune response: the role of immunogenetics and the emerging field of vaccinomics. *Clinical pharmacology and therapeutics*. 2007 Dec; 82(6):653–64. [PubMed: 17971814]
19. Nelson MR, Kardia SL, Ferrell RE, Sing CF. A combinatorial partitioning method to identify multilocus genotypic partitions that predict quantitative trait variation. *Genome research*. 2001 Mar; 11(3):458–70. [PubMed: 11230170]
20. Thornton-Wells TA, Moore JH, Haines JL. Genetics, statistics and human disease: analytical retooling for complexity. *Trends Genet*. 2004 Dec; 20(12):640–7. [PubMed: 15522460]
21. Dhiman N, Haralambieva IH, Kennedy RB, Vierkant RA, O'Byrne MM, Ovsyannikova IG, et al. SNP/haplotype associations in cytokine and cytokine receptor genes and immunity to rubella vaccine. *Immunogenetics*. 2010 Apr; 62(4):197–210. [PubMed: 20217072]
22. Ovsyannikova IG, Jacobson RM, Ryan JE, Vierkant RA, Pankratz VS, Jacobsen SJ, et al. HLA class II alleles and measles virus-specific cytokine immune response following two doses of measles vaccine. *Immunogenetics*. 2005 Feb; 56(11):798–807. [PubMed: 15712014]

23. Ovsyannikova IG, Ryan JE, Vierkant RA, Pankratz VS, Jacobson RM, Poland GA. Immunologic significance of HLA class I genes in measles virus-specific IFN-gamma and IL-4 cytokine immune responses. *Immunogenetics*. 2005 Dec; 57(11):828–36. [PubMed: 16331510]
24. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *American journal of human genetics*. 2004 Jan; 74(1):106–20. [PubMed: 14681826]
25. Sicotte H, Rider DN, Poland GA, Dhiman N, Kocher JP. SNPPicker: High quality tag SNP selection across multiple populations. *BMC bioinformatics*. 2011; 12:129. [PubMed: 21535878]
26. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Human mutation*. 2000; 15(1):7–12. [PubMed: 10612815]
27. Haralambieva IH, Ovsyannikova IG, Vierkant RA, Poland GA. Development of a novel efficient fluorescence-based plaque reduction microneutralization assay for measles virus immunity. *Clin Vaccine Immunol*. 2008 Jul; 15(7):1054–9. [PubMed: 18463223]
28. Ryan JE, Ovsyannikova IG, Dhiman N, Pinsky NA, Vierkant RA, Jacobson RM, et al. Inter-operator variation in ELISPOT analysis of measles virus-specific IFN-gamma-secreting T cells. *Scandinavian journal of clinical and laboratory investigation*. 2005; 65(8):681–9. [PubMed: 16319042]
29. Manly, B. *Randomization, Bootstrap and Monte Carlo Methods in Biology*. 2. Boca Raton, FL: Chapman & Hall/CRC; 1997.
30. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. *Science (New York, NY)*. 2002 Jun 21; 296(5576):2225–9.
31. Pankratz VS, Vierkant RA, O'Byrne MM, Ovsyannikova IG, Poland GA. Associations between SNPs in candidate immune-relevant genes and rubella antibody levels: a multigenic assessment. *BMC immunology*. 2010; 11(1):48. [PubMed: 20923569]
32. Dorig RE, Marciel A, Chopra A, Richardson CD. The human CD46 molecule is a receptor for measles virus (Edmonston strain). *Cell*. 1993 Oct 22; 75(2):295–305. [PubMed: 8402913]
33. Naniche D, Varior-Krishnan G, Cervoni F, Wild TF, Rossi B, Rabourdin-Combe C, et al. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *Journal of virology*. 1993 Oct; 67(10):6025–32. [PubMed: 8371352]
34. Tatsuo H, Ono N, Tanaka K, Yanagi Y. SLAM (CDw150) is a cellular receptor for measles virus. *Nature*. 2000 Aug 24; 406(6798):893–7. [PubMed: 10972291]
35. Genin P, Vaccaro A, Civas A. The role of differential expression of human interferon- α genes in antiviral immunity. *Cytokine & growth factor reviews*. 2009 Aug; 20(4):283–95. [PubMed: 19651532]
36. Theofilopoulos AN, Baccala R, Beutler B, Kono DH. Type I interferons (alpha/beta) in immunity and autoimmunity. *Annual review of immunology*. 2005; 23:307–36.
37. Jegou G, Palucka AK, Blanck JP, Chalouni C, Pascual V, Banchereau J. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity*. 2003 Aug; 19(2):225–34. [PubMed: 12932356]
38. Devaux P, Hodge G, McChesney MB, Cattaneo R. Attenuation of V- or C-defective measles viruses: infection control by the inflammatory and interferon responses of rhesus monkeys. *Journal of virology*. 2008 Jun; 82(11):5359–67. [PubMed: 18385234]
39. McAllister CS, Toth AM, Zhang P, Devaux P, Cattaneo R, Samuel CE. Mechanisms of protein kinase PKR-mediated amplification of beta interferon induction by C protein-deficient measles virus. *Journal of virology*. 2010 Jan; 84(1):380–6. [PubMed: 19846517]
40. Takaki H, Watanabe Y, Shingai M, Oshiumi H, Matsumoto M, Seya T. Strain-to-strain difference of V protein of measles virus affects MDA5-mediated IFN-beta-inducing potential. *Molecular immunology*. 2011 Jan; 48(4):497–504. [PubMed: 21071089]
41. Green HN, Mellanby E. Vitamin a as an Anti-Infective Agent. *British medical journal*. 1928 Oct 20; 2(3537):691–6. [PubMed: 20774205]
42. Glasziou PP, Mackerras DE. Vitamin A supplementation in infectious diseases: a meta-analysis. *BMJ (Clinical research ed)*. 1993 Feb 6; 306(6874):366–70.

43. Coutoudis A, Kiepiela P, Coovadia HM, Broughton M. Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles. *The Pediatric infectious disease journal*. 1992 Mar; 11(3):203–9. [PubMed: 1565535]
44. Benn CS, Aaby P, Bale C, Olsen J, Michaelsen KF, George E, et al. Randomised trial of effect of vitamin A supplementation on antibody response to measles vaccine in Guinea-Bissau, west Africa. *Lancet*. 1997 Jul 12; 350(9071):101–5. [PubMed: 9228962]
45. Benn CS, Balde A, George E, Kidd M, Whittle H, Lisse IM, et al. Effect of vitamin A supplementation on measles-specific antibody levels in Guinea-Bissau. *Lancet*. 2002 Apr 13; 359(9314):1313–4. [PubMed: 11965280]
46. Pelicano L, Li F, Schindler C, Chelbi-Alix MK. Retinoic acid enhances the expression of interferon-induced proteins: evidence for multiple mechanisms of action. *Oncogene*. 1997 Nov 6; 15(19):2349–59. [PubMed: 9393879]
47. Ross AC, Chen Q, Ma Y. Vitamin a and retinoic Acid in the regulation of B-cell development and antibody production. *Vitamins and hormones*. 2011; 86:103–26. [PubMed: 21419269]
48. Engedal N. Immune regulator vitamin a and T cell death. *Vitamins and hormones*. 2011; 86:153–78. [PubMed: 21419271]
49. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nature reviews*. 2008 Sep; 8(9):685–98.
50. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. *The American journal of clinical nutrition*. 2004 Dec; 80(6 Suppl):1717S–20S. [PubMed: 15585793]
51. Kriesel JD, Spruance J. Calcitriol (1,25-dihydroxy-vitamin D3) coadministered with influenza vaccine does not enhance humoral immunity in human volunteers. *Vaccine*. 1999 Apr 9; 17(15–16):1883–8. [PubMed: 10217585]
52. Enioutina EY, Visic D, McGee ZA, Daynes RA. The induction of systemic and mucosal immune responses following the subcutaneous immunization of mature adult mice: characterization of the antibodies in mucosal secretions of animals immunized with antigen formulations containing a vitamin D3 adjuvant. *Vaccine*. 1999 Aug 6; 17(23–24):3050–64. [PubMed: 10462240]
53. Moe SM, Zekonis M, Harezlak J, Ambrosius WT, Gassensmith CM, Murphy CL, et al. A placebo-controlled trial to evaluate immunomodulatory effects of paricalcitol. *Am J Kidney Dis*. 2001 Oct; 38(4):792–802. [PubMed: 11576883]
54. Van Der Stede Y, Verfaillie T, Cox E, Verdonck F, Goddeeris BM. 1alpha,25-dihydroxyvitamin D3 increases IgA serum antibody responses and IgA antibody-secreting cell numbers in the Peyer's patches of pigs after intramuscular immunization. *Clinical and experimental immunology*. 2004 Mar; 135(3):380–90. [PubMed: 15008969]
55. Ivanov AP, Dragunsky EM, Chumakov KM. 1,25-dihydroxyvitamin d3 enhances systemic and mucosal immune responses to inactivated poliovirus vaccine in mice. *The Journal of infectious diseases*. 2006 Feb 15; 193(4):598–600. [PubMed: 16425140]
56. Soye KJ, Trottier C, Richardson CD, Ward BJ, Miller WH Jr. RIG-I Is Required for the Inhibition of Measles Virus by Retinoids. *PloS one*. 2011; 6(7):e22323. [PubMed: 21811588]
57. Hu J, Nistal-Villan E, Voho A, Ganee A, Kumar M, Ding Y, et al. A common polymorphism in the caspase recruitment domain of RIG-I modifies the innate immune response of human dendritic cells. *J Immunol*. 2010 Jul 1; 185(1):424–32. [PubMed: 20511549]
58. Reich D, Patterson N, Ramesh V, De Jager PL, McDonald GJ, Tandon A, et al. Admixture mapping of an allele affecting interleukin 6 soluble receptor and interleukin 6 levels. *American journal of human genetics*. 2007 Apr; 80(4):716–26. [PubMed: 17357077]
59. Rodriguez-Rodriguez L, Lamas JR, Varade J, Lopez-Romero P, Tornero-Esteban P, Abasolo L, et al. Plasma soluble IL-6 receptor concentration in rheumatoid arthritis: associations with the rs192284 IL6R polymorphism and with disease activity. *Rheumatology international*. 2011 Mar; 31(3):409–13. [PubMed: 20661738]
60. Wu X, Gu J, Grossman HB, Amos CI, Etzel C, Huang M, et al. Bladder cancer predisposition: a multigenic approach to DNA-repair and cell-cycle-control genes. *American journal of human genetics*. 2006 Mar; 78(3):464–79. [PubMed: 16465622]

61. Delort L, Satih S, Kwiatkowski F, Bignon YJ, Bernard-Gallon DJ. Evaluation of breast cancer risk in a multigenic model including low penetrance genes involved in xenobiotic and estrogen metabolisms. *Nutrition and cancer*. 2010; 62(2):243–51. [PubMed: 20099199]
62. Beuten J, Gelfond JA, Franke JL, Weldon KS, Crandall AC, Johnson-Pais TL, et al. Single and multigenic analysis of the association between variants in 12 steroid hormone metabolism genes and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2009 Jun; 18(6):1869–80. [PubMed: 19505920]
63. Sun X, Jia Y, Zhang X, Xu Q, Shen Y, Li Y. Multi-locus association study of schizophrenia susceptibility genes with a posterior probability method. *Science in China*. 2005 Jun; 48(3):263–9. [PubMed: 16092759]
64. Ritchie MD, Bartlett J, Bush WS, Edwards TL, Motsinger AA, Torstenson ES. Exploring epistasis in candidate genes for rheumatoid arthritis. *BMC proceedings*. 2007; 1(Suppl 1):S70. [PubMed: 18466572]
65. Bell JT, Timpson NJ, Rayner NW, Zeggini E, Frayling TM, Hattersley AT, et al. Genome-wide association scan allowing for epistasis in type 2 diabetes. *Annals of human genetics*. 2011 Jan; 75(1):10–9. [PubMed: 21133856]
66. McKinney BA, Crowe JE, Guo J, Tian D. Capturing the spectrum of interaction effects in genetic association studies by simulated evaporative cooling network analysis. *PLoS genetics*. 2009 Mar. 5(3):e1000432. [PubMed: 19300503]
67. Oberg AL, Kennedy RB, Li P, Ovsyannikova IG, Poland GA. Systems biology approaches to new vaccine development. *Current opinion in immunology*. 2011 Jun; 23(3):436–43. [PubMed: 21570272]

Highlights

- 2008 saw the largest number of measles cases in Europe and U.S. in a decade
- Measles declared endemic in the UK
- Measles infection and vaccine response are complex biological processes
- Immunity to vaccine consists of a series of immune-response pathways

Figure 1. Gene view and linkage disequilibrium plot of DDX58

The top bar shows the intron/exon structure of the DDX58 gene, with exons being represented by the boxes. The chromosomal range covered by the LD plot is shown by the horizontal bars bordering the gene (Chromosome 9: 32,453,119 – 32,526,233). The triangle plot below the gene shows LD (darker shading = higher LD) and the number in each box represents the R^2 value. At the bottom right of the figure is an expanded view of a large LD block of interest. SNPs identified in the multigenic analysis (rs669260) and in our univariate analysis (rs11795343) are highlighted in boxes.

Figure 2. Gene view and linkage disequilibrium plot of IL6R
The top bar shows the intron/exon structure of the IL6R gene, with exons being represented by the boxes. The chromosomal range covered by the LD plot is shown by the horizontal bars bordering the gene (Chromosome 1: 154,381,103 – 154,444,209). The triangle plot below the gene shows LD (darker shading = higher LD) and the number in each box represents the R^2 value. SNPs identified in the multigenic analysis are highlighted in boxes.

Figure 3. Gene view and linkage disequilibrium plot of IL2RB

The top bar shows the intron/exon structure of the IL2RB gene, with exons being represented by the boxes. The chromosomal range covered by the LD plot is shown by the horizontal bars bordering the gene (Chromosome 22: 37,520,895 – 37,544,602). The triangle plot below the gene shows LD (darker shading = higher LD) and the number in each box represents the R^2 value. SNPs identified in the multigenic analysis are highlighted in boxes.

Table 1
Characteristics of the study population (n=563) and their associations with measles-specific immune outcomes (reported in IU/ml)
Multivariate model results.

Parameter	Level	IFN γ ELISPOT				Neutralizing Antibody					
		N	Median	Q1	Q3	p-value	N	Median	Q1	Q3	p-value
Overall		563	36.0	15.0	71.0		598	858.5	423.0	1762.0	
Age at enrollment (years)	11–12	115	34.0	16.0	78.0	0.238	121	981.0	491.0	1841.0	0.424
	13–14	141	33.0	12.0	56.0		150	863.5	487.0	1752.0	
	15–16	158	36.0	15.0	67.0		170	776.0	363.0	1695.0	
	17–22	149	39.0	15.0	75.0		157	872.0	438.0	1806.0	
Age at first measles vaccine (months)	15 months	331	38.0	16.0	75.0	0.297	351	866.8	440.0	1727.0	0.324
	16+ months	130	37.0	15.0	61.0		138	950.1	432.0	1841.0	
	<=14 months	102	30.0	10.0	69.0		109	736.6	386.0	1684.8	
	12+	104	43.0	19.5	82.0	0.051	112	783.5	427.5	1891.0	0.362
Age at second measles vaccine (years)	5	162	31.5	15.0	58.0		172	1003.5	515.1	1770.7	
	6–11	154	40.0	17.0	78.0		160	789.0	400.7	1611.0	
	<= 4	143	30.0	9.0	70.0		154	793.0	460.0	1841.0	
	Female	245	36.0	12.0	70.0	0.822	265	872.0	440.0	1718.0	0.982
Gender	Male	318	37.0	16.0	71.0		333	807.0	418.0	1762.0	
	New	254	26.5	9.0	51.0	<.001	268	1006.0	507.8	1885.2	0.004
New cohort	Old	309	46.0	22.0	83.0		330	764.5	391.0	1622.0	

Notes:

^a Q1 and Q3 represent the first (25th percentile) and third (75th percentile) quartiles, respectively.

^b p-value represents a test of the null hypothesis that there are no differences in the level of measles-specific antibody IgG levels among the categories represented.

Table 2
SNPs identified as being associated with variations in neutralizing antibody upon multigenic assessment

Multigenic SNP associations with IFN γ ELISPOT response

SNP ID	Gene Symbol	Location ^a	MAF ^b	0 MAs Median (IQR) ^c	1 MA Median (IQR) ^d	2 MAs Median (IQR) ^e	p-value, ordinal effect ^f	multigenic p-value
rs2724384	CD46	intron	23.5	996.4(464.5,1942.8)	782(401,1638)	533(274,706)	0.00084	0.0006
rs669260	DDX58	intron	15.3	809.5(393,1726)	896(528,1806)	1699.5(698,2096)	0.02521	0.0015
rs28383797	IFNA1	3UTR	24.0	811(423,1710.7)	997(441,1995.3)	1308.3(716.8,1806)	0.00779	<.0001
rs12409804	IKBKE	intron	21.3	834(436,1718)	869(392,1827)	867(497,1990.2)	0.71733	0.0032
rs1539241	IKBKE	intron	15.3	872.1(472.5,1772)	816(423,1752)	255.5(199.7,651)	0.03119	<.0001
rs3218266	IL2RB	intron	32.6	1019.5(456,1969.3)	799.5(432,1724.5)	583.5(297.5,1224.5)	0.00288	0.0002
rs2243248	IL4	5UTR	7.4	897(440,1798)	656(330.5,1561)	--	0.00253	<.0001
rs2069824	IL6	5UTR	5.4	803(409,1715)	1185(654,2558)	1440(872,2008)	0.01159	<.0001
rs2069835	IL6	intron	5.1	836(432,1747)	995.8(387,2139)	872(370,2008)	0.62872	0.0003
rs2854386	IL8RA	3UTR	6.0	826(418,1723)	1141(545.1,2119)	759(759,759)	0.02382	0.0044
rs791062	MAP3K7	intron	34.7	969.2(440,1990.2)	860(446,1718)	690(350,1333.8)	0.01242	0.0003
rs6800566	RARB	intron	32.3	702(386,1650)	996.5(458.5,1801)	1265(514.8,2154)	0.00113	0.0012
rs1286763	RARB	intron	47.0	995.8(474,1842)	802(401,1736.5)	836(409,1646.4)	0.26455	0.0011
rs3118523	RXRA	3UTR	20.2	891(446,1887.3)	795(396.2,1500.5)	531.5(355,1505.1)	0.03324	<.0001
rs4842196	RXRA	3UTR	26.0	844(432,1850)	904(438,1652)	629.5(394.9,1893.3)	0.48021	0.0003
rs1805343	RXRA	intron	35.6	868(489,1894.2)	872(402,1706.4)	772(386,1692.5)	0.10572	<.0001
rs1816702	TLR2	intron	13.2	782(401,1650)	1159(543,2096)	1550.2(708,2184)	0.00124	0.0001
rs11536897	TLR4	3UTR	5.4	880(434,1798)	646.3(365,1212)	2008(698,2563)	0.05079	0.0019
rs851178	TLR5	intron	41.9	997(528,1772.4)	796(401.4,1806)	721(361,1513)	0.01011	0.001
rs864058	TLR7	coding	7.9	826(423,1706.4)	1168(348,2206)	1589.3(610,2579)	0.03354	0.0017
rs7122620	TRIM5	3UTR	45.7	874(396.5,1608.2)	776(423,1750)	1163.4(514.8,2383)	0.01263	0.0049
rs6037678	VISA	intron	3.3	803.5(409,1718)	1418.6(880,2144)	--	0.00256	0.0016

^a Location of SNP relative to gene.

^b MAF = minor allele frequency.

^c Neutralizing antibody results expressed as the reciprocal of the serum dilution neutralizing 50% of viral activity for individuals not possessing any copies of the minor allele. IQR = interquartile range.

^d Neutralizing antibody results expressed as the reciprocal of the serum dilution neutralizing 50% of viral activity for individuals possessing a single copy of the minor allele.

Neutralizing antibody results expressed as the reciprocal of the serum dilution neutralizing 50% of viral activity for individuals possessing a two copies of the minor allele.

“-” indicates that there were no subjects with that genotype.

f p-value for the univariate genotypic trend test for each SNP.

Table 3
SNPs identified as being associated with IFN γ ELISPOT response upon multigenic assessment

Multigenic SNP associations with Neutralizing Antibody response.

SNP ID	Gene Symbol	Location ^a	MAF ^b	0 MAs Median (IQR) ^c	1 MA Median (IQR) ^d	2 MAs Median (IQR) ^e	p-value ordinal effect ^f	multigenic p-value
rs7625290	ACAA1	intron	4.3	37(15,71)	29,5(8,66)	--	0.10998	0.0029
rs7545126	CD46	intron	13.0	38(17,78)	27(8,5,52)	23(6,44)	0.00081	<.0001
rs131840	CSF2RB	coding	20.2	34,5(12,67)	41(16,78)	50(22,83)	0.02672	0.002
rs3770768	EIF2AK2	intron	12.1	33(12,5,66)	40(20,77)	83(57,94)	0.00239	<.0001
rs4648186	EIF2AK2	intron	13.9	35(14,69)	37(14,69)	83(57,94)	0.55787	<.0001
rs17860130	IFNAR2	intron	1.8	36(14,69)	56(24,93)	103(103,103)	0.00534	0.0001
rs6870828	IL12B	3'UTR	46.2	32(13,81)	39,5(17,66,5)	30,5(12,69,5)	0.57083	0.0037
rs2546893	IL12B	intron	49.4	40,5(17,78)	38(14,74)	28(9,5,56)	0.00383	<.0001
rs3218316	IL2RB	intron	10.4	35(14,69)	41(15,75)	72(26,78)	0.2087	<.0001
rs3218322	IL2RB	intron	33.0	37(17,73)	36,5(12,69)	31(8,5,58)	0.38332	<.0001
rs228937	IL2RB	3'UTR	21.7	36(12,66)	40(18,75)	43(8,81)	0.11568	0.0007
rs228960	IL2RB	intron	42.1	32(10,69)	37(16,69)	38(18,75)	0.6126	0.0026
rs228965	IL2RB	intron	42.4	33(11,69)	37(16,68)	38(17,75)	0.77077	0.0003
rs2243248	IL4	5'UTR	7.4	34(13,69)	42,5(24,87)	--	0.01697	0.0002
rs4379670	IL6R	3'UTR	22.4	36(12,73)	37(16,71)	36,5(14,51,5)	0.61356	0.0006
rs4845371	IL6R	intron	42.7	32(11,67)	36(15,68)	46(21,5,84,5)	0.00967	0.0004
rs2229238	IL6R	3'UTR	22.7	35,5(12,5,69,5)	37(16,72)	36,5(14,51,5)	0.83412	0.0016
rs4073	IL8	5'UTR	44.4	40(17,69)	38(15,76,5)	23,5(7,54,5)	0.01285	0.0033
rs16858784	IL8RA	3'UTR	4.4	37(14,72)	30,5(15,58)	21(15,27)	0.56184	0.0043
rs7280422	MX1	intron	19.8	39(16,74,5)	31,5(11,56)	16(7,85)	0.03456	<.0001
rs11569611	NFKB1A	5'UTR	3.8	37(15,71)	22(7,67)	--	0.01161	<.0001
rs2233407	NFKB1A	5'UTR	4.8	36(15,69)	35(11,5,81)	--	0.60811	0.0003

^a Location of SNP relative to gene.

^b MAF = minor allele frequency.

^c IFN γ ELISPOT results expressed as spot forming units per 200,000 cells for individuals possessing a no copies of the minor allele. IQR = interquartile range.

^d IFN γ ELISPOT results expressed as spot forming units per 200,000 cells for individuals possessing a single copy of the minor allele.

^e IFN γ ELISPOT results expressed as spot forming units per 200,000 cells for individuals possessing a two copies of the minor allele.

“..” indicates that there were no subjects with that genotype.

^f p-value for the univariate genotypic trend test for each SNP, all SNPs had p-values less than 0.01 when simultaneously included in the multi-SNP model.