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Associations between Single Nucleotide Polymorphisms and Haplotypes in Cytokine and Cytokine Receptor Genes and Immunity to Measles Vaccination

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Abstract

Identification of host genetic determinants of measles vaccine-induced immunity can be used to design better vaccines and ultimately predict immune responses to vaccination. We performed a comprehensive candidate gene association study across 801 genetic markers in 56 cytokine/cytokine receptor genes, in a racially diverse cohort of 745 schoolchildren after two doses of MMR vaccine. Using linear regression methodologies we examined associations between SNPs/haplotypes and measles virus-specific immunity.

Forty-eight significant SNP associations with variations in neutralizing antibodies and measles-specific IFN γ Elispot responses were identified ($p < 0.05$). Our study replicated an important previously found association of a functional *IL12B* genetic variant rs3212227 with variations in measles-specific humoral immunity ($p = 0.037$). Similarly, two previously reported promoter *IL10* and *IL2* polymorphisms (rs1800890 and rs2069762) demonstrated associations with measles-specific cellular immunity in Caucasians ($p \leq 0.034$). Multiple *IL7R* polymorphisms, including a non-synonymous functional SNP (rs6897932/Thr244Ile), were associated with humoral ($p \leq 0.024$) and/or cellular (IFN γ Elispot, $p \leq 0.023$) measles-specific immune responses in Caucasians, but not African-Americans. Haplotype level analysis confirmed the association of *IL7R* genetic variants with measles vaccine-induced immunity in the Caucasian group (global p -value = 0.003). Our results validate previous findings and identify new plausible genetic determinants, including *IL7R* polymorphisms, regulating measles vaccine-induced immunity in a race-specific manner.

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Disclosures

Dr. Poland is the chair of a DMSB for novel vaccines undergoing clinical study by Merck Research Laboratories. Dr. Jacobson recently served on a Safety Review Committee for a post-licensure study conducted by Kaiser-Permanente concerning Gardasil HPV vaccine funded by Merck & Co. Other authors declare that they have no conflict of interest.

Keywords

Measles vaccine; Immunity; Single Nucleotide Polymorphisms; Haplotypes; Cytokine; Cytokine receptor

1. Introduction

Recent advances in the field of immunogenetics have partly revealed the genetic basis of differential susceptibility to infectious diseases and inter-individual differences in vaccine response [1]. Studies (including ours) consistently implicate genetic polymorphisms in key immune response genes, such as cytokine and cytokine receptor genes as important determinants of adverse events and observed variations in immune responses to vaccines such as measles-mumps-rubella (MMR), influenza, smallpox, hepatitis A and B vaccines, pneumococcal conjugate vaccine and diphtheria-tetanus-pertussis vaccine [2-12]. Furthermore, racial, ethnic and gender differences have been associated with differences in immune responses to immunization and infection [2,3,13-18]. Previous studies have also demonstrated race-specific distributions of allelic variants in cytokine genes across populations [19,20]. Although much information has been accumulated in the field of measles immunity, the discovery and validation of genetic drivers of measles-specific immune responses still remains a challenge.

In the current study we performed a high-density, comprehensive, candidate gene genetic study to identify, replicate, and validate SNP associations across 56 key cytokine and cytokine receptor genes with measures of both humoral and cellular immunity to measles vaccine in a large racially diverse cohort of healthy schoolchildren and young adults after two doses of MMR vaccine.

2. Materials and Methods

2.1. Study subjects

The present study was based on a combined sample of 764 subjects from 2 independent age-stratified cohorts of randomly selected healthy schoolchildren and young adults from Olmsted County, MN: cohort 1 consisted of 388 subjects eligible for the current study (age 11-19 years, enrolled in 2006-2007 year) as previously reported [4], and cohort 2 consisted of 376 eligible subjects (age 11-22 years, enrolled in 2008-2009 year). Inclusion criteria required each participant to have documentation of receipt of two doses of measles-mumps-rubella vaccine (MMR). The race/ethnicity reported in the study were self-reported. The Institutional Review Board of the Mayo Clinic approved the study, and written, informed consent was obtained from subjects' parents/guardians as well as written assent from age-appropriate subjects at the time of enrollment.

2.2. Immune measures

Cytokine assays—The frequencies of measles-specific IFN γ -producing cells were quantified using commercially available IFN γ Elispot kits (R&D Systems, Minneapolis, MN) as previously described [21]. Seven secreted Th1, Th2, and innate/inflammatory cytokines were measured with commercial ELISA kits in PBMC cultures following in vitro virus stimulation using a response surface methodology approach to pre-optimize conditions for MOI and incubation time for each specific cytokine, as previously described [4]. We stimulated cell cultures with live measles virus using the following pre-optimized conditions for MOIs and incubation times: IFN α and TNF α MOI=1.0, 24 hours; IL-2 and IL-10 MOI=0.5, 48 hours; IL-6, IFN γ and IFN λ 1 MOI=1.0, 72 hours.

Plaque Reduction Microneutralization Assay (PRMN)—Measles-specific neutralizing antibodies were quantified using a high throughput fluorescence-based PRMN, as previously described [22,23] with the following modifications in the readout. The GFP-positive syncytia were scanned and counted on an automated Olympus IX71 Fluorescent microscope using the Image-Pro Plus Software Version 6.3 (MediaCybernetics). Neutralizing dose (ND₅₀) values, calculated using Karber's formula, were transformed into mIU/mL values using the 3rd WHO international anti-measles antibody standard as previously described [22,23].

2.3. SNP selection and genotyping

We selected tagSNPs within and 5kb upstream and downstream of 56 candidate genes, based on the approach of Carlson [4,24] on data from the source with the greatest number of SNPs from among the Hapmap Phase II (<http://www.hapmap.org>), Seattle SNPs (<http://pga.mbt.washington.edu/>), NIEHS SNPs (<http://egp.gs.washington.edu/>), and NCBI (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), as previously described [25], with SNP minor allele frequencies (MAF) ≥ 0.05 , and a linkage disequilibrium (LD) threshold of $r^2 \geq 0.90$. A total of 994 SNPs were selected based on this approach. The selected SNPs were genotyped as part of our population-based measles vaccine study using two custom Illumina GoldenGate SNP panels (Illumina Inc., San Diego, CA) for 1,536 and 768 SNPs, following the manufacturer's protocol, and as previously described [25].

2.6. Statistical methods

The statistical methods described herein are similar to those published for our previous genetic association studies [4,5,25]. Data were descriptively summarized using frequencies and percentages for categorical variables, and medians and inter-quartile ranges (IQR) for continuous variables. Assessments of cytokine secretion and IFN γ Elispot responses resulted in multiple recorded values per outcome both prior to and after stimulation with measles virus. For descriptive purposes, a single response measurement per individual was obtained by subtracting the median of the unstimulated values from the median of the stimulated values. Assessments of antibody levels resulted in only one recorded value per individual. Observed genotypes were used to estimate allele frequencies for each SNP and departures from Hardy-Weinberg equilibrium (HWE) were assessed using either a Pearson goodness-of-fit test or, for SNPs with a minor allele frequency (MAF) of less than 5%, a Fisher exact test [26]. Estimates of pair-wise linkage disequilibrium (LD) based on the r-squared statistic and D' statistic were obtained using Haploview software, version 3.32 [27].

SNP associations with immune response outcomes were evaluated using regression models. Simple linear regression was used for measles antibody levels. Repeated measures approaches were implemented for the cytokine secretion and IFN γ Elispot variables, simultaneously modeling the multiple observed measurements and using an unstructured within-person variance-covariance matrix to account for within-subject correlations. This was achieved by including the genotype variable in the regression model, together with a variable representing stimulation status. The resulting genotype-by-stimulation status interaction was then tested for statistical significance. Tests of association assumed an ordinal (log-additive) SNP effect using simple tests for trend.

To further explore genomic regions containing statistically significant single-SNP effects, we performed post-hoc haplotype analyses. Posterior probabilities of all possible haplotypes for an individual, conditional on the observed genotypes, were estimated using an expectation-maximization (EM) algorithm [28]. This information was used to define haplotype design variables that estimated the number of each of the haplotypes carried by an individual. Analyses were performed on all resulting common haplotypes (those with an

estimated frequency of greater than 1%) using simple least squares linear regression approach for antibody levels and repeated measures approaches for the cellular immune response (CMI) variables. Differences in immune response among common haplotypes were first assessed globally and simultaneously tested for statistical significance using a multiple degree-of-freedom test. Following these global tests, we examined individual haplotype effects. Each haplotype was included in a separate regression analysis, effectively comparing immune response levels for the haplotype of interest against all others combined. Due to phase ambiguity, haplotype-specific medians and inter-quartile ranges could not be calculated. Thus, descriptive summaries were represented using the corresponding t-statistics.

All of the association analyses adjusted for age at enrollment, race (in the combined analysis), gender, age at first and second measles vaccination, and cohort status (cohort 1 versus cohort 2). We used an inverse normal transformation for all cytokine secretion and CMI outcome variables, and a log transformation for the antibody response measure, in all formal tests to account for the skewed nature of the data. All statistical tests were two-sided and, unless otherwise indicated, all analyses were carried out using the SAS software system (SAS Institute, Inc., Cary, NC).

3. Results

3.1 Subjects demographics and immune responses

All demographic and clinical variables of the study cohort, and the immune response measures, are summarized in Table 1. Briefly, our study population had a median age of 15 years at the time of sampling with a median time from second MMR immunization to enrollment of 7.4 years. The cohort consisted of 417 males (55.97%), 598 Caucasians (80.27%), but included also 89 African-Americans (11.95%) and a limited number of other races/ethnicities.

3.2 Genotyping of SNPs in cytokine and cytokine receptor genes

Genotyping was performed on 764 subjects for 994 known SNPs in the cytokine: IL2, IFNG, IL12A, IL12B, IL4, IL5, IL10, IL1A, IL1B, IL6, IL8, IL18, TNFA, CSF2, IFNB1, IL28A/IFN λ 2, *IL28B*, *IL29/IFN λ 1*, and 13 *IFNA*, and cytokine receptor genes: IL2RA, IL2RB, IL2RG, IFNGR1, IFNGR2, IL12RB1, IL12RB2, IL4R, IL10RA, IL10RB, IL6R, IL6ST, IL7R, IL8RA, IL18R1, IL1R1, IL1R2, IL1RN, CSF2RA, CSF2RB, IFNAR1, IFNAR2, IL28RA, TNFRSF1A, TNFRSF1B. Our overall genotyping sample success rate was 98.75% (including replicate samples), the locus success rate was 94.55%, and reproducibility was 100%. Nineteen DNA samples failed because of low call rates (<95%), leaving 745 subjects for final analysis. In addition, fifty-five SNPs failed the genotyping, one hundred ten SNPs were excluded based on MAF<1%, one SNP was excluded based on having a low call rate (<0.95%), and twenty-seven SNPs were excluded based on being monomorphic. A total of 801 SNPs were used for the final analysis in a set of 745 subjects (including 598 Caucasians and 89 African-Americans).

3.3 Genetic associations

Associations between SNPs in cytokine and cytokine receptor genes and humoral immune responses after measles vaccination—Overall we found 19 significant associations between SNPs within the coding or regulatory gene regions and variations (29 to 72% increase/decrease) in measles-specific neutralizing antibody levels ($p<0.05$) (Table 2). Of note, in this study we were able to replicate the association of SNP rs3212227 (in LD with rs6859018, D' statistic, a pairwise measure of LD=0.98) located in the 3'UTR region of the *IL12B* gene with variations in measles-specific antibody levels

($p=0.037$) [5]. Other interesting findings include three *IL4* SNPs (including two promoter SNPs rs2243248 and rs2243247) associated with significant immune outcome variations (53 to 72% increase/decrease in antibody responses, $0.0064 \leq p \leq 0.0358$) (Table 2). The minor alleles of one coding SNP (rs6897932/Thr244Ile) and one promoter SNP (rs6890853) within the *IL7R* gene (in LD, $D'=0.99$), were associated with up to a 34% decrease in measles-specific antibody levels in an allele dose-related manner.

Race-specific analysis in the Caucasian subjects ($n=598$) revealed that many of the identified associations with measles-specific antibody levels still remained valid ($p<0.05$, Table 3), however SNPs rs3212227/*IL12B* displayed only a suggestive association with immune outcome ($p=0.08$, data not shown). In addition to the two already reported *IL7R* SNPs (rs6897932/Thr244Ile and rs6890853, $D'=0.99$), our analysis demonstrated four additional *IL7R* SNP associations (including one additional coding SNP rs3194051/Ile356Val; SNPs in LD, $D' \geq 0.99$) demonstrating a considerable (approximately 2-fold for rs3194051) allele dose-related increase in antibody responses ($p \leq 0.024$, Table 3).

We also performed a race-specific analysis for the African-American subjects ($n=89$). Only two SNP associations (rs2228046/*IL6ST* and rs2243292/*IL4*) from the combined analysis were significant in this racial group, and those were different from the SNPs that demonstrated significance in the Caucasian group (Table 3).

Associations between SNPs in cytokine and cytokine receptor genes and cellular immune responses after measles vaccination—Overall, we found 29 significant associations between genetic variations in the coding/regulatory regions of cytokine/cytokine receptor genes and measles virus-specific IFN γ Elispot responses after measles immunization ($p<0.05$) (Table 4). We found six *IL7R* genetic variants (all of which were also associated with antibody responses) associated with measles-specific cellular immunity (Table 4). The minor alleles of one coding SNP (rs6897932/Thr244Ile) and one promoter *IL7R* SNP (rs6890853), in LD ($D'=0.99$), were associated with up to a 21% increase in measles-specific IFN γ Elispot responses. The minor alleles of four other *IL7R* SNPs (one additional coding SNP rs3194051/Ile356Val and three SNPs in the 3' intergenic region, in LD, $D' \geq 0.93$) demonstrated up to a 40% allele dose-related decrease in measles-specific cellular responses ($p \leq 0.04$, Table 4). Two coding synonymous *IL10RA* SNPs (rs2229115/Thr324Thr and rs4252249/Ala40Ala) were also associated with allele dose-related variations in the immune outcome ($p \leq 0.011$). In addition, the minor alleles of two *IL4R* coding SNPs (rs1805011/Glu400Ala and rs2234900/Leu433Leu) and one *LTA* coding SNP (rs2229092/His51Pro) demonstrated up to a 72% (for rs2229092) decrease/increase in IFN γ Elispot responses ($p \leq 0.039$). Two *IL2* promoter SNPs (the previously reported rs2069762 [5] and rs4833248, in LD, $D'=1$) also demonstrated a significant increase in IFN γ Elispot responses with the representation of the minor allele genotype ($p \leq 0.037$).

A race-specific analysis in the Caucasian group confirmed many of the above associations found in the combined analysis (Table 5). In particular, the two *IL7R* SNPs (rs6897932/Thr244Ile and the promoter SNP rs6890853), the two coding *IL4R* SNPs (rs1805011/Glu400Ala and rs2234900/Leu433Leu) and the *LTA* coding SNP (rs2229092/His51Pro) reproduced their association, with up to an 87% variation in IFN γ Elispot responses between genotypes in the Caucasian group ($p \leq 0.037$). The two *IL2* promoter SNPs (rs2069762 and rs4833248) referred to above, also reproduced their association with measles-specific cellular immune responses ($p \leq 0.042$). In addition, one previously reported *IL10* promoter SNP rs1800890 [5] was associated with variations in IFN γ Elispot responses ($p=0.025$).

Analysis of the African-American group confirmed the associations (from the combined analysis) for the two coding *IL10RA* SNPs (rs2229115/Thr324Thr and rs4252249/

Ala40Ala), which were associated with significant variations in the immune outcome in this racial group ($p \leq 0.011$). One *IL4* SNP rs2243291 also reproduced its association in the African-American sample (Table 5). Two additional genetic variants in the promoter region of *IL4* (rs2243248 and rs2243250) demonstrated associations with substantial variations (3-fold decrease and 7-fold increase, respectively) in IFN γ Elispot responses (Table 5). One non-synonymous *IL4R* SNP (rs3024638/Thr256Thr) was associated with an allele dose-related increase in IFN γ Elispot response ($p=0.045$).

Associations between *IL7R* haplotypes and measles virus-specific humoral and cellular immunity—The *IL7R* haplotype level analysis revealed significant associations with measles-specific humoral immunity in Caucasians (global p -value=0.003, Table 6), but not in African-Americans (global p -value=0.259, data not shown). Particularly, the most common *IL7R* haplotype AAGCGAGAGAAAAAAGGA was associated with decrease in neutralizing antibodies (allele p -value=0.011), and also with increase in IFN γ Elispot responses (allele p -value=0.019). Another common haplotype GAGAGGGAGCGAGAGGCGA demonstrated significant association with increased antibody levels ($p=0.007$).

Associations between SNPs in cytokine and cytokine receptor genes and measles-specific secreted cytokines—We found 121 significant associations (p -value<0.01) between SNPs and measles virus-specific cytokine secretion (Supplementary Table 1). Of note, a non-synonymous *CSF2RB* polymorphism rs1801114/Val652Met demonstrated highly significant associations with the secretion of IL-2 ($p=0.00007$), IL-6 ($p=0.00003$) and IFN γ ($p=0.001$) in the African-American group. We also identified two highly significant intronic SNP associations, *IFNGR2* SNP rs9976572 ($p=0.000005$) and *IL1R2* SNP rs13408303 ($p=0.00001$) associated with measles-specific IL-6 and TNF α secretion (respectively) in the African-American group. (Supplementary Table 1).

4. Discussion

Cytokine and cytokine receptors are intrinsically linked to the generation and regulation of adaptive immune responses, and therefore are reasonable targets for candidate gene-based genetic studies [6,29-32]. We have previously sampled a smaller number of candidate cytokine and cytokine receptor gene polymorphisms/SNPs ($n=58$) in Caucasians ($n=118$) and African-Americans ($n=85$) and reported initial moderate associations between SNPs in *IL2*, *IL10*, *IL12B*, *IL4RA*, *IL12RB*, *IL6* and *TNFA* genes and measles vaccine-induced immunity [3,5]. The current comprehensive candidate gene association study aimed to replicate these initial findings in a racially diverse cohort of vaccinated individuals and to identify new plausible genetic variants/genes regulating measles vaccine-induced immunity.

Importantly, our current study confirmed and replicated a significant association of genetic variant rs3212227 located within the 3'UTR region of the *IL12B* gene with variations in measles-specific humoral immunity in our combined analysis ($p=0.037$) [5]. However, while discovered in a study of Caucasian subjects [5], this SNP demonstrated a suggestive association ($p=0.08$) in Caucasians in our current study. There are differences between the discovery and replication studies, which might explain the slightly different levels of significance in the Caucasian subjects, regardless, both findings were in the same direction. An important difference between the two studies is the measurement of the humoral immune outcome used in the analysis. The discovery study measured total measles-specific IgG using EIA, while the current study measured the clinically relevant measles-specific neutralizing antibody levels (anti-H and anti-F antibodies) using PRMN assay. In the discovery study the associations between SNPs and measles-specific humoral and cellular immune responses were assessed using χ^2 tests of significance that compared the

distribution of SNPs across four quadrants, defined by the extremes of humoral (antibody) and cellular (lymphoproliferative) immune responses [5], while the present study evaluated associations between SNPs and quantitative measures of humoral/cellular immune outcomes in an entire cohort using linear regression methodologies. While we speculate that these and/or other differences in the study design might account for the slightly different results found in Caucasian subjects between the two studies, we have planned subsequent validation of results in a new independent cohort.

Interleukin-12 (IL-12p70), a pivotal cytokine in the induction of Th1 immune responses is a dimer composed of two subunits p40 and p35, encoded by the *IL12B* and *IL12A* genes, respectively. Interestingly, the *IL12B* gene has a relatively high level of conservation among humans [33]. The replicated genetic variant rs321227 (in our combined analysis) that affects the *TaqI* restriction site within the 3'UTR gene region (*TaqI* polymorphism) is a known regulatory/functional polymorphism with confirmed effects on the expression of *IL12B* and the secretion of IL-12, and has been associated with a number of autoimmune diseases, immune/inflammatory disorders and infectious diseases [33-40]. More importantly, the *TaqI* polymorphism has been implicated in regulation of the antibody response to HBsAg after immunization [6]. The replication of this functional polymorphism in two independent measles vaccine studies with differences in the study design, cohort characteristics, analysis and in the humoral immune outcome (EIA vs. neutralizing antibodies), is highly suggestive of *IL12B* involvement in the immune response variations observed after measles vaccination. Similarly, one *IL2* promoter polymorphism (rs2069762) and one *IL10* promoter polymorphism (rs1800890), previously associated with measles-specific antibody and lymphoproliferative responses [5], demonstrated significant associations with IFN γ Elispot responses (as a measure of measles-specific cellular immunity) in the Caucasian group in this study. Particularly, *IL2* rs2069762 was also found to be associated with IL-2 secretion in our study ($p=0.006$, Supplementary Table 1), and is a plausible functional SNP with effects on gene regulation and IL-2 expression, previously known to regulate the response to HBV vaccination [12,41].

Other interesting findings include the multiple *IL7R* genetic variants that were associated, at the genotype and haplotype level, with both humoral and cellular measles-specific immune responses in the Caucasian sample, but not the African-American sample. The *IL7R* gene encodes one of the receptor subunits for the IL-7 receptor, IL7R α , which forms a receptor complex with IL-2RG. The ligand-receptor interaction and signaling induces somatic recombination of the immunoglobulin (Ig) and the T-cell-receptor (TCR) genes, promoting the survival and proliferation of B and T lymphocytes, as demonstrated in animal and human studies, where genetic aberrations lead to severe immune deficiencies [42,43]. Furthermore, as noted by Gregory et al., the presence of membrane-bound IL7R α is the factor that limits IL7 receptor formation with significant effects on IL-7 signaling, T cell regulation and maintenance [44]. One of the *IL7R* allelic associations identified in our study pertains to a known causal non-synonymous SNP rs6897932, found in the alternatively spliced exon 6 of *IL7R* (Chr5p13), with confirmed effects the expression of the gene and the amounts of membrane-bound and soluble protein isoforms [44]. This genetic variant and other polymorphisms in the *IL7R* locus have been implicated in the susceptibility of immune, autoimmune, inflammatory and degenerative disorders [43-47]. Interestingly, in our study, the minor alleles of the coding causal *IL7R* polymorphism rs6897932 and a promoter *IL7R* polymorphism rs6890853 were consistently associated with a substantial decrease (more than 30%) in measles-specific neutralizing antibody response and a modest increase (cross-regulation) in measles-specific cellular IFN γ Elispot response in our Caucasian group, while the other four identified coding/regulatory genetic variants in the same genetic locus were associated with increases and decreases in measles-specific humoral and cellular immunity, respectively. It is plausible that a gene with a critical role in virus-induced immunity (such

as *IL7R*), will likely demonstrate associations with both increases and decreases in adaptive immune response measures for polymorphisms affecting gene regulation/activation, transcriptional activity/gene expression, and/or translation and receptor binding, depending on the SNP specific location and function. Thus our findings, in concert with the observed haplotype effects, strongly suggest the involvement of *IL7R* α -related immune response mechanisms in the immune response heterogeneity observed after measles vaccination.

Among other findings, the results of the present study also highlight genetic variants within the *IL4* and *IL4R* genes that are plausible determinants of vaccine-induced immune responses as recognized by other studies [4-7]. Particularly interesting are our findings for a genetic variant in the promoter region of *IL4* (rs2243248), which has been associated with multiple measles-specific immune outcomes including neutralizing antibody levels in Caucasians ($p=0.003$). This same genetic variant has been associated with Herpesvirus-8 lytic antibody titers in HHV-8-positive adults [48]. Another *IL4* promoter polymorphism (rs2243250) with likely functional consequences (with effects on IL-4 activity by creating a transcription factor binding site for NFAT-1 [49]), and known to confer susceptibility to subacute sclerosing panencephalitis [50], demonstrated a 7-fold increase in measles-specific cellular immunity/IFN γ Elispot with the representation of the minor allele genotype in our African-American group ($p=0.026$). Two coding *IL4R* SNPs (rs2234900 and rs1805011), associated with measles-specific IL-4 secretion in our initial study [5], demonstrated associations with IFN γ responses in Caucasians in our current study, thus further confirming their importance for measles vaccine-induced immunity.

Genetic studies often report inconsistent findings on associations between interrogated genes/polymorphisms and complex traits such as vaccine-induced immunity. The replication data (analysis of previously reported significant SNP associations in independent cohorts) and discovery data (data from interrogation of new genes/SNPs, not studied in our previous studies) presented here are in accordance with the polygenic nature of vaccine response, where multiple genes and interactions are likely to contribute to phenotype. Our findings are more consistent with race-specific, rather than common genetic variants/genes associated with measles-specific immune outcomes in the studied racial groups. A major strength of our study is the ability to validate previous pilot findings on genetic determinants of measles immunity by replication in a relatively large racially-diverse cohort of healthy vaccinees after two doses of MMR vaccine sampled from a region with high vaccine coverage and no circulating wild type measles virus (hence immune measures reflect vaccination). The heterogeneity of the study cohort and the smaller sample size of the different racial groups (particularly the African-American racial group), limited our power, therefore we chose to use the less stringent cutoff value 0.05 in the belief that the risk of false negatives outweighs that of false positives. In addition we reported race-specific associations for the two predominant racial groups. The resultant findings were mostly restricted to the specific groups being analyzed and the results cannot be extrapolated to other races and ethnicities, due to differences in the allele frequencies across populations.

This study, to our knowledge, represents the largest genetic association effort ever performed in measles immunity on non-HLA candidate genes. This enabled us to replicate previous findings, as well as to probe a large number of additional genetic variants and genes. We acknowledge the multiple testing issues and the possibility of false-positives. We would expect approximately 80 associations with antibody and IFN γ Elispot response outcomes by chance alone (assuming independent tests of association at the $p=0.05$), while we observed 98 associations (48 coding/regulatory and 50 intronic SNPs), which increase our confidence that some of the observed effects are genuine. The replication of previous findings, along with the observed allele dose-response relationships and haplotype effects, cross-regulation of cellular and humoral immunity, the biologically plausible functionality

and substantial immune outcome variations for some of the genetic variants further strengthen the significance of our results. Replication of all newly identified associations in an independent cohort, followed by fine mapping and functional studies of the implicated genetic determinants are planned to further extend our findings.

In conclusion, the present study highlights the importance of replicated and newly recognized non-HLA-related genetic variants and genes with likely functional consequences for interindividual variations in immune response to measles vaccine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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***Highlights**

- ± We studied 801 SNPs in 56 genes, in a cohort of 745 MMR-vaccinated schoolchildren
- ± We found 48 significant associations with variations in vaccine immune responses
- ± We replicated an association of *IL12B* rs3212227 with variations in humoral immunity
- ± We replicated two *IL10* and *IL2* SNP associations with cellular immunity variations
- ± Single SNP and haplotype analysis revealed *IL7R* associations with vaccine immunity

Table 1

Demographic and immunological characteristics of the study cohort

Variable	Median (IQR) ^a
Median age at enrollment, years (IQR)	15.0 (13; 17)
Median age at first measles immunization, months (IQR)	15.0 (15; 16)
Median age at second measles immunization, years (IQR)	5.0 (4; 11)
Median time from second measles immunization to enrollment, years (IQR)	7.4 (5.6; 9.2)
Gender, N (%)	
Male	417 (55.97)
Female	328 (44.03)
Race, N (%)	
White	598 (80.27)
African-American	89 (11.95)
Other	58 (7.78)
Ethnicity, N (%)	
Not Hispanic or Latino	723 (97.05)
Hispanic or Latino	15 (2.01)
Unknown	7 (0.94)
Neutralizing antibody, mIU/mL	846 (418; 1,772)
Th1 cytokine response, pg/mL	
IL-2	38 (21; 64)
IFN γ	67 (35; 120)
Th2 cytokine response, pg/mL	
IL-10	18 (11; 28)
Innate/inflammatory cytokine response, pg/mL	
IL-6	355 (248; 461)
TNF α	14 (9; 19)
IFN α	551 (273; 1,025)
IFN γ 1	34 (14; 73)
Elispot IFN γ , cytokine-positive spots per 200,000 cells	36 (12; 69)

^aResults are in median (IQR, inter-quartile range with 25% and 75% quartiles), unless indicated otherwise.

SNPs in coding/regulatory regions of cytokine and cytokine receptor genes associated with measles virus-specific neutralizing antibody responses

Table 2

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median Ab level mIU/mL (IQR) ^b	p-value ^c
<i>IL4</i>	rs2243248	promoter (-1100)	AA	624	897 (433; 1,806)	0.0064
			AC	118	667 (370; 1,695)	
			CC	2	425 (339; 510)	
<i>IL4</i>	rs2243292	3' intergenic	AA	728	853 (423; 1,784)	0.0079
			AG	14	776 (183; 1,504)	
			GG	2	239 (220; 257)	
<i>IL7R</i>	rs6890853	promoter (-4770)	GG	408	945 (472; 1,953)	0.0081
			GA	274	759 (376; 1,622)	
			AA	52	628 (337; 1,398)	
<i>IL18</i>	rs1946519	promoter (-9732)	CC	262	717 (378; 1,638)	0.0109
			CA	359	872 (455; 1,827)	
			AA	121	1,016 (462; 2,084)	
<i>IL7R</i>	rs6897932	coding Thr244Ile	GG	433	918 (457; 1,944)	0.0133
			GA	265	794 (386; 1,622)	
			AA	45	624 (336; 1,359)	
<i>IFNA1</i>	rs28383797	3' intergenic	GG	387	818 (401; 1,715)	0.0134
			GA	268	932 (431; 1,987)	
			AA	55	1,080 (607; 1,901)	
<i>IL6ST</i>	rs2228046	coding Ile454Thr	AA	710	830 (416; 1,729)	0.0194
			AG	31	1333 (462; 3,092)	
			GG	3	951 (370; 2,981)	
<i>IFNA21</i>	rs2891157	promoter (-1696)	CC	289	990 (473; 1,841)	0.0204
			CG	337	796 (409; 1,810)	
			GG	106	700 (389; 1,418)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median Ab level mIU/mL (IQR) ^b	p-value ^c
<i>TNF</i>	rs1799724	promoter (-1038)	GG	639	794 (401; 1,750)	0.0235
			GA	100	1,188 (599; 1,850)	
			AA	5	553 (475; 2,500)	
<i>IL6</i>	rs2069824	promoter (-1651)	AA	652	800 (405; 1,721)	0.0239
			AG	88	1,099 (527; 2,320)	
			GG	4	606 (261; 1,440)	
<i>IFNA8</i>	rs1330321	promoter (-1802)	AA	658	822 (407; 1,715)	0.0243
			AG	84	1,340 (541; 2,480)	
			GG	2	1,318 (240; 2,396)	
<i>IFNA10</i>	rs10811505	promoter (-1628)	AA	418	785 (392; 1,695)	0.0267
			AC	259	1,030 (486; 1,991)	
			CC	53	710 (369; 1,810)	
<i>IL1R2</i>	rs4851531	3' intergenic	GG	269	692 (369; 1,772)	0.0298
			GA	342	913 (460; 1,832)	
			AA	131	928 (515; 1,616)	
<i>CSF2RB</i>	rs4821564	promoter (-3961)	GG	374	919 (438; 1,962)	0.0339
			GA	304	843 (414; 1,784)	
			AA	66	589 (345; 1,187)	
<i>IL4</i>	rs2243247	promoter (-1138)	GG	720	834 (420; 1,728)	0.0358
			GA	21	1,928 (329; 2,870)	
			AA	3	1,810 (566; 4,034)	
<i>IL12B</i>	rs3212227	3'UTR	AA	428	837 (402; 1,717)	0.0372
			AC	259	807 (401; 1,772)	
			CC	57	951 (531; 2,556)	
<i>IL12B</i>	rs6859018	3' intergenic	GG	427	841 (401; 1,715)	0.0394
			GA	259	807 (416; 1,772)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median Ab level mIU/mL (IQR) ^b	p-value ^c
			AA	58	940 (527; 2,556)	
			GG	665	872 (436; 1,772)	0.04
<i>TNF</i>	rs361525	promoter	GA	64	547 (323; 1,630)	
		(-419)	AA	2	316 (193; 438)	
			AA	648	881 (431; 1,835)	0.0449
<i>IL1RN</i>	rs17486819	promoter	AC	89	702 (345; 1,253)	
		(-3791)	CC	6	1,718 (497; 1,827)	

Bolded SNPs are associated with an allele dose-related increase/decrease of antibody levels.

^aValues are presented as homozygous major allele/heterozygous/homozygous minor allele.

^bResults are presented as median neutralizing antibody (Ab) levels in mIU/mL $\pm$ IQR (inter-quartile range), as measured by the fluorescence-based measles virus-specific PRMN assay.

^cOrdinal p-value from the linear regression analysis adjusting for age at enrollment, gender, race, age at first and second immunization, and cohort status.

Race-specific analyses for associations of SNPs in coding/regulatory regions of cytokine and cytokine receptor genes and measles virus-specific neutralizing antibody responses

Table 3

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median Ab level mIU/mL (IQR) ^b	p-value ^c
Analysis in Caucasians (n=598)						
<i>IL4</i>	rs2243248	promoter (-1100)	AA	510	897 (440; 1,798)	0.003
			AC	88	656 (331; 1,561)	
			CC	0	(-)	
<i>IL7R</i>	rs6890853	promoter (-4770)	GG	324	988 (506; 1,953)	0.004
			GA	220	732 (377; 1,563)	
			AA	44	587 (334; 1,398)	
<i>IFNA1</i>	rs28383797	3' intergenic	GG	328	811 (423; 1711)	0.008
			GA	207	997 (441; 1,995)	
			AA	33	1,308 (717; 1,806)	
<i>IL7R</i>	rs6897932	coding Thr244Ile	GG	333	969 (503; 1,931)	0.011
			GA	223	792 (376; 1,622)	
			AA	41	631 (336; 1,436)	
<i>IL6</i>	rs2069824	promoter (-1651)	AA	535	803 (409; 1,715)	0.012
			AG	61	1,185 (654; 2,558)	
			GG	2	1,440 (872; 2,008)	
<i>IFNGR2</i>	rs4986958	coding Thr58Arg	CC	589	841 (423; 1,750)	0.013
			CG	7	1,479 (1,160; 3,028)	
			GG	1	3,114 (3,114; 3,114)	
<i>IL18</i>	rs1946519	promoter (-9732)	CC	210	727 (389; 1,600)	0.014
			CA	286	890 (473; 1,827)	
			AA	100	1,027 (438; 2,072)	
			GG	329	796 (401; 1,638)	

Gene	SNP ID	Location/ Function	Genotype ^a	Median Ab level mIU/mL (IQR) ^b	N ^a	p-value ^c
<i>IL7R</i>	rs1494571	3' intergenic	GC	981 (440; 1,798)	235	0.017
			CC	1,525 (758; 2,456)	34	
<i>IL7R</i>	rs3194051	coding	AA	796 (401; 1,638)	329	0.020
			AG	981 (440; 1,798)	235	
			GG	1,570 (758; 2,456)	33	
<i>IL7R</i>	rs1053496	3' intergenic	AA	796 (401; 1,638)	329	0.020
			AG	981 (440; 1,798)	235	
			GG	1,570 (758; 2,456)	33	
<i>IL28B</i>	rs10853727	promoter	AA	807 (401; 1,715)	485	0.021
			AG	1,004 (491; 1,883)	106	
			GG	1,727 (759; 2,230)	7	
<i>TNF</i>	rs1799724	promoter	GG	795 (402; 1,722)	512	0.023
			GA	1,223 (599; 1,938)	84	
			AA	462 (371; 553)	2	
<i>IL8RA</i>	rs2854386	3' intergenic	GG	826 (418; 1,723)	527	0.024
			GC	1,141 (545; 2,119)	70	
			CC	759(759,759)	1	
<i>IL7R</i>	rs700179	3' intergenic	AA	802 (401; 1,697)	380	0.024
			AG	978 (492; 1,783)	184	
			GG	1,570 (857; 2,456)	33	
<i>IFNA8</i>	rs1330321	promoter	AA	828 (418; 1,718)	533	0.025
			AG	1,340 (557; 2,143)	64	
			GG	2,396 (2,396; 2,396)	1	
<i>IL8RA</i>	rs11318739	3' intergenic	GG	839 (423; 1,727)	516	0.031
			GT	1,080 (509; 2,096)	73	
			TT	2,563 (2,563; 2,563)	1	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median Ab level mIU/mL (IQR) ^b	p-value ^c
<i>IL8RA</i>	rs16858784	3' intergenic	GG	547	867 (438; 1,772)	0.031
			GA	49	669 (294; 1,554)	
			AA	2	370 (105; 636)	
<i>IFNA10</i>	rs10811505	promoter (-1628)	AA	353	794 (401; 1,695)	0.033
			AC	198	1,040 (497; 1,850)	
			CC	35	794 (369; 2,396)	
<i>IL8RA</i>	rs2671222	promoter (-2669)	GG	529	826 (422; 1,723)	0.034
			GA	68	1,141 (524; 2,108)	
			AA	1	759 (759; 759)	
<i>IL8RA</i>	rs1567868	3' intergenic	AA	528	827 (420; 1,725)	0.036
			AG	69	1,080 (545; 2,096)	
			GG	1	759 (759; 759)	
<i>IL8RA</i>	rs1467142	3' intergenic	AA	527	828 (418; 1,726)	0.036
			AG	69	1,080 (545; 2,096)	
			GG	1	759 (759; 759)	
<i>IL1A</i>	rs3783521	promoter (-2231)	GG	266	839 (432; 1,699)	0.041
			GA	268	806 (387; 1,721)	
			AA	64	1,304 (617; 2,481)	
<i>IFNA16</i>	rs10125010	3' intergenic	GG	365	794 (402; 1,700)	0.044
			GA	199	1,048 (492; 1,887)	
			AA	34	733 (369; 2,206)	
<i>IFNA21</i>	rs2891157	promoter (-1696)	CC	228	996 (484; 1,846)	0.044
			CG	273	794 (401; 1,769)	
			GG	87	707 (389; 1,418)	
<i>IL1R2</i>	rs7589525	3' intergenic	AA	348	764 (392; 1,740)	
			AC	203	932 (473; 1,812)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median Ab level mIU/mL (IQR) ^b	p-value ^c
			CC	47	939 (6,24; 1,556)	0.045
			GG	221	898 (457; 1,873)	
<i>IL1A</i>	rs3783516	Promoter (-4332)	GA	285	862 (441; 1,772)	
			AA	92	776 (321; 1,291)	0.048
			GG	225	692 (386; 1,772)	
<i>IL1R2</i>	rs4851531	3' intergenic	GA	271	932 (451; 1,798)	
			AA	100	935 (544; 1,555)	0.049
Analysis in African-Americans (n=89)						
			AA	62	575 (240; 1,690)	0.005
<i>IL6ST</i>	rs2228046	coding	AG	25	1,810 (430; 3,092)	
		Ile454Thr	GG	2	1,966 (951; 2,981)	
			AA	48	1,383 (470; 2,404)	0.006
<i>IFNGRI</i>	rs4896243	3' intergenic	AG	33	520 (251; 918)	
			GG	8	325 (166; 1,738)	
			AA	65	1,167 (327; 2,487)	0.009
<i>IL4R</i>	rs2074570	3'UTR	AG	19	547 (205; 848)	
			GG	4	444 (315; 869)	
			AA	54	1,059 (430; 2,556)	0.013
<i>IL2</i>	rs11575812	3' intergenic	AG	28	586 (319; 1,668)	
			GG	7	232 (183,649)	
			AA	63	583 (240; 1,967)	0.028
<i>IL2</i>	rs11932411	3' intergenic	AG	21	1,170 (430; 2,556)	
			GG	5	1,690 (712; 4,034)	
			AA	76	878 (334; 2,219)	0.033
<i>IL4</i>	rs2243292	3' intergenic	AG	11	547 (91; 1,504)	
			GG	2	239 (220; 257)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median Ab level mIU/mL (IQR) ^b	p-value ^c
<i>IL8</i>	rs2227532	promoter (-845)	AA	74	681 (257; 1,928)	0.034
			AG	15	1,690 (531; 4,120)	
			GG	0	(-)	
<i>IL18R1</i>	rs12998521	promoter (-4705)	CC	42	681 (339; 1,690)	0.035
			CA	37	520 (232; 2,272)	
			AA	10	1,848 (1,261; 2,811)	
<i>IL5</i>	rs739719	3' intergenic	CC	26	1,259 (240; 2,487)	0.042
			CA	41	928 (477; 2,272)	
			AA	22	470 (237; 716)	
<i>IL1B</i>	rs1143634	coding (Phe 105Phe)	GG	58	1,059 (486; 2,272)	0.044
			GA	25	477 (240; 1,347)	
			AA	6	199 (91; 624)	
<i>IFNGR2</i>	rs1059293	3'UTR	AA	48	1,304 (470; 2,896)	0.045
			AG	30	616 (279; 1,193)	
			GG	10	475 (240; 712)	
<i>IFNGR2</i>	rs17880053	promoter deletion/ insertion	-/-	44	549 (226; 1,770)	0.048
			-/C	33	908 (327; 1,832)	
			CC	12	2,620 (1153; 3,799)	

Bolded SNPs are associated with an allele dose-related increase/decrease of antibody levels.

^aValues are presented as homozygous major allele/heterozygous/homozygous minor allele.

^bResults are presented as median neutralizing antibody (Ab) levels in mIU/mL ± IQR (inter-quartile range), as measured by the fluorescence-based measles virus-specific PRMN assay.

^cOrdinal p-value from the linear regression analysis adjusting for age at enrollment, gender, age at first and second immunization, and cohort status.

SNPs in coding/regulatory regions of cytokine and cytokine receptor genes associated with measles virus-specific IFN γ Elispot responses

Table 4

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
<i>IL7R</i>	rs6897932	coding Thr244Ile	GG	414	33 (12; 68)	0.002
			GA	249	38 (13; 69)	
			AA	43	38 (18; 78)	
<i>IL10RA</i>	rs2229115	coding Thr324Thr	GG	680	35 (12; 68)	0.004
			GA	26	48 (21; 94)	
			AA	1	100 (100; 100)	
<i>IL1RI</i>	rs949963	promoter (-4400)	GG	436	34 (12; 68)	0.005
			GA	236	36 (12; 71)	
			AA	35	47 (20; 83)	
<i>IL10RB</i>	rs1058867	3'UTR	AA	248	40 (20; 79)	0.005
			AG	334	33 (11; 64)	
			GG	125	28 (8; 61)	
<i>IL1RN</i>	rs315949	3' intergenic	GG	263	38 (15; 78)	0.010
			GA	330	32 (11; 64)	
			AA	111	32 (11; 66)	
<i>IFNARI</i>	rs2850015	5'UTR	GG	407	37 (13; 69)	0.010
			GA	249	35 (13; 69)	
			AA	48	25 (8; 59)	
<i>IL10RA</i>	rs4252249	coding Ala40Ala	GG	560	36 (12; 71)	0.011
			GA	138	36 (17; 57)	
			AA	8	25 (3; 87)	
<i>IFNARI</i>	rs17875752	promoter (-865)	CC	208	29 (9; 60)	0.011
			CA	350	38 (14; 75)	
			AA	115	39 (22; 70)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
<i>IL4R</i>	rs1805011	coding Glu400Ala	AA	526	36 (15; 69)	0.017
			AC	159	34 (8; 67)	
			CC	21	29 (4; 56)	
<i>IL1B</i>	rs3917368	3' intergenic	GG	328	34 (11; 68)	0.019
			GA	295	33 (12; 69)	
			AA	84	43 (19; 73)	
<i>IL7R</i>	rs6890853	promoter (-4770)	GG	389	33 (11; 68)	0.021
			GA	258	35 (12; 67)	
			AA	50	40 (18; 71)	
<i>IL1RN</i>	rs397211	3' intergenic	AA	353	33 (11; 63)	0.021
			AG	290	37 (13; 73)	
			GG	63	37 (20; 84)	
<i>IL7R</i>	rs1494571	3' intergenic	GG	391	38 (12; 75)	0.021
			GC	272	33 (12; 60)	
			CC	44	25 (15; 70)	
<i>IL28RA</i>	rs10465895	3' intergenic	TT	533	36 (14; 72)	0.022
			TA	160	29 (10; 52)	
			AA	14	58 (13; 103)	
<i>IL7R</i>	rs1053496	3' intergenic	AA	377	38 (13; 75)	0.023
			AG	281	33 (12; 59)	
			GG	48	26 (11; 76)	
<i>IL4</i>	rs2243291	3' intergenic	GG	441	36 (12; 67)	0.023
			GC	219	36 (11; 71)	
			CC	45	36 (16; 64)	
<i>IL1RN</i>	rs17486819	promoter	AA	613	36 (14; 69)	0.024
			AC	87	30 (7; 62)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
		(-3791)	CC	6	47 (6; 61)	
			AA	296	38 (15; 76)	
<i>IL28RA</i>	rs10903035	3'UTR	AG	303	31 (11; 62)	0.028
			GG	108	35 (13; 68)	
			AA	376	34 (10; 67)	
<i>IL2</i>	rs2069762	promoter	AC	279	36 (15; 71)	0.029
		(-386)	CC	52	37 (14; 68)	
			GG	525	37 (14; 72)	
<i>IL2RB</i>	rs3218339	3' intergenic	GA	172	30 (11; 50)	0.030
			AA	10	24 (7; 62)	
			GG	483	34 (11; 69)	
<i>IL12RB1</i>	rs436857	5'UTR	GA	200	37 (15; 66)	0.031
			AA	24	45 (9; 100)	
			GG	474	37 (15; 71)	
<i>IL12B</i>	rs3181225	3' intergenic	GA	213	33 (8; 67)	0.032
			AA	18	30 (16; 54)	
			TT	417	35 (12; 69)	
<i>IL6R</i>	rs4379670	3'UTR	TA	256	36 (15; 67)	0.034
			AA	33	40 (11; 55)	
			AA	640	36 (13; 69)	
<i>LTA</i>	rs2229092	coding	AC	64	30 (10; 62)	0.036
		His51Pro	CC	3	10 (1; 36)	
			GG	377	33 (10; 66)	
<i>IL2</i>	rs4833248	promoter	GA	275	37 (15; 71)	0.037
		(-2811)	AA	47	39 (14; 69)	
			AA	424	38 (15; 73)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
<i>IL7R</i>	rs700179	3' intergenic	AG	234	30 (10; 59)	0.037
			GG	48	26 (11; 76)	
<i>IL4R</i>	rs2234900	Coding	AA	505	36 (15; 71)	0.039
			AG	174	31 (7; 61)	
		Leu433Leu	GG	26	39 (5; 64)	
<i>IL7R</i>	rs3194051	coding	AA	387	38 (12; 75)	0.040
			AG	274	33 (12; 59)	
		Ile356Val	GG	45	26 (15; 79)	
<i>IL28B</i>	rs8105790	3' intergenic	AA	437	36 (11; 68)	0.047
			AG	231	33 (15; 64)	
			GG	37	38 (12; 83)	

Bolded SNPs are associated with an allele dose-related increase/decrease of median IFN γ Elispot responses.

^aValues are presented as homozygous major allele/heterozygous/homozygous minor allele.

^bResults are presented as median IFN γ -positive spots per 2×10^5 cells $\pm$ IQR (inter-quartile range), as measured by the IFN γ Elispot assay.

^cOrdinal p-value from the repeated measures linear regression analysis adjusting for age at enrollment, gender, race, age at first and second immunization, and cohort status.

Race-specific analyses for associations of SNPs in coding/regulatory regions of cytokine and cytokine receptor genes and measles virus-specific IFN γ Elispot responses

Table 5

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
Analysis in Caucasians (n=598)						
IL7R	rs6897932	coding Thr244Ile	GG	315	35 (14; 69)	0.007
			GA	208	39 (15; 75)	
			AA	39	38 (16; 71)	
IL1B	rs3917368	3' intergenic	GG	244	35 (12; 68)	0.007
			GA	248	35 (14; 69)	
			AA	71	47 (23; 82)	
IFNAR1	rs2850015	5'UTR	GG	303	38 (16; 73)	0.010
			GA	214	37 (14; 71)	
			AA	43	23 (8; 58)	
IL1RN	rs17486819	promoter (-3791)	AA	476	37 (15; 73)	0.011
			AC	80	31 (8; 61)	
			CC	6	47 (6; 61)	
IL7R	rs6890853	promoter (-4770)	GG	306	34 (13; 68)	0.023
			GA	205	37 (15; 72)	
			AA	42	38 (16; 71)	
IL10	rs1800890	promoter (-3586)	TT	205	34 (10; 63)	0.025
			TA	264	34 (18; 72)	
			AA	94	43 (15; 78)	
IL10RB	rs1058867	3'UTR	AA	207	39 (22; 73)	0.025
			AG	262	35 (11; 69)	
			GG	94	29 (9; 62)	
			AA	247	38 (16; 78)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
<i>IL28RA</i>	rs10903035	3'UTR	AG	238	34 (12; 63)	0.027
			GG	78	34 (13; 67)	
<i>IL1R1</i>	rs949963	promoter (-4400)	GG	360	36 (15; 69)	
			GA	183	38 (14; 75)	0.029
			AA	20	40 (12; 53)	
<i>IL4R</i>	rs1805011	coding Glu400Ala	AA	453	37 (15; 71)	
			AC	104	37 (9; 70)	0.030
			CC	5	5 (1; 54)	
<i>IL6ST</i>	rs2228043	3'UTR	CC	444	33 (12; 69)	
			CG	111	46 (23; 76)	0.031
			GG	8	36 (23; 75)	
<i>IL28RA</i>	rs10465895	3'intergenic	TT	427	37 (15; 74)	
			TA	125	29 (11; 53)	0.032
			AA	11	70 (13; 106)	
<i>IL4R</i>	rs2234900	Coding Leu433Leu	AA	440	37 (16; 72)	
			AG	115	34 (8; 67)	0.033
			GG	6	55 (5; 72)	
<i>IL2</i>	rs2069762	promoter (-386)	AA	294	35 (11; 69)	
			AC	230	38 (16; 72)	0.034
			CC	39	39 (14; 79)	
<i>LTA</i>	rs2229092	coding His51Pro	AA	505	37 (15; 72)	
			AC	55	32 (10; 62)	0.037
			CC	3	10 (1; 36)	
<i>IL18R1</i>	rs1035130	coding (Phe251Phe)	GG	296	39 (15; 74)	
			GA	212	34 (10; 67)	0.040
			AA	53	31 (16; 56)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
<i>IL2</i>	rs4833248	promoter (-2811)	GG	294	35 (11; 69)	0.042
			GA	227	38 (16; 72)	
			AA	35	42 (14; 79)	
<i>IL28RA</i>	rs4648942	3' intergenic	GG	171	37 (16; 77)	0.043
			GA	288	37 (12; 73)	
			AA	103	31 (14; 60)	
<i>IL18RI</i>	rs1035129	3' intergenic	AA	159	37 (15; 62)	0.045
			AG	266	38 (14; 73)	
			GG	136	35 (15; 78)	
<i>IL6ST</i>	rs10471960	3' intergenic	AA	436	33 (12; 69)	0.045
			AG	118	46 (22; 75)	
			GG	9	42 (26; 82)	
<i>IL1RN</i>	rs397211	3' intergenic	AA	266	36 (15; 63)	0.047
			AG	244	35 (13; 76)	
			GG	53	42 (20; 84)	
Analysis in African-Americans (n=89)						
<i>IL10RA</i>	rs2229115	coding Thr324Thr	GG	69	17 (4; 40)	0.002
			GA	17	39 (21; 64)	
			AA	1	100 (100; 100)	
<i>IFNA7</i>	rs4977686	3' intergenic	CC	63	27 (4; 51)	0.005
			CA	16	11 (3; 46)	
			AA	7	7 (0; 30)	
<i>IFNARI</i>	rs17875752	promoter (-865)	CC	24	15 (2; 40)	0.006
			CA	46	21 (5; 43)	
			AA	14	34 (4; 92)	
			AA	65	30 (7; 54)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN _γ Elispot (IQR) ^b	p-value ^c
<i>IL4</i>	rs2243248	promoter (-1100)	AC	20	9 (3; 29)	0.008
			CC	2	9 (2; 16)	
<i>IFNA1</i>	rs28383797	3' intergenic	GG	32	14 (3; 30)	0.008
			GA	40	28 (4; 57)	
			AA	14	39 (29; 69)	
<i>IL28RA</i>	rs11579657	3' intergenic	AA	64	29 (6; 48)	0.008
			AG	22	7 (1; 39)	
			GG	1	9 (9; 9)	
<i>IL29</i>	rs8108392	promoter (-2991)	AA	34	14 (3; 30)	0.010
			AG	48	27 (4; 53)	
			GG	5	37 (33; 69)	
<i>IL10RA</i>	rs4252249	coding Ala40Ala	GG	64	26 (5; 47)	0.011
			GA	19	29 (2; 52)	
			AA	4	12 (1; 25)	
<i>IL4</i>	rs2243291	3' intergenic	GG	27	10 (2; 40)	0.012
			GC	43	19 (4; 43)	
			CC	17	47 (33; 79)	
<i>IL4</i>	rs2243231	Promoter (-4697)	GG	73	25 (4; 43)	0.013
			GA	14	28 (4; 52)	
			AA	0	(-)	
<i>IL12RB2</i>	rs3762316	promoter (-14030)	AA	57	11 (2; 39)	0.017
			AG	27	39 (29; 79)	
			GG	3	4 (0; 10)	
<i>IL4</i>	rs2243250	promoter (-590)	GG	21	6 (2; 29)	0.026
			GA	47	19 (5; 43)	
			AA	16	43 (25; 85)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
<i>IFNA5</i>	rs7031048	promoter (-1064)	AA	39	15 (4; 36)	0.026
			AG	37	29 (3; 51)	
			GG	9	91 (43; 92)	
<i>IL12A</i>	rs2243147	3' intergenic	AA	25	36 (7; 72)	0.032
			AG	40	14 (4; 45)	
			GG	22	25 (4; 39)	
<i>IFNA13</i>	rs594544	Promoter (-4118)	AA	61	27 (4; 51)	0.032
			AG	25	19 (4; 39)	
			GG	0	(-)	
<i>IFNA8</i>	rs1330321	promoter (-1802)	AA	68	20 (4; 42)	0.033
			AG	18	32 (7; 51)	
			GG	1	62 (62; 62)	
<i>IL12A</i>	rs2243143	3' intergenic	GG	25	36 (7; 72)	0.033
			GA	41	15 (4; 43)	
			AA	21	29 (4; 39)	
<i>IL1R2</i>	rs7589525	3' intergenic	AA	68	20 (4; 41)	0.034
			AC	19	40 (9; 78)	
			CC	0	(-)	
<i>IL12A</i>	rs2243151	3' intergenic	AA	24	37 (13; 76)	0.037
			AT	40	16 (4; 45)	
			TT	22	25 (4; 39)	
<i>IL29</i>	rs8107861	promoter (-2911)	GG	37	16 (5; 30)	0.038
			GA	46	32 (4; 61)	
			AA	4	35 (17; 69)	
<i>TNFRSF1A</i> rs4149570		promoter	CC	56	23 (4; 48)	0.038
			CA	23	29 (5; 61)	

Gene	SNP ID	Location/ Function	Genotype ^a	N ^a	Median IFN γ Elispot (IQR) ^b	p-value ^c
		(-611)	AA	8	23 (2; 34)	
			AA	74	23 (4; 49)	
<i>IL4</i>	rs2243292	3' intergenic	AG	11	15 (4; 39)	0.039
			GG	2	79 (39; 118)	
			GG	72	27 (4; 47)	
<i>IFNB1</i>	rs7033217	promoter	GA	14	16 (5; 52)	0.044
		(-3565)	AA	1	0 (0; 0)	
			GG	77	19 (4; 47)	
<i>IL4R</i>	rs3024638	coding	GC	7	33 (5; 51)	0.045
		Thr256Thr	CC	3	38 (20; 62)	
			GG	29	29 (11; 64)	
<i>IFNA2</i>	rs624704	3' intergenic	GA	44	16 (4; 45)	0.046
			AA	14	18 (2; 36)	
			AA	26	27 (3; 43)	
<i>IL28B</i>	rs12980275	3' intergenic	AG	43	19 (4; 51)	0.049
			GG	18	33 (7; 54)	

Bolded SNPs are associated with an allele dose-related increase/decrease of median IFN γ Elispot responses.

^aValues are presented as homozygous major allele/heterozygous/homozygous minor allele.

^bResults are presented as median IFN γ -positive spots per 2×10^5 cells $\pm$ IQR (inter-quartile range), as measured by the IFN γ Elispot assay.

^cOrdinal p-value from the repeated measures linear regression analysis adjusting for age at enrollment, gender, age at first and second immunization, and cohort status.

Table 6*IL7R* haplotype associations with measles-specific humoral and cellular immune responses in Caucasians

<i>IL7R</i> Haplotype ^a	Frequency	Test statistic (haplotype <i>t</i> -statistic) ^b	Allele <i>p</i> - value ^c	Global <i>p</i> - value
Associations with antibody levels				
AAGCGAGAGAAAAAAGGA	0.242	−2.56	0.011	0.003
GAACAGGGGAGTAGAAGGG	0.216	1.17	0.242	
GAGAGGGAGCGAGAGACGA	0.041	0.93	0.352	
GAGAGGGAGCGAGAGGCGA	0.198	2.71	0.007	
GAGCAGAGGAGTAGAAGGG	0.122	0.91	0.363	
GGGCGGGAAAGTAGAAGCA	0.129	−1.81	0.071	
Associations with IFNγ Elispot responses				
AAGCGAGAGAAAAAAGGA	0.242	2.35	0.019	0.142
GAACAGGGGAGTAGAAGGG	0.216	−0.17	0.866	
GAGAGGGAGCGAGAGACGA	0.041	−0.50	0.618	
GAGAGGGAGCGAGAGGCGA	0.198	−1.73	0.085	
GAGCAGAGGAGTAGAAGGG	0.122	−1.13	0.261	
GGGCGGGAAAGTAGAAGCA	0.129	0.43	0.665	

^a *IL7R* genetic variants from left to right: rs6890853, rs4869492, rs1353251, rs11567701, rs1494558, rs7717955, rs11567714, rs10941267, rs7737000, rs1494554, rs6897932, rs987106, rs3194051, rs10063294, rs1053496, rs700179, rs1494571, rs1389834, rs10058453.

^b Haplotype effects (for common haplotypes with estimated frequency of greater than 1%) are calculated using the haplotype *t*-statistic, which shows the direction and magnitude of the estimated haplotypic effect on the measles-specific neutralizing antibody or IFN γ Elispot measure.

^c Allele *p*-values compare individual haplotypes to all other haplotypes combined. Statistically significant *p*-values (*p*<0.05) are highlighted in bold. Analyses are adjusted for age at enrollment, gender, race, age at first and second MMR immunization and cohort status (cohort 1 or cohort 2).