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Associations between Polymorphisms in the Antiviral TRIM Genes and Measles Vaccine Immunity

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Abstract

The role of polymorphisms within the antiviral tripartite motif (TRIM) genes in measles vaccine adaptive immune responses was examined. A limited association was found between TRIM5 (rs7122620) and TRIM25 (rs205499) gene polymorphisms and measles-specific antibody levels. However, many associations were found between TRIM gene SNPs and variations in cellular responses (IFN-γ Elispot and secreted cytokines IL-2, IL-6, IL-10, IFN-γ, and TNF-α). TRIM22 rs2291841 was significantly associated with an increased IFN-γ Elispot response (35 vs. 102 SFC per 2×10^5 PBMC, p=0.009, q=0.71) in Caucasians. A non-synonymous TRIM25 rs205498 (in LD with other SNPs, $r^2\geq0.56$), as well as the TRIM25 AAAGGAAAGGAGT haplotype, was associated with a decreased IFN-γ Elispot response (t-statistic −2.32, p=0.02) in African-Americans. We also identified polymorphisms in the TRIM5, TRIM22, and TRIM25 genes that were associated with significant differences in cytokine responses.

Additional studies are necessary to replicate our findings and to examine the functional consequences of these associations.

Keywords

Single-nucleotide polymorphisms; measles virus; measles vaccine immunity; TRIM genes; antiviral; innate; antibody; cytokines; Elispot; Caucasians; African-Americans

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Conflict of interest Dr. Poland is the chair of a safety evaluation committee for novel non-measles vaccines undergoing clinical studies by Merck Research Laboratories. Dr. Poland offers consultative advice on new vaccine development to Merck & Co. Inc., Avianax, TheraClone Sciences, Liquida Technologies Inc., Dynavax, Sanofi Pasteur, Novartis Vaccines and Therapeutics and PAXVAX Inc. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

1. Introduction

We previously demonstrated that the heritability of measles vaccine-induced humoral immunity was very high (~90%) [1]. Measles virus (MV)-induced immunity is influenced by a multitude of host-genetic variants (SNPs) that, in part, explain inter-individual differences in humoral and cell-mediated immune (CMI) responses to live measles vaccine [2,3]. Several candidate-gene association studies have demonstrated that multiple genes/SNPs/haplotypes (HLA, cytokine, viral and innate receptors, and others) have significant effects on measles vaccine-induced immune responses [4-9]. Genetic variation and its effect on viral immune response may also be restricted by antiviral innate factors, such as members of the conserved tripartite motif (TRIM) protein family [10].

TRIM proteins (TRIM5, TRIM22, TRIM25, and others) have recently emerged as important cellular factors for innate immunity and antiviral defense, and are induced by type I interferons (IFNs) [10-12]. For example, TRIM5 is documented to play a role in host defense by inhibiting the replication of some retroviruses (e.g., HIV-1) through its contact with the HIV-1 capsid protein [13]. Human TRIM22 is induced by type I IFNs, can bind to HIV-Gag protein, and can inhibit HIV-1 replication [14]. A non-synonymous SNP (His43Tyr) in the TRIM5 gene was recently found to be correlated with rubella vaccine antibody response [15] after having been earlier described to have functional effects [16,17]. Experiments with Sendai and Newcastle disease viruses demonstrated that the ubiquitin ligase TRIM25 is involved in the retinoic-acid-inducible gene-I (RIG-I) signaling pathway, which is important for antiviral immunity [18]. In turn, a recent measles vaccine study found that neutralizing antibody, IFN-γ Elispot, and cytokine (IFN-γ and IL-2) immune responses were associated with RIG-I gene polymorphisms [8]. Finally, the important role of TRIM25 in antiviral host defense (by inhibiting RING-mediated E3 ligase) and IFN-β production in response to the nonstructural protein 1 (NS1) of influenza A virus was recently described [11].

However, no information is available regarding the role of TRIM gene polymorphisms in MV vaccine-induced adaptive immune response. Therefore, the goal of our study was to examine associations between individual SNPs/haplotypes in the TRIM5, TRIM22, and TRIM25 genes and variations in humoral (neutralizing antibody) and CMI (IFN-γ Elispot and secreted cytokines) immunity in healthy children following measles vaccination.

2. Materials and Methods

2.1. Study subjects

Subject enrollment for this study has been previously described in detail [7,9,19,20]. Briefly, we enrolled 764 children (11 to 22 years of age) in Rochester, MN, who received two age-appropriate doses of measles vaccine (Merck). Of these, genotype-phenotype data were available for a total of 745 study subjects. Our study population was predominantly Caucasian (n=598), with 89 African-Americans. The Mayo Clinic Institutional Review Board approved the study and written informed consent and/or assent were obtained from each subject and/or guardian.

2.2. Antibody measurement

Specifics of the antibody assay for the study subjects have been previously published [7,9,19,20]. MV-specific neutralizing antibody levels were measured by using a fluorescence-based plaque reduction microneutralization test **(**PRMN, mIU/ml), as previously described [19,21]. The coefficient of variation (CV) for this assay in our laboratory was 5.7% [19].

2.3. Elispot assay

Details of measurement of the IFN-γ Elispot responses (Elispot kits from R&D Systems, Minneapolis, MN) have been previously published [7,20,22]. The intraclass correlation coefficients (ICCs) comparing the multiple observations per individual were 0.94 for the MV-stimulated values, and 0.85 for the unstimulated values [23].

2.4. Cytokine measurements

Details of the measurement of the IL-2, IL-6, IL-10, IFN-γ, and TNF-α by enzyme-linked immunosorbent assays (Elisa) in supernatants of cultured **peripheral blood mononuclear cells (PBMC)** stimulated with MV are nearly identical to those we previously published [7-9,20]. Briefly, the Edmonston B vaccine strain of MV was grown in Vero cells supplemented with 5 % heat-inactivated fetal calf serum (FCS, Hyclone, Logan, UT). The MV was titrated, aliquoted, and stored at −80°C in stocks of 6×10^7 pfu/ml. The multiplicity of infection (MOI) and incubation time for each cytokine were as follows: IL-2, MOI=0.5, 48 hours; IL-6, MOI=1.0, 72 hours; IL-10, MOI=0.5, 48 hours; IFN-γ, MOI=1.0, 72 hours; and TNF-α, MOI=1.0, 24 hours. Cytokine-specific ICCs ranged from 0.65 (IL-2, unstimulated values) to 0.94 (IL-6, MV-stimulated values) [23].

2.5. SNP selection and genotyping

The description of the tagging SNP selection approaches **and genotyping methods** for this study has been previously described and is nearly identical to those we previously published [7-9,20]. SNPs from three candidate genes encoding TRIM5 (n=19 SNPs), TRIM22 (n=4 SNPs), and TRIM25 (n=13 SNPs) molecules were selected. SNPs within candidate genes, 5 kb upstream and downstream for each gene, were chosen based on **the linkage disequilibrium (LD)** tagSNP selection algorithm [24] from the Hapmap Phase II (http://www.hapmap.org), Seattle SNPs (http://pga.mbt.washington.edu/), and NIEHS SNPs (http://egp.gs.washington.edu/), with SNP minor allele frequencies (MAF) ≥0.05, LD threshold of r^2 ≥0.90, for the Caucasian sample and African-American sample separately, as previously described [7-9,20]. Thirty-six SNPs from the three candidate genes were included in the two custom Illumina GoldenGate SNP panels (Illumina Inc., San Diego, CA) for 1,536 and 768 SNPs as part of a larger measles vaccine immunogenetic study. All SNPs had an Illumina design score >0.4, and all DNA samples were genotyped following the manufacturer's procedure.

2.6. Statistical methods

The statistical methods for this study are nearly identical to those used and published by our group for previous candidate SNP studies [8,9,20]. Assessments of cytokine secretion and IFN-γ Elispot responses resulted in multiple recorded values per outcome both prior to and after stimulation with MV. 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.

Estimates of pair-wise linkage disequilibrium (LD) amongst SNP genotypes based on the r-squared statistic were obtained using Haploview software, version 3.32 [25]. 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 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 [26]. 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 a simple least squares linear regression approach for antibody levels and repeated measures approaches for the cytokine secretion and Elispot 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 (only in combined analyses), gender, age at first and second measles vaccination, and cohort status. We used an inverse normal transformation for all cytokine secretion and Elispot outcome variables, and a log transformation for the antibody response measure in all formal tests to account for the skewed nature of the data. Due to the relatively large number of statistical tests, we supplemented all p-values with corresponding q-values based on the false discovery rate (FDR) literature [27,28]. Q-values are specific to each outcome variable, and were calculated based on results from the entire set of candidate SNPs plated on the two Illumina panels and passing QC. 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

The median (inter-quartile range/IQR) MV-specific antibody titers and IFN-γ Elispot counts for all subjects was 846 mIU/ml (41; 1772) and 36 spot-forming cells (SFC)/200,000 cells (12; 69), respectively. The median (IQR) MV-specific IL-2, IL-6, IL-10, IFN-γ, and TNF-α secretion levels was 38 pg/ml (21; 64), 366 pg/ml (249; 461), 18 pg/ml (11; 29), 67 pg/ml (35; 121), and 14 pg/ml (9; 19), respectively [6-9].

Genotype data were examined independently for the whole cohort (n=745), Caucasians (n=598), and African-Americans (n=89). With regard to antibody response, TRIM5 promoter SNP (rs7122620, $p<0.02$, $q=0.84$) demonstrated an association with MV-specific antibodies in the combined cohort and among Caucasians (Table 1). In addition, TRIM25 intronic SNP (rs205499, $p<0.04$, $q=0.96$) demonstrated an association with measles antibodies in the combined cohort and among African-Americans.

With regard to cellular immune response, an increased carriage of a minor allele C for a promoter SNP rs2291841 in the TRIM22 gene in Caucasians was associated (and already identified in the combined cohort) with an allele dose-dependent increase in measles-specific IFN-γ Elispot counts (935 vs. 102 SFC per 2×10^5 PBMC, $p=0.009$, $q=0.71$) (Table 1). Pairwise LD analysis found a coding non-synonymous rs205498 ($p=0.02$, $q=0.51$) to be linked to rs2525993, rs2525994, rs2525996 and rs25259921 ($r^2 \geq 0.56$), located in the

TRIM25 gene, which was correlated with differences in IFN-γ Elispot response in African-Americans.

With regard to MV-induced cytokine response, TRIM5 promoter SNP rs7122620 demonstrated an association with IL-2 secretion in the combined cohort (p=0.01, q=0.55) and among Caucasians (p=0.05, q=0.77) (Table 2). We identified three TRIM5 gene SNP associations (rs3824949, p=0.004, q=0.29; rs10769175; and rs7124435, p=0.03, q=0.41) with variations in IL-6 secretion in African-Americans. Further, in the combined cohort, five significant associations were discovered between SNPs in the TRIM25 gene (coding rs205498, p=0.02, q=0.91; intronic rs2525996, p=0.006, q=0.79; rs2525992, p=0.009, q=0.79; rs2525993, p=0.009, q=0.79; and rs2525994, p=0.01, q=0.81; $r^2 \geq 0.97$), and production of IL-10 in response to MV stimulation. In the African-American subgroup, six significant associations ($p<0.05$) were found between SNPs in the TRIM25 gene and IL-10 secretion. The presence of homozygous genotype AA for a promoter SNP (rs885002) of the TRIM22 gene on chromosome 11 was associated with a 17-fold increase in median IL-10 secretion levels (12 vs. 200 pg/ml, p=0.02, q=0.50), as compared to the heterozygous variants.

The minor allele variant of a specific SNP (rs169530) in the TRIM25 gene was associated with a two-fold decrease in IFN-γ responses in the combined cohort (p=0.05, q=0.05) and among Caucasians (p=0.008, q=0.26). A coding TRIM5 variant (rs11601507, V112F, p=0.04, q=0.56) exhibited an association with IFN-γ secretion among African-Americans. Pair-wise LD analysis found another coding rs3740995 to be linked to rs3740994 located in the TRIM5 gene ($r^2=0.4$) that was associated with an allele dose-dependent decrease in IFN-γ production (6 vs. 60 pg/ml, p=0.02, q=0.55) among African-Americans. The observed MAF, for both the Caucasian and African-American subgroups, for all SNPs of interest are shown in a Supplementary Table 1.

Lastly, a haplotype analysis identified a significant association between IFN-γ Elispot responses and a TRIM25 haplotype among African-Americans (global p-value=**0.03**) (Table 3). Specifically, the TRIM25 haplotype AAAGGAAAGGAGT (rs11540270/ rs2525998/ rs2525997/rs205499/rs2525996/rs2525994/rs2525993/rs205498/r s7225205/rs9909750/ rs2525992/rs169530/rs11869863) was associated with lower IFN-γ Elispot counts (t-statistic −2.32, **p=0.02**) (Table 3).

4. Discussion

This study reports significant associations between polymorphisms in the innate ubiquitin ligase TRIM genes and adaptive immune responses (humoral and CMI) to measles vaccine. Human TRIM5, TRIM22, and TRIM25 molecules belong to the TRIM protein family and contain three signature domains: RING, B-box, and coiled-coil domains [10]. A number of TRIM proteins are inducible by IFNs and have a role in innate immunity to control viral replication [10]. It has been proposed that TRIM molecules interact with viral proteins, enabling viruses to escape recognition by host immunity and evade the host IFN system [11]. However, no detailed information regarding measles-induced immune response has been available for any of these (TRIM5, 22, and 25) genetic loci.

In this study, we observed significant associations between TRIM gene SNPs and variations in MV-induced CMI responses, such as IFN-γ Elispot and secreted cytokines. We identified only two polymorphisms (TRIM5 rs7122620 and TRIM25 rs205499) that were associated with differences in measles-specific antibody levels among both Caucasians and African-Americans, respectively. The same promoter TRIM5 rs7122620 was also associated with IL-2 secretion in Caucasians, though the exact mechanism by which these SNPs affect

immune response is currently unclear. Of importance, TRIM5 genetic coding functional variants (rs3740996 and rs10838525) [16,17] were previously found to be associated with production of rubella-specific cytokines (TNF-α, IL-2, and GM-CSF) [29]. The involvement of genetic variants within the innate RIG-I gene (through TRIM25-mediated ubiquitination) in measles vaccine-induced immunity has recently been reported [8,18].

Five SNPs, including likely functional coding non-synonymous rs205498 (Pro358Leu) belonging to the TRIM25 gene, were associated with IFN-γ Elispot responses in the African-American subgroup. As previously indicated, TRIM25 is a RING-finger E3 ubiquitin protein and is crucial for RIG-I-induced antiviral function [18]. It is plausible that a non-synonymous coding SNP rs205498, Pro358Leu, that is in close LD with other genetic variants, may affect the biological function of the TRIM25 protein. The effects of these SNPs were also observed at the multigenic (haplotype) level, where haplotype analysis showed a significant association between the TRIM25 AAAGGAAAGGAGT haplotype and lower measles-specific IFN-γ Elispot response. This suggests a potential role of TRIM25 gene locus/variants in the development of MV-induced adaptive cellular immunity and the importance of taking ethnicity into account in genetic studies such as this. Clearly, further studies designed **to clarify** the functional properties of these SNPs are needed.

We also identified several polymorphisms in the TRIM5, TRIM22 and TRIM25 genes that were associated with measles-specific cytokine responses (IL-2, IL-6, IL-10, IFN-γ, and TNF-α). Specifically, our data offer evidence for TRIM25 SNP and haplotype associations with IFN-γ Elispot counts, and IFN-γ and IL-10 secretion levels across Caucasian and African-American subgroups. Additionally, we identified a coding TRIM25 SNP rs205498 that appears to influence secretion of IL-10 in the combined cohort and among Caucasians. Human IL-10 is known to stimulate antibody-producing plasma cells and increase production of specific antibodies [30]. Particularly interesting are two significant associations in African-Americans that were discovered between coding polymorphisms in the TRIM5 (rs3740995 and rs11601507) gene and production of IFN-γ in response to MV stimulation; however, there were no subjects homozygous for the rs11601507 (V112F) AA genotype to observe a difference in IFN-γ secretion between the genotypes. One could speculate that these coding polymorphisms may affect TRIM5 protein structure and therefore antiviral function (IFN-γ production) in natural killer (NK) cells, CD4+ and CD8+ cytotoxic T-cells, and B-cells.

The limitations of our study include the relatively small sample of African-Americans and the potential for false-positive associations. We examined associations between 36 SNPs, seven measures of immune response, and across three racial groups, for 756 statistical tests. Hence, multiple testing issues are a legitimate concern, and the resulting modest false discovery rate q-values reflect some amount of uncertainty in the associations. Nevertheless, we observed a higher number of significant associations than we would anticipate by chance alone (45 vs. 38 significant associations assuming independent tests for association and a type I error rate of 0.05), suggesting that some of these associations are real. Due to the number of statistical tests run, corresponding q-values were modest; thus, additional independent studies are necessary to replicate our findings and to examine the functional consequences of these associations.

In conclusion, for the first time, we have shown that specific polymorphisms/haplotypes in the antiviral TRIM5, TRIM22 and TRIM25 genes **are associated with** variations in humoral and CMI responses to measles vaccine. Our findings point to polymorphisms in the innate antiviral TRIM genes as critical elements controlling the downstream adaptive immune responses to measles vaccine. Our data may also suggest racial genetic variations in immune responses to measles vaccine, which will require further studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MMR	measles/mumps/rubella
MV	measles virus
CMI	cell-mediated immunity
LD	linkage disequilibrium
SNP	single-nucleotide polymorphism
TRIM	tripartite motif
IQR	interquartile range
FDR	false discovery rate
EM	expectation-maximization
MAF	minor allele frequency
IFN	interferon
PBMC	peripheral blood mononuclear cells
SFC	spot-forming cells

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Table 1

Associations between SNPs in the TRIM genes and measles-specific humoral and IFN-γ Elispot immune responses.

Gene	SNP ID	Location/ Function	Genotype[a]	N[a]	Median (IQR)[b]	p value[c]	q value[d]
Antibody titer (mIU/ml)							
Combined cohort of subjects							
TRIM5	rs7122620	flanking_3UTR	AA AG GG	200 382 162	866 (391, 1685) 759 (402, 1762) 1086 (498, 1957)	0.0169	0.84
TRIM25	rs205499	intron	GG GA AA	465 236 36	931 (432, 1957) 787 (386, 1634) 599 (383, 1308)	0.0233	0.84
Caucasian subgroup							
TRIM5	rs7122620	flanking_3UTR	AA AG GG	168 313 117	874 (396, 1608) 776 (423, 1750) 1163 (515, 2383)	0.0126	0.75
African-American subgroup							
TRIM25	rs205499	intron	GG GA AA	54 27 8	1227 (329, 2536) 477 (232, 928) 512 (363, 1333)	0.0366	0.96
IFN-γ Elispot (SFC per 2 ×10^5 PBMC)							
Combined cohort of subjects							
TRIM22	rs2291841	flanking_5UTR	AA AC CC	598 100 9	34 (11, 66) 39 (21, 74) 35 (0, 105)	0.0260	0.67
Caucasian subgroup							
TRIM25	rs205498	coding/non-synonymous	AA AG GG	320 199 34	32 (12,68) 43 (20,79) 34 (13,66)	0.0259	0.71
TRIM22	rs2291841	flanking_5UTR	AA AC CC	477 82 4	35 (13, 69) 41 (22, 78) 102 (49,113)	0.0092	0.71
African-American subgroup							
TRIM5	rs12271333	intron	AA AC CC	61 24 2	26 (5, 47) 18 (4, 53) 21 (3, 39)	0.0214	0.49
TRIM25	rs205498	coding/non-synonymous	AA AG GG	50 30 5	37 (6, 52) 11 (3, 30) 15 (1, 27)	0.0254	0.51
TRIM25	rs2525996	intron	GG GC CC	65 20 2	32 (7, 52) 7 (2, 23) 15 (0, 29)	0.0019	0.37

Gene	SNP ID	Location/ Function	Genotype[a]	N[a]	Median (IQR)[b]	p value[c]	q value[d]
TRIM 25	rs2525992	intron	AA AG GG	63 20 4	29 (5, 52) 10 (3, 37) 19 (5, 28)	0.0034	0.37
TRIM 25	rs2525993	intron	AA AG GG	64 21 2	31 (7, 52) 9 (2, 26) 15 (0, 29)	0.0036	0.37
TRIM 25	rs2525994	intron	AA AC CC	57 28 2	33 (7, 52) 11 (3, 28) 15 (0, 29)	0.0096	0.45

A-Adenine, C-Cytosine, G-Guanine, FDR-false discovery rate, IFN-interferon, IQR-interquartile range, MMR-measles-mumps-rubella, SNP-single nucleotide polymorphism, **LD-linkage disequilibrium,** PBMC-peripheral blood mononuclear cells, **SFC-spot-forming cells.**

A total of 36 SNPs were examined; only those found to be statistically significant (P≤ 0.05) were included in the table.

Rs2525993, rs2525994 and rs2525996 are in LD (r^2=0.95).

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

[b]IQR values are median levels in mIU/ml, as measured by plaque reduction microneutralization assay, and at median levels in SFC per 2×10^5 PBMC as measured by IFN-γ Elispot.

[c]Test for trend P-value from the repeated measures linear regression analysis (for IFN-γ Elispot) and ordinary least squares regression analysis (for antibody levels). The P-values are adjusted for age, gender, age at 1st and 2nd MMR and cohort status using linear regression analysis.

[d]Corresponding q-values, adjusted for FDR.

Table 2

Associations between SNPs in the TRIM genes and measles-specific secreted cytokine immune responses.

Secreted cytokine	Gene	SNP ID	Location/ Function	Genotype[a]	N[a]	Median, pg/ml (IQR)[b]	p value[c]	q value[d]
IL-2	Combined cohort of subjects							
	TRIM5	rs7122620	flanking_3UTR	AA AG GG	198 378 163	38.3 (19.5, 64.4) 37.2 (19.8, 65.1) 37.8 (23.5, 60.8)	0.0152	0.55
	Caucasian subgroup							
	TRIM5	rs7122620	flanking_3UTR	AA AG GG	166 311 117	41.5 (21.1, 66.0) 41.9 (21, 67.0) 42.3 (26.6, 64.9)	0.0469	0.77
	African-American subgroup							
	None							
IL-6	Combined cohort of subjects							
	TRIM5	rs11820502	flanking_3UTR	CC CG GG	308 333 96	376.3 (254.3, 478.3) 351.3 (245.8, 454.6) 305.1 (237.4, 417.9)	0.0289	0.53
	Caucasian subgroup							
	TRIM5	rs7943397	flanking_3UTR	CC CG GG	212 277 102	340.7 (222.8, 458.2) 361.3 (269.6, 461.4) 363.4 (240.2, 458.3)	0.0323	0.99
	African-American subgroup							
	TRIM5	rs3824949	UTR	GG GC CC	8 31 49	379.8 (340.9, 542.7) 308.2 (233.4, 503.6) 318.4 (261.8, 403.1)	0.0037	0.29
	TRIM5	rs10769175	intron	GG GA AA	22 46 20	347.7 (295.5, 422.2) 340.9 (238.7, 461.5) 282.0 (12.9, 387.7)	0.0313	0.41
	TRIM5	rs7124435	intron	AA AG GG	71 15 2	340.2 (262.5, 440.6) 277.9 (124.0, 441.3) 191.7 (18.4, 364.9)	0.0351	0.44
IL-10	Combined cohort of subjects							
	TRIM25	rs2525996	intron	GG GC CC	427 273 40	17.0 (10.3, 28.4) 19.4 (12.1, 28.2)	0.0057	0.79

Secreted cytokine	Gene	SNP ID	Location/ Function	Genotype[a]	N[a]	Median, pg/ml (IQR)[b]	p value[c]	q value[d]
						20.0 (14.3, 35.4)		
	TRIM25	rs2525992	intron	AA AG GG	426 272 41	17.0 (10.3, 28.5) 19.6 (12.3, 28.2) 19.0 (14.2, 29.7)	0.0095	0.79
	TRIM25	rs2525993	intron	AA AG GG	428 273 39	17.0 (10.3, 28.2) 19.5 (12.3, 28.3) 19.0 (14.2, 32.3)	0.0097	0.79
	TRIM25	rs2525994	intron	AA AC CC	419 281 39	17.0 (10.4, 28.4) 19.4 (12.1, 28.3) 19.0 (14.2, 32.3)	0.0108	0.81
	TRIM25	rs205498	coding	AA AG GG	413 273 40	17.4 (10.5, 28.5) 19.4 (12.1, 28.3) 18.5 (13.9, 31.0)	0.0235	0.91
Caucasian subgroup								
	TRIM25	rs2525996	intron	GG GC CC	335 222 37	17.5 (11.7, 29.0) 19.6 (12.3, 28.2) 21.6 (14.5, 38.5)	0.0328	0.74
	TRIM25	rs205498	coding	AA AG GG	338 211 34	17.6 (11.5, 28.9) 19.9 (12.3, 28.4) 21.8 (14.9, 38.5)	0.0433	0.78
African-American subgroup								
	TRIM22	rs885002	flanking_5UTR	GG GA AA	70 17 1	11.7 (7.4, 21.2) 18.3 (8.5, 30.6) 200.4 (200.4, 200.4)	0.0202	0.51
	TRIM25	rs9909750	intron	GG GA AA	61 23 4	11.0 (7.4, 19.2) 18.3 (9.5, 35.8) 23.9 (12.7, 29.9)	0.0300	0.51
	TRIM25	rs2525992	intron	AA AG GG	64 20 4	12.8 (7.6, 25.9) 13.3 (7.3, 24.9) 15.7 (11.4, 21.0)	0.0356	0.52
	TRIM25	rs7225205	intron	GG GA AA	53 28 7	11.0 (7.0, 19.2) 15.2 (8.6, 33.5) 21.2 (12.3, 33.1)	0.0357	0.52
	RIM25	rs2525996	intron	GG GC CC	66 20 2	12.8 (7.8, 23.9) 13.2 (7.3, 31.8) 15.7 (13.3,	0.0417	0.53

Secreted cytokine	Gene	SNP ID	Location/ Function	Genotype[a]	N[a]	Median, pg/ml (IQR)[b]	p value[c]	q value[d]
						18.1)		
	TRIM25	rs2525997	intron	AA AC CC	65 22 1	13.6 (8.2, 25.6) 8.8 (5.1, 20.6) 144.9 (144.9, 144.9)	0.0428	0.53
	TRIM25	rs2525993	intron	AA AG GG	65 21 2	12.8 (7.8, 23.9) 12.1 (7.4, 28.6) 15.7 (13.3, 18.1)	0.0478	0.54
IFN-γ	Combined cohort of subjects							
	TRIM25	rs169530	intron	GG GA AA	499 219 19	71.8 (35.3, 125.1) 60.7 (35., 108.1) 30.2 (14.8, 108.8)	0.0495	0.57
Caucasian subgroup								
	TRIM25	rs169530	intron	GG GA AA	383 191 18	75.0 (38.0, 130.6) 60.7 (35.1, 108.1) 36.9 (14.8, 108.8)	0.0085	0.26
	TRIM25	rs205499	intron	GG GA AA	363 198 26	74.2 (37.3, 126.6) 64.9 (35.7, 115.2) 42.5 (18.9, 108.8)	0.0345	0.46
African-American subgroup								
	TRIM25	rs2525997	intron	AA AC CC	65 22 1	60.6 (30.2, 115.4) 56.9 (19.6, 65.1) 205.4 (205.4, 205.4)	0.0060	0.36
	TRIM25	rs2525998	flanking_3UTR	GG GA AA	30 44 14	52.1 (30.2, 116.5) 60.7 (26.8, 104.3) 60.4 (19.6, 84.6)	0.0341	0.56
	TRIM5	rs3740994	intron	AA AC CC	69 19 0	57.3 (26.0, 103.4) 60.6 (42.5, 105.2) ---	0.0175	0.55
	TRIM5	rs3740995	coding	GG GA AA	51 34 3	60.1 (30.1, 103.4) 59.8 (19.0, 105.2) 6.2 (−211.9, 457.5)	0.0205	0.55
	TRIM5	rs11601507	coding	CC CA AA	86 2 0	59.5 (26.1, 103.4) 118.4 (17.9, 219) ---	0.0437	0.56

Secreted cytokine	Gene	SNP ID	Location/ Function	Genotype[a]	N[a]	Median, pg/ml (IQR)[b]	p value[c]	q value[d]
TNF-α	Combined cohort of subjects							
	TRIM22	rs885002	flanking_5UTR	GG GA AA	668 61 3	13.5 (9.2, 18.8) 13.9 (9.1, 18.8) 47.0 (7.8, 71.2)	0.0287	0.92
Caucasian subgroup								
	TRIM5	rs7124435	intron	AA AG GG	561 24 2	13.7 (9.5, 18.8) 15.2 (11.8, 20.0) 27.4 (7.8, 47.0)	0.0327	1
	TRIM22	rs885002	flanking_5UTR	GG GA AA	550 35 2	13.7 (9.5, 18.8) 15.3 (9.8, 20.0) 27.4 (7.8, 47.0)	0.0405	1
African-American subgroup								
	None							

---no subject for that genotype, A-Adenine, C-Cytosine, G-Guanine, FDR-false discovery rate, IQR-interquartile range, MMR-measles-mumps-rubella, SNP-single nucleotide polymorphism, **LD-linkage disequilibrium.**

A total of 36 SNPs were examined; only those found to be statistically significant (P≤ 0.05) were included in the table.

Rs2525993, rs2525994 and rs2525996 are in LD (r^2=0.95).

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

[b]IQR, interquartile range, values are median levels in pg/ml as measured by Elisa.

[c]Test for trend P-value from the analysis of covariance adjusting for age, gender, race and age of immunization. The P-values are adjusted for age, gender, race (when necessary), age at 1st and 2nd MMR and cohort status using repeated measures linear regression analysis.

[d]Corresponding q-values, adjusted for FDR.

Table 3

TRIM25 gene haplotype associations with IFN-γ Elispot response to measles vaccine in African-Americans.

Locus TRIM25 haplotype	Allele[a] AAAGGAAAGGAGT	Frequency 0.148	Test statistic (haplotype *t* statistic) −2.32	Allele p value[b] 0.023	Global p value 0.028
	AACGGAAAGGAGT	0.100	1.52	0.133	
	AGAGGAAAAAAGT	0.106	1.66	0.102	

[a]TRIM25 genetic variants from left to right: rs11540270, rs2525998, rs2525997, rs205499, rs2525996, rs2525994, rs2525993, rs205498, rs7225205, rs9909750, rs2525992, rs169530, rs11869863.

Statistically significant p values (P≤0.05) are highlighted in bold.

Haplotype effects are estimated using the haplotype t-statistic, which reflects the direction and relative magnitude of the estimated haplotypic effect on the cytokine measure. Allele P-values compare individual haplotypes to all other haplotypes combined.

[b]One degree-of-freedom ordinal p value from the repeated measures regression analysis adjusting for age, gender, race, age at 1st and 2nd MMR vaccine and cohort status.