Polymorphisms in the Vitamin A Receptor and Innate Immunity Genes Influence the Antibody Response to Rubella Vaccination

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Background. Genetic polymorphisms play an important role in rubella vaccine-induced immunity.

Methods. We genotyped 714 healthy children after 2 age-appropriate doses of rubella-containing vaccine for 142 potential single-nucleotide polymorphisms (SNPs).

Results. Specific polymorphisms in the vitamin A receptor, retinoic acid-inducible gene I (RIG-I), and tripartite motif 5 and 22 (TRIM5 and TRIM22) genes were significantly associated with rubella vaccine humoral immunity. The minor allele of the rs4416353 in the vitamin A receptor gene was associated with an allele dose–related decrease in rubella antibody response. The minor allele of rs6793694, in the vitamin A receptor gene, was associated with an allele dose–related antibody decrease (P = .019). The minor variant of nonsynonymous SNP rs10813831 (Arg7Cys) in the RIG-I gene was associated with an allele dose–related decrease in rubella antibody level from 37.4 to 28.0 IU/mL (P = .039). The minor variant of nonsynonymous SNP rs3824949 (P = .015) in the antiretroviral TRIM5 gene was associated with an allele dose–related increase in rubella antibody. It is of particular interest that the nonsynonymous SNP rs3740996 (His43Tyr) in the TRIM5 gene was associated with variations in rubella antibody response (P = .016) after having been previously found to play a significant functional role.

Conclusions. These findings further expand our immunogenetic understanding of mechanisms of rubella vaccine–induced immunity.

Rubella epidemics occasionally occur in developing countries, and both rubella virus infection and congenital rubella syndrome remain major health concerns around the world. Although the importance of sustaining protective rubella humoral immunity is widely recognized, the host genetic influence on rubella vaccine–induced immunity remains incomplete. A previous study estimated the heritability for antibody response to rubella to be 46%, with a 1-sided 95% confidence interval lower limit of 4.9% [1].

Our group and others have shown that polymorphisms at the human leukocyte antigen (HLA) loci are associated with significant variations in immune responses to rubella vaccine, such as antibody level, lymphocyte proliferation, and cytokine production [2–6]. Studies with other viral vaccines, such as measles and mumps vaccines, have also demonstrated associations between cytokine and cytokine receptor gene polymorphisms and immune responses [7, 8]. The regulatory complexity of the human immune system ensures a high probability of functional redundancy in both cell-mediated and humoral immune responses [9, 10]. This is also true for loci/polymorphisms beyond those regulating HLA and cytokine and cytokine receptor groups. Several new genes (and molecules) have
Table 1. Characteristics of the Study Population and Their Associations with Rubella-Specific Immunoglobulin G Antibody Levels

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of subjects</th>
<th>Mean ± SD, IU/mL</th>
<th>Median (IQR), IU/mL</th>
<th>P*</th>
</tr>
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<tbody>
<tr>
<td>Total</td>
<td>714</td>
<td>49.8 ± 45.5</td>
<td>34.5 (19.2–63.7)</td>
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<td>Age at enrollment, years</td>
<td></td>
<td></td>
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<tr>
<td>11–13</td>
<td>212</td>
<td>50.1 ± 44.2</td>
<td>37.0 (19.1–67.1)</td>
<td>.967</td>
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<tr>
<td>14–15</td>
<td>190</td>
<td>50.9 ± 51.2</td>
<td>34.8 (19.4–58.5)</td>
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<tr>
<td>16–17</td>
<td>200</td>
<td>49.9 ± 44.1</td>
<td>33.3 (19.2–70.2)</td>
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<td>18–19</td>
<td>112</td>
<td>47.3 ± 40.7</td>
<td>34.5 (19.2–57.9)</td>
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<td>Age at first rubella vaccination, months</td>
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<tr>
<td>≤14</td>
<td>89</td>
<td>47.2 ± 38.4</td>
<td>35.4 (19.6–59.9)</td>
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<td>15</td>
<td>384</td>
<td>49.1 ± 47.0</td>
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<td>16–17</td>
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<td>&gt;18</td>
<td>122</td>
<td>54.1 ± 50.8</td>
<td>39.9 (21.8–66.2)</td>
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<td>Age at second rubella vaccination, years</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>27.6 (16.6–55.3)</td>
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<td>&gt;12</td>
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<td>56.0 ± 52.7</td>
<td>39.9 (22.1–69.7)</td>
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<tr>
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<td>49.5 ± 44.8</td>
<td>33.4 (17.7–62.9)</td>
<td>.903</td>
</tr>
<tr>
<td>White</td>
<td>649</td>
<td>49.8 ± 45.6</td>
<td>34.5 (19.2–63.7)</td>
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</tbody>
</table>

NOTE. IQR, interquartile range; SD, standard deviation.

* P value represents a test of the null hypothesis that the means of the categories are all equal versus the alternative hypothesis that at least 2 of the categories differ, with comparisons performed via analysis of variance on rubella antibody levels on the logarithmic scale.

been identified as candidate immune response genes. Studies in a variety of other models (viruses, bacteria, and microbial antigens) have recently demonstrated the importance of innate and vitamin receptor genes in regulating immune responses [11–13]. In this regard, the discovery of genetic variations caused by single-nucleotide polymorphisms (SNPs) has led to population-based immunogenetic studies intended to elucidate the potential relationship between host genomic variation and immune response [14].

In the present study, we investigated whether the humoral immune response to 2 doses of rubella vaccination is associated with polymorphisms of non-HLA candidate immune response genes, such as the retinoic acid (vitamin A) receptor and the vitamin D receptor (VDR), the innate immunity toll-like receptors (TLRs), the antiretroviral tripartite motif-containing (TRIM) factors, and the retinoic acid–inducible gene I (RIG-I) pathway genes.

SUBJECTS, MATERIALS, AND METHODS

Study subjects. The study cohort of 738 healthy children and young adults (age, 11–19 years) from Olmsted County in Minnesota has been described elsewhere [15]. All study subjects were immunized with 2 age-appropriate doses of live mea-sles-mumps-rubella (MMR-II) vaccine containing the Wistar RA 27/3-strain of rubella virus. The majority of the study population was white (91%), with 47% being female, and a median age at enrollment of 15 years (Table 1). The median ages at the first and second immunizations were 15 months and 11 years, respectively, and the median time between last rubella immunization and sample draw was 5.8 years. The participants lived in a community where no case of rubella infection had been reported during their lifetimes. Although 738 children and young adults were enrolled in the study, genotyping data were available for only 714 subjects. The Mayo Clinic Institutional Review Board granted approval for the study, and written informed consent (parental permission and assent for minors) was obtained.

Humoral immunity assays. Rubella-specific immunoglobulin G (IgG) antibodies after 2 doses of the rubella vaccine were detected in serum by a whole rubella virus–specific chemiluminescent immunoassay (Beckman Coulter Access) according to the manufacturer’s instructions. The limit of detection for this assay was 0.5 IU/mL, and the coefficient of variation in our laboratory was 6%.
TagSNP selection. We selected tagSNPs from innate immune response candidate genes ($n=12$) belonging to the vitamin A family (retinoic acid receptor $\alpha$, $\beta$, and $\gamma$ [RARA, RARB, and RARG] and retinoid X receptor $\alpha$ [RXRA]), VDR, TLR family (TLR3 and TLR4), RIG-I pathway (DDX58, CASP10, and VISA) and cellular antiretroviral TRIM factors (TRIM5 and TRIM22). The details of SNP selection were described elsewhere [16]. Briefly, we used a linkage-disease equilibrium (LD) tagSNPs selection approach [17] to generate a list of SNPs within and 10 kb upstream and downstream of these 12 genes using the Hapmap Phase II (http://www.hapmap.org), Seattle SNPs (http://pga.mbt.washington.edu/) and NIEHS SNPs (http://egp.gs.washington.edu/) as source databases. We included SNPs that had validation data, successful predictive genotyping scores for Illumina GoldenGate assays, a minor allele frequency (MAF) $\geq 0.05$, and a pairwise LD threshold of $r^2 < 0.90$ for whites. We selected 142 potential SNPs in our candidate genes of interest using the ldSelect algorithm. We used the nomenclature described by den Dunnen and Antonarakis [18] for all genotype variants.

Genotyping methods. Our genotyping methods were described in detail elsewhere [7]. Briefly, genomic DNA samples (738 samples, 250 ng each) obtained from frozen blood clots using the Puregene extraction kit (Genta Systems) were genotyped for 142 candidate SNPs using a custom designed 768-plex Illumina GoldenGate assay (Illumina), following the manufacturer’s instructions [16]. All the SNPs selected for the custom Illumina panel had design scores $\geq 0.4$. A Corriel Trio DNA (mother, NA11875; father, NA10859; daughter, NA10858) and 2 other genomic DNA controls were used as standards to review and refine clustering. These controls were genotyped on each plate, which allowed us to assess genotyping concordance of replicate subjects.

Illumina 10% GenCall scores $>0.4$ and call rates $>90\%$ were used as thresholds for the initial laboratory quality control. The data from genotype calls made by BeadStudio 2 software were transferred to SAS software for additional analysis. Our overall genotyping success rate for the Illumina 768-plex platform and Taqman platform was 94.53%. The study sample success rate was 96.75%. SNP-specific deviation from Hardy-Weinberg Equilibrium (HWE) was tested, and we excluded any SNP that displayed violations of HWE ($P<.001$). Subject exclusions were made on the basis of DNA quality ($n=6$), complete genotyping failure on both platforms ($n=4$), and low call rates below 95% ($n=14$), leaving a total of 714 subjects for the study.

We used PCR-based TaqMan assays (Applied Biosystems) as the secondary platform to genotype SNPs ($n=6$) that failed genotyping on the Illumina platform. All assays were performed according to the manufacturer’s instructions, and the results were analyzed on the ABI Prism 7900 using Sequence Detection software (Applied Biosystems). Of these 6 SNPs, 1 also failed Taqman genotyping, and 4 were excluded because the minor allele frequency was $\leq 5\%$. This resulted in 137 SNPs available for analysis in 714 subjects.

Statistical methods. The purpose of the efforts reported here was to assess associations between genetic variation in candidate SNPs and rubella antibody levels (measured in international units per milliliter). Characteristics of the study participants were categorized, and the numbers of subjects in these categories were tabulated, along with summaries of rubella antibody levels. We compared the mean rubella antibody levels, transformed on the logarithmic scale, among the various classes of these descriptive variables using linear models methodologies (analysis of variance). Participants’ genotypes were used to estimate allele frequencies for each SNP of interest. We assessed departures from HWE, using a Pearson goodness-of-fit test or, for SNPs with a minor allele frequency of $<5\%$, a Fisher exact test [19].

Individual SNP associations with antibody levels were formally evaluated using linear models approaches. Primary tests of association assumed no specific genetic model of the potential action of the SNPs on rubella antibody levels, although the degree to which the SNP’s association appeared to act in a dose-response fashion was also examined. Analyses were adjusted for the following set of covariates potentially associated with immune response: age at enrollment, race, gender, age at first rubella vaccination, age at second rubella vaccination, and cohort status. Due to data skewness, original antibody values were replaced with corresponding log-transformed values in all the linear regression models. All statistical tests were 2-sided, and unless otherwise indicated, all analyses were performed using the SAS software system (SAS Institute).

RESULTS

We performed a population-based study to investigate the contribution of polymorphisms in vitamin receptor and innate immunity genes to variations in antibody responses after rubella vaccination. A total of 8 significant SNP associations ($P<.05$) (in 4 of 12 candidate genes) were identified between the RARB (vitamin A), RIG-I (DDX58), and TRIM (TRIM5 and TRIM22) genes and humoral immunity after rubella vaccine (Table 2). These associations were identified while adjusting for several potential confounders, including gender and age at first and second vaccinations. Results were similar without adjusting for these covariates (data not shown). Of these, 3 SNPs were located in coding and regulatory regions of the genes corresponding to the RIG-I and TRIM5 molecules.

We found significant associations ($P = .019–.034$) between 3 intronic SNPs located in the retinoic acid receptor gene on chromosome 3 (3p24) and measures of rubella vaccine humoral immunity. Increased representation of the minor allele of an
intrinsic SNP (rs4416353) in the RARβ gene was associated with an allele dose–related decrease ($P = .019$) in rubella-specific antibody response (Table 2). Similarly, the minor allele of a SNP (rs6793694) in the RARβ gene was also associated with an allele dose–related decrease ($P = .039$) in rubella-induced antibody levels. In addition, an intronic SNP was significantly associated with variation in antibody levels; however, no allele dose–related association with rubella antibody levels was observed.

We also analyzed the relationship between SNPs belonging to the retinoic acid–inducible gene I (RIG-I/DDX58 gene) on chromosome 9 that encodes a pattern recognition receptor protein-RNA helicase, and rubella humoral immune responses. Increased representation of the minor allele of a nonsynonymous SNP in exon 1 of the RIG-I CARD domain (rs10813831, Arg$^*$Cys at amino acid 7) was associated with an allele dose–related decrease ($P = .035$) in rubella antibody response from 37.4 IU/mL (0 copies) to 28.0 IU/mL (2 copies). For another RIG-I SNP (rs669260, $P = .048$), the increased representation of the minor allele was associated with an allele dose–related increase in antibody response. None of the SNPs identified as associated with rubella antibody responses in RIG-I gene were found to be in LD.

Genotypes for SNPs from the antiretroviral TRIM5 and TRIM22 genes on chromosome 11 (11p15) were also analyzed, and 2 significantly associated SNPs located in the 5′UTR and coding regions of the TRIM5 gene are shown in Table 2. Increased representation of the minor allele of the 5′UTR SNP (rs3824949, $P = .015$) in the antiretroviral TRIM5 gene was associated with an allele dose–related increase in rubella antibody response from 31.5 IU/mL (0 copies) to 40.8 IU/mL (2 copies). The TRIM5 A/G nonsynonymous polymorphism (rs3740996, $P = .016$) in exon 2 (loop 2 region of the RING domain, leading to His$^*$Tyr change at amino acid 43) was associated with variations in rubella antibody level, although without a clear allele dose–related effect. Finally, increased representation of the minor allele of an intronic SNP (rs2179) in the TRIM22 gene was associated with an allele dose–related decrease ($P = .039$) in rubella-specific antibody response in our study subjects.

None of the SNPs identified as associated with rubella humoral immunity in the RARB, RIG-1, and TRIM5 genes were found to be in LD with one another. No significant associations were found with polymorphisms in the TLR family (TLR3 and TLR4), RIG-1 pathway (CASP10 and VISA), vitamin family (RARA, RARG, and RXRA), or VDR genes.

**DISCUSSION**

Because of the multigenic nature of immune responses to complex antigens, most genes that are important in influencing immune responses to vaccination are still unknown. In this study we examined the association of polymorphisms in vitamin A receptor, vitamin D receptor, and innate immune response genes to rubella vaccine–induced immunity. We assessed these candidate genes in the largest rubella vaccine immunogenetics study, to our knowledge, yet reported.
We previously reported that sex was a strong influence on antibody responses after MMR-II vaccine [4, 8]. In fact, in our study, girls demonstrated significantly higher rubella antibody levels than boys (median antibody level, 39.9 vs 30.9 IU/mL; P = .007). A better understanding of the factors that influence sex differences in the innate and adaptive immune responses to vaccines may lead to the identification of immune-related genes and pathways as targets for the development of more uniformly immunogenic vaccines.

The role played by vitamin and vitamin-receptor genes in rubella vaccine–specific immunity is still unclear. Retinoic acid, the biologically active form of vitamin A, has hormone-like properties and can affect both innate and adaptive immune responses [20]. Recent studies have demonstrated that vitamin A has an immunomodulatory role and can influence lymphocyte functions, such as T and B cell proliferation, T cell activation, cytotoxicity, and apoptosis [20]. Vitamin A can also activate monocytes, stimulate antigen presentation and cellular immune responses, suppress immunoglobulin production, lymphocyte proliferation, and modulate cytokine production [21, 22]. Dietary supplementation with vitamin A to children has been used to improve antibody responses to several vaccines, including measles, tetanus, diphtheria, and polio [22–25]. Benn et al [26] demonstrated that vitamin A supplementation with measles vaccine in West Africa at 9 months of age resulted in higher levels of measles-specific antibodies in children (especially in boys) at 18 months of age. Furthermore, concurrent administration of vitamin A and measles vaccine at 9 months of age had a lasting effect on measles-specific antibody concentrations [25]. No information is available regarding the impact of vitamin A on rubella vaccine–induced antibody levels.

We demonstrated that the presence of specific genetic variations in the RARB gene and a member of the thyroid-steroid hormone receptor superfamily of nuclear transcriptional regulators was associated with rubella-specific antibody responses. The RARB receptor binds retinoic acid, and this gene was first identified in hepatocellular carcinoma, where it surrounds a site of integration of hepatitis B virus [27]. Importantly, in our study, an allele dose–related decrease in rubella antibody response was observed with increased representation of the minor alleles for SNPs rs4416353 and rs6793694. Additional work will be needed to elucidate the exact mechanisms by which vitamin A receptor gene variations influence rubella vaccine-induced humoral immunity.

A large amount of work has been done investigating the effects of genes on innate immunity, including the discovery of pathogen recognition receptors and pathways [28]. A novel pathway of TLR-independent response to pathogens was described with the finding of RIG-like helicase proteins [29]. For example, interferon α/β (IFN-α/β) production in infected cells is important for resistance to viral infection and can be triggered through the cytoplasmic RNA helicase retinoic acid–inducible gene I (RIG-I) in a TLR-independent way [30, 31]. Known by its intracellular antiviral properties, it has been demonstrated that RIG-I is essential in triggering the host response to hepatitis C virus, influenza viruses, and paramyxoviruses [31, 32]. Our data show significant associations between coding (rs10813831) and intronic (rs669260) SNPs in the RIG-I (DDX58, DEAD [Asp-Glu-Ala-Asp] box polypeptide 58) gene, and rubella vaccine–specific antibody response in an allele dose–related manner. These 2 SNPs had an opposite effect on rubella antibody levels. For example, a nonsynonymous SNP located in exon 1 (rs10813831) of the RIG-I gene, leading to an amino acid change of arginine to cysteine at position 7, was associated with an allele dose–related decrease in rubella IgG level. On the other hand, the intronic SNP (rs669260) in the RIG-I gene was associated with an allele dose–related increase in rubella antibodies. The discovery of allele dose–response relationships for rs10813831 and rs669260 more strongly suggests evidence of a functional role of these SNPs, or of SNPs in high LD with them. Interestingly, the same nonsynonymous RIG-I SNP, rs10813831, was shown to be of functional importance and to influence the innate immune response to Newcastle disease viral infection in human dendritic cells by potentially affecting RIG-I folding or interaction with the mitochondrial antiviral signaling protein [33]. Although SNPs found in coding regions clearly could have functional impact, intronic SNPs may also alter the binding site of a transcription factor in an intronic region of the gene [34]. These data suggest that the innate immune response to viral infection (or live viral vaccination) may be influenced by a functional polymorphism in the RIG-I (DDX58) gene.

Recently, 2 groups [35, 36] reported the results of an analysis of gene expression in human fetal and adult fibroblasts and endothelial cells infected with rubella virus. Importantly, the retinoic acid receptor α (RARA), VDR (1.25-dihydroxyvitamin D3), DDX58, and TRIM5 genes were found to be upregulated by 3.20-, 3.16-, 19.76- and 3.37-fold, respectively, in fetal umbilical vein endothelial cells infected with rubella virus. The DDX58 gene also exhibited a 14.23-fold upregulation in rubella virus–infected adult Hs888Lu cells. Likewise, the TRIM22 gene was upregulated in both rubella-infected fetal and adult fibroblasts and endothelial cells [35, 36]. These observations cumulatively demonstrate how rubella virus infection alters host cells and offer functional biologic insight into our findings.

The antiviral activity and retroviral restriction of molecules within the TRIM family proteins has been previously recognized. Both TRIM5 and TRIM22 proteins are members of the TRIM family. TRIM5α has been identified as an important cellular antiretroviral restriction factor [37, 38]. It has been proposed that TRIM5α might neutralize retroviruses through...
a ubiquitin-mediated pathway requiring the RING finger motif [39]. TRIM22 is an E3 ubiquitin ligase that emerged as an IFN-induced protein involved in both innate and adaptive immunity; as a result, its expression leads to antiviral properties [40]. It has been speculated that polymorphisms in human TRIM5 genes may influence susceptibility to human immunodeficiency virus type 1 (HIV-1) infection [41]. However, data on TRIM5 protein’s activity against HIV in humans are lacking. We identified 2 nucleotide polymorphisms in the TRIM5 gene 5’UTR (rs3824949) and coding (rs3740996) regions, one of which (rs3824949) was associated with an allele dose–related increase in rubella antibody levels. Additionally, in our study, rs2179 in the TRIM22 gene appeared to be an important SNP that was significantly associated with lower rubella-specific antibody levels in an allele dose–related manner. Speelman et al [42] demonstrated that the same G to C polymorphism at position –2 in the 5’UTR of the TRIM5 gene, rs3824949 (in combination with a nonsynonymous SNP Arg136Gln), was associated with enhanced HIV-1 susceptibility and disease progression. Thus, this nonsynonymous coding polymorphism, rs3824949, emerged as a key SNP that was significantly associated with higher rubella specific–antibody response and also had an effect on human HIV-1 infection. We speculate that polymorphisms in the TRIM5 gene may possibly contribute to the overall human antiviral response.

The associations between TRIM5 polymorphisms and rubella vaccine–induced immunity are particularly intriguing, because a nonsynonymous SNP (rs3740996, His43Tyr) at the human TRIM5α locus has been found to have a significant functional consequence [39]. Sawyer et al [39] reported that this polymorphism lies in the RING loop 2 domain of the TRIM5α gene, may negatively affect E3 ubiquitin ligase activity, and may impair TRIM5α restriction of 2 retroviruses. The importance of this specific SNP with regard to the clinical course of HIV-1 infection was demonstrated by a recent study showing that an accelerated disease progression was observed for subjects who were homozygous for the 43Tyr genotype, compared with disease progression for subjects who were heterozygous or homozygous for the 43His genotype [43]. We found that the same TRIM5 nonsynonymous polymorphism in exon 2 (rs3740996, His43Tyr) was associated with variations in rubella-specific IgG antibody level, suggesting the functional importance of the TRIM5 gene in rubella vaccine–induced humoral immunity.

In conclusion, our data suggest significant associations between polymorphisms in the vitamin A receptor, RIG-I and antiretroviral TRIM innate immunity genes, and antibody responses to rubella vaccine. It is likely that other innate defense and immune regulation genes also contribute to the immunogenetic influence on rubella immunity. In this first study, to our knowledge, of genetic associations of candidate genes that are involved in vitamin A processing, we have studied a large number of SNPs and have identified a number of SNPs that appear to be associated with rubella vaccine–induced antibody levels. We have not performed corrections for these multiple tests because of the statistical power implications for analyses based on 714 subjects. Therefore, it is important that the results reported here be followed up in additional studies to validate these associations and further clarify the genetic contributions of these candidate genes to rubella vaccine–induced antibody levels. These findings require additional validation in an independent cohort. Understanding the mechanism by which vitamin A receptor and innate gene polymorphisms alter immune responses may provide important insights into new ways of modulating not only innate immune response but adaptive immune responses as well, and they may have implications for developing new vaccine adjuvants and immunostimulant molecules. This information might be used in future applications to improve human vaccine responses and to develop personalized vaccines [14].

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References


