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OAS single-nucleotide polymorphisms and haplotypes are associated with variations in immune responses to rubella vaccine

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Abstract

Interferon (IFN)-induced antiviral genes are crucial players in innate antiviral defense and potential determinants of immune response heterogeneity.

We selected 114 candidate SNPs from 12 antiviral genes using an LD tagSNP selection approach and genotyped them in a cohort of 738 schoolchildren immunized with two doses of rubella vaccine. Associations between SNPs/haplotypes and rubella virus-specific immune measures were assessed using linear regression methodologies.

We identified 23 significant associations ($p<0.05$) between polymorphisms within the 2′-5′-oligoadenylate synthetase (*OAS*) gene cluster, and rubella virus-specific IL-2, IL-10, IL-6 secretion and antibody levels. The minor allele variants of three *OAS1* SNPs (rs3741981/Ser162Gly, rs1051042/Thr361Arg, rs2660), located in a linkage disequilibrium block of functional importance, were significantly associated with an increase in rubella virus-specific IL-2/Th1 response ($p\leq0.024$). Seven *OAS1* and *OAS3* promoter/regulatory SNPs were similarly associated with IL-2 secretion. Importantly, two SNPs (rs3741981 and rs10774670), independently cross-regulated rubella virus-specific IL-10 secretion levels ($p\leq0.031$). Furthermore, both global tests and individual haplotype analyses revealed significant associations between *OAS1* haplotypes and rubella virus-specific cytokine secretion.

Our results suggest that innate immunity and *OAS* genetic variations are likely involved in modulating the magnitude and quality of the adaptive immune responses to live attenuated rubella vaccine.

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Keywords

Single Nucleotide Polymorphism; Haplotype; 2′,5′-Oligoadenylate Synthetase; Rubella vaccine; Immunity

1. Introduction

Significant research efforts have attempted to elucidate the importance and influence of immune response gene polymorphisms on infectious disease susceptibility and heterogeneity of humoral, cell-mediated, and even innate immune responses to vaccines at the individual and population level [1-4]. Our previous studies revealed associations between single-nucleotide polymorphisms (SNPs) in innate immune genes, such as toll-like receptors (TLRs) and related signaling molecules, the interferon (IFN) β gene (*IFNB1*), and type I IFN receptor gene *IFNAR2*, and immune responses to measles and rubella vaccines [5,6].

Innate immunity is the first line of defense against viral pathogens, playing an essential role in viral sensing, immediate control of viral replication and spread, and initiation and modulation of adaptive immunity. The biological effects of IFNs are determined primarily by a set of transcriptionally activated IFN-stimulated genes (ISG). A repertoire of antiviral factors and pathways, such as dsRNA-activated protein kinase R (PKR also known as EIF2αK2), the 2′-5′-oligoadenylate-synthetase (OAS) - ribonuclease L (RNaseL) pathway, Mx protein GTPases, adenosine deaminase, RNA-specific 1(ADAR1), the ISG15 ubiquitin-like pathway, 3′-5′ exonuclease ISG20 and others, play an important role in the outcome of viral infection [7-10]. The direct importance of these antiviral effectors in humans is supported by earlier genetic studies showing that polymorphisms in the *MxA*, *OAS1, OASL* and *PKR* genes correlate with response to IFN therapy and/or susceptibility to HCV, HBV, measles virus, WNV and SARS-coV [11-18].

Not studied in the context of viral vaccine immunity is the genetic diversity of antiviral effector genes that might contribute to the heterogeneity of vaccine-induced immune response. Our study aimed to evaluate host antiviral IFN-stimulated molecules and IFN-related transcription factors likely to be involved in controlling initial viral replication and in priming and shaping the adaptive immune response to live attenuated vaccines.

2. Materials and Methods

Study population

The study cohort was a large population-based, age-stratified random sample of 738 healthy children and young adults (aged 11 to 19 years), consisting of two independent random cohorts (342 and 396 subjects) from Olmsted County, Minnesota with clinical and demographic characteristics previously reported [19]. All subjects resided in a community where no cases of rubella infection had been reported during their lifetimes. All study participants had been previously immunized with two doses of MMR-II vaccine, containing the Wistar RA 27/3-strain of rubella virus. The Mayo Clinic Institutional Review Board granted approval for the study. Written, informed consent and assent (from minors) from subjects and/or parents/guardians was obtained at the time of enrollment.

Immune measures

Rubella-specific IgG antibody levels were determined using the Beckman Coulter Access® Rubella IgG assay (Beckman Coulter; Fullerton, CA). Antibodies levels were determined from a multi-point calibration curve standardized against the WHO reference serum with a limit of

detection of 0.5 IU/mL, a cut-off for seronegativity of 10 IU/mL (a cut-off for seropositivity of 15 IU/mL, equivocal 10-15 IU/mL) and a coefficient of variation (CV) of 6% in our laboratory. Human IFN-γ Elispot assays (R & D Systems, Minneapolis, MN, USA) and IL-10 Elispot assays (BD Biosciences, San Diego, CA, USA) were performed in PBMC cultures in triplicate after stimulation with the W-Therien strain of rubella virus and compared to unstimulated measures (also in triplicate), as previously described [20,21] and following the manufacturer's protocol. Rubella-specific secreted cytokines (IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IFN-γ, TNF-α and GM-CSF) were quantified by ELISA in PBMC cultures (unstimulated and stimulated measures in triplicate) after stimulation with rubella virus using pre-optimized conditions for time and MOI for the different cytokines [19].

Candidate genes and SNP selection

Twelve genes encoding IFN-induced antiviral effectors (n=9; MX1, MX2, OAS1, OAS2, OAS3, RNASEL, EIF2AK2/PKR, ADAR, ISG20) and key IFN regulatory factors (n=3; *IRF3, IRF7, IRF9/ISGF3G*) were identified from literature searches and public databases and included as part of our ongoing population genetics study on rubella vaccine response. SNPs within the 12 candidate genes, 10 kb upstream and downstream for each gene, were selected based on the LD tagSNP selection algorithm [22] from the Hapmap Phase II (http://www.hapmap.org), Seattle SNPs (http://pga.mbt.washington.edu/) and NIEHS SNPs (http://egp.gs.washington.edu/). We applied the ldSelect program [22] on each gene for each genotype source for the Caucasian samples in those public sources, to bin SNPs with a minor allele frequency (MAF) ≥0.05, a pairwise linkage disequilibrium (LD) threshold of r^2 ≥0.90, and successful Illumina predictive genotyping scores. We then used the SNPPicker program to post-process and refine the selection of computed tagSNPs in order to accommodate a set of platform-dependent design constraints. In addition, a list of putative functional "obligate" SNPs (coding: nonsynonymous, synonymous, 5′ or 3′ untranslated regions) with a MAF ≥0.05 was provided to SNPPicker to choose in preference to other tagSNPs or add to the final list. A total of 114 SNPs were selected based on this approach. The nomenclature used for the description of the variants follows that described by den Dunnen and Antonarakis [23].

Genotyping methods

One hundred and fourteen SNPs from the 12 candidate antiviral genes were included in the custom Illumina GoldenGate SNP panel (Illumina Inc., San Diego, CA) for 768 SNPs. Genotype calls were made using the Genotyping module of BeadStudio 2 software. For quality control we used control genomic DNA samples (CEPH family trio from Coriell Institute and two other genomic DNAs). The replicate and inheritance data were used to review and refine clustering. For the SNPs that failed the standard initial laboratory quality assurance, genotyping was successfully carried out using TaqMan (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Primers and probes were Assay-by Design (Applied Biosystems). Following PCR amplification, end reactions were read on the ABI Prism 7900ht using Sequence Detection Software (Applied Biosystems).

Statistical methods

Measurement of cytokine secretion resulted in six recorded values per individual for each outcome: three without in vitro viral stimulation (background control, unstimulated in triplicate) and three after in vitro PBMCs stimulation with rubella virus (virus-stimulated in triplicate). A single summary measurement per individual was obtained for each outcome by subtracting the median of the three unstimulated values from the median of the three stimulated values. Assessments of antibody levels resulted in only one recorded value per individual. Data were summarized across individuals using frequencies and percentages for categorical variables, and medians and inter-quartile ranges for continuous variables.

Observed genotypes were used to estimate allele frequencies for each SNP, and departures from Hardy-Weinberg equilibrium (HWE) were assessed using a Pearson goodness-of-fit test or, for SNPs with a MAF of less than 5%, a Fisher exact test [24]. Estimates of pair-wise LD based on the r-squared statistic were obtained using Haploview software, version 3.32 [25].

SNP associations with immune response outcomes were individually evaluated using linear regression models. Simple linear regression was used for rubella antibody levels, which had only one measured value per individual. Repeated measures approaches were implemented for the cytokine secretion variables, simultaneously modeling all six observed measurements (three unstimulated values and three virus-stimulated values). The primary test of significance assessed the degree to which the SNP was associated with stimulation-induced differences in the response, and was obtained from the covariate reflecting the genotype-by-stimulation status interaction. We accounted for within-subject correlations without imposing any constraints on the nature of the correlations. Primary tests of association assumed an ordinal SNP effect, based on the number of copies of the minor allele.

To further explore genomic regions containing statistically significant single-SNP effects for one or more outcomes of interest, we performed post-hoc haplotype analyses. Posterior probabilities of all haplotypes consistent with the genotypes of each individual were estimated using an expectation-maximization (EM) algorithm, similar to the method outlined by Schaid et al [26]. This information was used to define haplotype design variables that reflected the number of each of the haplotypes that were expected to be carried by each subject. Analyses were performed on these haplotype design variables using the simple least squares regression approach for antibody levels and the repeated measures approach for the cytokine secretion described above. Because of the imprecision involved in estimating the effects of low-frequency haplotypes, we considered only those occurring with an estimated frequency of greater than 1%. Due to phase ambiguity, haplotype-specific medians and inter-quartile ranges could not be calculated. Thus, descriptive summaries were represented using the t-statistics corresponding to the haplotype main effect term for antibody levels or the haplotype-by-stimulation status interaction term for all immune measures.

All analyses adjusted for race, gender, age at enrollment, age at first and second rubella vaccination, and cohort status, thus accounting for time since last immunization, waning of immune response and time of immune response measurements. Data transformations were used to correct for data skewness in all linear regression models. An inverse-normal transformation was used for all cytokine secretion outcome variables, and a logarithmic transformation was used for the antibody response measure. 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

Subjects demographics and immune response

The study subjects were primarily Caucasian (91%), and 46% were females. The subjects had a median age at the time of enrollment of 15 years. The median age at first and second MMR-II immunization was 15 months and 11 years respectively, and the median time since last immunization to enrollment (measurement of immune status) was 5.8 years. All characteristics of the study cohort have been described previously [19]. Immune variables for the study subjects are summarized in Table 1, including median cytokine levels of unstimulated samples (background levels), median cytokine levels of rubella virus-stimulated samples and corrected final levels. The median rubella-specific IgG antibody response for the cohort was 34 IU/mL, while rubella-specific IFN-γ and IL-10 Elispot T cell memory responses were hardly detectable. Cytokine secretion patterns were skewed towards a predominant inflammatory

response, characterized by high levels of IL-6 and modest levels of TNF-α and GM-CSF. Th1 cytokines (IFN-γ and IL-2) were detected at a lower level, while IL-12p40, Th2 cytokines (IL-4, IL-5) and IL-10 were suppressed, although still detectable in the case of IL-10.

Genotyping of SNPs in interferon-related transcription factors and antiviral genes

Seven hundred and thirty eight DNA samples (n=738, 250 ng each) were genotyped following the Illumina protocol. Overall, twenty four samples failed the genotyping due to insufficient/inadequate DNA quality (n=6), complete genotyping failure on both platforms (n=4) or low call rates < 95% (n=14). Of the 114 SNPs included, 107 (93.86%) yielded genotype data on the Illumina platform and 6 SNPs failed the standard initial laboratory quality assurance. For the failed SNPs genotyping was successfully carried out using TaqMan. No significant SNP-specific deviation ($p<0.001$) from the HWE was observed. Two SNPs had MAF < 5% and were excluded from analyses. This resulted in a final sample size of 714 subjects and 112 SNPs in the candidate antiviral genes. The frequencies of genetic variants in the *OAS1* gene region of interest for the population cohort in the study are shown in Supplementary Table 1.

Genetic associations

Associations between SNPs in antiviral genes/transcription factors and rubella virus-specific antibodies—Overall, we found four significant associations ($p<0.05$) between SNPs located in the coding or regulatory regions of antiviral genes / transcription factors and rubella-specific measures of humoral immunity (Table 2). The presence of a homozygous minor allele genotype or heterozygous genotype for two regulatory SNPs (rs1732778 and rs2464288), in strong LD ($r^2=1$), belonging to the *OAS2* gene, was associated with an increase in rubella-specific antibody levels ($p = 0.036$). Increased representation of the minor alleles of a regulatory SNP (rs17256713, $p=0.014$) in *IRF9/ISGF3G* and a nonsynonymous SNP (rs3743477/Pro15Leu, $p=0.048$) in *ISG20L1* were associated with a decrease/increase in median rubella-specific antibody levels respectively.

Associations between SNPs in antiviral genes and rubella virus-specific Th1 cytokine responses—Overall, we found 14 SNPs in coding and regulatory gene regions significantly associated ($p<0.05$) with an increase/decrease of rubella virus-specific IFN-γ or IL-2 secretion levels (Table 3). Minor allele variants of one coding, nonsynonymous SNP in exon 2 (rs2229857, Arg384Lys) and one regulatory SNP (rs1127317) in the 3′UTR of the *ADAR* gene were associated ($p\leq0.021$) with an allele dose-related increase in secreted IFN-γ in response to rubella virus (these SNPs were in LD, $r^2=0.94$). Similarly, the minor allele of a synonymous SNP in exon 2 (rs3743476, Glu58Glu) and the minor allele of a promoter SNP (rs11073795) in the *ISG20L1* gene and *ISG20* gene respectively also demonstrated allele dose relationships towards higher IFN-γ response ($p \leq0.045$). The most striking associations were observed between polymorphisms in genes belonging to the OAS gene cluster (ordered *OAS1*, *OAS3* and *OAS2* on 12q24.2) and levels of rubella virus-specific IL-2, a crucial T cell growth factor and essential mediator of immune response to antigenic stimuli (Table 3). We found 10 significant associations ($p\leq0.05$) in coding or regulatory SNPs, all belonging to genes of the OAS system and 7 belonging to *OAS1* (although the SNPs were clustered in two LD blocks, Fig. 1). The minor alleles of three *OAS1* SNPs (a nonsynonymous SNP rs1051042 in exon 6 Thr361Arg; SNP rs2660 in the 3′UTR region; and a nonsynonymous SNP rs3741981 in exon 3 Ser162Gly), located in a LD block of functional importance (Fig.1), were significantly associated with an increase of rubella virus-specific IL-2 (Th1) response ($p\leq0.024$, Table 3). Furthermore, two of these SNPs (rs1051042 and rs2660), in tight LD with a previously described functional SNP (rs10774671) altering splicing and enzyme/antiviral activity of *OAS1* [27,28], demonstrated an allele dose-response relationship. In addition, we identified four SNPs in the promoter region of *OAS1* (rs10774670, rs10774669, rs10492028 and rs1557865 clustered in a LD block, Fig.1) associated with variations in rubella virus-specific

IL-2 levels ($p \leq 0.026$). Increased representation the minor allele for all of these SNPs was associated with an allele dose-dependent increase of IL-2 secretion (Table 3). Three SNPs located in the *OAS3* promoter region, downstream the *OAS1* gene (rs2384071 and rs2384072, in LD, Fig.1) and in *OAS3* 3′UTR (rs2010604) also demonstrated significant associations ($p \leq 0.047$) with rubella virus-induced IL-2 levels (Table 3).

All genotype-specific cytokine measures (background unstimulated levels, virus-stimulated levels and corrected levels are presented in Supplementary Table 2.

Associations between SNPs in antiviral genes and rubella-specific IL-10 cytokine responses—We were not able to test for SNP associations with classical Th2 cytokines, since extremely low IL-4, IL-5 secretion levels were detected in response to rubella virus stimulation. Instead we tested for SNP associations with rubella virus-specific IL-10, a key immunoregulatory cytokine that can be produced by different cell types. Four of the five identified significant SNP associations were also within genes of the OAS/RNaseL pathway (Table 4). The heterozygous and homozygous minor allele genotypes of *OAS1* SNP (rs3741981, Ser162Gly) were significantly associated with a decrease of rubella-specific IL-10 levels (p=0.031) and an increase in IL-2 levels (p=0.024) (Tables 3 and 4). Two SNPs within the promoter region of the *OAS1* gene (rs10774670 and rs2240193) were associated with variations in rubella virus-specific IL-10 levels. The *OAS1* promoter SNP rs10774670 demonstrated the most significant associations both with IL-2/Th1 (p=0.002) and IL-10 (p=0.009) secretion. Increased representation the minor allele of a nonsynonymous SNP in exon 3 of the *RNASEL* gene (rs627928, Glu541Asp) was associated with an allele dose-related decrease in IL-10 levels (p=0.01).

All genotype-specific cytokine measures (background unstimulated levels, virus-stimulated levels and corrected levels are presented in Supplementary Table 2.

Associations between SNPs in antiviral genes and rubella-specific inflammatory cytokine responses—Our analysis revealed an allele dose-related increase of TNF-α with the representation the minor allele of a SNP in the promoter region of the *ADAR* gene (rs1552902, p=0.007). SNP rs9427092 located in the 3′ intergenic region downstream in the same gene was also significantly associated with variations of TNF-α (p=0.015) (Table 5). We found four SNPs (rs8127664, rs13433394 and rs8127290 -$r^2 \geq 0.96$, and rs456298) located downstream of the *MX1* gene, associated with variations in rubella virus-specific TNF-α secretion ($p \leq 0.046$). We observed 8 significant SNP associations with the level of rubella virus-specific IL-6 secretion and 5 of them were within genes of the OAS/RNaseL pathway (Table 5). Similar to the regulation of IL-10 secretion (Table 4), the minor allele of a nonsynonymous SNP in the *RNASEL* gene (rs627928, Glu541Asp) was also associated with an allele dose-related decrease in IL-6 levels (p=0.033, Table 5). Interestingly, the two regulatory *OAS2* SNPs (rs1732778 and rs2464288, $r^2=1$) associated with variations in rubella virus-specific antibody level (Table 2), were also significantly associated with an allele dose-related decrease in IL-6 secretion levels (p=0.041)(Table 5). Similarly, the minor allele of a promoter SNP (rs12815666) in the *OAS2* gene was associated with a decrease in IL-6 levels (p=0.049). A SNP in the 3′UTR of the same gene (rs13311) was associated with variation in IL-6 secretion (p=0.02). We found a significant association between a coding nonsynonymous SNP in the *MX1* gene (rs469390/Ile379Val) and variations in IL-6 levels (p=0.044). In addition, two SNPs in the *ADAR* gene (rs2229857/Arg384Lys and rs9616) were also associated with variations in IL-6 secretion ($p \leq 0.045$)(Table 5).

All genotype-specific cytokine measures (background unstimulated levels, virus-stimulated levels and corrected levels are presented in Supplementary Table 2.

Associations between *OAS1* haplotypes and rubella virus-specific IL-2 and IL-10 secretion—Since most of our significant SNP associations were within the *OAS1* gene region we performed focused haplotype analysis including all genotyped SNPs in the specific gene region of interest. We were able to identify 7 *OAS1* haplotypes with frequencies ≥1% in our study cohort (Table 6). The global tests from *OAS1* haplotype analyses demonstrated statistically significant associations between haplotypes and rubella virus-specific IL-2 (p=0.008) and IL-10 (p=0.042) secretion (Table 6). The most common haplotype GGGCACAGG (major alleles of all genetic variants) was associated with lower rubella virus-specific IL-2 secretion levels (*t*-statistic −2.49, *p*=0.013) as well as with higher IL-10 levels (*t*-statistic 2.68, *p*=0.008). Similarly, the minor allele haplotype AAAAGGGAA (minor alleles of all genetic variants) was associated with higher rubella virus-specific IL-2 levels (*t*-statistic 2.11, *p*=0.035), but its association with IL-10 secretion was only suggestive and did not reach the level of significance (*t*-statistic −1.42, *p*=0.157). In addition, haplotype GGACGCAGG (minor alleles for rs10774670 and rs3741981 and major alleles for all other genetic variants) also showed evidence of association with decrease in IL-10 levels (*t*-statistic of −2.36, *p*=0.019).

4. Discussion

The present study highlights the important role of host genetic factors, and, in particular, variations in the OAS gene cluster (including functional polymorphisms in the *OAS1* gene) and OAS/RNaseL pathway in the modulation of rubella vaccine-induced immune response.

Upon binding to dsRNA OAS1 catalyses the oligomerization of ATP into 2′-5′-linked oligoadenylates and activates RNaseL, which breaks down viral and cellular RNA. The OAS system is also involved in other cellular processes such as growth and differentiation, gene regulation and apoptosis [29].

The functional relevance of the OAS system to rubella virus infection was suggested by earlier *in vivo* studies in humans demonstrating elevated OAS1 enzyme activity after immunization with rubella vaccine and robust *OAS1*, *OAS2* and *OAS3* gene expression following infection of human fibroblasts with rubella virus [30,31].

As part of our study we extensively genotyped 31 genetic variants/SNPs located in the OAS gene cluster and 7 genetic variants in the functionally related *RNASEL* gene. Our data provide evidence for 23 OAS SNP associations with different measures of rubella-virus specific immune response. As demonstrated in the literature [27,28] and evidenced from our data (Fig. 1), SNPs at the *OAS1* region cluster into two LD blocks and at least six SNPs (including rs10774671) cluster in a LD block with functional importance. Among these are rs3741981 in exon 3, rs10774671 in intron 5 (at the splice-acceptor site), rs1051042 in exon 6, and rs2660 in the 3′UTR. SNPs rs10774671, rs1051042 and rs2660 are in virtually complete LD and form two haplotypes affecting differential splicing and highly associated with the OAS1 enzyme activity [27,28]. The functional SNP is believed to be rs10774671, for which the more common A allele is predicted to ablate the specific splice site resulting in isoforms with lower enzyme activity, while the G allele is predicted to allow splicing resulting in the production of the p46 isoform with higher enzyme activity. Heterozygotes were reported to have intermediate OAS activity [27]**.** Notably, recent work with West Nile virus provides compelling functional evidence that rs10774671 is a risk factor for WNV infection in humans and that early WNV replication in human lymphoid tissue may be regulated by the *OAS1* SNP genotype [28]. We genotyped two tag SNPs (rs1051042 and rs2660, located in the same bin with the splicing variant rs10774671) that were in complete LD and correlated perfectly with the presumably causal SNP. Thus were able to uniquely identify the genotypes/haplotypes associated with high

and low OAS1 enzyme/antiviral activity and establish their association with variations in rubella virus-specific IL-2 secretion.

Of note, the nonsynonymous A/G polymophism (rs3741981) occurs in an evolutionarily conserved DNA region in all OAS1 isoforms near the dsRNA binding domain. This same polymorphisms is suggested to be functional in a genetic study of type 1 diabetes [32]. In this respect it is worth noting that the only viral infection proven to cause type 1 diabetes in humans is congenital rubella. In our study, the OAS1 Ser/Gly candidate functional polymorphism cross-regulated rubella virus-specific Th1/IL-2 and IL-10 secretion levels. IL-2 is a multifunctional Th1 cytokine with a wide spectrum of effects on the immune system including immune activation [33]. IL-10, a key immunoregulatory cytokine during infection, is produced by different cell types, mainly antigen presenting cells and T cells, and plays a critical role in immune regulation inhibiting the activity of Th1 cells, NK cells and macrophages [34,35]. It is biologically plausible to suggest that one or more linked causal polymorphisms in the OAS gene cluster can finely tune the cytokine balance and thereby influence the adaptive immune response to a live viral vaccine. Finally, potentially interesting findings in our study include polymorphisms in the promoter and regulatory regions of *OAS1, OAS2* and *OAS3* (some in LD), associated with virus-specific IL-2, IL-10 and IL-6 secretion and antibody levels. Since complex trait associations are more likely dependent on several/multiple genetic variants, it is reasonable to suggest that the observed effects in our study may be a result of several functional genetic variants. Accordingly, the global tests and individual haplotypic analyses revealed significant correlations between *OAS1* haplotypes and rubella virus-specific cytokine secretion. The most common major allele haplotype GGGCACAGG, (which identifies the major allele A of the splicing variant SNP rs10774671), was associated with lower rubella virus-specific IL-2 secretion and higher IL-10 levels, while the minor allele haplotype AAAAGGGAA, (which identifies the G allele of SNP rs10774671), was significantly associated with higher IL-2 secretion. Thus, haplotype analyses provide additional evidence that OAS gene polymorphisms might be involved in the mechanisms underlying immune response heterogeneity to rubella vaccine.

Consistent with the known OAS antiviral effector pathway are the observed associations for C/A nonsynonymous polymorphism within the protein kinase domain of the *RNASEL* gene (rs627928). Although this genetic variant was shown to produce similar levels of RNaseL activity [36], the evidence for cross-regulation and an allele dose-related decrease of IL-6 and IL-10 is suggestive of the possible functional importance in cytokine regulation.

The major strength of our work is the well-characterized study cohort (primarily Caucasians −91%, which is representative of US white population) with documented MMR vaccine coverage and no known wild type rubella viruses circulating in the community. Additional analyses of phenotype/genotype SNP associations in Caucasians only revealed similar results as those presented for the whole study cohort. Another benefit is that the LD tagSNP selection approach we used [22], selects a maximally informative set of SNPs for analyses using LD, which allowed us to infer genotypes/haplotypes and associations for SNPs of interest (like splicing variant rs10774671, recently implicated in antiviral response [28]) with a high degree of confidence. The precise quantitative immune profiling of the study subjects allowed us to look for allele dose-related variations as well as cross-regulation patterns for the genetic variants, which increased our confidence in the observed associations. However, we are aware of some limitations to the present study. The results cannot be extrapolated to other ethnic groups given that these primarily concern Caucasians. The issues of multiple testing and possible false-positive associations are also of concern in any study like this. Assuming independent tests of association we would expect 39 associations to be statistically significant by chance alone (at the p=0.05 level), while we observed 48 associations (including intronic) which is suggestive that at least some of the effects are real. We choose the less stringent cutoff

value of 0.05 since our study is the first to examine the effect of antiviral gene polymorphisms on vaccine-induced immunity and we believe that the risk of false negatives outweighs that of false positives. As with any statistical association, the study needs to be replicated in an independent cohort to validate the findings, which we are currently planning. Another legitimate concern is that some of the observed differences in immune measures associated with different SNP alleles appear modest and it is difficult to assign biological or clinical importance to them. Although the effect of a single functional polymorphism (that either alters gene expression or protein structure) on immunity as a complex trait outcome is more likely to be small, there remains doubt over functionality of certain genetic variants. At this point in our understanding of the cytokine network as a highly regulated system, there is still far too much complexity in the interplay between cytokines and other components of immunity, to be able to define or predict the effect of even modest variations on cell-mediated immunity or humoral immunity. Validation studies with increasing sample size or meta-analyses in concert with functional studies are key to resoling questions regarding SNP functionality [37].

Nevertheless, the observed allele-dose relationships, cytokine cross-regulation pattern, pathway-focused findings and haplotype analyses for some genetic variants, as well as the high biological plausibility of the *OAS1* associations in light of what is already known suggest the significance of our results.

In conclusion, the present study highlights for the first time the importance of antiviral effector genes and in particular OAS gene cluster functional polymorphisms and haplotypes in regulating the immune response to rubella vaccine in humans. Our work supports the novel concept that innate immunity is likely involved in controlling initial viral replication for priming and modulating the magnitude and quality of the adaptive immune response to a live attenuated viral vaccine. Both follow-up replication and functional studies are needed to confirm the plausibility of the associations and delineate the mechanisms by which OAS genetic variants influence rubella virus infection and subsequent immune response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

SNP	single-nucleotide polymorphism
IFN	interferon
ISG	interferon-stimulated genes
PRK	dsRNA-activated protein kinase R
OAS	2′-5′-oligoadenylate-synthetase
RNaseL	ribonuclease L
ADAR1	adenosine deaminase, RNA-specific 1

MMR	measles-mump-rubella
IRF	interferon regulatory factor
HWE	Hardy-Weinberg equilibrium
MAF	minor allele frequency
LD	linkage disequilibrium

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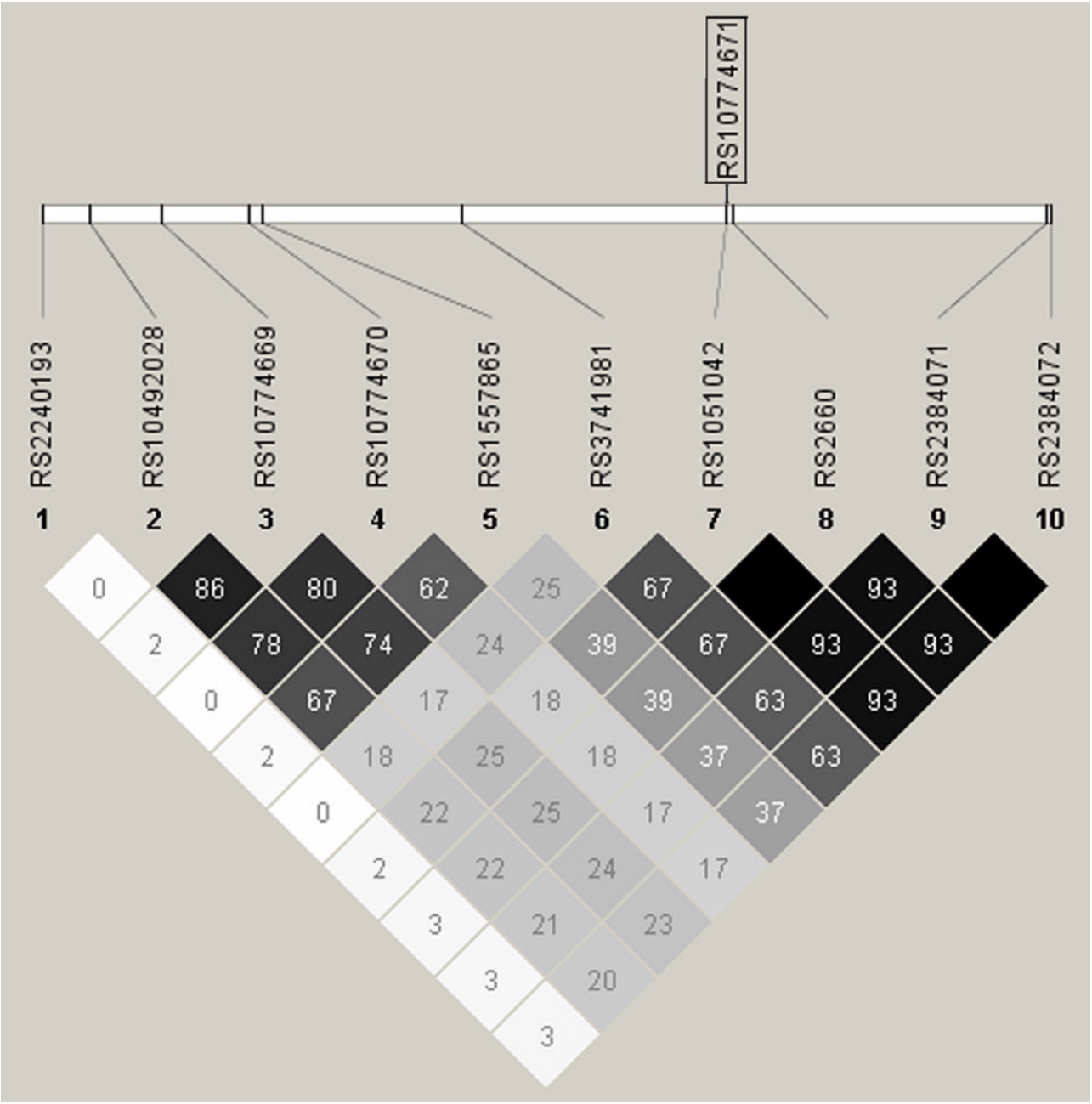

Fig.1.

Haplotype block structure of the *OAS1* gene region in the study cohort. The LD block structure was analyzed using Haploview software, version 3.32. SNPs in the *OAS1* region clustered into two blocks of linkage disequilibrium. The location of the splicing variant rs10774671 (boxed; not genotyped but in one bin/tight LD with rs1051042 and rs2660) is shown. The r^2 color scheme is: white ($r^2=0$), shades of grey ($0<r^2<1$), black ($r^2=1$).

Table 1

Immune measures summary for the study cohort

Immune variable	No. of Subjects	Median Cytokine Level, Stimulated	Median Cytokine Level, Unstimulated	Median Final Level (IQR[a])	Positive, %[e]
Antibody [b]					
	738	N/A	N/A	34.4 (19.2, 63.7)	644 (87.3)
Elispot [c]					
IL-10	725	46.0	45.0	1.0 (−7.0, 9.0)	380 (52.4)
IFN-γ	719	15.0	20.0	−4.0 (−12.0, 0.0)	141 (19.6)
Cytokine [d]					
IL-2	713	33.8	15.9	17.6 (7.7, 30.5)	652 (91.4)
IL-4	691	−1.2	−1.5	0.3 (−0.3, 1.0)	392 (56.7)
IL-5	691	−0.6	−1.1	0.5 (0.0, 1.1)	482 (69.8)
IL-6	713	3952.4	83.2	3681 (3160, 4052)	707 (99.2)
IL-10	713	10.8	5.6	4.2 (2.3, 6.7)	641 (89.9)
IL-12p40	711	−2.0	−2.3	0.0 (−7.1, 7.2)	326 (45.9)
IFN-γ	713	9.7	−1.1	8.5 (3.0, 23.4)	644 (90.3)
TNF-α	713	176.7	129.9	29.7 (−7.0, 89.2)	490 (68.7)
GM-CSF	711	27.7	−0.8	28.0 (23.6, 32.6)	711 (100)

[a] IQR, interquartile range

[b] In UI/mL, cut-off for seropositivity of 15 IU/mL.

[c] Rubella-specific IL-10 or IFN-γ producing cells per 2×10^5 PBMCs; Median of rubella virus-specific stimulated response (measured in triplicate) minus the median unstimulated response (in triplicate). and Due to differences in the distributional shapes of the stimulated secretion values and the unstimulated values, it is possible that the difference in the medians of the stimulated and unstimulated values will not equal the median of the differences in the values.

[d] In pg/mL; Median background levels of cytokine secretion in cultures not stimulated with rubella virus were subtracted from the median levels of rubella virus-specific stimulated responses to produce the "corrected" final secretion values. Negative values indicate that unstimulated levels were, on average, higher than stimulated secretion levels. Subjects are considered to have a positive cytokine response if the median of the stimulated cells (measured in triplicate for each subject) is larger than the median of the unstimulated cells (also in triplicate). Due to differences in the distributional shapes of the stimulated secretion values and the unstimulated values, it is possible that the difference in the medians of the stimulated and unstimulated values will not equal the median of the differences in the values.

Table 2

Associations between SNPs in antiviral genes and rubella virus-specific antibody responses

Gene	SNP ID	Location /Relative position	Function	Geno type	N[a]	Median Ab level IU/mL (IQR)[b]	p value[c]
IRF9 (ISGF3G)	rs17256713	3′ intergenic		GG	582	35.9 (19.7,67.5)	0.014
		8345 C>T		GA	123	33.1 (17.2,59.1)	
				AA	9	17.9 (13.3,52.4)	
OAS2	rs1732778[d]	3′intergenic		GG	400	32.9 (19.2,57.4)	0.036
		40511 G>A		GA	270	38.4 (19.1,71.7)	
				AA	44	37.8 (19.3,74.8)	
OAS2	rs2464288[d]	3′intergenic		AA	400	32.9 (19.2,57.4)	0.036
		42965 A>C		AC	270	38.4 (19.1,71.7)	
				CC	44	37.8 (19.3,74.8)	
ISG20L1	rs3743477	Coding		GG	633	33.3 (19.0,61.7)	0.048
		43 C>T	Pro15Leu	GA	78	44.4 (24.3,81.3)	
				AA	2	37.4 (12.8,62.0)	

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

[b] IQR, interquartile range, values are in IU/mL measured by the Beckman Coulter Access® Rubella IgG assay.

[c] One degree-of-freedom ordinal p-value from the linear regression analysis, modeling the single antibody response measure obtained per individual, adjusting for age of enrollment, gender, race, age at first and second MMR immunizations, and cohort status. Only statistically significant associations ($p<=0.05$) are presented.

[d] rs1732778 and rs2464288 are in strong LD ($r^2=1$) with each other.

Table 3

Associations between SNPs in antiviral genes and rubella-specific Th1 cytokine responses

Secreted Cytokine SNP ID (Gene)	Location /Relative position	Function	Geno type	N[a]	Median level pg/mL (IQR)[b]	p valuer[c]
IFN-γ						
rs1127317[d](*ADAR*)	3′UTR		AA	382	8.3 (2.7, 23.5)	
	24442 A>C		AC	251	8.4 (3.0, 23.2)	0.014
			CC	59	12.4 (3.8, 32.6)	
rs3743476 (*ISG20L1*)	Coding		AA	311	7.0 (2.5, 23.4)	
	173A>G	Glu58Gl	AG	309	9.0 (3.5, 23.5)	0.019
		u	GG	72	10.8 (3.9, 27.2)	
rs2229857[d](*ADAR*)	Coding		GG	376	8.3 (2.7, 23.5)	
	6515 C>T	Arg384L	GA	253	8.5 (3.0, 23.2)	0.021
		ys	AA	63	11 (3.8, 32.6)	
rs11073795 (*ISG20*)	5′intergenic	promoter	GG	248	6.7 (2.6, 19.3)	
	−1820 G>A		GA	334	9.0 (3.1, 29.2)	0.045
			AA	110	11.1 (3.7, 21.9)	
IL-2						
rs10774670[e] (*OAS1*)	5′intergenic	promoter	GG	353	16.7 (5.9, 30.4)	0.002
	−2683 G>A		GA	274	17.5 (8.8, 30.2)	
			AA	46	21.7 (11, 34.9)	
rs10774669[e] (*OAS1*)	5′intergenic	promoter	GG	404	16.4 (6.2, 29.5)	0.003
	−5418 C>T		GA	253	18.1 (8.7, 32.4)	
			AA	34	23.6(13.1,47.2)	

Secreted Cytokine SNP ID (Gene)	Location /Relative position	Function	Geno type	N[a]	Median level pg/mL (IQR)[b]	p valuer[c]
rs10492028[e] (*OAS1*)	5′intergenic	promoter	GG	410	16.3 (6.1, 29.5)	0.004
	−7682 G>A		GA	243	17.7 (8.7, 32.4)	
			AA	39	23 (13.8, 47.2)	
rs1051042[f] (*OAS1*)	Coding		CC	292	16.0 (5.7, 29.4)	0.018
	12392 C>G	Thr361A	CG	327	18.1 (8.7, 31.1)	
		rg	GG	72	18.9 (8.9, 37.9)	
rs2660[f] (*OAS1*)	3′UTR		AA	293	16.0 (5.7, 29.3)	0.019
	12597 A>G		AG	327	18.1 (8.7, 31.1)	
			GG	72	18.9 (8.9, 37.9)	
rs2384071[f] (*OAS3*)	5′intergenic	promoter	GG	278	15.4 (5.7, 29.3)	0.023
	−8993 G>A		GA	328	18.1 (8.7, 30.6)	
			AA	76	18.9 (8.9, 37.3)	
rs3741981[f](*OAS1*)	Coding		AA	224	16.3 (5.7, 29.5)	0.024
	4025 A>G	Ser162Gl	AG	345	17.9 (7.9, 30.5)	
		y	GG	123	17.7 (9.5, 34.6)	
rs1557865[e] (*OAS1*)	5′intergenic	promoter	CC	449	17.0 (7.6, 30.3)	0.026
	−2247G>T		CA	222	17.7 (8.4, 32.2)	
			AA	21	24.1(13.1,47.2)	
rs2384072[f](*OAS3*)	5′intergenic	promoter	GG	281	15.9 (5.8, 29.3)	0.036
	−8914 C>T		GA	333	18.1 (8.7, 30.7)	
			AA	78	18.9 (9.5, 37.1)	

Secreted Cytokine SNP ID (Gene)	Location /Relative position	Function	Geno type	N[a]	Median level pg/mL (IQR)[b]	p valuer[c]
rs2010604 (*OAS3*)	3′UTR		GG	326	15.9 (5.9, 29.5)	0.047
	31872 G>C		GC	309	18.2 (8.7, 30.5)	
			CC	57	18.4(10.5,37.6)	

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

[b] IQR, interquartile range, values in pg/mL measured by ELISA.

[c] One degree-of-freedom ordinal p-value from the repeated measures regression analysis, simultaneously modeling the three stimulated and three unstimulated cytokine secretion values per individual, adjusting for age of enrollment, gender, race, age at first and second MMR immunizations, and cohort status. Only statistically significant associations ($p<=0.05$) are presented.

[d] SNPs rs1127317 and rs2229857 are in LD ($r^2=0.94$).

[e] SNPs rs10774670, rs10774669, rs10492028 and rs1557865 are in a LD block (Fig. 1).

[f] SNPs rs1051042, rs2660, rs3741981, rs2384071 and rs2384072 are in a LD block (Fig. 1).

Table 4

Associations between SNPs in antiviral genes and rubella-specific IL-10 cytokine responses

Secreted Cytokine SNP ID (Gene)	Location /Relative position	Function	Geno type	N[a]	Median level pg/mL (IQR)[b]	p value[c]
IL-10						
rs10774670 (*OAS1*)	5′intergenic	promoter	GG	353	4.5 (2.4, 7.0)	0.009
	−2683 G>A		GA	274	4.0 (1.7, 6.2)	
			AA	46	5.2 (3.3, 7.7)	
rs627928 (*RNASEL*)	Coding		CC	234	4.6 (2.7, 7.1)	0.01
	4604 C>A	Glu541A	CA	306	4.2 (1.9, 6.9)	
		sp	AA	152	3.9 (2.1, 5.9)	
rs3741981 (*OAS1*)	Coding		AA	224	4.6 (2.5, 7.7)	0.031
	4025 A>G	Ser162Gl	AG	345	4.1 (2.0, 6.5)	
		y	GG	123	4.1 (2.3, 6.2)	
rs2240193 (*OAS1*)	5′intergenic	promoter	CC	585	4.1 (2.2, 6.5)	0.033
	−9184 C>A		CA	99	4.6 (2.7, 8.9)	
			AA	8	4.7 (3.1, 6.9)	
rs8038865 (*ISG20*)	5′intergenic	promoter	CC	369	4.2 (2.2, 6.7)	0.037
	−1496 C>G		CG	265	4.3 (2.6, 6.7)	
			GG	58	2.9 (1.1, 6.1)	

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

[b] IQR, interquartile range, values in pg/mL measured by ELISA

[c] One degree-of-freedom ordinal p-value from the repeated measures regression analysis, simultaneously modeling the three stimulated and three unstimulated cytokine secretion values per individual, adjusting for age of enrollment, gender, race, age at first and second MMR immunizations, and cohort status. Only statistically significant associations (p<=0.05) are presented.

Table 5

Associations between SNPs in antiviral genes and rubella-specific inflammatory cytokine responses

Secreted Cytokine SNP ID (Gene)	Location /Relative position	Function	Geno type	N[a]	Median level pg/mL (IQR)[b]	p value[c]
TNF-α						
rs1552902 (*ADAR*)	5′intergenic	promoter	GG	331	25.6 (−14.5,73.9)	0.007
	−34591G>C		GC	285	31.3 (−4.2, 89.3)	
			CC	76	41.9 (−0.3, 139.9)	
rs8127664[d] (*MX1*)	3′intergenic		GG	497	35.0 (−3.0, 97.4)	0.008
	28640 C>T		GA	180	21.6 (−18.9,73.8)	
			AA	14	47.0 (−14.5, 95.9)	
rs13433394[d] (*MX1*)	3′intergenic		GG	493	35.0 (−3.0, 97.4)	0.013
	27912 C>T		GA	182	22.2 (−18.9, 74.5)	
			AA	17	36.5 (−14.5, 75.6)	
rs8127290[d] (*MX1*)	3′intergenic		GG	493	35.0 (−3.0, 97.4)	0.013
	28166 G>A		GA	182	22.2 (−18.9, 74.5)	
			AA	17	36.5 (−14.5, 75.6)	
rs9427092 (*ADAR*)	3′intergenic		AA	414	28.3 (−3.4, 88.7)	0.015
	26760 T>C		AG	239	36.7 (−8.9, 95.6)	
			GG	38	1.0 (−21.6, 56.9)	
rs456298 (*MX1*)	3′intergenic		AA	477	35.4 (−3.6, 96.0)	0.046
	32753 A>T		AT	192	25.1 (−16.5, 82.3)	
			TT	23	18.7 (−37.7, 73.0)	
IL-6						

Secreted Cytokine SNP ID (Gene)	Location /Relative position	Function	Geno type	N[a]	Median level pg/mL (IQR)[b]	p value[c]
rs13311 (*OAS2*)	3′UTR		CC	270	3667.4 (3167.4,4031.3)	0.02
	32238 C>A		CA	314	3697.1 (3140.4,4090.8)	
			AA	108	3657.8 (3087.8,4083.1)	
rs627928 (*RNASEL*)	Coding		CC	234	3708.0 (3204.1,4095.1)	0.033
	4604 C>A	Glu541A	CA	306	3662.0 (3099.0,4038.5)	
		sp	AA	152	3636.0 (3077.7,4045.4)	
rs9616 (*ADAR*)	3′UTR		TT	366	3713.1 (3216.4,4105.7)	0.034
	24749 T>A		TA	267	3659.3 (3090.5,4030.4)	
			AA	59	3668.3 (2931.9,4061.3)	
rs1732778 (*OAS2*)[e]	3′intergenic		GG	388	3739.5 (3178.1,4070.6)	0.041
	40511G>A		GA	263	3609.8 (3067.1,4062.4)	
			AA	41	3517.9 (3226.6,4061.3)	
rs2464288 (*OAS2*)[e]	3′intergenic		AA	388	3739.5 (3178.1,4070.6)	0.041
	42965 A>C		AC	263	3609.8 (3067.1,4062.4)	
			CC	41	3517.9 (3226.6,4061.3)	
rs469390 (*MX1*)	Coding		AA	226	3649.1 (3093.9,4020.6)	0.044
	13932 A>G	Ile379Va	AG	347	3693.6 (3098.7,4121.6)	
		l	GG	119	3684.3 (3248.5,4071.3)	
rs2229857 (*ADAR*)	Coding		GG	376	3659.8 (3072.0,4061.9)	0.045
	6515 C>T	Arg384L	GA	253	3703.0 (3187.9,4083.5)	
		ys	AA	63	3698.0 (3099.0,3969.3)	
rs12815666 (*OAS2*)	5′intergenic	promoter	GG	536	3692.8 (3131.7,4076.6)	0.049

Secreted Cytokine SNP ID (Gene)	Location /Relative position	Function	Geno type	N[a]	Median level pg/mL (IQR)[b]	p value[c]
	−660 C>T		GA	146	3666.8 (3131.1,4030.0)	
			AA	10	3613.3 (3366.6,3900.6)	
GM-CSF						
rs3743476 (*ISG20L1*)	Coding		AA	311	29.5 (24.4, 33.1)	0.021
	173 A>G	Glu58Gl	AG	307	27.4 (23.0, 31.9)	
		u	GG	72	26.1 (20.9, 31.6)	

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

[b] IQR, interquartile range, values in pg/mL measured by ELISA

[c] One degree-of-freedom ordinal p-value from the repeated measures regression analysis, simultaneously modeling the three stimulated and three unstimulated cytokine secretion values per individual, adjusting for age of enrollment, gender, race, age at first and second MMR immunizations, and cohort status. Only statistically significant associations (p<=0.05) are presented.

[d] SNPs rs8127664, rs13433394 and rs8127290 are in LD ($r^2 \geq 0.96$).

[e] SNPs rs1732778 and rs2464288 are in LD ($r^2 = 1$).

Table 6

OAS1 haplotype associations with rubella-specific IL-2 and IL-10 cytokine secretion

***OAS1* Haplotype**	**Frequency**	**Test statistic (haplotype *t*-statistic**	**Allele *p*-value**	**Global *p*-value**	**Test statistic (haplotype *t*-statistic**	**Allele *p*-value**	**Global *p*-value**
			IL-2			**IL-10**	
GGGCACAGG[a]	0.511	−2.49	**0.013**		2.68	**0.008**	
GGGCACAAA[a]	0.012	−1.57	0.116		0.14	0.887	
GGGCGCAGG[a]	0.049	−1.64	0.101		−0.82	0.414	
GGGCGGGAA[a]	0.156	0.67	0.506	**0.008**	0.21	0.836	**0.042**
GGACGCAGG[a]	0.025	1.55	0.122		−2.36	**0.019**	
AAACACAGG[a]	0.034	1.40	0.161		−0.29	0.774	
AAAAGGGAA[a]	0.168	2.11	**0.035**		−1.42	0.157	

[a] *OAS1* genetic variants from left to right: rs10492028, rs10774669, rs10774670, rs1557865, rs3741981, rs1051042, rs2660, rs2384071, rs2384072