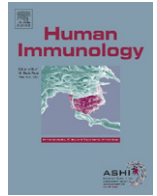




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2'-5'-Oligoadenylate synthetase single-nucleotide polymorphisms and haplotypes are associated with variations in immune responses to rubella vaccine

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ABSTRACT

Interferon-induced antiviral genes are crucial players in innate antiviral defense and potential determinants of immune response heterogeneity. We selected 114 candidate single-nucleotide polymorphisms (SNPs) from 12 antiviral genes using an LD tagSNP selection approach and genotyped them in a cohort of 738 school children 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/T_H1 response ($p \leq 0.024$). 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 ($p \leq 0.031$). 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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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 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 biologic 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 EIF2 α K2), 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 hepatitis C virus (HCV), hepatitis B virus (HBV), measles virus, West Nile virus (WNV), and Severe Acute Respiratory Syndrome coronavirus (SARS-coV) [11–18].

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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

2.1. Study population

The study cohort was a large population-based, age-stratified random sample of 738 healthy children and young adults (aged 11–19 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 measles-mumps-rubella II (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.

2.2. 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) and IL-10 Elispot assays (BD Biosciences, San Diego, CA) were performed in peripheral blood mononuclear cell (PBMC) cultures in triplicate after stimulation with the W-Therien strain of rubella virus and compared with unstimulated measures (also in triplicate), as described previously [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 preoptimized conditions for time and MOI for the different cytokines [19].

2.3. 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 linkage disequilibrium (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 LD threshold of $r^2 \geq 0.90$, and successful Illumina predictive genotyping scores. We then used the SNPPicker program to postprocess and refine the selection of computed tagSNPs to accommodate a set of platform-dependent design constraints. In addition, a list of putative functional "obligate" SNPs (coding: non-synonymous, 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 on the basis of this approach. The nomenclature used for the description of the variants follows that described by den Dunnen and Antonarakis [23].

2.4. Genotyping methods

One hundred 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). After PCR amplification, end reactions were read on the ABI, Prism 7900ht using Sequence Detection Software (Applied Biosystems).

2.5. 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 an MAF of $< 5\%$, 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 measure 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 earlier. 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%. Because of 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

3.1. Subjects demographics and immune response

The study subjects were primarily Caucasian (91%), and 46% were females. The subjects had a median age of 15 years at the time of enrollment. 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, whereas rubella-specific IFN- γ and IL-10 Elispot T cell memory responses were hardly detectable. Cytokine secretion patterns were skewed toward a predominant inflamma-

tory response, characterized by high levels of IL-6 and moderate levels of TNF- α and GM-CSF. T_H1 cytokines (IFN- γ and IL-2) were detected at a lower level, whereas IL-12p40, T_H2 cytokines (IL-4, IL-5), and IL-10 were suppressed, although still detectable in the case of IL-10.

3.2. Genotyping of SNPs in IFN-related transcription factors and antiviral genes

Seven hundred thirty-eight DNA samples ($n = 738$, 250 ng each) were genotyped after the Illumina protocol. Overall, 24 samples failed the genotyping because of insufficient or 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 six 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 S1.

3.3. Genetic associations

3.3.1. 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.

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, % ^b |
|--------------------------|-----------------|-----------------------------------|-------------------------------------|--|--------------------------|
| Antibody ^c | 738 | N/A | N/A | 34.4 (19.2, 63.7) | 644 (87.3) |
| Elispot ^{d,e,f} | | | | | |
| 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 ^{e,f} | | | | | |
| 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) |

^aIQR, interquartile range.

^bNumber of positive, % positive.

^cIn IU/ml, cut-off for seropositivity of 15 IU/ml.

^dRubella-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).

^eIn 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).

^fDue 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 | Genotype | N ^a | Median Ab level IU/ml (IQR) ^b | p-value ^c |
|---------------|------------------------|----------------------------|----------|----------|----------------|--|----------------------|
| IRF9 (ISGF3G) | rs17256713 | 3' intergenic 8345 C > T | | GG | 582 | 35.9 (19.7, 67.5) | 0.014 |
| | | | | GA | 123 | 33.1 (17.2, 59.1) | |
| | | | | AA | 9 | 17.9 (13.3, 52.4) | |
| OAS2 | rs1732778 ^d | 3' intergenic 40,511 G > A | | GG | 400 | 32.9 (19.2, 57.4) | 0.036 |
| | | | | GA | 270 | 38.4 (19.1, 71.7) | |
| | | | | AA | 44 | 37.8 (19.3, 74.8) | |
| OAS2 | rs2464288 ^d | 3' intergenic 42,965 A > C | | AA | 400 | 32.9 (19.2, 57.4) | 0.036 |
| | | | | AC | 270 | 38.4 (19.1, 71.7) | |
| | | | | CC | 44 | 37.8 (19.3, 74.8) | |
| ISG20L1 | rs3743477 | Coding 43 C > T | Pro15Leu | GG | 633 | 33.3 (19.0, 61.7) | 0.048 |
| | | | | GA | 78 | 44.4 (24.3, 81.3) | |
| | | | | AA | 2 | 37.4 (12.8, 62.0) | |

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

^bIQR, interquartile range, values are in IU/ml measured by the Beckman Coulter Access® Rubella IgG assay.

^cOne 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.

^drs1732778 and rs2464288 are in strong LD (*r*² = 1) with each other.

3.3.2. Associations between SNPs in antiviral genes and rubella virus-specific T_h1 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* ≤ 0.021) 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 toward higher IFN- γ response (*p* ≤ 0.045). 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* ≤ 0.05) in coding or regulatory SNPs, all belonging to genes of the OAS system and seven 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 (T_h1) response (*p* ≤ 0.024, 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* ≤ 0.026). Increased representation of 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* ≤ 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 S2.

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

We were not able to test for SNP associations with classical T_h2 cytokines, because 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/RNase L 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/T_h1 (*p* = 0.002) and IL-10 (*p* = 0.009) secretion. Increased representation of 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, such as background unstimulated levels, virus-stimulated levels and corrected levels, are presented in Supplementary Table S2.

3.3.4. 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 of 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 with *r*² ≥ 0.96, and rs456298) located downstream of the *MX1* gene, associated with variations in rubella virus-specific TNF- α secretion (*p* ≤ 0.046). We observed eight significant SNP associations with the level of rubella virus-specific IL-6 secretion, and five of them were within genes of the OAS/RNase L 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).

Table 3
Associations between SNPs in antiviral genes and rubella-specific T_H1 cytokine responses

| Secreted cytokine | SNP ID (gene) | Location/relative position | Function | Genotype | N ^a | Median level pg/ml (IQR) ^b | p-value ^c |
|-----------------------------|---|----------------------------|-----------|----------|-------------------|---------------------------------------|----------------------|
| IFN- γ | rs1127317 ^d (<i>ADAR</i>) | 3'UTR 24,442 A > C | | AA | 382 | 8.3 (2.7, 23.5) | 0.014 |
| | | | | AC | 251 | 8.4 (3.0, 23.2) | |
| | | | | CC | 59 | 12.4 (3.8, 32.6) | |
| | rs3743476 (<i>ISG20L1</i>) | Coding 173A > G | Glu58Glu | AA | 311 | 7.0 (2.5, 23.4) | 0.019 |
| | | | | AG | 309 | 9.0 (3.5, 23.5) | |
| | rs2229857 ^d (<i>ADAR</i>) | Coding 6515 C > T | Arg384Lys | GG | 72 | 10.8 (3.9, 27.2) | 0.021 |
| | | | | GA | 376 | 8.3 (2.7, 23.5) | |
| | rs11073795 (<i>ISG20</i>) | 5'intergenic – 1820 G > A | Promoter | AA | 63 | 11 (3.8, 32.6) | 0.045 |
| | | | | GG | 248 | 6.7 (2.6, 19.3) | |
| | | | | GA | 334 | 9.0 (3.1, 29.2) | |
| rs11073795 (<i>ISG20</i>) | 5'intergenic – 1820 G > A | Promoter | AA | 110 | 11.1 (3.7, 21.9) | | |
| | | | GA | 334 | 9.0 (3.1, 29.2) | | |
| IL-2 | rs10774670 ^e (<i>OAS1</i>) | 5'intergenic – 2683 G > A | Promoter | GG | 353 | 16.7 (5.9, 30.4) | 0.002 |
| | | | | GA | 274 | 17.5 (8.8, 30.2) | |
| | | | | AA | 46 | 21.7 (11, 34.9) | |
| | rs10774669 ^e (<i>OAS1</i>) | 5'intergenic – 5418 C > T | Promoter | GG | 404 | 16.4 (6.2, 29.5) | 0.003 |
| | | | | GA | 253 | 18.1 (8.7, 32.4) | |
| | | | | AA | 34 | 23.6 (13.1, 47.2) | |
| | rs10492028 ^e (<i>OAS1</i>) | 5'intergenic – 7682 G > A | Promoter | GG | 410 | 16.3 (6.1, 29.5) | 0.004 |
| | | | | GA | 243 | 17.7 (8.7, 32.4) | |
| | | | | AA | 39 | 23 (13.8, 47.2) | |
| | rs1051042 ^f (<i>OAS1</i>) | Coding 12,392 C > G | Thr361Arg | CC | 292 | 16.0 (5.7, 29.4) | 0.018 |
| | | | | CG | 327 | 18.1 (8.7, 31.1) | |
| | | | | GG | 72 | 18.9 (8.9, 37.9) | |
| | rs2660 ^f (<i>OAS1</i>) | 3'UTR 12,597 A > G | | AA | 293 | 16.0 (5.7, 29.3) | 0.019 |
| | | | | AG | 327 | 18.1 (8.7, 31.1) | |
| | | | | GG | 72 | 18.9 (8.9, 37.9) | |
| | rs2384071 ^f (<i>OAS3</i>) | 5'intergenic – 8993 G > A | Promoter | GG | 278 | 15.4 (5.7, 29.3) | 0.023 |
| | | | | GA | 328 | 18.1 (8.7, 30.6) | |
| | | | | AA | 76 | 18.9 (8.9, 37.3) | |
| | rs3741981 ^f (<i>OAS1</i>) | Coding 4025 A > G | Ser162Gly | AA | 224 | 16.3 (5.7, 29.5) | 0.024 |
| | | | | AG | 345 | 17.9 (7.9, 30.5) | |
| | | | | GG | 123 | 17.7 (9.5, 34.6) | |
| | rs1557865 ^e (<i>OAS1</i>) | 5'intergenic – 2247G > T | Promoter | CC | 449 | 17.0 (7.6, 30.3) | 0.026 |
| | | | | CA | 222 | 17.7 (8.4, 32.2) | |
| | | | | AA | 21 | 24.1 (13.1, 47.2) | |
| | rs2384072 ^f (<i>OAS3</i>) | 5'intergenic – 8914 C > T | Promoter | GG | 281 | 15.9 (5.8, 29.3) | 0.036 |
| | | | | GA | 333 | 18.1 (8.7, 30.7) | |
| | | | | AA | 78 | 18.9 (9.5, 37.1) | |
| | rs2010604 (<i>OAS3</i>) | 3'UTR 31,872 G > C | | GG | 326 | 15.9 (5.9, 29.5) | 0.047 |
| GC | | | | 309 | 18.2 (8.7, 30.5) | | |
| CC | | | | 57 | 18.4 (10.5, 37.6) | | |

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

^bIQR, interquartile range, values in pg/ml measured by ELISA.

^cOne 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 ($p \leq 0.05$) are presented.

^dSNPs rs1127317 and rs2229857 are in LD ($r^2 = 0.94$).

^eSNPs rs10774670, rs10774669, rs10492028 and rs1557865 are in a LD block (Fig. 1).

^fSNPs rs1051042, rs2660, rs3741981, rs2384071 and rs2384072 are in a LD block (Fig. 1).

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, such as background unstimulated levels, virus-stimulated levels and corrected levels, are presented in Supplementary Table S2.

3.3.5. Associations between *OAS1* haplotypes and rubella virus-specific IL-2 and IL-10 secretion

Because 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 seven *OAS1* haplotypes with frequencies $\geq 1\%$ 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 , $p = 0.019$).

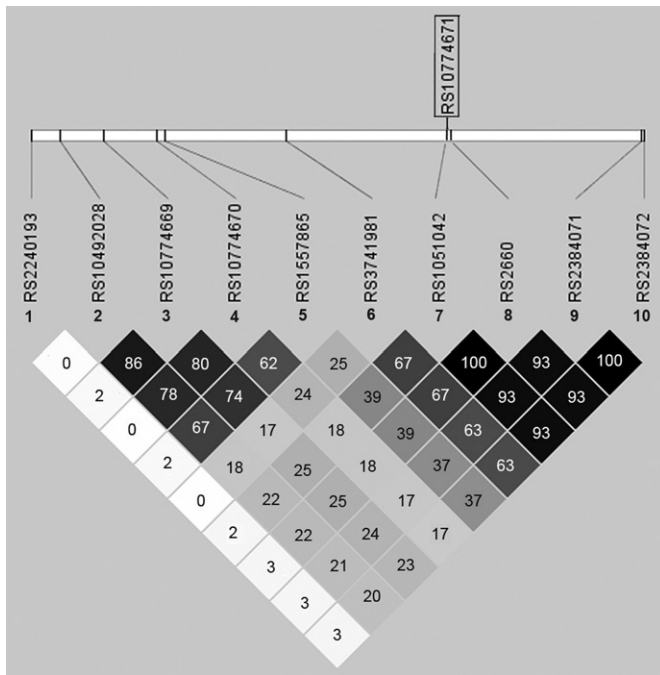


Fig. 1. Haplotype block structure of the *OAS1* gene region in the study cohort. The LD block structure was analyzed using Haploview software, version 3.32. SNPs in the *OAS1* region clustered into two blocks of linkage disequilibrium. The location of the splicing variant rs10774671 (boxed; not genotyped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

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*/*RNase L* pathway in the modulation of rubella vaccine-induced immune response.

On binding to dsRNA *OAS1* catalyses the oligomerization of ATP into 2'-5'-linked oligoadenylates and activates *RNase L*, 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].

Table 4

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

| Secreted cytokine | SNP ID (gene) | Location/relative position | Function | Genotype | N ^a | Median level pg/mL (IQR) ^b | p-value ^c |
|----------------------------|----------------------------|----------------------------|-----------|----------|----------------|---------------------------------------|----------------------|
| IL-10 | rs10774670 (<i>OAS1</i>) | 5' intergenic -2683 G > A | Promoter | GG | 353 | 4.5 (2.4, 7.0) | 0.009 |
| | | | | GA | 274 | 4.0 (1.7, 6.2) | |
| | | | | AA | 46 | 5.2 (3.3, 7.7) | |
| | rs627928 (<i>RNaseL</i>) | Coding 4604 C > A | Glu541Asp | CC | 234 | 4.6 (2.7, 7.1) | 0.01 |
| | | | | CA | 306 | 4.2 (1.9, 6.9) | |
| | | | AA | 152 | 3.9 (2.1, 5.9) | | |
| rs3741981 (<i>OAS1</i>) | Coding 4025 A > G | Ser162Gly | AA | 224 | 4.6 (2.5, 7.7) | 0.031 | |
| | | | AG | 345 | 4.1 (2.0, 6.5) | | |
| | | | GG | 123 | 4.1 (2.3, 6.2) | | |
| rs2240193 (<i>OAS1</i>) | 5' intergenic -9184 C > A | Promoter | CC | 585 | 4.1 (2.2, 6.5) | 0.033 | |
| | | | CA | 99 | 4.6 (2.7, 8.9) | | |
| | | | AA | 8 | 4.7 (3.1, 6.9) | | |
| rs8038865 (<i>ISG20</i>) | 5' intergenic -1496 C > G | Promoter | CC | 369 | 4.2 (2.2, 6.7) | 0.037 | |
| | | | CG | 265 | 4.3 (2.6, 6.7) | | |
| | | | GG | 58 | 2.9 (1.1, 6.1) | | |

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

^bIQR, interquartile range, values in pg/ml measured by ELISA.

^cOne 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 ($p \leq 0.05$) are presented.

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 after 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 seven 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 published data [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-accepter 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, whereas 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 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 polymorphism (rs3741981) occurs in an evolutionarily conserved DNA region in all *OAS1* isoforms near the dsRNA binding domain. This same polymorphism 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 T_H1 /IL-2 and IL-10 secretion

Table 5
Associations between SNPs in antiviral genes and rubella-specific inflammatory cytokine responses

| Secreted cytokine | SNP ID (gene) | Location /relative position | Function | Genotype | N ^a | Median level pg/ml (IQR) ^b | p-value ^c |
|----------------------------|--|-----------------------------|-----------|----------|-------------------------|---------------------------------------|----------------------|
| TNF- α | rs1552902 (<i>ADAR</i>) | 5'intergenic -34,591G > C | Promoter | GG | 331 | 25.6 (-14.5, 73.9) | 0.007 |
| | | | | GC | 285 | 31.3 (-4.2, 89.3) | |
| | | | | CC | 76 | 41.9 (-0.3, 139.9) | |
| | rs8127664 ^d (<i>MX1</i>) | 3'intergenic 28,640 C > T | | GG | 497 | 35.0 (-3.0, 97.4) | 0.008 |
| | | | | GA | 180 | 21.6 (-18.9, 73.8) | |
| | | | | AA | 14 | 47.0 (-14.5, 95.9) | |
| | rs13433394 ^d (<i>MX1</i>) | 3'intergenic 27,912 C > T | | GG | 493 | 35.0 (-3.0, 97.4) | 0.013 |
| | | | | GA | 182 | 22.2 (-18.9, 74.5) | |
| | | | | AA | 17 | 36.5 (-14.5, 75.6) | |
| | rs8127290 ^d (<i>MX1</i>) | 3'intergenic 28,166 G > A | | GG | 493 | 35.0 (-3.0, 97.4) | 0.013 |
| | | | | GA | 182 | 22.2 (-18.9, 74.5) | |
| | | | | AA | 17 | 36.5 (-14.5, 75.6) | |
| | rs9427092 (<i>ADAR</i>) | 3'intergenic 26,760 T > C | | AA | 414 | 28.3 (-3.4, 88.7) | 0.015 |
| | | | | AG | 239 | 36.7 (-8.9, 95.6) | |
| | | | | GG | 38 | 1.0 (-21.6, 56.9) | |
| rs456298 (<i>MX1</i>) | 3'intergenic 32,753 A > T | | AA | 477 | 35.4 (-3.6, 96.0) | 0.046 | |
| | | | AT | 192 | 25.1 (-16.5, 82.3) | | |
| | | | TT | 23 | 18.7 (-37.7, 73.0) | | |
| IL-6 | rs13311 (<i>OAS2</i>) | 3'UTR 32,238 C > A | | CC | 270 | 3667.4 (3167.4, 4031.3) | 0.02 |
| | | | | CA | 314 | 3697.1 (3140.4, 4090.8) | |
| | | | | AA | 108 | 3657.8 (3087.8, 4083.1) | |
| | rs627928 (<i>RNASEL</i>) | Coding 4604 C > A | Glu541Asp | CC | 234 | 3708.0 (3204.1, 4095.1) | 0.033 |
| | | | | CA | 306 | 3662.0 (3099.0, 4038.5) | |
| | | | | AA | 152 | 3636.0 (3077.7, 4045.4) | |
| | rs9616 (<i>ADAR</i>) | 3'UTR 24,749 T > A | | TT | 366 | 3713.1 (3216.4, 4105.7) | 0.034 |
| | | | | TA | 267 | 3659.3 (3090.5, 4030.4) | |
| | | | | AA | 59 | 3668.3 (2931.9, 4061.3) | |
| | rs1732778 (<i>OAS2</i>) ^e | 3'intergenic 40,511G > A | | GG | 388 | 3739.5 (3178.1, 4070.6) | 0.041 |
| | | | | GA | 263 | 3609.8 (3067.1, 4062.4) | |
| | | | | AA | 41 | 3517.9 (3226.6, 4061.3) | |
| | rs2464288 (<i>OAS2</i>) ^e | 3'intergenic 42,965 A > C | | AA | 388 | 3739.5 (3178.1, 4070.6) | 0.041 |
| | | | | AC | 263 | 3609.8 (3067.1, 4062.4) | |
| | | | | CC | 41 | 3517.9 (3226.6, 4061.3) | |
| rs469390 (<i>MX1</i>) | Coding 13,932 A > G | Ile379Val | AA | 226 | 3649.1 (3093.9, 4020.6) | 0.044 | |
| | | | AG | 347 | 3693.6 (3098.7, 4121.6) | | |
| | | | GG | 119 | 3684.3 (3248.5, 4071.3) | | |
| rs2229857 (<i>ADAR</i>) | Coding 6515 C > T | Arg384Lys | GG | 376 | 3659.8 (3072.0, 4061.9) | 0.045 | |
| | | | GA | 253 | 3703.0 (3187.9, 4083.5) | | |
| | | | AA | 63 | 3698.0 (3099.0, 3969.3) | | |
| rs12815666 (<i>OAS2</i>) | 5'intergenic -660 C > T | Promoter | GG | 536 | 3692.8 (3131.7, 4076.6) | 0.049 | |
| | | | GA | 146 | 3666.8 (3131.1, 4030.0) | | |
| | | | AA | 10 | 3613.3 (3366.6, 3900.6) | | |
| GM-CSF | rs3743476 (<i>ISG20L1</i>) | Coding 173 A > G | Glu58Glu | AA | 311 | 29.5 (24.4, 33.1) | 0.021 |
| | | | | AG | 307 | 27.4 (23.0, 31.9) | |
| | | | | GG | 72 | 26.1 (20.9, 31.6) | |

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

^bIQR, interquartile range, values in pg/ml measured by ELISA.

^cOne 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 ($p \leq 0.05$) are presented.

^dSNPs rs8127664, rs13433394 and rs8127290 are in LD ($r^2 \geq 0.96$).

^eSNPs rs1732778 and rs2464288 are in LD ($r^2 = 1$).

levels. IL-2 is a multifunctional T_H1 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 T_H1 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. Because complex trait associations are more likely dependent on several/multiple genetic variants, it is reasonable to suggest that the ob-

served 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, whereas 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

Table 6
OAS1 haplotype associations with rubella-specific IL-2 and IL-10 cytokine secretion

| <i>OAS1</i> haplotype | Frequency | IL-2 | | | IL-10 | | |
|------------------------|-----------|--|------------------------|------------------------|--|------------------------|------------------------|
| | | Test statistic (haplotype <i>t</i> -statistic) | Allele <i>p</i> -value | Global <i>p</i> -value | Test statistic (haplotype <i>t</i> -statistic) | Allele <i>p</i> -value | Global <i>p</i> -value |
| 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 | |

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. Statistically significant *p*-values ($p < 0.05$) are highlighted in bold.

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

within the protein kinase domain of the *ASEL* gene (rs627928). Although this genetic variant was shown to produce similar levels of RNase L 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 U.S.A. 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 because 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 planning. Another legitimate concern is that some of the observed differences in immune measures associated with different SNP alleles appear moderate and it is difficult to assign biologic 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 moderate 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 resolving 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 biologic 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.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.humimm.2010.01.004.

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