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Single nucleotide polymorphisms/haplotypes associated with multiple rubella-specific immune response outcomes post-MMR immunization in healthy children

Inna G. Ovsyannikova[1] · Hannah M. Salk[1] · Beth R. Larrabee[2] ·
V. Shane Pankratz[2] · Gregory A. Poland[1]

Abstract The observed heterogeneity in rubella-specific immune response phenotypes post-MMR vaccination is thought to be explained, in part, by inter-individual genetic variation. In this study, single nucleotide polymorphisms (SNPs) and multiple haplotypes in several candidate genes were analyzed for associations with more than one rubella-specific immune response outcome, including secreted IFN-γ, secreted IL-6, and neutralizing antibody titers. Overall, we identified 23 SNPs in 10 different genes that were significantly associated with at least two rubella-specific immune responses. Of these SNPs, we detected eight in the *PVRL3* gene, five in the *PVRL1* gene, one in the *TRIM22* gene, two in the *IL10RB* gene, two in the *TLR4* gene, and five in other genes (*PVR*, *ADAR*, *ZFP57*, *MX1*, and *BTN2A1/BTN3A3*). The *PVRL3* gene haplotype GACGGGGGCAGCAAAAAGAAGAGGAAAGAACAA was significantly associated with both higher IFN-γ secretion (*t*-statistic 4.43, *p*<0.0001) and higher neutralizing antibody titers (*t*-statistic 3.14, *p*=0.002). Our results suggest that there is evidence of multigenic associations among identified gene SNPs and that polymorphisms in these candidate genes contribute to the overall observed differences between individuals

in response to live rubella virus vaccine. These results will aid our understanding of mechanisms behind rubella-specific immune response to MMR vaccine and influence the development of vaccines in the future.

Introduction

The morbidity associated with rubella virus (RV) infection remains of great concern. Although typical childhood or adulthood infection can be benign (signified by rash, fever, lymphadenopathy, and malaise), rubella infection is particularly dangerous in pregnant women, resulting in congenital defects of the fetus (Plotkin 2001) or, in severe cases, perinatal death (Sydnor and Perl 2014). The most effective way to prevent rubella infection and reduce the morbidity associated with congenital rubella syndrome is maternal immunization with the measles-mumps-rubella (MMR) virus vaccine, which has successfully reduced rubella infection by >99 % since its introduction in 1971 in the USA (Lievano et al. 2012).

While many individuals develop protective immunity against rubella after MMR vaccination (~95 %), others remain susceptible to infection for several reasons, including waning rubella-specific immune memory and primary vaccine nonresponse to RV (Sydnor and Perl 2014), both of which are influenced by genetic factors. Our previous study aimed to

✉ Gregory A. Poland
poland.gregory@mayo.edu

1 Mayo Clinic Vaccine Research Group, Mayo Clinic, Guggenheim 611C, 200 1st Street S.W., Rochester, Minnesota 55905, USA

2 Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55905, USA

identify genetic influences on response to rubella vaccine (Ovsyannikova et al. 2007, 2009a, 2014a). Twenty-seven genes, including genes in the HLA-A and HLA-B loci, were found to differ between low and high antibody responders after stimulation with RV (Haralambieva et al. 2013), suggesting that genetics play a large role in one's ability to develop protective immunity against rubella after immunization. In this study, we identified several single-nucleotide polymorphisms (SNPs)/haplotypes in candidate immune response genes that are significantly associated with multiple rubella vaccine-induced immune response outcomes after MMR immunization.

To our knowledge, this study is the first study of its kind to identify polymorphisms in several candidate genes that are significantly associated with *more than one* rubella-specific immune response (i.e., secreted IFN-γ, IL-6, and neutralizing antibody titers) post-MMR vaccination. Developing an understanding of the function of genetic variability on immune response to rubella immunization is critical for designing more effective vaccines in the future.

Materials and methods

The methods described in this study are similar to those published for our previous studies (Haralambieva et al. 2011a, 2014; Lambert et al. 2013, 2014; Ovsyannikova et al. 2011a, b).

Study subjects and immunization

Subjects from a previously described cohort were utilized for this study (Haralambieva et al. 2010; Ovsyannikova et al. 2004, 2005, 2010a, 2011b). The study cohort comprised a combined sample of 1052 subjects from three independent cohorts of healthy children in Rochester, MN. Specifically, 368 healthy children, ranging in age from 12 to 18 years, were recruited from Rochester, MN, between the years 2001 and 2002. In 2006–2007, we enrolled an additional 396 healthy children (age 11–19 years) as part of our original MMR vaccine studies. In 2008–2009, additional subjects, ranging in age from 11 to 22 years, were added to this cohort, resulting in a cohort of 1052 subjects. Prior to participation in these studies, subjects provided documentation of receiving two doses of rubella-containing vaccine. After excluding subjects without genotyping data, 1039 subjects remained for analysis. Each subject provided a written record of receiving two age-appropriate doses of MMR vaccine. Permission to conduct this study was granted by the institutional review board of Mayo Clinic.

Antibody measurement

Rubella-specific neutralizing antibody (NA) titers were quantified for each subject using a method that has been previously published (Lambert et al. 2014). In brief, a modified soluble immunocolorimetric (ICA)-based neutralization assay (sICNA) was optimized for high-throughput measurement and analysis. Measurements were reported as the highest dilution at which there was a 50 % reduction in viral activity (NT_{50}).

Secreted cytokine measurement

Secreted rubella-specific IFN-γ and IL-6 were measured by conducting enzyme-linked immunosorbent assays (ELISAs). The complete protocol for this methodology has been previously published (Dhiman et al. 2010; Lambert et al. 2013; Ovsyannikova et al. 2009b). To summarize, cryopreserved peripheral blood mononuclear cells (PBMCs) from each subject were cultured, in triplicate (2×10^5 cells per well in 96-well plates), with either media (control wells) or the W-Therien strain of RV (MOI=5). PHA (5 μg/ml) was used as a positive control. Cell cultures were incubated based upon previous optimization results: 48 h for maximal IFN-γ secretion and 24 h for maximal IL-6 secretion. ELISAs were performed using the manufacturer's recommendations (BD Pharmingen), and plates were read at 450 nm on a microplate reader (Molecular Devices Corporation, Sunnyvale, CA).

Candidate gene SNP genotyping

The description of the tagging SNP selection strategies and genotyping methods has been previously described (Haralambieva et al. 2011a, 2014; Ovsyannikova et al. 2011a, b). SNPs within candidate genes, 5 kb upstream and downstream for each candidate gene, were chosen based on the linkage disequilibrium (LD) tagSNP selection algorithm (Yen et al. 2006) from the Hapmap Phase II (http://www. hapmap.org), Seattle SNPs (http://pga.mbt.washington.edu/), and NIEHS SNPs (http://egp.gs.washington.edu/), with SNP minor allele frequencies≥0.05, LD threshold of r^2≥0.90. Overall, 768 SNPs in 92 candidate genes were analyzed as part of this study (Haralambieva et al. 2014; Pankratz et al. 2010). The 768 SNPs were genotyped using a custom-designed 768-plex Illumina GoldenGate™ assay (Illumina Inc., San Diego, CA) following the manufacturer's instructions. The BeadStudio 2 software was used to call genotypes.

Statistical analysis

Our goal was to determine whether there were genetic variants that shared associations with multiple immune response phenotypes for rubella. We have reported many of these

associations previously, and the analytical methods used to assess the associations are outlined in those publications (Haralambieva et al. 2011a; Ovsyannikova et al. 2010b, 2011b, 2012). Briefly, to assess the additive genetic association with the logarithmic transformation of neutralizing antibody NT50 titer, simple linear regression models were employed. All other phenotypes had multiple observations per subject and were evaluated with repeated measures approaches, including an unstructured within-person variance-covariance matrix to account for within-subject correlations. A test of the ordinal genotype by stimulation status interaction assessed the effect of genotype on the average difference between stimulated and unstimulated PBMC samples. Our primary tests of association for each of these phenotypes were adjusted for a number of potentially important covariates, including age at enrollment, ages at first and second vaccinations, sex, time between most recent vaccination and study participation, batch/run number of assay, and the first three population stratification eigenvectors. The q-values were then computed. The p-values from these primary tests of association were compared on a SNP-by-SNP basis to identify polymorphisms significant ($p<0.05$) for more than one phenotype. Six-hundred and five SNPs were used in these comparisons. Summaries of the measures of virus-specific immune responses were obtained for these SNPs, broken by allelic category, as noted in our previously published work (Haralambieva et al. 2011a; Ovsyannikova et al. 2010a, b, 2011b).

Further exploration of these data then commenced via post hoc haplotype estimation and analysis. In the absence of pedigree data and known linkage phase, there may be multiple possible haplotypes for an observed genotype. To address this complication, the expectation-maximum (EM) algorithm, as per Schaid et al. (Schaid et al. 2002), was used to establish the conditional posterior probabilities of a given haplotype with an observed genotype. From these probabilities, a design matrix of expected haplotype counts was constructed and used to investigate associations with phenotype. The above techniques were applied utilizing the Haplo.Stats package with the default settings for batch size, maximum number of iterations and convergence criteria. Haplotypes with less than a 1 % frequency were eliminated to help reduce the error associated with estimation.

Results

Demographics

In total, 1039 subjects participated in this study. The median age at the time of enrollment was 15.1 years with a range of 11–22 years. Slightly over half (55.2 %) of the cohort consisted of male subjects, while the remaining 44.8 % were female. Caucasian-Americans accounted for 85.0 % of the cohort, and 8.1 % of subjects identified as either Black or African-American. Quantification of NA titers, IL-6 secretion, and IFN-γ secretion within the cohort yielded median values of 57.4 (range 17.0–2391.2), 3595.7 ng/μl (range −957.3–5831.8 ng/μl), and 6.1 ng/μl (range −239.0–579.4 ng/μl), respectively. See Table 1 for a summary of subject demographics.

Associations between SNPs and immune measures

Since the study subjects were racially diverse, genotype-phenotype data were analyzed for a combined cohort of 1039 subjects (Table 2) and, in addition, separately for 883 Caucasian subjects (Supplementary Table S1).

PVRL3 (Nectin-3) gene associations

Overall, we identified 23 SNPs in 10 genes that were significantly associated with more than one rubella-specific immune phenotype post-MMR vaccination, including eight SNPs in the PVRL3 gene. Of these eight SNPs, five were significantly associated with rubella-specific IL-6 and IFN-γ secretion: rs4682233 ($p<0.0007$), rs72935984 ($p<0.004$), rs72937914 ($p<0.04$), rs10433385 ($p<0.01$), and rs78545860 ($p<0.01$). Interestingly, the major alleles of these SNPs were all associated with an increase in IL-6 secretion, and enhanced IFN-γ secretion was observed in four of the five. The remaining three SNPs identified in the PVRL3 gene (rs75054607, rs76464851, and rs78157313) were significantly associated with IFN-γ secretion and NA titers ($p<0.002$, $p<0.002$, and $p<0.002$, respectively). Though relatively rare (only one homozygous minor allele GG genotype was observed), increasing copies of the minor alleles in all three of these SNPs resulted in a greater than 80-fold increase in IFN-γ secretion from baseline and a greater than 3-fold increase in NA titers from baseline (Table 2). These associations remained statistically significant when excluding the homozygous minor allele genotype observed only in one subject (IFN-γ, $p=0.004$; NT$_{50}$, $p=0.0007$).

Several of the SNPs in the PVRL3 gene are in high linkage disequilibrium (LD) and are likely to be inherited together. SNPs in high LD ($r^2 \geq 0.92$) include rs72935984 and rs4682233; rs75054607, rs78157313, and rs76464851; and rs10433385 and rs78545860 (Fig. 1).

PVRL1 (Nectin-1) gene associations

Five SNPs in the PVRL1 gene were also found to be significantly associated with multiple rubella-specific immune response outcomes. The homozygous major alleles of the rs11820364 ($p<0.008$; one homozygous minor allele genotype was observed) and rs61247604 ($p<0.0005$; no

Table 1 Subject demographics and immune outcomes summary

Subject demographics and immune outcomes summary

	Total (N=1039)	p value
Age		<0.0001
N	1039	
Mean (SD)	15.1 (2.2)	
Median	15.0	
Q1, Q3	13.0, 17.0	
Range	(11.0–22.0)	
Age at first vaccination (months)		0.0002
N	1039	
Mean (SD)	20.1 (20.9)	
Median	15.0	
Q1, Q3	15.0, 16.0	
Range	(11.0–185.0)	
Age at second vaccination (years)		<0.0001
N	1039	
Mean (SD)	8.4 (3.5)	
Median	9.0	
Q1, Q3	5.0, 12.0	
Range	(1.0–17.0)	
Time from second vaccination to enrollment (years)		<0.0001
N	1039	
Mean (SD)	6.7 (2.9)	
Median	6.4	
Q1, Q3	4.6, 8.6	
Range	(0.4–16.8)	
Gender		0.6255
Male	574 (55.2 %)	
Female	465 (44.8 %)	
Race		<0.0001
American Indian, Alaska Native	4 (0.4 %)	
Asian, Hawaiian, Pacific Islander	27 (2.6 %)	
Black or African American	84 (8.1 %)	
Caucasian-American	883 (85.0 %)	
Multiple	28 (2.7 %)	
Other	7 (0.7 %)	
Unknown	6 (0.6 %)	
Ethnicity		0.0089
Not Hispanic or Latino	1012 (97.4 %)	
Hispanic or Latino	20 (1.9 %)	
Don't know	7 (0.7 %)	
Neutralizing antibody (NT_{50})		0.0034
N	1029	
Mean (SD)	81.6 (123.5)	
Median	57.4	
Q1, Q3	34.9, 95.5	
Range	(17.0–2391.2)	
IL-6 (ng/µl)		<0.0001
N	988	
Mean (SD)	3435.4 (908.0)	

Table 1 (continued)

Subject demographics and immune outcomes summary

Median	3595.7	
Q1, Q3	3027.1, 4005.1	
Range	(−957.3–5831.8)	
IFN-γ (ng/µl)		<0.0001
N	969	
Mean (SD)	24.4 (70.0)	
Median	6.1	
Q1, Q3	1.5, 20.0	
Range	(−239.0–579.4)	

Negative cytokine values indicate that the unstimulated secretion levels were, on average, higher than the rubella virus-stimulated secretion levels.

IL interleukin, *IFN* interferon, *SD* standard deviation, *Q1* first quartile, *Q3* third quartile, NT_{50} neutralizing titer

homozygous minor allele genotype was found) SNPs were associated with a decrease in both IL-6 secretion and NA titers. Both associations remained significant when excluding the homozygous minor allele genotype from analysis (rs11820364, IL-6, p=0.002; NT_{50}, p=0.02; rs61247604, IL-6, p=0.0003; NT_{50}, p=0.0005). An increase in the count of major alleles of the rs73571285 (p<0.02) and rs73571287 (p<0.02) SNPs were associated with an increase in IL-6 secretion and a decrease in secreted IFN-γ; however, rs73571285 demonstrated no association with neither IL-6 nor IFN-γ secretion, and rs73571287 displayed no association with IFN-γ secretion when excluding the homozygous minor allele genotypes from statistical analysis (data not shown). The three remaining SNPs in the PVRL1 gene (rs79849521, p<0.03; rs4936489, p<0.03; and rs73578845, p<0.04) were significantly associated with both IL-6 secretion and NA titers. Likewise, when examining associations without the homozygous minor genotypes that have few (0–2) subjects in that category, IL-6 association with the PVRL1 rs73578845 becomes more significant (p<0.0001), but the NA titer association becomes less significant (p=0.104). Decreasing the count of the major alleles of the rs79849521 resulted in an increase from baseline in IL-6 secretion, as well as an increase in NA titers. Conversely, the major allele of rs4936489 tended to correlate with enhanced IL-6 (p=0.04) secretion and decreased NA titers (p=0.03). These associations remained statistically significant when excluding the homozygous minor allele genotype observed only in one individual (IL-6, p= 0.009; NT_{50}, p=0.04) (Table 2).

Several intronic SNPs in the *PVRL1* gene have a moderate likelihood of being inherited together. SNPs with an LD of $r^2 \geq 0.33$ include rs11820364 with rs79849521 and rs61247604, rs79849521 with rs61247604, and rs73571285 with rs73571287 (Fig. 2).

Table 2 SNPs associated with more than one rubella-specific immune response outcome after MMR vaccination.

Cohort	SNP ID	Chromosome/position	Gene	Location	Immune measure	Number	Genotype	Number	Immune response outcome (IQR)
Rubella Vaccine Study	rs4682233	3/1.11×10^8	PVRL3	Intron	Secreted IL-6 (ng/µl)	969	AA AG GG	953 16 0	3609.7 (3049.2, 4006.3) 3372.2 (1952.7, 4094.1)
Rubella Vaccine Study	rs72935984	3/1.11×10^8	PVRL3	Intron	Secreted IL-6 (ng/µl)	962	CC CA AA	944 18 0	3609.8 (3048.0, 4007.3) 3608.4 (2134.9, 4123.8)
Rubella Vaccine Study	rs75054607+NA	3/1.11×10^8	PVRL3	Intron	Secreted IFN-γ (ng/µl)	969	AA AG GG	946 22 1	6.1 (1.5, 19.7) 11.6 (3.9, 33.6) 508.8 (508.8, 508.8)
Rubella Vaccine Study	rs76464851+NA	3/1.11×10^8	PVRL3	Intron	Secreted IFN-γ (ng/µl)	969	AA AG GG	946 22 1	6.1 (1.5, 19.7) 11.6 (3.9, 33.6) 508.8 (508.8, 508.8)
Rubella Vaccine Study	rs7815313+NA	3/1.11×10^8	PVRL3	Intron	Secreted IFN-γ (ng/µl)	967	AA AG GG	944 22 1	6.1 (1.5, 19.8) 11.6 (3.9, 33.6) 508.8 (508.8, 508.8)
Rubella Vaccine Study	rs7293914+IFN-γ	3/1.11×10^8	PVRL3	Intron	Secreted IL-6 (ng/µl)	969	AA AC CC	942 27 0	3612.3 (3056.4, 4008.4) 2917.9 (2076.4, 4064.3)
Rubella Vaccine Study	rs10433385+IFN-γ	3/1.11×10^8	PVRL3	Intergenic	Secreted IL-6 (ng/µl)	968	AA AG GG	929 36 3	3613.2 (3056.4, 4020.6) 3398.5 (2754.5, 3870.5) 2717.6 (2617.3, 4306.3)
Rubella Vaccine Study	rs78545860+IFN-γ	3/1.11×10^8	PVRL3	Intergenic	Secreted IL-6 (ng/µl)	969	AA AG GG	930 36 3	3614.0 (3056.4, 4020.6) 3398.5 (2754.5, 3870.5) 2717.6 (2617.3, 4306.3)
Rubella Vaccine Study	rs11820364+NA	11/1.2×10^8	PVRL1	Intron	Secreted IL-6 (ng/µl)	969	GG GA AA	953 15 1	3595.6 (3036.5, 4000.4) 4328.9 (3759.0, 4494.6) 2271.6 (2271.6, 2271.6)
Rubella Vaccine Study	rs61247604	11/1.2×10^8	PVRL1	Intron	Secreted IL-6 (ng/µl)	968	GG GA AA	956 12 0	3596.0 (3037.7, 4003.1) 4178.1 (3605.9, 4384.3)
Rubella Vaccine Study	rs73571285[a]+IFN-γ	11/1.2×10^8	PVRL1	Intron	Secreted IL-6 (ng/µl)	969	AA AG GG	953 14 2	3600.2 (3049.7, 4003.7) 4011.0 (2630.3, 4439.8) −450.2 (−957.3, 56.9)
Rubella Vaccine Study	rs73571287[a]+IFN-γ	11/1.2×10^8	PVRL1	Intron	Secreted IL-6 (ng/µl)	968	GG GA AA	959 8 1	3598.9 (3043.4, 4003.9) 4011.0 (1079.8, 4310.4) 56.9 (56.9, 56.9)
Rubella Vaccine Study	rs79849521[c]	11/1.2×10^8	PVRL1	Intron	Secreted IL-6 (ng/µl)	969	GG GA AA	959 10 0	3598.9 (3036.5, 4003.9) 4099.9 (3356.6, 4328.9)
Rubella Vaccine Study	rs4936489[c]	11/1.2×10^8	PVRL1	Intergenic	Secreted IL-6 (ng/µl)	968	GG GA AA	936 31 1	3605.0 (3041.1, 4005.1) 3514.8 (2803.7, 4126.2) 3696.9 (3696.9, 3696.9)
Rubella Vaccine Study	rs73578845+NA	11/1.19×10^8	PVRL1	Intergenic	Secreted IL-6 (ng/µl)	969	GG GC CC	956 11 2	3596.0 (3041.1, 4003.1) 4078.1 (2299.1, 4439.8) 4099.9 (3970.0, 4229.9)
Rubella Vaccine Study	rs2291842+NA	11/5719667	TRIM22	Coding	Secreted IFN-γ (ng/µl)	969	AA AG GG	639 285 45	5.9 (1.5, 19.9) 6.1 (1.9, 18.0) 11.5 (2.7, 42.5)

Table 2 (continued)

Cohort	SNP ID	p value	Chromosome/position	Gene	Location	Immune measure	Number	Genotype	Number	Immune response outcome (IQR)
Rubella Vaccine Study	rs2291842+NA	0.000675	11/5719667	TRIM22	Coding	Secreted IL-6 (ng/μl)	969	AA	639	3619.8 (3056.4, 4002.4)
								AG	285	3595.8 (3024.7, 4038.5)
								GG	45	3350.8 (3025.7, 3868.0)
Rubella Vaccine Study	rs2291842+IFN-γ		11/5719667	TRIM22	Coding	Secreted IL-6 (ng/μl)	969	AA	639	3619.8 (3056.4, 4002.4)
								AG	285	3595.8 (3024.7, 4038.5)
								GG	45	3350.8 (3025.7, 3868.0)
Rubella Vaccine Study	rs962859[d]+IFN-γ		21/34648123	IL10RB	Intron	Secreted IL-6 (ng/μl)	954	AA	323	3660.3 (3064.3, 4061.3)
								AC	483	3569.1 (3023.7, 4000.9)
								CC	148	3533.8 (3066.0, 3900.4)
Rubella Vaccine Study	rs2284552[d]+IFN-γ		21/34644082	IL10RB	Intron	Secreted IL-6 (ng/μl)	968	CC	600	3535.6 (2995.2, 3975.5)
								CA	321	3668.8 (3160.0, 4044.1)
								AA	47	3666.1 (3064.3, 4150.4)
Rubella Vaccine Study	rs5030728[c]		9/1.2×10^8	TLR4	Intron	Secreted IL-6 (ng/μl)	965	GG	489	3619.4 (3066.3, 4016.0)
								GA	398	3597.3 (3015.3, 3984.0)
								AA	78	3569.5 (2943.2, 4033.6)
Rubella Vaccine Study	rs2770150+IFN-γ		9/1.2×10^8	TLR4	Intergenic	Secreted IL-6 (ng/μl)	968	AA	516	3615.4 (3064.5, 4005.1)
								AG	389	3600.2 (3018.3, 4008.4)
								GG	63	3542.9 (2943.2, 4085.3)
Rubella Vaccine Study	seq-rs112723153+IFN-γ		19/45178636	PVR	Intron	Secreted IL-6 (ng/μl)	969	GG	961	3609.8 (3049.2, 4008.4)
								GA	8	2936.2 (2438.8, 3855.6)
								AA	0	
Rubella Vaccine Study	rs2229857[d]+IFN-γ	0.001363	1/1.55×10^8	ADAR	Coding	Secreted IL-6 (ng/μl)	968	GG	505	3595.6 (3058.5, 4006.3)
								GA	374	3594.7 (3036.5, 4033.3)
								AA	89	3732.8 (3023.6, 3969.3)
Rubella Vaccine Study	rs3870968+IFN-γ		6/29647149	ZFP57	Intron	Secreted IL-6 (ng/μl)	969	CC	866	3619.6 (3038.9, 4024.9)
								CA	102	3523.0 (3032.0, 3900.6)
								AA	1	3643.5 (3643.5, 3643.5)
Rubella Vaccine Study	rs469483[d]+IFN-γ	0.000028	21/42818515	MX1	Intron	Secreted IL-6 (ng/μl)	969	AA	308	3528.1 (2975.4, 3968.9)
								AG	485	3641.8 (3064.3, 4080.6)
								GG	176	3598.0 (3096.5, 3975.8)
Rubella Vaccine Study	rs2393657[c]		6/26457688	BTN2A1/BTN3A3	Intergenic	Secreted IL-6 (ng/μl)	961	CC	854	3619.9 (3064.3, 4023.2)
								CA	107	3398.9 (2750.9, 3832.4)
								AA	0	

Cohort	Immune measure	Number	Genotype	Number	Immune response outcome (IQR)	p value
Rubella Vaccine Study	Secreted IFN-γ (ng/μl)	969	AA	953	6.2 (1.5, 20.2)	0.000498
			AG	16	3.7 (−5.8, 10.7)	
			GG	0		
Rubella Vaccine Study	Secreted IFN-γ (ng/μl)	962	CC	944	6.2 (1.5, 20.0)	0.004264
			CA	18	5.4 (−0.4, 27.3)	
			AA	0		
Rubella Vaccine Study	NA (NT_{50})	1029	AA	1004	56.6 (34.6, 93.9)	0.001575
			AG	23	112.5 (65.5, 159.4)	
			GG	2	192.1 (31.2, 353.0)	

Table 2 (continued)

Cohort	p value	Immune measure	Number	Genotype	Number	Immune response outcome (IQR)	p value
Rubella Vaccine Study	0.000028	NA (NT$_{50}$)	1029	AA AG GG	1004 23 2	56.6 (34.6, 93.9) 112.5 (65.5, 159.4) 192.1 (31.2, 353.0)	0.001575
Rubella Vaccine Study	0.000029	NA (NT$_{50}$)	1027	AA AG GG	1002 23 2	56.6 (34.6, 93.8) 112.5 (65.5, 159.4) 192.1 (31.2, 353.0)	0.001575
Rubella Vaccine Study	0.000386	Secreted IFN-γ (ng/μl)	969	AA AC CC	942 27 0	6.1 (1.5, 19.9) 6.1 (−0.4, 27.3)	0.040073
Rubella Vaccine Study	0.008086	Secreted IFN-γ (ng/μl)	968	AA AG GG	929 36 3	6.3 (1.6, 20.8) 3.5 (0.1, 7.7) 3.4 (2.6, 16.6)	0.012882
Rubella Vaccine Study	0.008063	Secreted IFN-γ (ng/μl)	969	AA AG GG	930 36 3	6.3 (1.6, 20.8) 3.5 (0.1, 7.7) 3.4 (2.6, 16.6)	0.012874
Rubella Vaccine Study	0.000102	NA (NT$_{50}$)	1029	GG GA AA	1010 18 1	57.4 (34.9, 95.3) 67.3 (32.5, 126.5) 75.8 (75.8, 75.8)	0.00822
Rubella Vaccine Study	0.000332	NA (NT$_{50}$)	1028	GG GA AA	1015 13 0	57.4 (34.8, 95.8) 63.1 (36.2, 74.5)	0.000477
Rubella Vaccine Study	0.000144	Secreted IFN-γ (ng/μl)	969	AA AG GG	953 14 2	6.2 (1.5, 20.0) 4.8 (−0.9, 62.3) −51.9 (−60.5, −43.3)	0.022627
Rubella Vaccine Study	9×10^{-7}	Secreted IFN-γ (ng/μl)	968	GG GA AA	959 8 1	6.2 (1.5, 20.2) 3.7 (−1.1, 38.4) −43.3 (−43.3, −43.3)	0.02229
Rubella Vaccine Study	0.026416	NA (NT$_{50}$)	1029	GG GA AA	1018 11 0	57.4 (34.9, 95.7) 63.1 (33.4, 75.8)	0.004395
Rubella Vaccine Study	0.042337	NA (NT$_{50}$)	1028	GG GA AA	992 35 1	57.4 (34.7, 95.2) 65.5 (41.0, 101.3) 77.3 (77.3, 77.3)	0.03046
Rubella Vaccine Study	0.000315	NA (NT$_{50}$)	1029	GG GC CC	1015 12 2	57.4 (34.6, 95.4) 62.5 (52.7, 112.3) 116.5 (74.5, 158.6)	0.037341
Rubella Vaccine Study	0.014516	NA (NT$_{50}$)	1029	AA AG GG	683 299 47	56.6 (34.6, 95.7) 58.9 (35.1, 94.8) 61.0 (34.9, 97.4)	0.029181
Rubella Vaccine Study	0.002321	NA (NT$_{50}$)	1029	AA AG	683 299	56.6 (34.6, 95.7) 58.9 (35.1, 94.8)	0.029181

Table 2 (continued)

Cohort	p value	Immune measure	Number	Genotype	Number	Immune response outcome (IQR)	p value
Rubella Vaccine Study	0.002321	Secreted IFN-γ (ng/µl)	969	GG	47	61.0 (34.9, 97.4)	0.014516
				AA	639	5.9 (1.5, 19.9)	
				AG	285	6.1 (1.3, 18.0)	
Rubella Vaccine Study	0.008451	Secreted IFN-γ (ng/µl)	954	GG	45	11.5 (2.7, 42.5)	0.027823
				AA	323	6.9 (1.8, 22.8)	
				AC	483	4.9 (1.2, 18.2)	
				CC	148	7.5 (2.5, 21.4)	
Rubella Vaccine Study	7.89×10^{-5}	Secreted IFN-γ (ng/µl)	968	CC	600	5.9 (1.2, 19.2)	0.018961
				CA	321	6.2 (1.9, 21.7)	
				AA	47	7.9 (1.6, 30.7)	
Rubella Vaccine Study	0.018059	Secreted IFN-γ (ng/µl)	965	GG	489	5.5 (0.8, 19.7)	0.037292
				GA	398	6.4 (1.9, 20.0)	
				AA	78	6.5 (1.4, 25.2)	
Rubella Vaccine Study	0.01256	Secreted IFN-γ (ng/µl)	968	AA	516	5.9 (1.1, 20.0)	0.017476
				AG	389	6.3 (1.7, 19.2)	
				GG	63	6.6 (1.1, 27.5)	
Rubella Vaccine Study	0.011401	Secreted IFN-γ (ng/µl)	969	GG	961	6.1 (1.5, 20.0)	0.041896
				GA	8	10.4 (0.3, 34.5)	
				AA	0		
Rubella Vaccine Study	0.000435	Secreted IFN-γ (ng/µl)	968	GG	505	6.0 (1.4, 20.0)	0.01358
				GA	374	5.9 (1.5, 19.2)	
				AA	89	8.4 (2.3, 22.1)	
Rubella Vaccine Study	0.006571	Secreted IFN-γ (ng/µl)	969	CC	866	5.9 (1.3, 19.2)	0.018177
				CA	102	9.0 (3.2, 38.2)	
				AA	1	16.1 (16.1, 16.1)	
Rubella Vaccine Study	0.002902	Secreted IFN-γ (ng/µl)	969	AA	308	5.0 (0.9, 18.5)	0.019999
				AG	485	6.6 (1.8, 21.8)	
				GG	176	6.4 (1.6, 19.5)	
Rubella Vaccine Study	0.031523	Secreted IFN-γ (ng/µl)	961	CC	854	6.2 (1.6, 20.8)	0.034371
				CA	107	6.1 (0.4, 15.2)	
				AA	0		

NA neutralizing antibody, *SNP ID* single nucleotide polymorphism identification, *IL* interleukin, *IFN* interferon, *IQR* interquartile range, NT_{50} neutralizing titer, *A* adenine, *C* cytosine, *G* Guanine

Negative values indicate that the unstimulated secretion levels were higher than the rubella virus-stimulated secretion levels.

[a] Loss of significant association when homozygous minor allele genotype (observed only in few subjects) was excluded from analysis

[b] One phenotype failed to remain significantly associated with genotype as determined by having a q-value greater than 0.1.

[c] Two phenotypes failed to remain significantly associated with genotype as determined by having a q-value greater than 0.1.

[d] IL-6 remained significantly associated with genotype as determined by having a q-value greater than 0.1 in the Caucasian subset.

TRIM22 gene associations

One coding SNP in the tripartite motif containing 22 (TRIM22) gene, rs2291842 ($p<0.01$), was significantly associated with all three rubella-specific immune response outcomes in this study (IFN-γ, IL-6, and NA titers). For this SNP, increasing numbers of the minor allele were associated with an increase in NA titers (1.1-fold) and IFN-γ secretion (2.0-fold); however, the major allele seemed to correlate with enhanced secretion of IL-6 (1.1-fold) (Table 2).

Other gene associations

We identified nine other associations between multiple rubella-specific immune phenotypes and SNPs from the following genes: interleukin 10 receptor beta (IL10RB; two SNPs), toll-like receptor 4 (TLR4; two SNPs), poliovirus receptor (PVR; one SNP), adenosine deaminase (ADAR; one non-synonymous SNP), zinc finger protein 57 (ZFP57; one SNP), MX dynamin-like GTPase 1 (MX1; one SNP), and butyrophilin, subfamily 2/3, member A1/3 (BTN2A1/BTN3A3; 1 SNP). The two SNPs in the TLR4 gene (rs5030728, $p<0.04$; and rs2770150, $p<0.02$) are both significantly associated with both IL-6 secretion and IFN-γ secretion. The major alleles in both SNPs are associated with increased secretion of IL-6; however, subjects with minor alleles of these SNPs tended to secrete increased levels of IFN-γ (Table 2).

Associations between PVRL1 and PVRL3 haplotypes and rubella immune response outcomes

The Haploview output for the PVRL3 and PVRL1 gene SNPs that were genotyped and were significant in the study is shown in Figs. 1 and 2. Among the seven PVRL1 haplotypes with frequencies ≥1 % in the study cohort, the global statistical tests revealed a significant association (global $p=0.017$) between lower IFN-γ secretion and the PVRL1 haplotype GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCAGCGCAGGGGTCCGGG (t-statistic −2.49, $p=0.01$) (Table 3). This specific PVRL1 haplotype was not associated with rubella-specific IL-6 secretion or NA titers. In addition, we identified six haplotypes (with frequencies ≥1 %) in the PVRL 3 gene in our study cohort (Table 3). The global tests demonstrated highly significant associations between IFN-γ secretion (global $p=0.0001$) and NA titers (global $p=0.037$) and the PVRL3 haplotype. Specifically, the PVRL3 haplotype GACGGGGGCAGCAAAAAGAAGAGGAAAGAACAA was significantly associated with both higher IFN-γ production (t-statistic 4.43, $p<0.0001$) and higher NA titers (t-statistic 3.14, $p=0.0018$). PVRL1 and PVRL3 gene haplotype associations with rubella-specific immune response outcomes in the Caucasian cohort are shown in Supplementary Table S2.

Discussion

Previous studies have identified a broad collection of SNPs/haplotypes identified from HLA and non-HLA genes significantly associated with humoral and cellular immune responses to rubella vaccine (Haralambieva et al. 2010, 2014; Ovsyannikova et al. 2004, 2005, 2009b, 2010a, b; Pankratz et al. 2010). The purpose of this exploratory study was to identify SNPs in several candidate genes that were significantly associated with more than one rubella-specific immune response post-MMR vaccination. To our knowledge, this is the first study that has identified genes associated with multiple immune response outcomes. Because the identified genotypes are significantly associated with more than one RV-specific outcome, it may be suggested that they have a greater impact on overall immune response to vaccination (Ovsyannikova et al. 2014b). Overall, we identified 23 SNPs in 10 genes that were associated with at least two rubella-specific immune response outcomes, including secreted IL-6, secreted IFN-γ, or neutralizing antibody titers, suggesting joint effects of various genes/genetic variants in the control of the vaccine-induced immune response.

Our statistical analysis results demonstrated evidence for the role of multiple SNPs (some in LD) in the poliovirus receptor (PVR), poliovirus receptor-related 1 (PVRL1), and poliovirus receptor-related 3 (PVRL3) genes for being immunologically relevant to the development of both antibody and cytokine immune responses to rubella vaccine. Little is known about the role of genetic variants within these genes in the genetic control of immune response to rubella vaccination; however, it has been demonstrated that the PVR (CD155) gene encodes a transmembrane glycoprotein that belongs to the immunoglobulin (Ig) superfamily and serves as a cellular entry receptor for poliovirus that mediates cell-cell adhesion and cell migration (He et al. 2000). While evidence for PVR's contribution to RV vaccine-induced immunity is not strong, CD155/PVR was demonstrated to play a role in regulation of Th2 phenotype polarization, natural killer (NK) cell activation, secretion of lytic granules and IFN-γ, and modulation of antigen-specific IgG antibodies in response to TLR agonists (Fuchs et al. 2004; Kamran et al. 2013).

We detected eight SNPs in the PVRL3 (Nectin-3, CD113) gene that were significantly associated with more than one rubella-specific immune response post-MMR vaccination. The effects of these SNPs were also detected at the haplotype level, where haplotype analysis showed a significant association between the PVRL3 GACGGGGGCAGCAAAAAGAAGAGGAAAGAACAA haplotype and both higher rubella-specific NA titers and IFN-γ production. This increases our confidence that the PVRL3 gene locus/allelic variants play a role in the development of RV-induced humoral and cellular immune response. The Nectin family is considered a member of
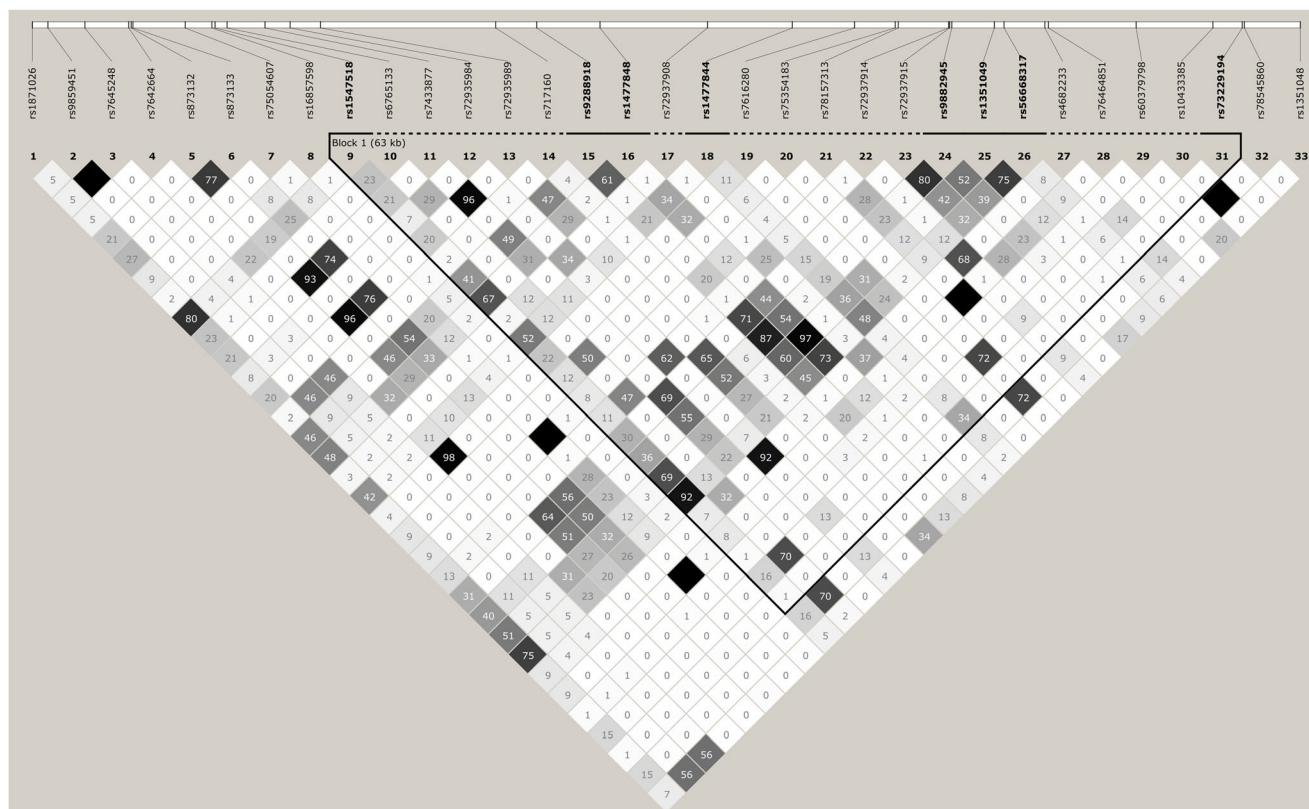
Fig. 1 Haplotype block structure of the PVRL3 genetic variants, analyzed using Haploview software, version 4.2 (all SNPs presented were genotyped and ut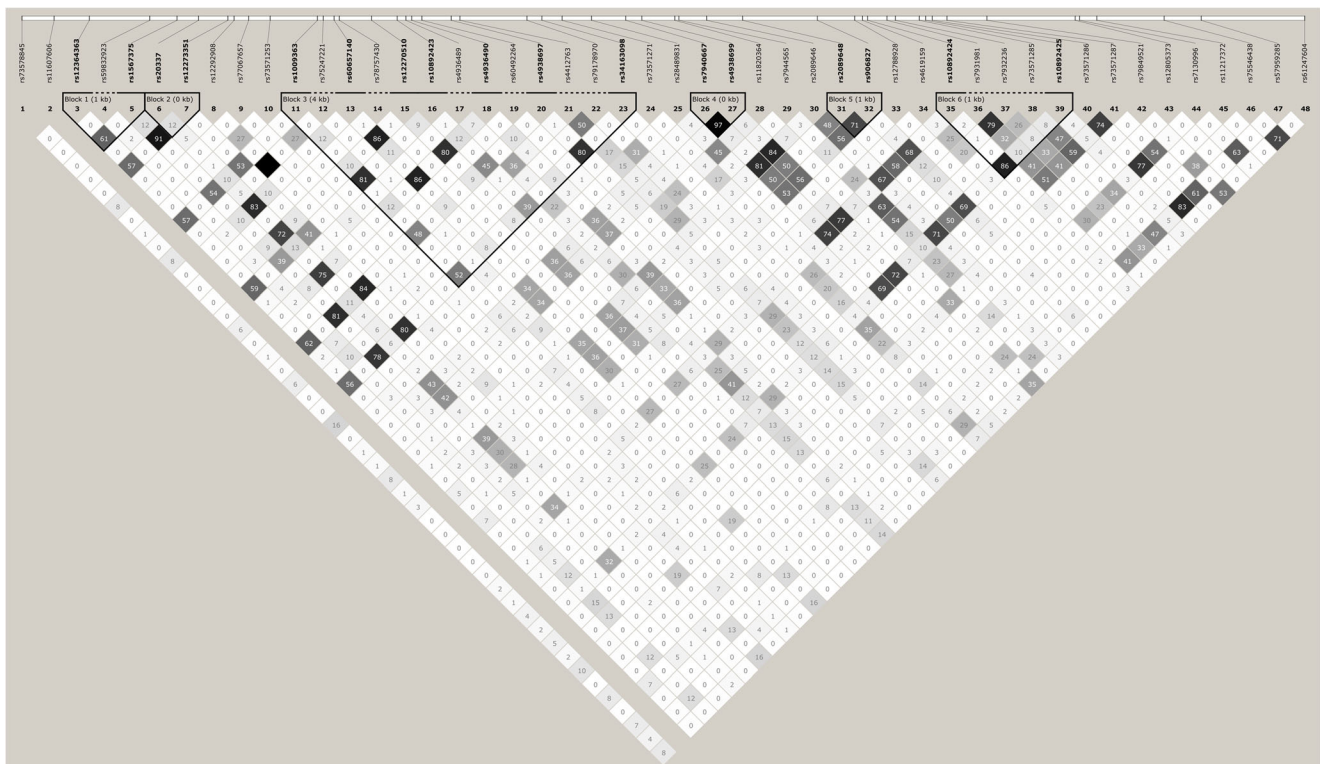ilized in the construction of haplotypes). The r^2 color scheme is *white* ($r^2=0$), *shades of gray* ($0<r^2<1$), and *black* ($r^2=1$). The numbers report the r^2 value multiplied by 100

the immunoglobulin super family due to structural similarities (Takai et al. 2008). Nectin-3 has been shown to play a critical role in the control of junctions between endothelial cells, which is important for the transmigration of immune cells during infection (Devilard et al. 2013). Once expressed on the surface of T lymphocytes, Nectin-3 binds to Nectin-2 expressed on endothelial cells. Results from previous studies suggest that this trans-interaction induces the opening of endothelial cell junctions and is required for efficient and effective extravasation of lymphocytes from the blood to sites of infection (Devilard et al. 2013).

Additionally, we identified several SNPs in the *PVRL1* (Nectin-1, CD111) gene that were significantly associated with multiple rubella-specific immune response phenotypes. As another member of the Nectin family, Nectin-1 has been shown to influence viral infection. A study conducted by Geraghty et al. identified Nectin-1 (referred to as HveC) as the primary receptor allowing for initial mucosal infection of herpes simplex virus 1 (HSV-1) and HSV-2 and subsequent entry into epithelial and neuronal cells (Geraghty et al. 1998; Satoh-Horikawa et al. 2000). It is unknown how the *PVRL1* gene and Nectin gene family relate specifically to immune response to RV although speculations can be made.

Based on the functional role of Nectins in lymphocyte transmigration, HSV infection (Geraghty et al. 1998; Satoh-Horikawa et al. 2000), and measles virus infection (Muhlebach et al. 2011), it might be speculated that they also play a role in the propagation and/or elimination of RV. Since RV replicates in mucosal cells of the nasopharynx, lymphocyte transmigration is essential for terminating viral replication. Therefore, polymorphisms in the Nectin-3 gene could significantly impact the ability to evade rubella infection. Likewise, Nectin-1 has been shown to mediate cellular infection of HSV and pseudorabies virus (PRV) as well suggesting that a polymorphism in the Nectin-1 gene may enhance or decrease one's susceptibility to rubella viral infection via downstream effects in viral receptor-mediated entry into host cells and control following immune response. Future studies should, therefore, be aimed at determining if such a relationship between Nectin genes and live RV vaccine exists.

In addition, we identified a coding SNP, rs2291842, in the *TRIM22* gene coding region that was significantly associated with all three immune response outcomes. TRIM22 is a member of the tripartite motif family, which is involved in a various array of cellular processes, including differentiation, regulation, and apoptosis (Reymond et al. 2001). For example, rs2291841 in the TRIM22 gene was previously associated

Fig. 2 Haplotype block structure of the PVRL1 genetic variants, analyzed using Haploview software, version 4.2 (all SNPs presented were genotyped and utilized in the construction of haplotypes). The r^2 color scheme is *white* ($r^2=0$), *shades of gray* ($0<r^2<1$), and *black* ($r^2=1$). The numbers report the r^2 value multiplied by 100. Blocks are missing when the minor allele frequency for at least one SNP is 0

with a higher IFN-γ ELISPOT response to measles virus vaccine, while TRIM22 rs885002 was associated with a diminished IL-10 and TNF-α measles virus-specific response (Ovsyannikova et al. 2013). Previous studies also identify TRIM22 as a key regulator of signaling pathways in the innate immune system, especially the antiviral response (McNab et al. 2011). These studies also suggest that IFN-γ induces *TRIM22* expression; this leads to an increase in the transcription factor nuclear factor-κB, which has been shown to stimulate pro-inflammatory cytokine secretion, including IL-6 secretion (Yu et al. 2011). The minor allele variant (GG) of the coding rs2291842 identified in this study was associated with an increase from baseline in both rubella-specific IFN-γ secretion and rubella-specific NA titers. Conversely, the major allele variant (AA) of this SNP was associated with secreted IL-6. We speculate that this coding polymorphism may affect TRIM22 protein structure and hence antiviral function and IFN-γ, IL-6 and antibody production by NK/T cells, T cells/ macrophages and B cells, respectively. Our earlier studies on live RV vaccine identified associations between SNPs in the *TRIM22* gene and rubella-specific IFN-γ, IL-2, and IL-6 secretion levels (Ovsyannikova et al. 2010a), as well as humoral immunity after rubella vaccination (Ovsyannikova et al. 2010b). Therefore, a polymorphism in the *TRIM22* gene may impact the response to live RV vaccine due to its significance in innate antiviral immune response.

Two SNPs, in the *IL10RB* gene, rs962859, and rs2284552, were found to be significantly associated with both rubella-specific IFN-γ and IL-6 secretion. Expression of IL10RB is essential for signal transduction induced by IL-10. The interaction between IL10RB, IL10RA, and IL-10 has been shown to impede the secretion of many cytokines, including IL-6 (Dokter et al. 1996). Interestingly, in this study, the major allele variant of the rs962859 SNP and the minor allele variant of the rs2284552 SNP were associated with a significant increase in secreted IL-6 levels, suggesting that polymorphisms in this gene alter this sensitive IL-10 pathway.

Finally, we identified two SNPs in the *TLR4* gene that were significantly associated with rubella-specific IL-6 and IFN-γ secretion. Toll-like receptor protein 4 is encoded by the *TLR4* gene, and it is important for pathogen recognition and activation of innate immune response pathways (Kopp and Medzhitov 1999). The major allele variants of the rs5030728 and rs2770150 SNPs of the *TLR4* gene were found to be significantly associated with increases in rubella-specific IL-6 secretion; however, they were also associated with decreases in rubella-specific IFN-γ secretion. Cytokines IL-6 and IFN-γ are known to have functionally distinct roles and regulate many biological processes, including antiviral immune response. However, data suggest that IL-6 and IFN-γ induce overlapping sets of genes and both signal through a common regulatory JAK/STAT signaling pathway (Qi et al.

Table 3 PVRL1 and PVRL3 gene haplotype associations with rubella-specific immune response outcomes in the study cohort

PVRL1 (Nectin-1) Gene Allele[a]	Immune outcome	Frequency	Test statistic	Allele p value	Global p value
					0.0174
GAGGCACGGCAGAGAGGAGAGGGGGCCGGAGCGGCGCAGGGGTCCGGG	IFN-γ	0.0377	0.7327	0.4640	
GAGGAGAGGCAGCGGGGGGGGGAGGCCGGAGCGGCGCAGGGGTCCGGG	IFN-γ	0.3452	−0.8671	0.3861	
GAGGAGAGGCAGCGGAGGGGGGGAGGCCGGAGCGGCGCAGGGGTCCGGG	IFN-γ	0.0168	−0.4096	0.6822	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCGGCGCAGGGGTCCGGG	IFN-γ	0.2571	−1.6004	0.1099	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCGGCGCAGGGGTCGGGG	IFN-γ	0.0682	0.3797	0.7042	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCAGCGCAGGG GTCCGGG	**IFN-γ**	**0.0662**	**−2.4912**	**0.0129**	
GAAGCACGGCAGAGAGGAGAGGGGGAGGGGAAGGAGCAAGGGTCCGGG	IFN-γ	0.0498	1.3947	0.1635	
					0.2050
GAGGCACGGCAGAGAGGAGAGGGGGCCGGAGCGGCGCAGGGGTCCGGG	IL-6	0.0377	1.6917	0.0911	
GAGGAGAGGCAGCGGGGGGGGGAGGCCGGAGCGGCGCAGGGGTCCGGG	IL-6	0.3452	1.3917	0.1643	
GAGGAGAGGCAGCGGAGGGGGGGAGGCCGGAGCGGCGCAGGGGTCCGGG	IL-6	0.0168	1.7540	0.0798	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCGGCGCAGGGGTCCGGG	IL-6	0.2571	1.1243	0.2612	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCGGCGCAGGGGTCGGGG	IL-6	0.0682	1.2159	0.2243	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCAGCGCAGGGGTCCGGG	IL-6	0.0662	−0.8107	0.4178	
GAAGCACGGCAGAGAGGAGAGGGGGAGGGGAAGGAGCAAGGGTCCGGG	IL-6	0.0498	−0.0479	0.9618	
					0.9084
GAGGCACGGCAGAGAGGAGAGGGGGCCGGAGCGGCGCAGGGGTCCGGG	NA	0.0377	0.4669	0.6407	
GAGGAGAGGCAGCGGGGGGGGGAGGCCGGAGCGGCGCAGGGGTCCGGG	NA	0.3452	0.2172	0.8281	
GAGGAGAGGCAGCGGAGGGGGGGAGGCCGGAGCGGCGCAGGGGTCCGGG	NA	0.0168	−0.2991	0.7649	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCGGCGCAGGGGTCCGGG	NA	0.2571	0.8906	0.3734	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCGGCGCAGGGGTCGGGG	NA	0.0682	1.0419	0.2977	
GAAGCGCGGCGGCGGAGGGAGGGGGCCGGAGCAGCGCAGGGGTCCGGG	NA	0.0662	0.7988	0.4246	
GAAGCACGGCAGAGAGGAGAGGGGGAGGGGAAGGAGCAAGGGTCCGGG	NA	0.0498	0.7432	0.4575	
PVRL3 (Nectin-3) Gene Allele[b]					
					0.0001
CACGGGAGAAGCAAGCACAAAAACGGAAAACAA	IFN-γ	0.7719	1.4267	0.1540	
CACGGGAGAAGCAAGCAGAAAAACGGAAAAAAA	IFN-γ	0.0939	1.4610	0.1444	
CACAGGAGCAGCAAGAAGGAAAACAAAAAACAA	IFN-γ	0.0160	−0.0068	0.9946	
GACGGGGGCAGCAAAAAGAAGAGGAAAGAACAA	**IFN-γ**	**0.0119**	**4.4292**	**<0.0001**	
GACGAAAGCAACCAAAAGAAAAGGAAAAAACAA	IFN-γ	0.0294	1.5462	0.1224	
GACAGGAGCAGCAAGAAGGAAAACAAAAAACAA	IFN-γ	0.0186	−1.2745	0.2028	
					0.6698
CACGGGAGAAGCAAGCACAAAAACGGAAAACAA	IL-6	0.7719	−0.7554	0.4502	
CACGGGAGAAGCAAGCAGAAAAACGGAAAAAAA	IL-6	0.0939	−0.1292	0.8973	
CACAGGAGCAGCAAGAAGGAAAACAAAAAACAA	IL-6	0.0160	−0.2601	0.7949	
GACGGGGGCAGCAAAAAGAAGAGGAAAGAACAA	IL-6	0.0119	−1.3743	0.1697	
GACGAAAGCAACCAAAAGAAAAGGAAAAAACAA	IL-6	0.0294	−0.9995	0.3178	
GACAGGAGCAGCAAGAAGGAAAACAAAAAACAA	IL-6	0.0186	−1.2313	0.2185	
					0.0372
CACGGGAGAAGCAAGCACAAAAACGGAAAACAA	NA	0.7719	−0.2135	0.8310	
CACGGGAGAAGCAAGCAGAAAAACGGAAAAAAA	NA	0.0939	−0.3169	0.7514	
CACAGGAGCAGCAAGAAGGAAAACAAAAAACAA	NA	0.0160	−0.3080	0.7581	
GACGGGGGCAGCAAAAAGAAGAGGAAAGAACAA	**NA**	**0.0119**	**3.1359**	**0.0018**	

Table 3 (continued)

PVRL1 (Nectin-1) Gene Allele[a]	Immune outcome	Frequency	Test statistic	Allele p value	Global p value
GACGAAAGCAACCAAAAGAAAAGGAAAAAACAA	NA	0.0294	−0.5452	0.5858	
GACAGGAGCAGCAAGAAGGAAAACAAAAAACAA	NA	0.0186	−0.7710	0.4409	

p-values calculated using linear (for antibodies) and linear mixed models (secreted cytokines) while adjusting for the potentially confounding variables of sex, age at enrollment, vaccination history (age at first immunization, age at second immunization, and time from last immunization to blood draw), batch/run number of immune assay used to measure immune outcome, and differences in population genetics (eigenvalues). Allelic p-values compare individual haplotypes to all other haplotypes combined. Statistically significant p-values ($p<0.05$) are in boldface type

[a] PVRL1 genetic variants from left to right: rs73578845, rs11607606, rs12364363, rs59832923, rs1567375, rs20337, rs12273351, rs12292908, rs77067657, rs73571253, rs1009363, rs75247221, rs60657140, rs78757430, rs12270510, rs10892423, rs4936489, rs4936490, rs60492264, rs4938697, rs4412763, rs79178970, rs34163098, rs73571271, rs28489831, rs7940667, rs4938699, rs11820364, rs7944565, rs2089646, rs2089648, rs906827, rs12788928, rs4619159, rs10892424, rs7931981, rs7932236, rs73571285, rs10892425, rs73571286, rs73571287, rs79849521, rs12805373, rs7130996, rs11217372, rs75546438, rs57959285, and rs61247604

[b] PVRL3 genetic variants from left to right: rs1871026, rs9859451, rs7645248, rs7642664, rs873132, rs873133, rs75054607, rs16857598, rs1547518, rs6765133, rs7433877, rs72935984, rs72935989, rs717160, rs9288918, rs1477848, rs72937908, rs1477844, rs7616280, rs75354183, rs78157313, rs72937914, rs72937915, rs9882945, rs1351049, rs56668317, rs4682233, rs76464851, rs60379798, rs10433385, rs73229194, rs78545860, and rs1351048

2013; Yuan et al. 1994). The role of TLR4 in the immune response to viral pathogens (i.e., viral envelope glycoprotein) has been widely studied (Barton 2007; Ovsyannikova et al. 2011b; Puthothu et al. 2006; Zhou et al. 2011), and our results support the importance of this gene in the immune response to live RV vaccine. We also identified one SNP in each of the following genes that were significantly associated with more than one rubella-specific immune response post-MMR vaccination: *PVR*, *ZFP57*, *BTN2A1/BTN3A3*, and IFN-γ-induced antiviral *MX1* and *ADAR*. For example, a non-synonymous rs2229857 (Lys384/Arg) in the antiviral RNA-specific *ADAR* gene, known to be involved in RNA editing and gene regulation, demonstrated an allele dose-related increase in IL-6 and IFN-γ secretion with the representation of a minor allele. Consistent with the current results, our earlier vaccine study with measles demonstrated an association between *ADAR* SNP rs2229857 and measles virus-specific IFN-γ ELISPOT responses (Haralambieva et al. 2011b). We speculate that this genetic variant is likely to be involved in regulation of virus-induced cellular immune mechanisms. This study is strengthened by several factors. The recruitment and utilization of a relatively large sample population (1039 subjects, 85 % Caucasian) with documented vaccine coverage and no circulating wild-type RV enhances our confidence that the immune outcomes measured reflected rubella immunization alone and not disease. Additionally, the relatively small range of age at time of enrollment limits additional factors, such as immunosenescence and waning immune response. The chief limitation of this study is the sole use of candidate genes for statistical analysis and the possibility of detecting potential false-positive associations. This study could be improved upon by having a more genetically diverse population, and future studies will explore whether these associations are observed in other genetically distinct populations. The associations we report herein cannot be generalized to other ethnic groups not examined in this study. We observed very small or no representation of a minor allelic variant for some SNPs in the *PVRL1*, *PVRL3*, *PVR*, and other candidate genes that may have skewed the IL-6, IFN-γ, and NA immune responses. Multiple statistical tests were completed for this rubella vaccine study; thus, it is possible that a number of false-positive associations with immune response outcomes have been found. It is important to note that after controlling for multiple testing via q-value, some of the SNP-specific tests failed to remain significant at the $p<0.1$ level. These SNPs have been noted in Table 2. These associations require confirmation in a separate cohort to understand their functional significance. Future replication studies are necessary to validate all of these results.

In conclusion, this is the first study that analyzed and identified SNPs/haplotypes significantly associated with more than one rubella-specific immune response post-MMR vaccination. Our results provided additional insights into multigenic and haplotypic associations between candidate gene SNPs and rubella vaccine-specific NA titers and cytokine production. The results from this study suggest that polymorphisms in these genes contribute to the overall heterogeneity in rubella-specific immune response phenotypes in individuals after being immunized with the MMR vaccine. In the future, these results could aid the prediction of immune response phenotypes in patients pre-vaccination, as well as influence the design of better vaccines through generation of new knowledge and the identification of targets and biomarkers for vaccine response (Poland et al. 2009, 2011a, b).

Conflict of interest Dr. Poland is the chair of a Safety Evaluation Committee for novel non-rubella investigational vaccine trials being conducted by Merck Research Laboratories. Dr. Poland offers consultative advice on vaccine development to Merck & Co. Inc., CSL Biotherapies, Avianax, Sanofi Pasteur, Dynavax, Novartis Vaccines and Therapeutics, PAXVAX Inc., Emergent Biosolutions, Adjuvance, and Vaxess. Drs. Poland and Ovsyannikova hold two patents related to measles and vaccinia peptide research. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

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