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ORIGINAL INVESTIGATION

Genetic polymorphisms associated with rubella virus-specific cellular immunity following MMR vaccination

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Abstract Rubella virus causes a relatively benign disease in most cases, although infection during pregnancy can result in serious birth defects. An effective vaccine has been available since the early 1970s and outbreaks typically do not occur among highly vaccinated (≥2 doses) populations. Nevertheless, considerable inter-individual variation in immune response to rubella immunization does exist, with single-dose seroconversion rates ~95 %. Understanding the mechanisms behind this variability may provide important insights into rubella immunity. In the current study, we examined associations between single nucleotide polymorphisms (SNPs) in selected cytokine, cytokine receptor, and innate/antiviral genes and immune responses following rubella vaccination in order to understand genetic influences on vaccine response. Our approach consisted of a discovery cohort of 887 subjects aged 11–22 at the time of enrollment and a replication cohort of 542 older adolescents and young adults (age 18–40). Our data indicate that SNPs near the butyrophilin genes (BTN3A3/BTN2A1) and cytokine receptors (IL10RB/IFNAR1) are associated with variations in IFNγ secretion and that multiple SNPs in the PVR gene, as well as SNPs located in the ADAR gene, exhibit significant associations with rubella virus-specific IL-6 secretion. This information may be useful, not only in furthering our understanding immune responses to rubella vaccine, but also in identifying key pathways for targeted adjuvant use to boost immunity in those with weak or absent immunity following vaccination.

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Introduction

Rubella virus, a member of the *Togaviridae* family, typically causes a relatively benign illness, although arthritis and arthralgia have been observed in adults. Less frequent complications include both encephalitis and thrombocytopenia (Reef and Plotkin 2013). Infection during pregnancy, especially early in pregnancy, can cause congenital rubella syndrome (CRS), leading to devastating fetal abnormalities. Since the advent of rubella vaccines and their widespread use in the 1970s, the number of cases of both rubella and CRS has dropped dramatically. Nevertheless, rubella remains a significant public health concern due to inadequate vaccine coverage (Metcalf et al. 2012), which leads to yearly outbreaks including a current nationwide outbreak in Japan with over 5,000 cases in (2013) It is estimated that over 100,000 CRS cases occurred globally in 2010 (Reef and Plotkin 2013).

In the US, rubella vaccines are given as part of multicomponent formulations that also include measles and mumps (MMR) or measles, mumps, and varicella (MMRV) (Jacobsen et al. 2009; Klein et al. 2010; Marin et al. 2010). These vaccines are highly effective, eliciting protective immunity in >95 % of recipients after a single dose, and in >99 % of recipients after two doses (Reef and

Plotkin 2013; Vesikari et al. 2012). Both humoral and cellular immune responses are elicited following immunization with rubella-containing vaccines and both contribute to protection against subsequent infection (Dhiman et al. 2010b; Honeyman et al. 1974; Mitchell et al. 1999; Reef and Plotkin 2013; Vesikari and Buimovici-Klein 1975). At the population level, there is considerable inter-individual variation in immune responses to vaccines, including those containing rubella. We have previously identified a number of genetic polymorphisms associated with variations in rubella vaccine response (Dhiman et al. 2010a; Haralambieva et al. 2010; Kennedy et al. 2010; Ovsyannikova et al. 2004, 2005, 2009a, b, 2010a, b, c).

Here, we report a meta-analysis combining data from two independent cohorts in order to identify genetic polymorphisms associated with rubella virus-specific cellular immune responses.

Methods

Subject recruitment and demographics

The study set was a large sample of 1,429 healthy children, older adolescents, and healthy adults (age 11–40 years), consisting of two independent cohorts: a Rochester cohort and a San Diego cohort. The methods described herein are similar or identical to those published for our previous studies (Dhiman et al. 2010a; Haralambieva et al. 2010; Kennedy et al. 2010; Ovsyannikova et al. 2004, 2005, 2009a, b, 2010a, b, c). Similarly, clinical and demographic characteristics of these cohorts have been previously reported. This study reports our findings among the Caucasian subset of each cohort.

The Rochester cohort comprised 1,145 individuals enrolled into three age-stratified cohorts of healthy, school-age children and young adults from all socioeconomic strata in Rochester, MN. Specifically, between December 2001 and August 2002, we enrolled 346 healthy children (age 12–18 years, cohort #1) (Ovsyannikova et al. 2004, 2005). Between December 2006 and August 2007, we enrolled 440 healthy children (age 11–18 years, cohort #2), as previously published (Haralambieva et al. 2010; Ovsyannikova et al. 2010a). In November 2008–September 2009, we enrolled 388 healthy children, enriched with African-American youth (age 11–22 years, cohort #3) (Ovsyannikova et al. 2011a, 2012b). Parental consent was obtained for all participants and each subject had written records of having received two doses of measles–mumps–rubella (MMR, Merck) vaccine. Of the 1,145 individuals recruited into these three studies, 1,039 (90.7 %) had sufficient samples and were successfully assayed for the measures of immune response. This cohort was predominantly of Caucasian descent, and after removing non-Caucasian individuals, the analysis set comprised 887 (85.4 % of 1,039) participants.

In July 2005–September 2006, we enrolled an additional 1,076 healthy older adolescents and healthy adults (age 18–40 years) from armed forces personnel in San Diego, CA (Kennedy et al. 2009; Ovsyannikova et al. 2011b). As members of the US military, they represent a cross section of the US population with proven vaccine-induced immunity to MMR. Of the 1,076 subjects recruited into this study, 997 (92.7 %) subjects had provided consent for use of their samples and data in other studies, and had sufficient samples which were successfully assayed for rubella neutralizing antibody levels. Of those subjects, 542 (54.4 %) were of Caucasian descent and were included in this study. The Institutional Review Boards of the Mayo Clinic and the Naval Health Research Center (NHRC) approved the study, and written informed consent was obtained from each subject, from the parents of all children who participated in the study, as well as written assent from age-appropriate participants.

Rubella virus-specific cytokine secretion

The levels of secreted cytokines following stimulation of PBMCs with live rubella virus were measured, as previously described by our group (Dhiman et al. 2010b; Ovsyannikova et al. 2009b). Briefly, 2×10^5 PBMCs were stimulated with the W-Therien strain of rubella virus (a gift from Dr. Teryl Frey, Georgia State University, Atlanta, GA) with optimized MOI and incubation times depending on the specific cytokine measured. For the measurement of IL-2, IL-6, and IFN-γ, PBMCs were stimulated with an MOI of 5. The levels of secreted TNF-α were determined after viral stimulation with an MOI of 0.05. The supernatants were removed post-stimulation at 24 h for IL-6, 48 h IFN-γ, 8 days for IL-2 and TNF-α, and all samples were stored at −80 °C until assayed. Cytokine levels were quantified using BD OptEIA™ Human ELISA kits, and absorbance levels were measured using a Molecular Devices SpectraMax 340PC.

SNP selection and candidate SNP genotyping methods

The SNP selection and genotyping methods described herein are similar or identical to those published for our previous genetic association studies (Haralambieva et al. 2010; Ovsyannikova et al. 2010a, b). The replication effort included follow-up on 299 previously identified (Rochester cohort) genetic associations ($p < 0.05$) between SNPs in immune response genes (cytokine and cytokine receptor genes, Toll-like receptor genes, vitamin D and vitamin A receptor family genes, antiviral effector and other innate genes) and immune measures after rubella vaccination. In addition, another 571 candidate tag SNPs selected from eight newly discovered viral receptor and attachment factor

genes (MOG, PVR, PVRL1, PVRL2, PVRL3, PVRL4, BTN2A1, BTN3A1) were identified using the tagSNP selection approach (Carlson et al. 2004) based on linkage disequilibrium (LD) and included on the genotyping panel. Genotypes for 102 of these SNPs were already available for the replication (San Diego) cohort from a previously performed genome-wide SNP genotyping using the Illumina Infinium HumanHap550 or HumanHap650Y BeadChip arrays. All previously discovered significant SNPs not present on the Illumina 550/650 arrays ($n = 197$ SNPs) were genotyped using a custom Illumina GoldenGate 768-plex genotyping assay. The selection criteria included: SNPs with validation data; successful predictive genotyping scores for Illumina GoldenGate assays; a minor allele frequency (MAF) $\geq$0.05; and a pairwise linkage disequilibrium (LD) threshold of $r^2 \geq 0.90$. SNP-specific deviation from Hardy–Weinberg equilibrium (HWE) was tested and we excluded any SNP that displayed violations of HWE ($p < 0.001$). The SNP list was further post-processed and refined using the SNPPicker program (Sicotte et al. 2011) in order to accommodate a set of Illumina platform constraints and to pick tagSNPs optimally across the multiple population groups. A total of 768 SNPs (including 197 replication SNPs not previously genotyped in the San Diego cohort, and 571 SNPs selected from eight new candidate genes) were selected based on this approach.

Our genotyping methods have been previously described (Dhiman et al. 2007). Genomic DNA was extracted from blood using the Puregene extraction kit (Gentra Systems Inc., Minneapolis, MN). DNA was quantified using the Picogreen method (Molecular Probes). 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. Corriel Trio DNA samples (mother: 1347-02 NA11875, father: 1347-01 NA10859, daughter: 1347-08 NA11875) and two other genomic DNA controls were used for quality control and to test for genotyping reproducibility. All data were transferred electronically to SAS for further analysis. The genotyping quality using the Illumina GoldenGate assay was high, with a total genotyping success rate >99.7 % in both cohorts and 96–100 % reproducibility rate for controls and replicate samples. For the Caucasian-only analyses, we used data on 555 SNPs from 887 Rochester subjects and 565 SNPs from 542 San Diego subjects.

Statistical analyses

Subject demographics were summarized separately for the combined Rochester cohorts and the San Diego cohort. Summaries comprised counts and percentages for categorical variables and medians and 25th and 75th percentiles for continuous variables. A principal components analysis was performed to confirm racial groupings using data available from prior genome-wide analyses. Because non-Caucasian samples comprised few individuals, and genetic and phenotypic differences among racial groups can bias association studies, we focused on the Caucasian participants in this analysis.

Linear mixed effects models were used to assess associations between the various cellular immune response measures and SNP genotypes within the Rochester and San Diego cohorts. Our assessments of the cellular immunity measures were measured in multiple wells, some with and some without stimulation with rubella virus. We included the results from each assay well in our analyses, and accounted for within-subject effects by modeling an unstructured covariance matrix within the linear mixed effects regression models. In these analyses, we assumed an additive genetic model and tested for differential SNP associations on the stimulation effect through a per-SNP genotype variable representing the number of minor alleles carried. In order to ensure that the cellular immune response measures conformed to linear models assumptions, an inverse normal transformation was applied to each phenotype prior to analysis. Individual SNP associations were tested for significance after adjustment for potential differences due to gender, age at enrollment, immunization age, and time from last immunization to enrollment.

After testing for associations between SNPs and phenotypes within the two cohorts, a fixed effects meta-analysis was performed for each SNP and for each phenotype (Greenland and O'Rourke 2008). Pooled estimates were obtained and tested for significance, and tests for heterogeneity of effect between cohorts were obtained. Q values, which estimate the probability that a p value reflects a false-positive finding, were computed separately for the p values from the different phenotypes using the methods of (Storey 2002, 2003) for SNPs that did not display evidence of significant heterogeneity between cohorts ($p > 0.10$). SNPs with meta-analysis p values below 0.01 were considered to be of potential interest, and SNPs with q values below 0.1 were considered to be significantly associated with the corresponding phenotype.

Results

Because of the different mix of races in each cohort, and the effect of race on immune outcome, we confined this report to the Caucasian subset of each cohort (Table 1), as this was the largest racial subset available for each cohort. We initially tested secretion of four cytokines (IL-2, IL-6, IFNγ, TNFα) in response to in vitro rubella virus stimulation in our immunized subjects. Both IL-2 and TNFα

Table 1 Subject demographics

	Rochester cohort ($n = 887$)	San Diego cohort ($n = 542$)
Median age in years at enrollment	15 (13–17)	23 (22–27)
Median age in years at most recent vaccination	10 (5–12)	19 (18–21)
Years since most recent vaccination	6.4 (4.6–8.5)	3.0 (2.2–3.9)
Gender		
Male	487 (55 %)	394 (73 %)
Female	400 (45 %)	148 (27 %)

secretion were largely undetectable in San Diego subjects and therefore were not included in this report. The Rochester cohort ($n = 887$) had a median IL-6 response of 3,629.3 pg/ml (IQR = 3,083.5–4,002.4) and the San Diego cohort ($n = 542$) had a median IL-6 response of 4,128.9 pg/ml (IQR = 3,514.5–4,816.3). This difference in IL-6 secretion was significant ($p < 0.0001$). IFNγ secretion had a median value of 6.3 pg/ml (IQR = 1.7–19.7) in the Rochester cohort and a median value of −1.8 pg/ml (IQR = −6.4 to −2.9) in the San Diego cohort. These negative values likely indicate that rubella virus exerts a suppressive effect on basal IFNg levels in most of the San Diego cohort subjects. The differential IFNγ response was also significant ($p < 0.0001$).

Individually, the two cohorts are fairly small for genetic association studies and may have lacked sufficient power to detect the small effects typically expected from individual SNPs. This is readily apparent in the San Diego cohort p values (Tables 2, 3). With this in mind, we performed a meta-analysis using data from both cohorts. The genotyping panel contained SNPs located within or near a variety of cytokine and cytokine receptor genes, as well as innate and antiviral genes (Supplemental Table 1). We identified a number of SNPs that are potentially associated with variations in IFNγ secretion levels. While not meeting thresholds set to control false discovery, the most significant SNPs ($p < 0.01$) are shown in Table 2. These included: two intergenic SNPs located between the IL10RB and IFNAR1 genes; an SNP in TOP2A, encoding a DNA topoisomerase; and another intergenic SNP located between two butyrophilin genes, BTN3A3 and BTN2A1.

Furthermore, we identified multiple significant SNP:IL-6 response associations. The most significant SNP associations ($p < 0.01$) are shown in Table 3. All but one of these met our false-positive threshold (q value < 0.10). These SNPs are located in the following genes: ADAR, encoding the RNA-specific adenosine deaminase; IL10RB; IL6R; PVR, the poliovirus receptor; PVRL1 and PVRL2, the poliovirus receptor-related proteins that also mediate herpesvirus entry; and TNFRSF1B, encoding the p75 TNFα receptor. The list also includes: five SNPs located in or near the PVR gene (Fig. 1), which encodes the poliovirus receptor; an intergenic SNP located in between BCAM, the basal cell adhesion molecule gene and PVRL2; as well as a single SNP near the TLR4 and astrotactin 2 genes.

Discussion

Immune responses to antigenic stimuli, either infection or vaccination, are influenced, in part, by host genetics. Our work has focused on the role of genetic polymorphisms on immune responses to viral vaccines. We have previously identified a number of genetic variants (mainly HLA alleles and SNPs) associated with variations in cellular and/or humoral immunity to rubella vaccine (Dhiman et al. 2008, 2010a; Haralambieva et al. 2010; Jacobson et al. 2009; Kennedy et al. 2010; Ovsyannikova et al. 2004, 2005, 2006, 2009a, b, 2010b, c; Pankratz et al. 2010). Our current study design incorporates several important elements: the use of SNP data to define race/ethnicity, and the inclusion of two separate cohorts for SNP discovery and replication. Our study also has several limitations that include: the chance for rubella exposure and/or disease in our San Diego cohort; relatively small cohort sizes (especially when compared to cancer gene association studies with tens of thousands of subjects); and response outcomes that reflect complex immunologic processes controlled by multiple genes and pathways, where individual SNPs have minor contributions to the spectrum of immune response. These limitations are especially evident in the San Diego cohort-specific data, as demonstrated by the larger p values observed for both the IFNγ and IL-6 associations. Potential confounding variables were the notable differences in age, time since vaccination, and gender composition between our two cohorts, and these were accounted for in our statistical analyses. In spite of these limitations, our meta-analysis did identify a number of statistically significant genetic associations across two independent cohorts.

We identified four SNPs significantly associated with variations in IFNγ response in both of our cohorts. Three of the SNPs are intergenic and one is intronic; it is possible that they are not the causal variant, but are in close LD to the causal variant. However, most of the variations found with each genotype are extremely small (1–2 pg/ml) and the majority of the San Diego cohort had minimal to

Table 2 SNPs associated with variations in IFNγ response to Rubella

SNP ID[a]	Chr[b]	Gene[c]	Location	Rochester cohort					San Diego cohort					Meta	
				MAF[d]	Genotype	N[e]	Median (IQR)[f]	Cohort *p* value	MAF[d]	Genotype	*N*[e]	Median (IQR)[f]	Cohort *p* value	*p* value[g]	*q* value[h]
rs471692	17	TOP2A	Intergenic	20.18	GG	521	6.6 (1.9, 20.8)	0.150	19.13	GG	309	−1.3 (−5.7, 3.2)	0.002	0.0007	0.237
					GA	277	6.0 (1.5, 18.2)			GA	147	−2.7 (−7.4, 1.2)			
					AA	31	5.0 (1.1, 13.1)			AA	17	−1.7 (−7.1, 1.4)			
rs7278931	21	IL10RB/IFNAR1	Intergenic	17.45	GG	555	5.9 (1.6, 17.6)	0.011	18.50	GG	310	−1.9 (−6.3, 2)	0.040	0.0018	0.315
					GA	231	6.6 (2, 21.7)			GA	143	−2 (−6.5, 3.2)			
					AA	24	8.8 (2.9, 35.9)			AA	15	2.6 (−10.5, 4.2)			
rs8134731	21	IL10RB/IFNAR1	Intergenic	18.15	AA	561	6 (1.6, 18)	0.053	18.82	AA	312	−1.9 (−6.3, 2)	0.053	0.0069	0.650
					AG	238	7.1 (2, 22.1)			AG	147	−2.0 (−6.5, 3.3)			
					GG	30	8.8 (2.9, 33.6)			GG	15	2.6 (−10.5, 4.2)			
rs10484440	6	BTN3A3/BTN2A1	Intergenic	13.00	AA	614	6.6 (1.7, 21.8)	0.071	13.03	AA	357	−2 (−6.5, 2.8)	0.055	0.0091	0.650
					AG	202	5.9 (1.9, 16.6)			AG	111	−1.7 (−6.6, 2.5)			
					GG	7	0.0 (−0.6, 5.3)			GG	5	−2.5 (−4.4, −0.8)			

[a] rs SNP identification number

[b] Chromosomal location

[c] Gene or genetic region containing the indicated SNP

[d] Minor allele frequency

[e] Number of subjects with a given genotype

[f] Median outcome measurement for each genotype group. Results expressed as pg/ml. The interquartile range (IQR) is shown in parentheses

[g] *P* values were adjusted for demographic and clinical variables as well as inflation of significance described in the Methods section

[h] *Q* values computed using the methods of (Storey 2002, 2003) from the distribution of *p* values from SNPs whose heterogeneity tests did not reach significance

Table 3 SNPs associated with variations in IL-6 response to rubella

SNP ID[a]	Chr[b]	Gene[c] Location	Rochester cohort					San Diego cohort					Meta *p* value[g]	*q* value[h]
			MAF[d]	Genotype	*N*[e]	Median (IQR)[f]	Cohort *p* value	MAF[d]	Genotype	N[e]	Median (IQR)[f]	Cohort *p* value		
rs474247	1	TNFRSF1B	19.5	GG	539	3,615 (3,099–4,041)	4.2×10^{-6}	23.1	GG	278	4,152 (3,539–4,909)	0.2771	4.9×10^{-6}	0.001
		Intron		GA	258	3,637 (3,120–3,969)			GA	167	4,050 (3,364–4,674)			
				AA	31	3,694 (3,025–3,985)			AA	28	4,096 (3,411–4,715)			
rs9393725	6	BTN1A1/BTN2A1	1.6	AA	803	3,634 (3,097–4,008)	0.0008	1.5	AA	459	4,141 (3,491–4,820)	0.0806	0.00014	0.014
		Intergenic		AC	26	3,469 (2,997–3,797)			AC	14	3,829 (3,559–4,702)			
				CC	0	(,)			CC	0	(–)			
rs7260482	19	PVR/LOC147710	25.8	AA	452	3,665 (3,131–3,997)	0.0004	28.8	AA	240	4,130 (3,503–4,824)	0.1717	0.00017	0.014
		Intergenic		AC	313	3,547 (3,025–4,052)			AC	195	4,181 (3,548–4,876)			
				CC	55	3,458 (3,099–3,881)			CC	35	3,698 (3,175–4,282)			
rs2229857	1	ADAR	28.2	GG	442	3,617 (3,081–4,003)	0.0003	30.4	GG	235	4,031 (3,448–4,783)	0.0003	0.00026	0.016
		Coding		GA	308	3,634 (3,120–4,027)			GA	189	4,267 (3,533–4,914)			
				AA	78	3,744 (3,099–3,975)			AA	49	4,076 (3,541–4,611)			
rs9616	1	ADAR	28.3	TT	429	3,654 (3,135–4,034)	0.0001	28.6	TT	245	4,198 (3,574–4,828)	0.0003	0.00038	0.019
		UTR		TA	326	3,605 (3,065–3,964)			TA	185	4,002 (3,376–4,803)			
				AA	69	3,642 (2,981–3,978)			AA	43	4,127 (3,530–4,880)			
rs10410651	19	PVR	25.3	AA	465	3,665 (3,127–3,999)	0.0015	28.8	AA	241	4,133 (3,509–4,828)	0.1467	0.00046	0.019
		Intron		AG	307	3,561 (3,024–4,063)			AG	197	4,174 (3,548–4,847)			
				GG	56	3,458 (3,105–3,874)			GG	35	3,698 (3,175–4,282)			
rs203709	19	PVR	25.5	AA	464	3,663 (3,127–3,997)	0.002	28.9	AA	240	4,130 (3,503–4,860)	0.1405	0.00059	0.021
		Intron		AT	307	3,554 (3,010–4,063)			AT	198	4,178 (3,548–4,847)			
				TT	57	3,458 (3,110–3,881)			TT	35	3,698 (3,175–4,282)			
rs7250339	19	PVR/LOC147710	24.1	AA	470	3,650 (3,130–3,994)	0.0058	26.2	AA	262	4,164 (3,533–4,905)	0.0531	0.0008	0.025
		Intergenic		AG	300	3,572 (3,044–4,047)			AG	183	4,151 (3,503–4,813)			
				GG	47	3,396 (3,058–3,900)			GG	28	3,633 (3,152–4,259)			
rs1466435	19	BCAM/PVRL2	5.2	CC	748	3,633 (3,092–4,006)	0.004	3.2	CC	441	4,117 (3,497–4,787)	0.1661	0.0014	0.037
		Intergenic		CG	76	3,620 (3,124–3,989)			CG	32	4,299 (3,541–5,051)			
				GG	5	3,353 (3,077–3,834)			GG	0	(–)			
rs28483039	19	PVR	23.8	GG	482	3,658 (3,127–3,999)	0.0103	26.2	GG	262	4,164 (3,533–4,905)	0.0531	0.0015	0.037
		Intron		GA	299	3,576 (3,025–4,061)			GA	183	4,151 (3,503–4,813)			
				AA	48	3,364 (3,063–3,891)			AA	28	3,633 (3,151–4,259)			
rs1127317	1	ADAR	27.9	AA	446	3,625 (3,084–4,021)	0.0021	29.8	AA	238	4,041 (3,448–4,711)	0.3959	0.0018	0.039
		UTR		AC	306	3,634 (3,125–4,013)			AC	187	4,276 (3,533–4,914)			
				CC	77	3,694 (3,024–3,975)			CC	47	4,076 (3,541–4,611)			
rs7104562	11	PVRL1	2.5	AA	788	3,631 (3,079–4,004)	0.0006	2.3	AA	452	4,129 (3,500-4,810)	0.9782	0.0019	0.039

Table 3 continued

SNP ID[a]	Chr[b]	Gene[c] Location	Rochester cohort					San Diego cohort					Meta *p* value[g]	*q* value[h]
			MAF[d]	Genotype	*N*[e]	Median (IQR)[f]	Cohort *p* value	MAF[d]	Genotype	N[e]	Median (IQR)[f]	Cohort *p* value		
		Intron		AG	41	3,637 (3,247-3,963)			AG	21	4,155 (3,418–5,043)			
				GG	0	(,)			GG	0	(–)			
rs2770150	9	ASTN2/TLR4	27.7	AA	427	3,634 (3,135–4,001)	0.0094	24.8	AA	270	4,191 (3,450–4,830)	0.1888	0.0035	0.069
		Intergenic		AG	342	3,625 (3,039–4,004)			AG	167	4,076 (3,584–4,771)			
				GG	59	3,640 (3,024–4,121)			GG	36	3,859 (3,226–4,801)			
rs2228145	1	IL6R	37.5	AA	323	3,611 (3,120–3,999)	0.0069	39.8	AA	178	4,159 (3,574–4,847)	0.3192	0.0043	0.077
		Coding		AC	394	3,629 (3,066–4,023)			AC	211	4,076 (3,460–4,888)			
				CC	111	3,670 (3,065–3,992)			CC	84	4,006 (3,366–4,676)			
rs3852861	19	PVRL2	39.4	CC	295	3,681 (3,245–4,030)	0.0043	40.7	CC	167	3,980 (3,435–4,828)	0.602	0.0053	0.090
		Intron		CA	408	3,554 (3,038–4,003)			CA	230	4,209 (3,539-4,820)			
				AA	126	3,594 (3,127-3,987)			AA	75	4,155 (3,455–4,683)			
rs962859	21	IL10RB	42.7	AA	257	3,683 (3,183–4,052)	0.0023	42.9	AA	165	4,042 (3,425–4,720)	0.8688	0.0063	0.100
		Intron		AC	424	3,611 (3,048–4,002)			AC	200	4,168 (3,539–4,927)			
				CC	135	3,540 (3,090.5–3,889)			CC	106	4,154 (3,522–4,683)			
rs12416901	11	PVRL1	2.8	AA	785	3,631 (3,081–4,004)	0.0063	2.2	AA	453	4,127 (3,497–4,807)	0.8519	0.01	0.146
		Intron		AG	44	3,574 (3,221–3,932)			AG	20	4,258 (3,476–5,084)			
				GG	0	(,)			GG	0	(–)			

[a] rs SNP identification number

[b] Chromosomal location

[c] Gene or genetic region containing the indicated SNP

[d] Minor allele frequency

[e] Number of subjects with a given genotype

[f] Median outcome measurement for each genotype group. Results expressed as pg/ml. The interquartile range (IQR) is shown in parentheses

[g] *P* values were adjusted for demographic and clinical variables as well as inflation of significance described in the "Methods" section

[h] *Q* values computed using the methods of (Storey 2002, 2003) from the distribution of *p* values from SNPs whose heterogeneity tests did not reach significance

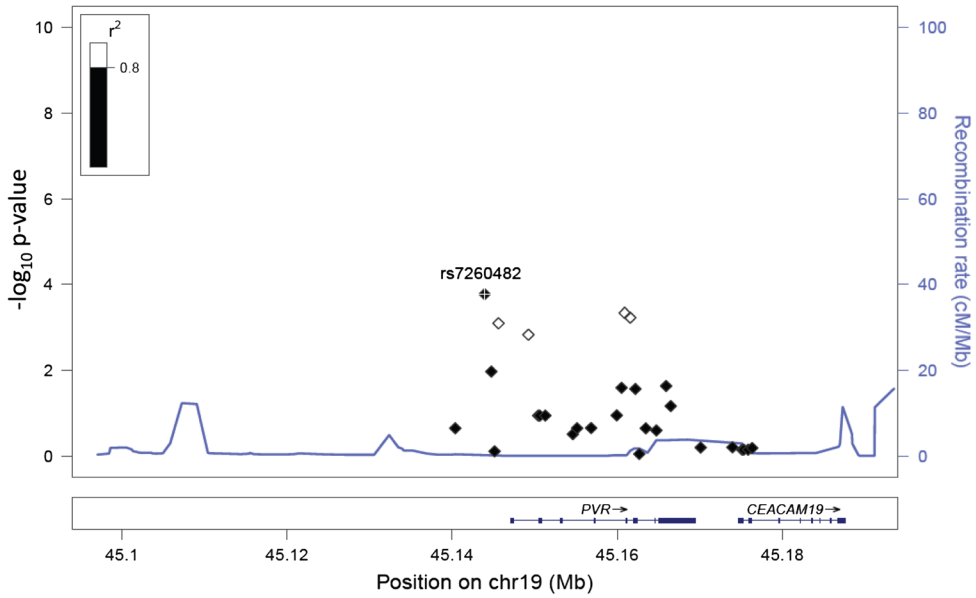

Fig. 1 Locus-zoom plot of selected region of Chromosome 19. A genetic region encompassing the PVR and CEACAM19 genes contained multiple SNPs significantly associated with rubella-specific IL-6 secretion following vaccination (Table 3). *P* value is depicted on the left *Y* axis. Each SNP is marked by the *solid diamonds* and LD (with respect to rs7260482) is indicated by the *grey color* of the diamond (there are no SNPs with r^2 between 0.6 and 0.8 or between 0.2 and 0.4). The recombination rate is mapped as the *grey line* at the bottom of the plot and on the right *Y* axis. Underneath the plot each gene and its chromosomal position is indicated. Thick segments of each gene indicate exons, while the *arrows* indicate the direction of transcription

negative values (indicating that in vitro stimulation with rubella virus suppressed IFNγ secretion), making the biological relevance unclear. The one exception is rs10484440 where, in the Rochester cohort, there was no IFNγ response (median IFNγ = 0 pg/ml) for individuals homozygous for the G allele. Homozygous A and heterozygous individuals had median IFNγ secretion levels of ~6 pg/ml. Two of the SNPs were located near the IL10RB gene and genetic polymorphisms in this region have also been associated with chronic hepatitis B infection (Romporn et al. 2013), cytokine responses to smallpox vaccine (Ovsyannikova et al. 2012a), HIV infection outcomes (Shrestha et al. 2010), immune responses following MMR vaccination (Dhiman et al. 2008) and malaria susceptibility (Khor et al. 2007).

Among the SNPs associated with IL-6 secretion after rubella virus stimulation, one was located within the TNFRSF1B gene, three SNPs were located in the ADAR gene, and five SNPs were located in or near the PVR gene. Each of the genetic associations demonstrated stronger signals in the Rochester cohort than in the San Diego cohort. Several factors may contribute to this: better vaccination dates in the Rochester cohort medical records, which allowed us to more accurately capture time since last vaccination; the smaller size of the San Diego cohort; and the potential impact of undocumented, chance rubella exposure or additional vaccinations in the San Diego cohort. This is especially apparent with rs474247 in TNFRSF1B, with a Rochester cohort p value of 4.2×10^{-6} and a San Diego cohort *p* value of 0.2771. In a previous study, we reported cytokine and cytokine receptor SNPs associated with rubella-specific cytokine secretion in a subset ($n = 738$) of the Rochester cohort. In that study, we identified multiple TNFRSF1B SNPs associated with variations in IL-6 secretion (Dhiman et al. 2010a). In the meta-analysis, the A allele of rs474247, an intronic SNP in TNFRSF1B, was associated with an allele dose-dependent increase in IL-6 secretion. Binding of TNFα to TNFR1 and TNFR2 leads to activation of NF-kB and pro-inflammatory responses, including IL-6 production. Polymorphisms affecting TNFRSF1B expression and/or function can lead to variations in TNFR pathway activation, leading to alterations in pro-inflammatory cytokine secretion (Till et al. 2005).

ADAR encodes an RNA-specific adenosine deaminase involved in pre-mRNA splicing, RNA stability and other RNA structure-related activities. The two ADAR-specific SNPs associated with IL-6 response were among the most significant SNPs in the Rochester cohort and were the only SNPs with strong evidence of association in the San Diego cohort. ADAR has been shown to exert antiviral properties by RNA editing of a number of viral RNA genomes including: vesicular stomatitis virus, measles virus, hepatitis C

virus, hepatitis delta virus, and HIV. Two of these three SNPs (rs2229857, rs1127317) were also associated with variations in cytokine secretion after measles stimulation (Haralambieva et al. 2011). The non-synonymous SNP, rs2229857 encoding a K > R change, is also associated with sustained response to IFN therapy for chronic HCV infection (Hwang et al. 2006; Welzel et al. 2009).

The second genetic region with multiple significant SNP associations was found on chromosome 19 near the PVR and PVRL2 genes (Fig. 1). PVR encodes for CD155, a transmembrane glycoprotein that mediates NK cell adhesion and effector function (Sakisaka and Takai 2004). Multiple T cell and NK cell receptors (TIGIT, DNAM-1, CD96) interact with PVR and its related proteins and these interactions modulate T and NK cell activity (Bottino et al. 2003; Fuchs et al. 2004; Yu et al. 2009). In fact, PVR and related proteins interact with a wide variety of ligands and participate in multiple immunologic functions and serve as cellular receptors for poliovirus, rhinovirus, and reovirus (Xu and Jin 2010). SNPs rs3852861 and rs1466435 are located in or near the neighboring PVRL2 gene that encodes for a component of adherens junctions and serves as a cellular receptor for herpes simplex virus and pseudorabies virus. As illustrated in Fig. 1, multiple SNPs located within these genetic regions were associated with differential IL-6 production. The presence of so many SNPs associated with immune response makes the TNFRSF1B and PVR regions excellent candidates for a fine mapping effort to assess correlations between these and other regional SNPs. This effort will be necessary to narrow down the possible causal variants. For example, rs203709 (Table 2) is in close LD ($r^2 = 0.959$) with another PVR SNP (rs7255066) exhibiting moderately significant associations with multiple sclerosis in a large collaborative GWAS involving over 9,000 European individuals with multiple sclerosis (Sawcer et al. 2011).

The differential evidence for genotype–phenotype associations between the two cohorts emphasizes the need to examine multiple populations in order to identify genetic regions expected to influence immune response. Furthermore, it highlights the importance of fine mapping studies that inform the selection of likely causal SNPs for targeted experiments designed to discover the underlying biology behind the variation in immune response. Our data so far highlight the importance of genetic association studies focused on vaccine response and suggest that genetic control of rubella vaccine-induced immunity may be mediated, in part, by novel genes and gene families such as PVR and butyrophilin genes. These findings, when combined with detailed studies elucidating the mechanisms behind identified genotype–phenotype associations, can then pave the way for potentially novel immunostimulatory therapies to improve viral vaccine efficacy and treat viral infections.

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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, and Emergent Biosolutions. Drs. Poland and Ovsyannikova hold two patents related to vaccinia and measles 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. The other authors do not have any conflicts of interest.

Ethical Standards All experiments described in this study comply with current, applicable US laws.

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