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Replication of Rubella Vaccine Population Genetic Studies: Validation of HLA Genotype and Humoral Response Associations

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Abstract

Purported genetic associations found in population studies require validation for confirmation. We previously reported rubella vaccine-induced immune responses and HLA associations in 346 adolescents, age 12–18 years (1st cohort), following two doses of a rubella-containing vaccine. We sought to replicate the associations discovered in that work by verifying these associations in a new cohort of 396 subjects, age 11–19 years (2nd cohort), all having had two doses of a rubella-containing vaccine. We found that B*2705 (median 1st cohort 20.9 IU/ml, $p=0.028$; 2nd cohort 20.5 IU/ml, $p=0.001$) and DPA1*0201 (median 1st cohort 32.5 IU/ml, $p=0.048$; 2nd cohort 25.8 IU/ml, $p=0.025$) alleles were consistently associated with lower rubella-induced antibodies. Further, DPB1*0401 (median 1st cohort 43.5 IU/ml, $p=0.021$; 2nd cohort 36.2 IU/ml, $p=0.002$) alleles were associated with higher antibody levels in both populations. The association of DRB1*04-DQB1*03-DPB1*03 (mean 1st cohort 25.2 IU/ml, $p=0.011$; 2nd cohort 21.4 IU/ml, $p=0.032$) and DRB1*15/16-DQB1*06-DPB1*03 (1st cohort 16.3 IU/ml, $p=0.043$; 2nd cohort 19.1 IU/ml, $p=0.023$) haplotypes with lower rubella-specific antibodies were observed in both studies. This study provides confirmatory evidence for an association between specific class I and II HLA markers and haplotypes with rubella vaccine-induced humoral responses and lends further weight to their influence on rubella immune responses.

Keywords

Rubella vaccine; HLA antigens; Antibody formation; Adolescent

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Conflict of Interest Statement

Dr. Poland is the chair of a safety evaluation committee for novel non-rubella vaccines undergoing clinical studies by Merck Research Laboratories. Dr. Jacobson serves on a Safety Review Committee for a post-licensure study of Gardasil for Kaiser-Permanente.

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1. Introduction

Immunity to rubella is an important public health issue world-wide. While it is well appreciated that polymorphic products of immune response genes, including the HLA region, determine variations in host susceptibility to infectious diseases [1], it is less appreciated that such genetic factors also determine variations in host immune responses to vaccines, including rubella [2]. We previously reported rubella vaccine-induced immune responses and HLA associations in 346 healthy adolescents after two doses of rubella vaccine [3–5]. These studies demonstrated that antibody responses to rubella vaccination are influenced by polymorphisms of the class I and II HLA genes. We sought to replicate the associations discovered in that work by verifying these associations in a new cohort of individuals. To confirm these findings, we undertook an independent study with a new cohort of 396 adolescents who received two doses of rubella vaccine and tested the hypothesis that the HLA associations discovered in the previous study would be replicated in a new cohort of similar subjects.

The lack of replication studies of allelic associations, including HLA genes, among different populations continues to cause difficulties in the identification of relationships between immune response gene polymorphisms and variations in adaptive immune responses to vaccines. Indeed, a large proportion of genetic associations fail to replicate in follow-up studies [6]. Although well recognized and accepted, the basis of such inter-population differences is not completely understood [7]. Validation of genetic associations is important not only for understanding mechanisms by which polymorphisms influence immune responses to vaccines, but also for development of novel vaccine targets [7]. Still, it has not been easy to determine whether specific HLA alleles are consistent or reliable determinants of humoral (and cellular) immune responses to vaccines in different populations [8]. In addition to extreme HLA gene polymorphism, discordances, for instance, have come from disparities in ethnicity, sample sizes, laboratory assays for measures of vaccine-induced immunity, statistical methods and other factors [8]. Thus, replication studies in different populations are crucial for better understanding the importance of genotype-phenotype associations and to differentiate true positive from false positive associations [9].

To address the basic question of whether these same HLA alleles, including HLA supertypes and haplotypes, are associated with antibody responses following two doses of rubella vaccine in an independent population, we took advantage of information obtained from two separate cohorts of adolescents who had participated in two studies of rubella vaccine-induced immunity and HLA gene associations.

2. Materials and methods

2.1. Study patients

As previously described, between December 2001 and August 2002, we enrolled 346 healthy adolescents (age 12 to 18 years) in Rochester, Minnesota (cohort 1) [3]. Three hundred and forty-two parents agreed to allow their adolescents to take part in the current rubella vaccine study. From December 2006 to August 2007, we enrolled a new cohort of 396 healthy adolescents and young adults (age 11 to 19 years) in Rochester, Minnesota (cohort 2). All 738 participants (combined cohort) had documentation of having received two doses of measles-mumps-rubella (MMR) vaccine containing the attenuated RA27/3 Wistar strain of rubella virus (Merck). No known circulating rubella virus was observed since the earliest year of birth for any subject in our geographic area. The Institutional Review Board of the Mayo Clinic approved the study. Written informed consent was obtained from the adolescents aged 18 and 19. Written parental permission from the parents of adolescents less than 18 years of age was provided, as well as assent from the adolescents themselves.

2.2. Antibody measurement

Original quantitative levels of rubella virus-specific IgG antibodies for cohort 1 were determined by the Enzygnost anti-rubella virus /IgG EIA (Dade Behring, Germany). To ensure consistency in antibody measures across both cohorts, rubella-specific IgG antibodies after two doses of the rubella vaccine in both cohorts were re-assayed by whole virus rubella-specific chemiluminescent immunoassay (Beckman Coulter Access, Fullerton, CA) according to the manufacturer's instructions. The limit of detection for this assay was 0.5 IU/ml and the coefficient of variation of this assay in our laboratory was 6%.

2.3. Molecular genotyping

Cohort 1 genotyping was performed several years prior to that of cohort 2, but genotyping methods were the same for both cohorts. Genomic DNA was extracted from fresh heparinized blood samples by conventional techniques using the Puregene® extraction kit (Gentra Systems). Class I HLA-A, -B and -C alleles typing was performed using High Resolution SSP (sequence-specific primer) A, B, and C UniTray typing kits, respectively. Any ambiguities were resolved using the Forensic Analytical sequencing kit and AmbiSolv™ when needed (Invitrogen). Class II HLA typing was performed with high resolution DRB1 SSP, DQB1 SSP, DPA1 SSP, and DPB1 SSP Unitray® typing kits with the entire locus on a single tray (Invitrogen). PCR was followed by AmbiSolv™ when needed and analyzed using MatchTools software. All PCR amplifications were carried out on an ABI 377 and analyzed using MatchTools software. All reactions were run with negative controls, and every fiftieth PCR reaction was repeated for quality control.

2.4. Statistical analysis

The purpose of the efforts reported here was to validate associations of individual HLA alleles, supertype alleles, and haplotypes with rubella antibody levels that were reported previously in our original rubella cohort (cohort 1), using data from an additional new cohort of 396 children (cohort 2). Published results on cohort 1 subjects were based on 346 individuals. However, only 342 of these subjects provided consent for future research and so are included in the current report. Also, previous cohort 1 reports used the Dade Behring Enzygnost Anti-Rubella/IgG enzyme immunoassay kit to determine rubella-specific antibody levels. This assay could not be obtained for cohort 2 subjects, so the Beckman Coulter Access Rubella IgG assay was used in its place. For consistency, cohort 1 subjects were re-assayed using the Beckman assay. A comparison of the two assays found high levels of agreement. We found that the two assays had a high qualitative (95%) and quantitative correlation 0.93 (0.92, 0.95) [Greenwood NP, Ovsyannikova IG, Vierkant RA, O'Byrne MM, and Poland GA, submitted]. Nevertheless, we wanted to ensure that the associations reported previously remained using the new assay and the smaller set of 342 subjects. Thus, all haplotype and allelic analyses described below proceeded in the following three-step fashion. We first re-analyzed the 342 cohort 1 subjects using the Beckman assay and compared results to the original 346 subjects based on the Dade Behring assay. If the original and updated results were similar, we then attempted to validate the statistically significant or suggestive cohort 1 results (based on the 342 subjects and the Beckman assay) using cohort 2 subjects. We examined the same associations after combining cohorts 1 and 2 into one overall study group. To minimize the possibility of false negative results and a corresponding missed opportunity for validation, we relaxed criteria for designating cohort 1 associations as statistically interesting. All genetic effects associated with antibody levels with a p-value less than 0.2 in cohort 1 were followed up with association tests in cohort 2 and in the combined study group.

HLA four-digit alleles and supertype alleles were grouped by locus, and summaries of antibody levels were obtained using medians and inter-quartile ranges. Individuals contributed two observations to these descriptive summaries, one for each of their two alleles. Antibody levels

were expressed in the original sampling units of IU/ml. Associations between HLA alleles and antibody measures were then formally evaluated using linear regression models. In contrast to the descriptive comparisons, each subject contributed one observation to these analyses, based on an observed genotype. Regression variables were created for each allele of interest and coded as 0, 1, or 2 according to the number of copies of the allele that a subject carried. Each allele variable was included in a separate linear regression analysis, effectively comparing antibody levels for the allele of interest against all other alleles combined. Due to data skewness, original immune response values were replaced with corresponding log-transformed values in the linear regression models.

Haplotype frequencies were created using a maximum likelihood approach. Due to the polymorphic nature of the HLA loci, examination of haplotypes using four-digit alleles was not possible. Thus, all haplotype analyses are based on two-digit HLA allele variables. Two sets of haplotypes were considered: one for the three class I loci (A, C, and B), and one for the three class II loci (DRB, DQB, and DPB). Because each individual's linkage phase is unknown, there may be multiple pairs of haplotypes which are consistent with the observed HLA alleles. Posterior probabilities of all possible haplotypes for an individual, conditional on the observed genotypes, were estimated using an expectation-maximization (EM) algorithm, similar to the method outlined by Schaid *et al.* [10]. Haplotype design variables were then created by first generating a matrix composed of the possible haplotypes for each person, weighting by the posterior probability of those possible haplotypes per person, and then collapsing back to a single row per person. As with the HLA allelic variables, values for each haplotype variable range from 0 to 2 and can be considered the estimated number of that haplotype carried by the individual after accounting for phase ambiguity. Because of the imprecision involved in estimating the effects of low-frequency haplotypes, we considered only those occurring in cohort 1 subjects with an estimated frequency of greater than 1%. Due to phase ambiguity, haplotype-specific medians and inter-quartile ranges could not be calculated. Thus, haplotype-specific descriptive associations were represented using means and corresponding 95% confidence intervals for individuals with one copy of the haplotype of interest, based on the parameter estimates from the linear regression models. As with the HLA allelic analyses, haplotype analyses were carried out using log-transformed antibody response values. Resulting means and confidence intervals were then back-transformed into original sampling units. All analyses described above were adjusted for covariates potentially associated with rubella antibody levels. These variables were: age at enrollment, race, gender, age at first rubella vaccination, and age at second rubella vaccination. Analyses combined across both cohorts also adjusted for cohort status (cohort 1 vs. cohort 2). All statistical tests were two-sided, and all analyses were carried out using the SAS (SAS Institute, Inc., Cary, NC) and S-Plus (Insightful, Inc., Seattle, WA) software systems.

3. Results

3.1. Demographics and antibody responses

Characteristics of the study cohorts 1 and 2 were similar - a little more than half of each cohort were male (52.9% and 54.3%), and the majority of the study subjects were Caucasian (93.8% and 88.6%) (Table 1). The median ages of the study subjects at the time of the rubella vaccinations were also similar. However, the children in cohort 1 had higher overall median antibody levels than those in cohort 2 ($p=0.008$), although at a level unlikely to be clinically significant. Median rubella-specific IgG antibody levels for the cohorts 1 ($n=342$), cohort 2 ($n=396$), and a combined cohort ($n=738$) were 38.8 IU/ml (IQR 19.7–69.7), 32.8 IU/ml (IQR 18.5–59.3) and 34.4 IU/ml (IQR 19.2–63.7), respectively. In cohort 1, females showed significantly higher antibody responses than males (median 43.5 IU/ml versus 31.5 IU/ml,

p=0.020). In a combined cohort, females also demonstrated significantly higher antibody responses than males (median 39.9 IU/ml versus 30.9 IU/ml, p=0.007).

3.2. Associations of HLA genotypes with rubella antibody levels

The associations between HLA alleles and rubella antibody levels were examined in all study cohorts. Only alleles found to be statistically or marginally significant in cohort 1 (p<0.20) are presented. Table 2 summarizes the strongest associations between individual class I and class II HLA alleles and rubella vaccine-induced humoral immune responses across each separate and combined cohort.

In cohort 1, individual alleles with the strongest association were B*2705 (median 20.9 IU/ml, p=0.028), B*4501 (median 74.4 IU/ml, p=0.039), DPA1*0201 (median 32.5, p=0.048), DPB1*0401 (median 43.5 IU/ml, p=0.021), DPB1*1301 (median 24.8 IU/ml, p=0.046), DPB1*1501 (median 70.8 IU/ml, p=0.018), and DRB1*1101 (median 56.2 IU/ml, p=0.033). Specifically, B*2705, DPA1*0201, and DPB1*1301 alleles were associated with lower median levels of rubella vaccine-induced antibodies. Among these statistically significant alleles, the HLA-B*2705 (median 20.5 IU/ml, p=0.001), DPA1*0201 (median 25.8 IU/ml, p=0.025) and DPB1*0401 (median 36.2 IU/ml, p=0.002) alleles were also associated with variations in antibody responses among rubella-vaccinated subjects in cohort 2, and in the same direction as the associations found in cohort 1. Associations were even stronger when combining data across cohorts. We found that HLA-B*2705 and HLA-DPA1*0201 alleles were significantly associated with lower rubella-induced antibody levels in both cohorts, and the DPB1*0401 alleles were significantly associated with higher antibody levels in both populations.

In cohort 1, individual alleles with marginally significant association (p-value between 0.05 and 0.20) were B*4002 (median 65.4 IU/ml, p=0.09), B*5701 (median 24.2 IU/ml, p=0.135), C*0704 (median 25.3 IU/ml, p=0.064), DPA1*0104 (median 64.2 IU/ml, p=0.098), DPB1*0202 (median 19.5 IU/ml, p=0.109), DPB1*0301 (median 26.2 IU/ml, p=0.128), and DQB1*0303 (median 26.8 IU/ml, p=0.095). Among these, B*5701, DPB1*0202 and DPB1*0301 alleles were also associated with variations in antibody responses among rubella-vaccinated subjects in cohort 2, and in the same direction as the associations found in cohort 1. In the final analysis, associations for the B*5701 and DPB1*0301 alleles were significant at the p<0.05 level when combining across cohorts. The B*4002, C*0704, DPB1*0202, DQB1*0303 alleles found to be interesting in cohort 1 were not validated in cohort 2. Finally, we were unable to examine A*2901, DPA1*0104, and DPB1*1501 alleles in cohort 2 due to low allele frequencies.

3.3. Associations of HLA supertypes with rubella antibody levels

Associations between the most common HLA class I A (A1, A2, A3 and A24) and B (B7, B27, B44, B58 and B62) supertypes and rubella-specific IgG antibody levels for all study cohorts were also examined (Table 2). The B27 supertype (median 1st cohort 31.8 IU/ml, p=0.072; 2nd cohort 25.9 IU/ml, p=0.051) was marginally associated with lower rubella-specific antibodies in both cohorts 1 and 2. When combining across cohorts, this association reached statistical significance (median 29.6 IU/ml, p=0.008). Further, the A3 supertype (median 35.0 IU/ml, p=0.196) found to be marginally associated with lower antibody levels in cohort 1 was not validated in cohort 2.

3.4. Associations of HLA haplotypes with rubella antibody levels

We also analyzed potential relationships between HLA haplotypes and mean antibody levels in rubella vaccine study populations (Table 3). Mean rubella-specific IgG antibody levels for cohort 1 (n=342), cohort 2 (n=396), and a combined cohort (n=738) were 55.0 IU/ml, 45.4 IU/ml and 49.9 IU/ml, respectively. Importantly, the association of DRB1*04-DQB1*03-

DPB1*03 (mean 1st cohort 25.2 IU/ml, $p=0.011$; 2nd cohort 21.4 IU/ml, $p=0.032$) and DRB1*15/16-DQB1*06-DPB1*03 (mean 1st cohort 16.3 IU/ml, $p=0.043$; 2nd cohort 19.1 IU/ml, $p=0.023$) haplotypes with lower rubella-specific antibodies was observed in both studies. In a combined cohort, the DRB1*04-DQB1*03-DPB1*03 (mean 23.6 IU/ml, $p=0.012$) and DRB1*15/16-DQB1*06-DPB1*03 (mean 18.3 IU/ml, $p=0.005$) haplotypes were associated with lower levels of IgG antibodies. Further, the DRB1*04-DQB1*03-DPB1*04 (mean 42.3 IU/ml, $p=0.065$) haplotype found to be interesting in cohort 1 was not validated in cohort 2.

Although our two cohorts have a fairly homogeneous racial composition, and although we adjusted for race in all statistical analyses, we were concerned about the possibility of residual confounding due to race. Thus, we ran a series of sensitivity analyses subset to Caucasian individuals. These results were virtually identical to those reported in Table 1–Table 3 (data not shown).

4. Discussion

Validation of population genetic associations is important for confidence that such associations are not spurious and for determining immunogenetic mechanisms by which polymorphisms influence immune responses to vaccines. A major shortcoming of many population-based studies involving a candidate gene approach, including HLA genes, is the lack of validation of the findings generated [7,11,12]. The present study is one of the few to examine the relationship between individual HLA alleles, HLA supertypes and HLA haplotypes and variations in humoral immune responses in a large group of healthy children after two doses of rubella vaccine and the first to validate the strongest associations found in our previous work in a new cohort of healthy children. The main reason for follow-up replication studies, such as this, is to rule out the possibility that results from the original rubella study were chance findings (i.e. false positives or type I errors).

We successfully validated several allelic associations found in the initial cohort. Notably, the B*2705 and DPA1*0201 alleles were associated with lower rubella-induced antibody levels, and the DPB1*0401 allele was associated with higher antibody production in both studies. In addition, the B27 supertype appeared to be independently associated with the lower rubella antibody responses in both cohorts. In contrast to our analyses in both populations, in a combined cohort of 738 subjects, we found more statistically significant associations between the HLA alleles and rubella humoral immunity, likely due to improved statistical power with a larger sample size. We saw consistent associations with several other alleles (B*4501, B*5701, DPB1*0301, DPB1*1301, and DRB1*1101), albeit at a lower level of significance. This could be considered our second tier of successful validations.

To examine the independent associations of the HLA alleles presented in our manuscript with rubella antibody levels, we ran a series of multivariate linear regression analyses within our combined cohort of 738 subjects. We first re-assessed the association of B*2705 after adjustment for all other common B alleles (including all observed Bw4 alleles). After adjustment, B*2705 was still significantly associated with lower antibody levels ($p=0.025$). We then ran one final linear regression model, simultaneously including the nine alleles found to be statistically significant in the combined cohort as reported in Table 2. All post-adjustment associations were in the same direction as reported in Table 2, and p -values were as follows: B*2705 ($p<0.001$), B*4501 ($p<0.001$), B*5701 ($p=0.07$), DPA1*0201 ($p=0.31$), DPB1*0401 ($p=0.02$), DPB1*1301 ($p=0.25$), DPB1*1501 ($p=0.02$), and DRB1*1101 ($p=0.03$). Thus, each of these alleles, with the possible exception of DPA1*0201 and DPB1*1301, seem to be independently associated with rubella antibody levels after rubella vaccination.

A striking finding was the consistent association of HLA class II haplotypes with rubella-specific antibodies. Two HLA haplotypes were found to be consistently associated with rubella vaccine-induced humoral immunity. The association of DRB1*04-DQB1*03-DPB1*03 and DRB1*15/16-DQB1*06-DPB1*03 haplotypes with lower rubella-specific antibodies were observed in both studies. Our rubella vaccine study suggests that class II polymorphisms dominated the HLA associations observed. Consistent with this interpretation, these results provide evidence of possible multi-genic HLA class II associations with antibody levels after rubella vaccination.

There were other HLA associations that were found in one cohort but not validated in the other – these associations are more likely to be false-positive associations than those that were validated. These false-positive associations are perhaps due to statistical associations arising by chance, underlying systematic biases due to study design and technical artifacts [12]. In general, false-positive associations can be minimized both by using methods designed to control the false discovery rate and by utilization of a validation cohort. However, a sample size of 342 subjects in the first study and subsequent validation cohort of 396 subjects allowed us to define the most important relationships between HLA gene polymorphisms and humoral immune responses following rubella vaccine. Thus, performance of two independent studies of a single rubella vaccine allowed us the unique opportunity to assess and validate the contribution of HLA genes in immune responses to rubella vaccination. Given the importance of understanding the mechanisms behind differential vaccine-induced antibody responses, data from this study will also inform directed and rational development of novel rubella vaccines. Knowing and understanding genetic elements that control (or influence) variations in vaccine-induced immunity allows prediction of individual and population-level immune responses to vaccine, including rubella [2,13].

In one of our earlier studies, we examined 100 twin pairs (45 monozygotic and 55 dizygotic) who had received one to two doses of MMR vaccine. We examined the heritability of immune responses to MMR and found that the heritability for rubella was 46%, whereas, the heritability for measles and mumps was 90% and 39%, respectively [14]. This suggests a strong genetic contribution to variation in rubella vaccine immune response. We were not able to estimate the total variance explained by the HLA system as a whole, because the number of polymorphisms across all the loci would overwhelm the number of enrolled subjects in any regression analysis. However, we were able to fit a series of locus-specific models to calculate the following r-squared values: HLA-A, 0; HLA-B, 0.0128; HLA-C, 0; DRB1, 0.0075; DQA1, 0.0052; DQB1, 0.0138; DPA1, 0.0152; DPB1, 0.0347. The sum of these r-squared statistics (0.0892) provides an upper bound for HLA contribution: the HLA system explains no more than (and most likely less than) 8.9% of the total variation in rubella antibody levels. Since the heritability for rubella was approximately 46%, this indicates that variation in the HLA loci only accounted for approximately 19% of the overall genetic variation in rubella antibodies.

Our study has several strengths. Both cohorts had documented MMR vaccination and no known wild type rubella viruses circulated in the community during their lifetimes. Both cohorts have adequate sample sizes to detect small to moderate genetic effects, thus lowering the risk of false negative results. Demographic and clinical characteristics were similar in the two cohorts, minimizing the possibility of discrepant results due to confounding effects of these attributes. Both cohorts have a fairly homogeneous racial composition (primarily Caucasians), minimizing the possibility of spurious results due to population stratification. However, this latter attribute also limits the inferential ability of our results to primarily Caucasian populations. Similar studies are needed in non-Caucasian populations to further validate these findings.

In conclusion, we report consistent associations with rubella vaccine-specific IgG antibody levels that validate our previously reported associations of HLA genetic polymorphisms on rubella vaccine antibody responses. A previously recruited sample and subsequent validation cohort allowed us to identify HLA markers associated with humoral immune responses following rubella vaccine. Replication of several associations allows confidence that specific individual HLA alleles and HLA haplotypes can now be considered as determinants of rubella vaccine-induced immunity. Findings from this study could inform and advance the design of improved viral vaccines and help to identify approaches for personalized vaccination [15]. Further, this information informs our understanding of genetic restrictions to immunity after vaccination and advances the knowledgebase in the new field of vaccinomics and adversomics [13,16].

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Table 1

Characteristics of the study cohorts by rubella antibody levels.

Variable	Level	Cohort 1 (n=342)				Cohort 2 (n=396)				Combined (n=738)			
		N	Median IU/ml	Q1 IU/ml	Q3 IU/ml	N	Median IU/ml	Q1 IU/ml	Q3 IU/ml	N	Median IU/ml	Q1 IU/ml	Q3 IU/ml
Overall		342	38.8	19.7	69.7	396	32.8	18.5	59.3	738	34.4	19.2	63.7
Age at enrollment	11–13	72	40.1	20.5	78.5	147	32.8	18.0	60.1	219	36.7	19.1	66.7
	14–15	95	39.2	21.9	65.4	101	31.7	18.3	52.5	196	33.7	19.6	57.8
	16–17	97	40.3	19.5	71.7	108	31.0	19.1	62.4	205	33.4	19.2	69.3
	18–19	78	34.5	19.2	58.9	40	38.3	22.6	66.6	118	36.7	19.6	59.9
Age at first rubella vaccination	<= 14 months	32	37.3	19.6	50.0	59	33.2	19.6	68.3	91	35.0	19.6	59.9
	15 months	171	31.8	19.6	76.7	223	31.1	18.3	55.3	394	31.4	19.1	62.2
	16–17 months	66	47.0	18.9	74.3	62	33.0	19.2	71.5	128	41.8	19.0	72.9
	>= 18 months	73	39.9	22.8	65.4	52	39.9	16.5	65.6	125	39.9	22.0	65.4
Age at second rubella vaccination	<= 5 years	31	20.7	15.9	46.7	178	28.0	16.6	58.5	209	27.8	16.6	55.3
	6–10 years	33	40.3	23.0	80.1	80	33.6	19.6	58.4	113	37.0	20.6	69.6
	11 years	84	38.0	19.2	66.9	45	30.6	17.3	48.2	129	33.6	18.9	65.4
	>= 12 years	194	42.2	22.1	70.7	93	38.1	24.3	64.6	287	39.9	22.4	69.7
Gender	Female	161	43.5	22.2	72.3	181	37.0	19.2	64.5	342	39.9	20.3	69.7
	Male	181	31.5	18.9	59.9	215	29.0	18.1	55.1	396	30.9	18.6	56.6
Race	Other	21	33.1	17.5	85.4	45	33.4	18.6	59.9	66	33.3	17.7	62.9
	White	321	39.5	20.4	69.5	351	32.6	18.4	59.3	672	34.5	19.4	64.0

Q1 and Q3 represent the first (25th percentile) and third (75th percentile) quartiles, respectively. N represents number of subjects studied.

Table 2

HLA allelic and HLA class I supertype associations with rubella vaccine-induced humoral responses.

HLA locus	Allele	Cohort 1 (n=342)						Cohort 2 (n=396)						Combined (n=738)			
		N	Median IU/ml	Q1 IU/ml	Q3 IU/ml	p-value	N	Median IU/ml	Q1 IU/ml	Q3 IU/ml	p-value	N	Median IU/ml	Q1 IU/ml	Q3 IU/ml	p-value	
Overall																	
HLA-A	*2901	5	38.8	19.7	69.7			32.8	18.5	59.3		6	34.4	19.2	63.7	0.218	
HLA-A	*2705	28	47.9	43.6	63.1	0.134	1	-	-	-	-	6	53.5	43.6	63.1	<0.001	
HLA-B	*4002	9	20.9	14.7	55.5	0.028	33	20.5	14.3	42.1	0.001	61	20.5	14.6	47.8	0.223	
	*4501	6	65.4	55.8	127.6	0.090	9	30.6	24.3	48.2	0.934	18	52.0	24.3	67.6	0.223	
	*5701	19	74.4	52.6	129.9	0.039	4	62.7	40.2	110.4	0.178	10	66.0	52.6	129.9	0.020	
HLA-C	*0704	16	24.2	18.8	44.2	0.135	20	25.0	16.5	42.1	0.098	39	24.2	18.1	44.2	0.031	
HLA-DPA1	*0104	7	25.3	15.1	43.1	0.064	19	57.4	16.3	125.3	0.370	35	29.0	16.3	78.9	0.520	
	*0201	112	64.2	44.2	87.7	0.098	0	-	-	-	-	7	64.2	44.2	87.7	0.111	
HLA-DPB1	*0202	5	32.5	19.6	62.4	0.048	104	25.8	16.8	53.9	0.025	216	29.0	18.2	58.5	0.005	
	*0301	69	19.5	14.9	33.1	0.109	3	73.2	38.0	99.8	0.180	8	35.6	17.2	67.5	0.592	
	*0401	268	26.2	17.6	53.3	0.128	78	26.2	15.2	42.7	0.057	147	26.2	17.4	48.5	0.013	
	*1301	15	43.5	21.7	79.6	0.021	334	36.2	20.9	64.5	0.002	602	40.0	21.0	69.6	<0.001	
	*1501	8	24.8	18.9	42.6	0.046	12	20.8	14.5	45.7	0.290	27	23.8	17.7	42.6	0.038	
HLA-DQB1	*0303	23	70.8	49.0	103.2	0.018	1	-	-	-	-	9	64.2	44.2	87.7	0.036	
HLA-DRB1	*1101	28	26.8	18.1	46.7	0.095	40	31.9	17.0	52.2	0.368	63	30.6	17.9	48.5	0.075	
	HLA supertype		56.2	27.2	85.8	0.033	36	39.9	18.6	70.6	0.268	64	47.7	22.2	76.2	0.022	
Class IA	A3	201	35.0	19.1	64.2	0.196	216	30.8	19.2	59.6	0.503	417	32.7	19.2	61.6	0.225	
Class IB	B27	73	31.8	19.1	63.7	0.072	84	25.9	16.5	53.7	0.051	157	29.6	17.5	58.9	0.008	

Linear regression analysis. P-values for antibody levels were based on log-transformed data, using simple linear regression. Only alleles found to be statistically or marginally significant in cohort 1 ($p < 0.20$) are presented. Analyses adjusted for age at blood draw, gender, race, age at first rubella, and age at second rubella vaccine. Statistically significant findings ($p < 0.05$) are bolded.

Table 3

HLA haplotype associations with rubella vaccine-induced humoral responses.

HLA Class	Haplotype	Cohort 1 (n=342)				Cohort 2 (n=396)				Combined (n=738)			
		Haplotype Frequency	Mean IU/ml	95% CI	p-value	Haplotype Frequency	Mean IU/ml	95% CI	p-value	Haplotype Frequency	Mean IU/ml	95% CI	p-value
Overall			55.0				45.4				49.9		
Class I (A-C-B)	*01-07-07	0.013	62.5	(33.8,115.6)	0.131	0.010	49.3	(26.6,91.1)	0.324	0.011	52.9	(34.6,80.9)	0.044
	*03-07-07	0.059	28.4	(20.8,38.8)	0.149	0.059	31.5	(24.3,40.8)	0.190	0.059	30.6	(25.2,37.1)	0.041
	*68-07-44	0.010	20.9	(10.9,40.3)	0.109	0.005	29.6	(12.9,67.8)	0.584	0.007	24.5	(14.8,40.5)	0.112
Class II (DRB-DQB-DPB)	*04-03-03	0.021	25.2	(15.4,41.3)	0.011	0.020	21.4	(13.4,34.0)	0.032	0.022	23.6	(16.9,33.0)	0.012
	*04-03-04	0.101	42.3	(32.1,55.7)	0.065	0.113	39.8	(32.3,49.0)	0.333	0.102	40.7	(34.6,47.9)	0.059
	*15/16-06-03	0.012	16.3	(6.9,38.3)	0.043	0.011	19.1	(9.0,40.7)	0.023	0.009	18.3	(10.4,32.0)	0.005

Only haplotypes statistically or marginally significant ($p < 0.20$) and relatively common (estimated frequencies ≥ 0.01) in cohort 1 are presented.

Predicted means (95% confidence interval [CIs]) for individuals who carry one copy of the haplotype of interest. Analyses were carried out on log-transformed values; the values were then back-transformed to obtain appropriate means and CIs. P-value comparing haplotype of interest to all other haplotypes combined. Statistically significant findings ($p < 0.05$) are bolded.