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Consistency of HLA Associations between Two Independent Measles Vaccine Cohorts: A Replication Study

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Abstract

Associations between HLA genotypes and measles vaccine humoral and cellular immune responses were examined to better understand immunogenetic drivers of vaccine response. Two independent study cohorts of healthy schoolchildren were examined: cohort one, 346 children between 12–18 years of age; and cohort two, 388 children between 11–19 years of age. All received two age-appropriate doses of measles-containing vaccine. The purpose of this study was to identify and replicate associations between HLA genes and immune responses following measles vaccination found in our first cohort. Associations of comparable magnitudes and with similar p-values were observed between B*3503 (1st cohort p=0.01; 2nd cohort p=0.07), DQA1*0201 (1st cohort p=0.03; 2nd cohort p=0.03), DQB1*0303 (1st cohort p=0.10; 2nd cohort p=0.02), DQB1*0602 (1st cohort p=0.07; 2nd cohort p=0.10), and DRB1*0701 (1st cohort p=0.03; 2nd cohort p=0.07) alleles and measles-specific antibody levels. Suggestive, yet consistent, associations were observed between the B7(1st cohort p=0.01; 2nd cohort p=0.08) supertype and higher measles antibody levels in both cohorts. Also, in both cohorts, the B*0801 and DRB1*0301 alleles, C*0802 and DPA1*0202 alleles, and DRB1*1303 alleles displayed consistent associations with variations in IFN- γ , IL-2 and IL-10 secretion, respectively. This study emphasizes the importance of replicating HLA associations with measles vaccine-induced humoral and cellular immune responses and increases confidence in the results. These data will inform strategies for functional studies and novel vaccine development, including epitope-based measles vaccines. This is the first HLA association replication study with measles vaccine-specific immune responses to date.

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Disclosures

Dr. Poland is the chair of a safety evaluation committee for novel non-measles 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. **Dr. Poland and Dr. Ovsyannikova hold a patent for the discovery of novel measles peptides potentially useful in developing new diagnostic assays and vaccines.**

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Keywords

Measles vaccine; HLA genotypes; Haplotypes; Antibodies; Cytokines; Replication study

Introduction

The mechanism(s) for inter-individual variations in measles vaccine-induced immune responses are unclear, but are important in understanding the immunogenetics of vaccine response, and for newly directed vaccine development using personalized “predictive vaccinology” and systems biology approaches [1;2]. We have hypothesized that immunogenetic markers could explain such differences. As such, we conducted two studies in independent study cohorts in the same setting to initially discover, then replicate, statistically significant associations between human leukocyte antigen (HLA) alleles and immune responses following measles vaccine. Our studies were informed by an initial study we performed in monozygotic and dizygotic twins in an attempt to separate out genetic factors from other factors [3]. This twins study estimated that the genetic contribution to the overall variation in antibody levels after measles vaccination is as high as 90% [3]. This evidence strongly supports the hypotheses of the genetic heritability of measles vaccine immune response.

A large number of population-based association studies have been conducted to establish relationships between HLA gene polymorphisms and vaccine immune response [4–7]. Although the role of HLA gene polymorphisms in immune response to vaccines, including measles-mumps-rubella (MMR) has been demonstrated [8;9], association studies in independent cohorts are still lacking. Further, non-replication and inconsistent results in candidate and genome-wide association population-based genetic studies have often been reported in subsequent attempted replication studies [10]. Replication of immunogenetic vaccine studies in different cohorts is essential for correctly understanding of the relationship between HLA and other genes and immune responses to vaccines and to be confident of results. In turn, such independent population-based association vaccine studies in different cohorts may help identify genetic markers and/or non-HLA genes and polymorphisms in linkage disequilibrium with the causative variant and, importantly, serves to more completely understand which polymorphisms are likely to have functional consequences in immune response.

Our initial study in a cohort of 346 children identified candidate associations between HLA alleles (as well as HLA supertypes and haplotypes) with variations in both humoral and cellular responses following measles vaccination [8;9;11–14]. To replicate these findings, we conducted an independent study in a separate cohort of 388 children after MMR vaccine, where associations between HLA alleles and measures of measles vaccine-induced immunity were examined. The goal of the study was to examine HLA associations in a new cohort of subjects following measles vaccine and to replicate our previous results, comparing the consistency of HLA associations between two independent study cohorts.

Materials and methods

Study subjects

Cohort 1—We leveraged work we previously accomplished by including subjects who had participated in our prior measles vaccine study [8;11–13]. Details of recruitment of this study cohort (n=346, 12–18 years of age) are described in our previous HLA association publications [8;9;13;15].

Cohort 2—We enrolled 388 healthy children and young adults (11–19 years of age) in Rochester, Minnesota, as previously described [16;17].

Antibody measurement

Cohort 1—Details of the antibody measurement for the 346 subjects have been previously described [8;9;18]. Measles virus (MV)-specific IgG antibody levels in serum samples were assessed by using the Enzygnost (Dade Behring, Germany) enzyme immunoassay (specificity, 100%; sensitivity, 99.6%).

Cohort 2

Plaque reduction microneutralization assay: Measles-specific neutralizing antibody levels were assessed using a fluorescence-based plaque reduction microneutralization assay (PRMN), as previously described [19]. The use of the 3rd WHO international anti-measles standard (3,000 IU/ml, NIBSC code no. 97/648) enabled quantitative ND₅₀ values to be transformed into mIU/ml.

Cytokine response measurement

Cohort 1—Details of the measurement of the IL-2, IL-10 and IFN- γ cytokine levels in cell culture supernatants of PBMCs stimulated with MV have been described in our previous publications [12;13;20]. The intra-assay coefficient of variation for IL-2, IL-10 and IFN- γ assays were 42%, 48%, and 44%, respectively.

Cohort 2—Enzyme-linked immunosorbent assays (ELISAs) were performed to measure the level of three cytokines (IL-2, IL-10, and IFN- γ) secreted by PBMCs following *in vitro* stimulation with MV, as previously described [20]. Cytokine-specific ICCs ranged from 0.65 (IL-2, unstimulated values) to 0.89 and 0.87 (IL-10 and IFN- γ , respectively, stimulated values).

HLA genotyping

Cohort 1—Details of PCR-based HLA allele (A, B, C, DQA1, DQB1, DPA1, and DPB1) typing have been published elsewhere [11;12].

Cohort 2—HLA class I (A, B, C) and class II (DRB1, DQA1, DQB1, DPA1, and DPB1) genotyping was carried out using high resolution SSP Unitrays typing kits (Invitrogen) with the whole locus on a single tray, as previously described [16].

Statistical methods

The statistical methods described herein are similar to those performed for our previous HLA association manuscripts [8;11;12;15]. We summarized the characteristics of the two study cohorts within defined categories of demographic characteristics, and compared these characteristics with chi-squared tests. We summarized the measures of measles vaccine immune response, both humoral and cellular, with medians and inter-quartile ranges (IQR). Where multiple measurements were obtained for each subject, per laboratory protocol, we used the median of the observed values, or the difference in the medians between stimulated and unstimulated states, as the individual level summary. We obtained these summaries overall, as well as within categories defined by HLA alleles and groups of HLA alleles grouped into HLA supertypes. In these HLA allelic summaries, each subject contributed two observations to the summaries, one for each allele carried.

We applied mixed effects linear models approaches to formally assess HLA associations with measures of measles vaccine immune response within each study cohort. In these

analyses, each participant contributed one observation per observed genotype for each of the multiple lab-based measurements obtained for the assay. An unstructured correlation structure was used in the linear mixed model to account for the repeated measurements obtained for each subject that were used in the analyses. For use as covariates in these linear mixed models, we created ordinal regression variables that represented the number of copies of each allele carried by each individual. We used these variables to perform tests for ordinal effects of the HLA alleles and HLA supertypes on the outcomes of interest. For analyses in the first cohort, we simultaneously included all but one of the allele variables in a linear regression model and examined global differences in immune response among all alleles of a given locus prior to assessing associations with individual alleles. For these tests, individual allele effects were examined in the spirit of Fisher's protected least significant difference test, only considering individual allelic associations to be statistically significant if we found global significance. As the focus of this effort was on replication of specific HLA allele associations, we performed specific tests of significance that focused on the HLA alleles of interest for each immune outcome in the second study cohort.

In addition to performing tests of significance for individual HLA alleles and supertypes, we performed a series of analyses to confirm possible HLA haplotype associations with the same immune response measures. To achieve this, we computed the posterior probabilities of all possible haplotypes for an individual, conditional on the observed genotypes, using an expectation-maximization algorithm [21]. We used these probabilities to define haplotype design variables that estimated the number of each of the haplotypes carried by an individual. We performed analyses on all common haplotypes (those with an estimated frequency of greater than 1%) using the linear mixed models approach used for the single-locus analyses using the haplotype design variables in the place of the ordinal SNP covariates. We examined individual haplotype effects by including each haplotype in a separate regression analysis, effectively comparing immune response levels for the haplotype of interest against all others combined. Due to phase ambiguity, haplotype-specific medians and inter-quartile ranges could not be calculated. Therefore, we estimated means and 95% confidence intervals for antibody levels associated with each haplotypes. For the other immune phenotypes, we used rank-based transformations, making it impractical to map the predicted ranks to observed immune measures. Thus, descriptive summaries for these measures were represented using the corresponding t-statistics.

In addition to performing the individual tests of significance, we tallied the number of significant tests observed in the replication phase, and tested whether more individual associations replicated in the follow-up stage than would be expected by chance. We achieved this by forming the ratio of observed to predicted number of significant results, and tested whether this ratio was significantly different from 1.0, recognizing that the variance of the logarithm of this ratio is equal to one over the number of tests significant in the follow-up stage.

We applied data transformations to the individual immune response measures in order to ensure that the outcome data conformed to the assumptions required by linear mixed effects models. When we tested for genetic associations with measles immune responses, we adjusted for potential confounding variables including age, race, gender, age at first measles vaccination, and age at second measles vaccination. We performed all analyses using the SAS version 9 (SAS Institute, Inc., Cary, NC) and S-Plus version 8 (Insightful Corporation, Seattle, WA) software systems.

Results

Characteristics of study cohorts

Detailed descriptions of the two study cohorts have been published previously [12;13;16;20]. There were a total of 346 and 388 healthy children in cohort 1 and cohort 2, respectively, with the majority of the subjects being Caucasian (Table 1). There were large differences between the two cohorts on a demographic level, such as age at first ($p=0.001$) and at second ($p<0.001$) measles vaccine. There were also differences between cohorts and time from vaccination to sampling ($p<0.001$). Nearly 57% of the cohort 1 children were vaccinated at 12+ years compared with 29% of the cohort 2 children ($p<0.001$). Overall, cohort 1 (median 1,556 IU/ml; IQR 746–2,677) demonstrated much higher measles antibody levels than cohort 2 (median 759 mIU/ml; IQR 391–1,563; $p<0.001$), as measured by IgG ELISA and PRMN, respectively. We found no significant differences in measles antibody levels between females and males in the cohorts.

Associations of HLA genotypes with measles antibodies

As previously reported, we examined associations between HLA alleles and measles IgG antibody levels in 346 children after two doses of MMR vaccine (first cohort) [8;9;22]. We also examined a separate cohort of 388 children who were HLA genotyped after receiving two doses of MMR (second cohort). Table 2 shows the results for the association ($p\leq 0.10$) between HLA alleles (and HLA supertypes) and measles-specific antibody levels across each study cohort. Certain previously identified HLA associations in first cohort were found to be associated with measles antibodies in second cohort. Specifically, the allele B*3503 was associated with increased antibody levels in both studies (median 1st cohort 2,703 IU/ml, $p=0.01$; 2nd cohort 1,207 mIU/ml, $p=0.07$). Class II DRB1*0701 (median 1st cohort 1,060 IU/ml, $p=0.03$; 2nd cohort 563 mIU/ml, $p=0.07$), DQA1*0201 (median 1st cohort 1,060 IU/ml, $p=0.03$; 2nd cohort 529 mIU/ml, $p=0.03$), and DQB1*0303 (median 1st cohort 1,129 IU/ml, $p=0.10$; 2nd cohort 492 mIU/ml, $p=0.02$) alleles were consistently associated with decreased measles antibodies in both cohorts. The B7 (*0702, *0704, *0705, *3501, *3502, *3503, *5101, *5301, *5501, *5601) supertype (median 1st cohort 1,848 IU/ml, $p=0.01$; 2nd cohort 900 mIU/ml, $p=0.08$) was associated with higher measles antibodies in both cohorts.

Associations of HLA haplotypes with measles antibodies

Previously we reported associations between HLA haplotypes and measles-specific IgG antibody levels in 346 subjects (first cohort) [8]. Associations between HLA haplotypes and measles-specific neutralizing antibody levels were also examined in 388 subjects (second cohort) (Table 3). Suggestive association of the DRB1*15/16-DQB1*06-DPB1*03 (1st cohort mean 2,259 IU/ml, $p=0.09$; 2nd cohort mean 298 mIU/ml, $p=0.07$) haplotype with variations in MV antibody levels was detected in both study cohorts; however, these associations were in the opposite direction.

Associations of HLA genotypes with measles-specific cytokines

Associations between HLA alleles and measles-specific cytokine immune responses for first cohort have been published elsewhere [11–13]. The results for the strongest association ($p\leq 0.10$) between HLA alleles and MV-specific IFN- γ , IL-2 and IL-10 secretion levels for cohorts 1 and 2 are summarized in Supplemental Table 1. Median measles-specific IFN- γ secretion levels for the first cohort and second cohort were 40.7 pg/ml (IQR 8.1–176.7) and 60.7 pg/ml (IQR 32.7–104.3), respectively. The allele B*0801 was associated with lower MV-specific IFN- γ responses in both first cohort (median 29.88 pg/ml, $p=0.04$) and second cohort (median 53.56 pg/ml, $p=0.03$). Similar, the common DRB1*0301 allele was

consistently associated with decreased IFN- γ secretion (median 1st cohort 25.41 pg/ml, $p=0.05$; 2nd cohort 50.59 pg/ml, $p=0.08$).

Median IL-2 secretion levels for the first cohort and second cohort were -2.5 pg/ml (IQR -14.9 – 13.8) and 34.6 pg/ml (IQR 18.9 – 56.5), respectively. The relatively rare allele C*0802 was associated with extremely high levels of IL-2 secretion in second cohort (median 46.7 pg/ml, $p=0.06$), and in the similar direction as the association observed in first cohort (median 14.0 pg/ml, $p=0.06$). Further, the DPA1*0202 allele (median 1st cohort -5.3 pg/ml, $p=0.09$; 2nd cohort 33.1 pg/ml, $p=0.06$) demonstrated suggestive allelic association with lower IL-2 secretion in both measles vaccine study cohorts.

Median IL-10 secretion levels for the first cohort and second cohort were 29.0 pg/ml (IQR 10.0 – 72.5) and 19.8 pg/ml (IQR 13.8 – 29.1), respectively. The association of DRB1*1301 (median 1st cohort 14.5 pg/ml, $p=0.06$; 2nd cohort 16.8 pg/ml, $p=0.04$) allele with variation in IL-10 secretion levels was observed in both cohorts.

Discussion

This study emphasizes the importance of HLA association replication studies with measles vaccine-induced immune responses and supports the development of a paradigm of “Discover–Replicate–Validate–Apply” in new vaccine development [23], by allowing the identification of the critical genetic determinants of vaccine-induced immunity, and highlighting those polymorphisms, which should undergo further functional studies. By understanding critical genetic determinants of immune response, it is feasible to uncover the basis for an individualized approach to vaccination to enhance immune response, for example, in vaccine low-responders [1;24]. Further, it is imperative that genetic association findings be replicated in independent cohorts to minimize the risk of false discovery, and enhance confidence in identification of the most promising genetic markers [25;26]. However, few HLA allelic and HLA haplotype association replication studies have been conducted [6]. This is one of the few -- if not the only -- HLA association replication studies with measles vaccine-induced humoral and cellular immune responses to date. Because such studies are often non-reproducible due to chance associations of statistical significance [27;28] we designed our studies to meet the rigorous NCI-NHGRI Working Group criteria for replicating genotype-phenotype associations [29].

Keeping these issues in mind, HLA allelic associations were compared between two independent cohorts after receiving two doses of measles-containing vaccine. We observed a number of replicated allelic associations with measles antibody levels that warrant further functional studies. The HLA-B7 supertype that was carried by 28.2% of study subjects was associated with increased antibody levels in both cohorts (1.18-fold higher in 1st cohort; 1.19-fold higher in 2nd cohort). The B*3503 allele belonging to the B7 supertype was associated with high antibody levels (1.74-fold greater than the overall median in 1st cohort; 1.59-fold higher than the overall median in 2nd cohort). The common DRB1*0701 (0.68-fold lower in 1st cohort; 0.74-fold lower in 2nd cohort), DQA1*0201 (0.68-fold lower in 1st cohort; 0.70-fold lower in 2nd cohort), and DQB1*0303 (0.72-fold lower in 1st cohort; 0.65-fold lower in 2nd cohort) alleles were associated with diminished antibody responses. It is possible that the B*3503 allele presents a quantitatively or qualitatively different set of peptides than DRB1*0701, DQA1*0201, or DQB1*0303 alleles, and is thus able to induce more strong MV-specific T-cell responses, which, in turn, elicit stronger humoral immunity. Of interest was the association observed between the DRB1*15/16-DQB1*06-DPB1*03 haplotype and measles antibody levels in both cohorts; however, the association was in the opposite direction. This specific haplotype appears to be important, since this haplotype was previously also associated with lower antibodies to rubella vaccine [6]. Thus, the important

outcome of this study is that a number of the individual HLA allelic associations with measles vaccine antibody responses were replicated, whereas none of the HLA haplotype associations were confirmed.

As genetic associations with humoral and cellular immunity to vaccines are assessed, it is necessary to consider the methods and standardized assays by which the response to vaccination is studied. The MV PRMN assay is considered to be the gold standard in measuring neutralizing antibody levels. A serum antibody titer of 120–200 mIU/ml is linked with protection from measles disease [30], whereas cellular correlates of vaccine immunity are more difficult to quantify and standardize [31].

As such, it is important to mention the differences in immune response measurement between the cohorts. Instead of using circulating measles-specific IgG antibody levels as a proxy for measles vaccine-induced humoral immunity, the second study utilized a PRMN assay as an indicator of humoral response. Differences between the two assays could account for apparent higher measles antibody levels in first cohort compared to second cohort. The PRMN assay measures the antibodies capable of neutralizing the virus, while the ELISA measures MV-specific circulating IgG levels. The differences in the cell culture conditions to test for MV-specific secreted cytokines explain the lack of consistency for cytokine levels observed between the two cohorts. It is likely we would have observed even greater consistency in findings across cohorts had identical outcome response measures been used for each.

Genetic and non-genetic (environmental) factors may influence immune response to vaccines in the human population [3;32]. The major role of HLA molecules is to present antigenic epitopes to T-cells, thereby initiating adaptive immune responses. Differential epitope presentation by HLA molecules may elicit distinctive CD4+ and CD8+ T-cell populations with varied specificities and/or functionalities. It is believed that these differences in repertoire presentation are likely the basis for some amount of variation in vaccine immune response outcome. With regard to measles, in our twins study we found the heritability (the ratio of genetic variance to total variance) of vaccine-induced humoral immune response to be nearly 90% ($p < 0.0001$) when compared to other vaccines [3]. We also calculated a number summarizing the amount of variation in measles antibody levels that is explained by HLA alleles that are consistently associated with this outcome across our two studies. The HLA alleles that are consistently associated with antibody levels between the two studies explain about 3.9% of the variation in this phenotype. Thus, population-based association studies and follow-up replication studies, such as this, have been very helpful in uncovering HLA and immune response gene polymorphisms and their role in vaccine-induced immune response [8;9;14].

Cytokines play critical roles in the modulation of immune response to measles vaccine [33;34]. HLA alleles may also vary in their ability to stimulate cytokine secretion, clonal expansion, or cytolytic activity. Consistent associations were found between the B*0801 and DRB1*0301 alleles and lower production of a major Th1-like cytokine-IFN- γ [35]. The C*0802 and the DPA1*0202 alleles were associated with higher and lower IL-2 levels, respectively, in both cohorts. Class I C*0802 molecules likely present a different spectrum of MV epitopes with different epitope densities and specificities than DPA1*0202 molecules. Importantly, the DQA1*0201 (and to some extent DRB1*1303) alleles were significantly associated with low levels of measles antibody after measles vaccine. In this regard, human IL-10 is known to enhance antibody production by B-cells, promote immunoglobulin class switching, and lymphocyte proliferation and cytokine secretion [36–38].

The strengths of our study include two well-characterized independent and large study cohorts with documented evidence of measles vaccination, no circulation wild-type MV strains in the population, and quality-controlled laboratory techniques to assess immunity to measles vaccine. Our analyses were adjusted for all key variables including race, gender, age, and vaccination status (time since last immunization, waning of immunity). Limitations of our study include differences in immune measures across cohorts and multiple testing issues. Despite these differences, we successfully replicated several associations between HLA alleles and immune responses after two doses of measles vaccine.

Replication studies are one of the most effective ways to control the number of false-positive findings. However, due to the number of statistically significant results originally reported in first cohort, some limited multiple testing issues remain. We observed 58 statistically significant or suggestive HLA associations ($p \leq 0.10$) in first cohort. Under the complete null hypothesis, we would expect 5% of these associations, or about 3, to have $p \leq 0.10$ and have associative effect in the same direction as the original results. Of the 58 alleles, we observed 14 statistically significant or suggestive results ($p \leq 0.10$) in the second cohort, 11 of which were in the same direction as before, significantly more than we would expect by chance alone ($p = 0.0001$).

In conclusion, replicated associations with the B*3503, DQA1*0201, DQB1*0303, DQB1*0602, and DRB1*0701 alleles, including the HLA-B7 supertype, with antibody levels provide further support for the effects of HLA gene polymorphisms on the humoral response to measles vaccine. Furthermore, these data instruct us on the genetic correlates of protective measles vaccine immunity and offer significant information for mechanistic studies and novel vaccine development. This study provides leads for functional studies to determine the direct effects and/or downstream functional consequences on measles vaccine immune outcomes of replicated HLA polymorphisms. Naturally processed MV-derived peptides isolated from these specific HLA alleles associated with vaccine low- and hyper-response can be helpful for constructing a synthetic epitope-based vaccine [39;40]. Additionally, the association of B*0801, C*0802, DRB1 (*0301, *1303), and DPA1*0202 alleles with MV-specific cytokine levels was found in both cohorts. If these associations are replicated in yet other independent populations, further studies will be warranted to identify MV epitopes presented by these HLA alleles. By identifying such genetically restricted peptides, and using knowledge regarding peptide/HLA promiscuity and HLA supertypes, the next generation of multi-epitope measles vaccines delivered with specific adjuvants could be prepared and tested for immunogenicity. Finally, such replication studies could serve to identify molecular mechanisms that influence immune responses, and provide new vaccine approaches and therapeutics to protect humans from measles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Two independent study cohorts of healthy school children were examined
- Purpose of study to identify associations between HLA genes and immune responses
- Purpose of study to replicate associations between HLA genes and immune responses
- First HLA association replication study with measles vaccine-specific immune responses to date

Table 1

Characteristics of the study cohorts.

	Cohort 1 (n=346)		Cohort 2 (n=388)		p-value
	N	%	N	%	
Age at enrollment (years)					
11-12	11	3.2	96	24.7	<0.001
13-14	110	31.8	92	23.7	
15-16	94	27.2	102	26.3	
17+	131	37.9	98	25.3	
Age at first measles vaccine (months)					
≤14 months	32	9.2	58	14.9	0.001
15 months	174	50.3	219	56.4	
16+ months	140	40.6	111	28.6	
Age at second measles vaccine (years)					
≤4	11	3.2	92	23.7	<0.001
5	20	5.8	82	21.1	
6-11	118	34.1	124	32.0	
12+	197	56.9	90	28.6	
Gender					
Male	185	53.5	212	54.6	0.808
Female	161	46.5	176	45.4	
Race					
Not white	21	6.1	43	11.1	0.023
White	325	93.9	345	88.9	

N represents number of subjects studied. P-values based on chi-square tests of significance.

Table 2

HLA allelic associations with measles virus-specific humoral immune responses.

HLA locus	Allele	Cohort 1 (n=346)					Cohort 2 (n=388)				
		N	Median, IU/ml (EIA IgG)	Q1 IU/ml	Q3 IU/ml	p-value	N	Median, mIU/ml (PRMN)	Q1 mIU/ml	Q3 mIU/ml	p-value
	Overall	692	1556	746	2677		744	759	391	1563	
HLA-A	*0301	108	1808	1088	3205	0.019	111	735	409	1556	0.870
	*2902	18	695	377	2335	0.111	29	440	210	884	0.031
HLA-B	* 3503	18	2703	1585	3781	0.012	14	1207	553	2059	0.073
	*5701	20	1062	509	2128	0.060	19	494	205	1747	0.104
HLA-C	*1203	34	1312	574	2180	0.149	30	1167	475	2059	0.063
HLA-DPA1	*0104	7	2970	2367	7245	0.020	-	-	-	-	-
HLA-DPB1	*1301	15	967	646	1543	0.008	12	987	421	1547	0.570
	*1501	8	2900	2522	5760	0.005	-	-	-	-	-
HLA-DQB1	* 0303	24	1129	848	1742	0.100	38	492	329	970	0.019
	* 0602	91	1885	765	3634	0.068	115	880	512	1600	0.100
HLA-DRB1	* 0701	73	1060	533	2335	0.026	88	563	296	1602	0.068
	*1303	10	1346	368	2237	0.203	7	1641	560	2692	0.057
	*1501	88	1977	853	3656	0.013	119	799	438	1570	0.380
HLA-DQA1	* 0201	73	1060	533	2335	0.030	88	529	283	1602	0.034
	HLA supertype										
Class IB	B7	195	1848	971	2987	0.01	234	900	423	1699	0.08

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 cohorts 1 and 2 ($p \leq 0.10$) are presented. Analyses adjusted for age at blood draw, gender, race, age at first measles, and age at second measles vaccine. Statistically or marginally significant findings ($p \leq 0.10$) are bolded.

Table 3

HLA haplotype associations with measles virus-specific humoral immune responses.

HLA Class	Haplotype	Cohort 1 (n=346)				Cohort 2 (n=388)			
		Haplotype frequency	Mean, IU/ml (EIA IgG)	95% CI	p- value	Haplotype frequency	Mean, mIU/ml (PRMIN)	95% CI	p-value
Overall			1391	(1261–1535)			753	(683–829)	
Class I (A-C-B)	*24-*03-*15	0.015	838	(443–1587)	0.04	0.008	1029	(435–2434)	0.398
	*02-*04-*35	0.034	1801	(1154–2811)	0.08	0.014	977	(518–1840)	0.352
	*02-*03-*40	0.035	964	(611–1523)	0.09	0.036	597	(402–888)	0.299
	*29-*16-*44	0.019	899	(508–1592)	0.08	0.026	526	(335–828)	0.134
	*02-*07-*08	0.013	1701	(829–3491)	0.78	0.017	1182	(684–2042)	0.094
Class II (DRB1-DQB1-DPB1)									
	*07-*02-*02	0.013	716	(373–1374)	0.05	0.010	522	(227–1201)	0.426
	*15/16-*06-*03	0.014	2259	(1054–4841)	0.09	0.011	298	(127–670)	0.067
	*08-*04-*04	-	-	-	-	0.018	392	(231–666)	0.018
	*07-*03-*04	0.012	492	(252–962)	0.001	0.017	561	(302–1040)	0.351
	*15/16-*06-*04	0.084	1888	(1366–2610)	0.02	0.108	838	(652–1077)	0.133
	*11-*05-*03	-	-	-	-	0.017	1329	(743–2379)	0.082
	*13-*05-*03	-	-	-	-	0.007	1455	(625–3386)	0.071
*15-*01-*06	-	-	-	-	0.011	372	(174–797)	0.041	
*15-*01-*06	-	-	-	-	0.111	899	(735–1098)	0.078	

Only haplotypes statistically or marginally significant ($p \leq 0.10$) and relatively common (estimated frequencies ≥ 0.1) in cohorts 1 and 2 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 or marginally significant findings ($p \leq 0.10$) are bolded.