MAJOR ARTICLE

Polymorphisms in HLA-DPB1 Are Associated With Differences in Rubella Virus–Specific Humoral Immunity After Vaccination

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Vaccination with live attenuated rubella virus induces a strong immune response in most individuals. However, small numbers of subjects never reach or maintain protective antibody levels, and there is a high degree of variability in immune response. We have previously described genetic polymorphisms in HLA and other candidate genes that are associated with interindividual differences in humoral immunity to rubella virus. To expand our previous work, we performed a genome-wide association study (GWAS) to discover single-nucleotide polymorphisms (SNPs) associated with rubella virus–specific neutralizing antibodies. We identified rs2064479 in the HLA-DPB1 genetic region as being significantly associated with humoral immune response variations after rubella vaccination ($P = 8.62 \times 10^{-8}$). All other significant SNPs in this GWAS were located near the HLA-DPB1 gene ($P \leq 1 \times 10^{-7}$). These findings demonstrate that polymorphisms in HLA-DPB1 are strongly associated with interindividual differences in neutralizing antibody levels to rubella vaccination and represent a validation of our previous HLA work.

Keywords. genome-wide association study; polymorphism; genetic; humoral; neutralizing antibody; immunity; measles-mumps-rubella vaccine.

Rubella is usually mild, unless it occurs during pregnancy and infection spreads to the fetus. During the first trimester, up to 90% of cases of rubella virus infection can lead to fetal defects, including stillbirth [1]. Newborn infants diagnosed with congenital rubella syndrome can present with multiple ophthalmic, auditory, cardiac, and craniofacial defects [2]. On average, there are 100 000 worldwide cases of congenital rubella syndrome reported annually [3]. Humans are the only known host for rubella virus, making the disease a logical target for global eradication. However, incomplete vaccination strategies have led to recent outbreaks in Poland, Romania, and Japan [4–6]. These outbreaks are concerning because of the potential risk of subsequent exposure to mother and fetus.

The rubella virus vaccine strain currently licensed in the United States is RA 27/3. It is administered in a 2-dose series as a component of the measles-mumps-rubella (MMR II) vaccine. Seroprotective levels are as high as 98% after the second dose [7,8]. Protective levels of humoral immunity are observed 20 years after vaccination [9]. Although vaccination may lead to lifetime protection, there is evidence of waning immunity, and we have previously reported a broad spectrum of interindividual differences in humoral responses to rubella vaccination, including subjects with antibody responses below the protective threshold [10–13].

Our laboratory has focused on explaining the genetic contributions to variations in rubella vaccine–induced immunity [14]. We have demonstrated that HLA genes play an important role in immune responses to rubella vaccine, accounting for up to 20% of the overall genetic variation observed in rubella virus–specific antibody levels [15]. In regard to humoral immunity, we have identified associations between HLA class I

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and II alleles, as well as polymorphisms in *TNFA/TNFRSF1B*, *IL2B*, *RARB*, *DDX58*, *TRIM5*, *TRIM22*, *IRF9*, and *OAS2* [15–18], with interindividual differences in response to rubella vaccination. The biological relevance of the HLA-DPB1 locus for immune response to rubella vaccination is not well understood. We have reported several HLA allelic (DPB1*0401) and haplotypic (DRPB1*04-DQB1*03-DPB1*03 and DRB1*15/16-DQB1*06-DPB1*03) associations with rubella vaccine–induced antibodies that were verified in separate study cohorts [15]. We also demonstrated that HLA-DPB1 (*0401) homozygosity was significantly associated with rubella virus antibody levels [19]. Here, we extend our previous work and report the first genome-wide association study (GWAS) in children and younger adults who received live rubella virus vaccine. We identified a significant association between rs2064479 in the HLA-DPB1 gene and the levels of neutralizing antibody response. This work validates the growing database that demonstrates differences in responses to vaccination and viral infection associated with genetic polymorphisms in this HLA class II locus.

METHODS

Study Participants

The study cohort was a large population-based sample of 1174 healthy children and younger adults (age, 11–22 years) from all socioeconomic strata in Rochester, Minnesota. The total cohort consists of 3 separate recruitment efforts, and detailed descriptions of these cohorts have been published elsewhere [18, 20–24]. For 1101 children, a parent agreed to let their child join the current rubella vaccine study, and from these children we obtained a blood sample. All 1101 participants had written records of having received 2 doses of MMR II vaccine (Merck). The Institutional Review Board of the Mayo Clinic approved the study. Written informed consent was obtained from each adult subject and from the parents of all children who participated in the study.

Rubella Virus-Specific Neutralizing Antibodies

The description for assaying the levels of neutralizing antibodies against live rubella virus is nearly identical to that in our previous report [11]. Briefly, serial dilutions of subjects' sera were incubated with the rubella vaccine virus strain HPV77. After incubation, the virus/sera mixture was added to Vero cells cultured in a flat-bottomed 96-well plate and incubated for 72 hours at 37°C in 5% CO_2. Cells were then fixed in cold methanol for 10 minutes, blocked with phosphate-buffered saline (PBS) supplemented with 5% skim milk (Difco; BD, New Jersey) and 0.1% Tween 20 for 30 minutes, and washed 3 times with PBS supplemented with 0.05% Tween 20 (PBS-T). Fixed cells were incubated with anti-E1 glycoprotein (Centers for Disease Control and Prevention, Atlanta, Georgia) for 30 minutes and washed 3 times with PBS-T. The secondary goat anti-mouse horseradish peroxidase–conjugated antibody (Invitrogen; Carlsbad, California) was added for 30 minutes. Plates were washed again, and antibody conjugate was visualized by adding NeA-Blue TMB substrate (Clinical Science Products; Mansfield, Massachusetts) for 10 minutes. The ODs were read at 450 nm/630 nm on an Eon microplate spectrophotometer (BioTek; Winooski, Vermont). The Loess method of statistical interpolation was used to estimate the median infectious dose from observed values [25]. The intraclass correlation coefficient for rubella virus–specific neutralizing antibody (NT_{50}) measurements was 0.89.

GWAS

The genome-wide SNP typing method used for this study is essentially identical to that used in our previously published reports [26–28]. Briefly, DNA was extracted from each subject's blood specimen, using the Gentra Puregene Blood kit (Gentra Systems; Minneapolis, Minnesota) and quantified by Picogreen (Molecular Probes; Carlsbad, California). The genome-wide SNP typing was performed using the Infinium Omni 1 M-Quad SNP array (Illumina; San Diego, California). DNA samples underwent amplification, fragmentation, and hybridization onto each BeadChip, which were imaged on an Illumina BeadArray reader. Genotype calls based on clustering of the raw intensity data were made using BeadStudio 2 software. The resulting genotype data on SNPs were exported into SAS for analysis. Quality-control checks included genotyping reproducibility, sex checks, cryptic relatedness to identify similar/identical subjects, removal of SNPs when typing failed in samples for

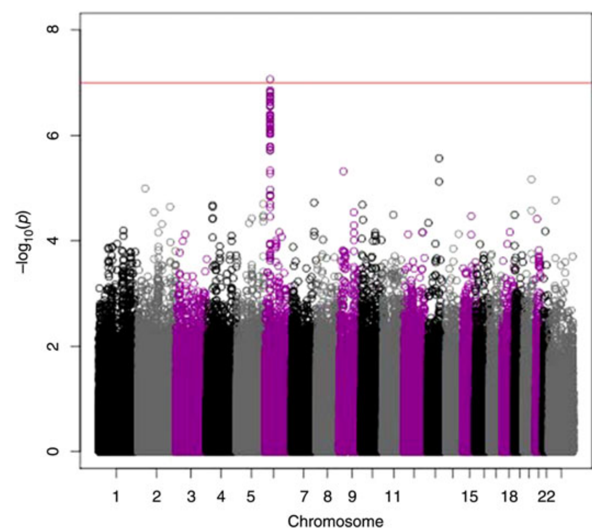

Figure 1. Genome-wide Manhattan plot of significant single-nucleotide polymorphisms (SNPs) associated with a neutralizing antibody response to rubella virus identified a majority of hits in chromosome 6, including rs2064479 ($P = 8.62 \times 10^{-8}$). The horizontal line near the top of the plot marks P values of $<1 \times 10^{7}$. The functional location of this SNP maps to the HLA-DPB1 gene.

Table 1. Single-Nucleotide Polymorphisms (SNPs) Associated With Variations in Neutralizing Antibody Response to Rubella Virus

SNP ID	Chr	Gene[a]	Location[b]	Genotype	No.[c]	Interpolated NT_{50}, Median (IQR)	*P* Value[d]
rs2064479	6	HLA-DPB1	Intergenic	GG	485	61.3 (37.7–100.7)	8.62×10^{-8}
				GA	336	51.5 (31.15–79.6)	
				AA	66	43.3 (32.6–77.5)	
rs3128925	6	HLA-DPB1	Intergenic	CC	519	60.4 (37.7–99.1)	1.39×10^{-7}
				CA	317	51.0 (31.2–80.3)	
				AA	51	40.1 (32.6–75.1)	
rs9277359	6	HLA-DPB1	Intronic	CC	444	60.8 (39.5–100.6)	1.48×0^{-7}
				CA	364	51.8 (31.4–82.8)	
				AA	80	43.3 (31.6–79.6)	
rs2064478	6	HLA-DPB1	Intergenic	GG	519	60.4 (37.7–99.1)	1.52×10^{-7}
				GA	318	51.0 (31.2–80.4)	
				AA	51	40.1 (32.6–75.1)	
rs3117230	6	HLA-DPB1	Intergenic	AA	519	60.4 (37.7–99.1)	1.52×10^{-7}
				AG	318	51.0 (31.2–80.4)	
				GG	51	40.1 (32.6–75.1)	
rs3117233	6	HLA-DPB1	Intergenic	GG	519	60.4 (37.7–99.1)	1.52×10^{-7}
				GA	318	51.0 (31.2–80.4)	
				AA	51	40.1 (32.6–75.1)	
rs3117239	6	HLA-DPB1	Intergenic	GG	519	60.4 (37.7–99.1)	1.52×10^{-7}
				GA	318	51.0 (31.2–80.4)	
				AA	51	40.1 (32.6–75.1)	
rs9277357	6	HLA-DPB1	Intronic	AA	443	60.8 (39.5–100.6)	1.56×10^{-7}
				AG	365	51.5 (31.5–83.8)	
				GG	80	40.9 (30.15–75.3)	
rs3117240	6	HLA-DPB1	Intergenic	AA	519	60.4 (37.7–99.1)	1.77×10^{-7}
				AG	317	51.0 (31.2–80.4)	
				GG	51	40.1 (32.6–75.1)	
rs2064473	6	HLA-DPB1	Intergenic	GG	602	59.5 (36.7–98.0)	1.86×10^{-7}
				GA	251	50.8 (30.9–75.3)	
				AA	35	39.4 (31.0–71.0)	
rs3117211	6	HLA-DPB1	Intergenic	CC	602	59.5 (36.7–98.0)	1.86×10^{-7}
				CA	251	50.8 (30.9–75.3)	
				AA	35	39.4 (31.0–71.0)	
rs3117218	6	HLA-DPB1	Intergenic	GG	602	59.5 (36.7–98.0)	1.86×10^{-7}
				GA	251	50.8 (30.9–75.3)	
				AA	35	39.4 (31.0–71.0)	
rs3130189	6	HLA-DPB1	Intergenic	AA	602	59.5 (36.7–98.0)	2.06×10^{-7}
				AG	250	50.7 (30.9–75.5)	
				GG	36	39.4 (31.5–69.0)	
rs2281389	6	HLA-DPB1	Intergenic	AA	603	59.5 (36.6–98.0)	2.29×10^{-7}
				AG	250	50.8 (31.1–75.5)	
				GG	35	39.4 (31.0–71.0)	
rs3128965	6	HLA-DPB1	Intergenic	GG	603	59.5 (36.6–98.0)	2.29×10^{-7}
				GA	250	50.8 (31.1–75.5)	
				AA	35	39.4 (31.0–71.0)	
rs3128966	6	HLA-DPB1	Intergenic	GG	603	59.5 (36.6–98.0)	2.29×10^{-7}
				GA	250	50.8 (31.1–75.5)	
				AA	35	39.4 (31.0–71.0)	
rs9277379	6	HLA-DPB1	Intronic	AA	603	59.5 (36.6–98.0)	2.29×10^{-7}
				AC	250	50.8 (31.1–75.5)	
				CC	35	39.4 (31.0–71.0)	

Table 1 continued.

SNP ID	Chr	Gene[a]	Location[b]	Genotype	No.[c]	Interpolated NT_{50}, Median (IQR)	P Value[d]
rs9277381	6	HLA-DPB1	Intronic	GG	603	59.5 (36.6–98.0)	2.29×10^{-7}
				GA	250	50.8 (31.1–75.5)	
				AA	35	39.4 (31.0–71.0)	
rs9277384	6	HLA-DPB1	Intronic	CC	603	59.5 (36.6–98.0)	2.29×10^{-7}
				CG	250	50.8 (31.1–75.5)	
				GG	35	39.4 (31.0–71.0)	
rs3097650	6	HLA-DPB1	Intergenic	GG	444	60.7 (38.9–100.6)	2.69×10^{-7}
				GA	364	52.3 (31.5–82.8)	
				AA	80	43.3 (31.6–79.6)	
rs3128961	6	HLA-DPB1	Intronic	GG	444	60.7 (38.9–100.6)	2.69×10^{-7}
				GA	364	52.3 (31.5–82.8)	
				AA	80	43.3 (31.6–79.6)	
rs9277386	6	HLA-DPB1	Intronic	AA	444	60.7 (38.9–100.6)	2.69×10^{-7}
				AG	364	52.3 (31.5–82.8)	
				GG	80	43.3 (31.6–79.6)	
rs9277378	6	HLA-DPB1	Intronic	AA	444	60.7 (38.9–100.6)	2.72×10^{-7}
				AG	363	52.1 (31.5–82.4)	
				GG	80	43.3 (31.6–79.6)	
rs1042544	6	HLA-DPB1	UTR	AA	432	61.1 (38.4–101.1)	2.76×10^{-7}
				AG	374	52.6 (31.6–83.8)	
				GG	82	43.3 (31.0–77.5)	
rs3130210	6	HLA-DPB1	Intergenic	CC	519	60.4 (38.4–101.1)	2.82×10^{-7}
				CA	318	51.0 (31.2–80.4)	
				AA	50	42.7 (33.4–75.2)	

Abbreviations: Chr, chromosome; ID, identification number; IQR, interquartile range; NT_{50}, neutralizing titer; UTR, untranslated region.

[a] Gene or genetic region.

[b] Predicted function.

[c] Number of subjects for each genotype.

[d] Adjusted for demographic and clinical variables, as well as for inflation of significance, as described in "Methods" section. All identified SNPs with $P \leq 2.64 \times 10^{-6}$ were associated with the HLA-DPB1 gene. The top 25 SNPs are listed above ($P \leq 2.82 \times 10^{-7}$).

>1% of subjects, removal of subjects when typing failed for >1% of SNPs, elimination of monomorphic SNPs, removal of duplicate samples, and a Hardy–Weinberg Equilibrium check (SNPs with $P < 1 \times 10^{-7}$ were flagged as having poor genotyping quality). We assessed population substructure by means of the principal components approach implemented in EIGENSTRAT, using SNPs spanning the genome that were in low linkage disequilibrium (LD; defined as an r^2 value of < 0.1) and had HWE *P* values of $>1 \times 10^{-3}$ [29]. We removed all subjects whose genetic background was farther than 15% of the way between the predominant White cluster and the other genetic clusters identified by the first two principal axes of genetic admixture.

Statistical Analysis

Demographic and vaccination history data were summarized for all study participants, using counts and percentages for categorical features and medians and interquartile ranges for quantitative features, including neutralizing antibody levels. Linear regression analyses were performed for each SNP to assess its association with neutralizing antibody levels. In these regression analyses, the primary test of significance evaluated an ordinal association between the genotypes of each SNP and log-transformed neutralizing antibody levels, with adjustment for sex, ages at enrollment and at vaccination, time between immunization and blood sample collection, assay batch, and population stratification eigenvectors. We assessed the degree to which the significance levels were inflated due to unmeasured confounding and adjusted our level of significance according to the estimated inflation factor [30]. For SNPs most strongly associated with neutralizing antibody levels, we summarized the genotype counts and the per-genotype medians and interquartile ranges of the interpolated NT_{50} values and derived a LocusZoom of the *P* values in the genomic region harboring the primary genetic signal [31].

RESULTS

The cohort consisted of 1052 subjects with appropriate data. As the participants were predominantly White (85.3%), all analyses

focused on the 897 subjects who were members of this racial group. From this group, we obtained neutralizing antibody and genotype information on a sample size of 897. The sex distribution was 490 males (54.6%) and 407 females (45.4%). The median age at enrollment was 15.0 years (interquartile range [IQR], 13.0–17.0 years), the median age at first vaccination was 15.0 months (IQR, 15.0–16.0 months), the median age at second vaccination was 10.0 years (IQR, 5.0–12.0 years), and median time since the last vaccination was 6.4 years (IQR, 4.6–8.5 years). The median interpolated NT_{50} was 55.4 (IQR, 34.4–91.3).

Genotyping assays performed using the Omni 1 M were attempted for 1063 potential participants. Only 10 (0.94%) of these attempted samples had SNP call rates of <99%. After removing these subjects and other subjects with inconsistent data on sex (3) or other characteristics (8), a total of 1052 potential subjects were available for analysis. Of the 934 149 candidate SNPs with call rates of >99%, 793 644 had minor allele frequencies of >1% and were included in the primary analysis. A total of 5656 of these SNPs were flagged as having HWE P values of $<1 \times 10^{-7}$. Of these SNPs, there were 70 742 with HWE P values of $> 1 \times 10^{-3}$ and with pairwise r^2 values of < 0.1 that were used in the assessment of population stratification. After removing subjects with >15% genetic admixture beyond the primary White genetic cluster, 897 subjects remained for analysis. Quality control measures were repeated on this subset, leaving 752 869 SNPs with minor allele frequencies of >1% for analysis. Three race-specific eigenvectors were obtained to control for residual genetic differences within this White subset.

For this study, we set the threshold of significance at $P \leq 1 \times 10^{-7}$ [32]. Our GWAS revealed that the most significant SNP (rs2064479) associated with variations in rubella virus–specific neutralizing antibody response was located in chromosome 6 (Figure 1), within a cluster of neighboring SNPs that approached significance. Table 1 lists the characteristics of all significant SNPs with P values of $\leq 2.82 \times 10^{-7}$. There were 118 SNPs within the HLA-DPB1 genetic region with P values of $\leq 1 \times 10^{-5}$. To obtain a graphical illustration of all SNPs in LD with rs2064479, we generated a LocusZoom plot representing the genetic region on 6p21.3, showing LD structure, SNP-associated P values, and the recombination rate (Figure 2). There is an obvious clustering of associated SNPs around the HLA-DPB1 gene. There were 81 SNPs in high LD ($r^2 \leq 0.8$) with rs2064479. Of these, 28 were located in the 3' untranslated region, downstream (hereafter, the "3' UTR/downstream region"), of the HLA-DPB1 gene (data not shown).

To determine whether SNPs located in the 3' UTR/downstream region of the HLA-DPB1 gene that are in high LD with rs2064479 may be located in microRNA (miRNA) binding

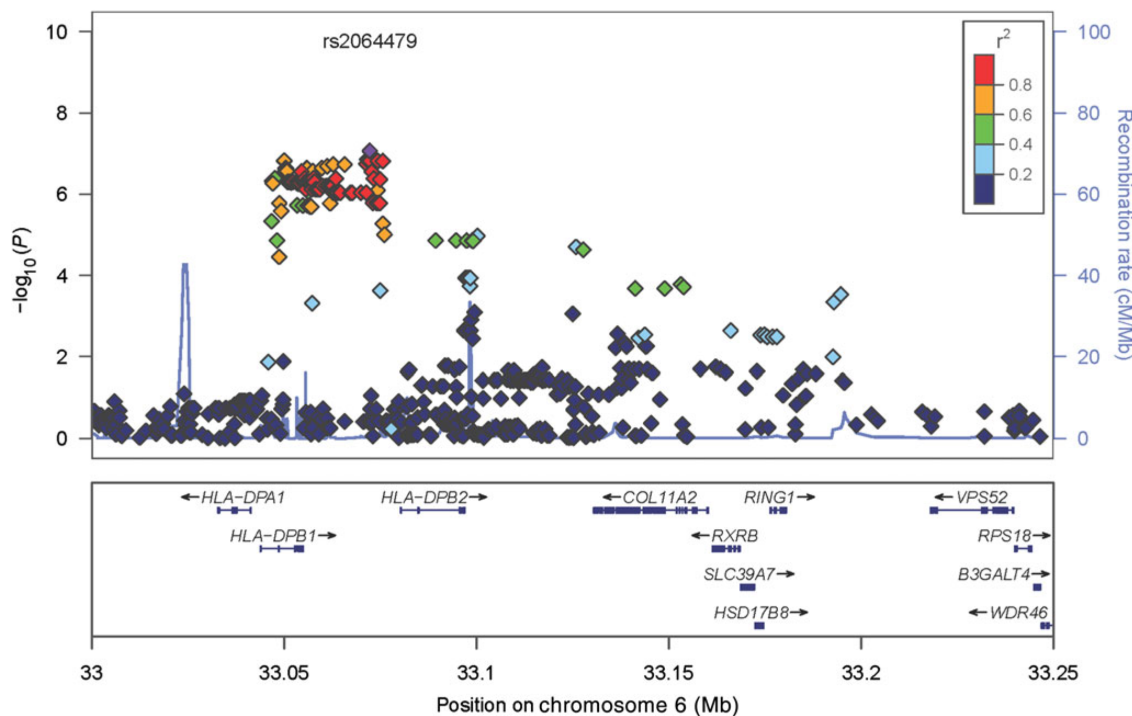

Figure 2. LocusZoom plot of single-nucleotide polymorphisms (SNPs) in high linkage disequilibrium (LD) with rs2064479. The LocusZoom plot allows for a visual depiction of the high-level LD of multiple SNPs with index SNP rs2064479 and the clustering of these SNPs near HLA-DPB1. A total of 82 SNPs were within an r^2 value of $\geq$0.8. Of those SNPs in high LD, 28 had a predicted function in the 3′ untranslated region, downstream, of HLA-DPB1.

sites, we queried the PolymiRTS (Polymorphism in microRNAs and their TargetSites) database [33]. Ten SNPs were identified as potentially influencing miRNA binding within the 3' UTR/downstream region of the HLA-DPB1 gene (Table 2).

We have previously characterized genome-wide transcription patterns between individuals with low and high antibody responses to rubella vaccine [34]. In the current study, we did not find any associations between differential gene expression and GWAS findings. This may be due to the difference in the antibody measure used to characterize these cohorts. Haralambieva et al used a rubella virus–specific total immunoglobulin G assay, while we used the levels of neutralizing antibodies for this association study. Also, the genome-wide transcriptional pattern work was also performed in a rather small cohort (n = 30). This may not have been a large enough cohort to detect the HLA associations.

DISCUSSION

To our knowledge, this is the first GWAS to investigate genetic influences on interindividual variations in rubella virus–specific neutralizing antibody response after vaccination. In a cohort of vaccinated individuals, we identified rs2064479, an intergenic SNP in the HLA-DPB1 genetic region, as having genome-wide significance in association with antibody levels. Interestingly, all SNPs that were significant ($P \leq 1 \times 10^{-7}$) were located near the HLA-DPB1 gene. Further analyses revealed 82 SNPs in high LD ($r^2 \geq 0.8$) with rs2064479. Twenty-eight of these SNPs were located in the 3' UTR/downstream region of the HLA-DPB1 gene, and 10 of those may be involved in miRNA binding sites.

The highly polymorphic nature of the HLA genes and their essential role in host response and antigen-specific adaptive immunity help to explain the large amount of data linking specific HLA variants to differences in susceptibility to infection and vaccine response. Specific variants in the HLA-DPB1 gene have been associated with clinical outcomes in hepatitis B virus (HBV) and hepatitis C virus infections and in vaccine responses against hepatitis B virus, rubella virus, measles virus, influenza virus, and malarial parasites [21, 35–41].

Although our most significant HLA-DPB1 finding (rs2064479) has yet to be identified as a causal variant with functional consequences in other genetic studies, it is in high LD ($r^2 = 0.82$) with rs9277534, which has a predicted function in the 3' UTR of HLA-DPB1. The 496 GG genotype of the latter SNP is associated with susceptibility to HBV persistence and an increase in HLA-DP protein and gene expression [36]. The authors hypothesized in that study that susceptibility to HBV persistence is due to high levels of HLA-DPB1 expression and not to differences in peptide repertoire. Our data revealed that the GG genotype of rs9277534 (minor allele) is associated with an allele dose-related decrease in rubella virus neutralizing antibodies, compared with the AA genotype (AA = 60.83, and GG = 43.35; $P = 5.01 \times 10^{-7}$). The mechanism behind the

Table 2. High–Linkage Disequilibrium (LD) Single-Nucleotide Polymorphisms (SNPs) in Potential microRNA (miRNA) Binding Sites of HLA-DPB1

SNP ID	r^{2a}	miRNA ID	Context + Score Change[b]
rs3117228	0.822797	hsa-miR-16-2-3p	0.22
		hsa-miR-195-3p	0.21
rs9277533	0.822797	hsa-miR-3607-5p	−0.077
		hsa-miR-568	−0.004
		hsa-miR-6516-3p	−0.118
rs9277538	0.822797	hsa-miR-432-3p	−0.087
rs9277539	0.926541	hsa-miR-3165	−0.109
		hsa-miR-8071	−0.157
rs9277542	0.822797	hsa-miR-6730-5p	0.024
rs9277547	0.822797	hsa-miR-4493	−0.035
		hsa-miR-4499	0.006
		hsa-miR-605-3p	−0.121
		hsa-miR-6126	−0.094
		hsa-miR-873-3p	−0.037
		hsa-miR-124-3p	−0.005
		hsa-miR-3714	0.009
		hsa-miR-3910	−0.055
		hsa-miR-506-3p	−0.012
rs9277549	0.822797	hsa-miR-552-3p	0.067
		hsa-miR-764	0.023
		hsa-miR-6789-3p	−0.183
rs9277550	0.822797	hsa-miR-6077	0.032
		hsa-miR-7-5p	0.005
		hsa-miR-1185-5p	−0.066
		hsa-miR-3679-5p	−0.066
		hsa-miR-4534	0.046
		hsa-miR-4802-5p	−0.028
		hsa-miR-6894-5p	−0.111
		hsa-miR-7154-3p	−0.043
		hsa-miR-765	−0.026
		hsa-miR-766-5p	−0.032
		hsa-miR-8082	0.028
rs9277554	0.822797	hsa-miR-1271-3p	No change
		hsa-miR-4297	0.03
		hsa-miR-550a-3-5p	0.003
		hsa-miR-550a-5p	0.003
		hsa-miR-550b-2-5p	−0.02
		hsa-miR-5581-5p	0.027
		hsa-miR-4724-3p	0.032
rs9277555	0.964201	hsa-miR-345-5p	−0.033

The PolymiRTS (Polymorphism in microRNAs and their TargetSites) database allows a query of SNPs in potential miRNA seed and binding sites.

Abbreviation: ID, identification number.

[a] Correlation value between 2 loci.

[b] More-negative values indicate that a given SNP has an increased likelihood of disrupting miRNA binding. Ten SNPs in high LD with rs2064479 ($r^2 > 0.8$) may influence miRNA binding sites in the 3′ untranslated region of HLA-DPB1.

significant differences in antibody levels observed in association with rs2064479 remains unclear. The next step is to perform additional fine-mapping panels to identify the true causal variant. SNPs in the 3' UTR/downstream region of the HLA C gene may interfere with the ability of regulatory miRNAs to bind and are associated with control of human immunodeficiency virus [29]. The multiple SNPs we identified in predicted miRNA binding targets may influence rubella vaccine–induced humoral immunity through a similar mechanism.

The strength of our study is the use of a well-characterized viral vaccine. Rubella virus contains a stable genome with very similar immune responses across strains [42, 43]. We also chose this well-defined study cohort because of the written documentation of having received 2 doses of MMR II vaccine and a lack of circulating virus in Rochester. These data allow us to assume that the measured differences in antibody response against rubella virus are from vaccination and not from infection.

We acknowledge that the cohort might be considered small for a GWAS. However, recruitment and vaccine administration in a larger cohort is not economically or logistically feasible. The overwhelming number of SNPs that displayed a trend toward significance associated with regions near HLA-DPB1, and the potential of certain SNPs to influence miRNA binding sites, increases our confidence that this is a valid finding. If fine-mapping analyses and a proposed validation GWAS identify the causal variant as associated with HLA-DPB1, then functional studies will be designed to measure differences in HLA-DP protein and gene expression in variants. These data will contribute immensely to studies aimed at elucidating genetic variants associated with differences in immunity to live viral vaccines.

Notes

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This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

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Potential conflicts of interest. G. A. P. is the chair of a safety evaluation committee for novel investigational vaccine trials being conducted by Merck Research Laboratories and offers consultative advice on vaccine development to Merck, CSL Biotherapies, Avianax, Sanofi Pasteur, Dynavax, Novartis Vaccines and Therapeutics, PAXVAX, Emergent Biosolutions, Adjuvance, and Vaxness. G. A. P. and I. G. O. hold 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. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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