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Genetic variants within the MHC region are associated with immune responsiveness to childhood vaccinations☆

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Abstract

The influence of genetic variability within the major histocompatibility complex (MHC) region on variations in immune responses to childhood vaccination was investigated. The study group consisted of 135 healthy infants who had been immunized with hepatitis B (HBV), 7-valent pneumococcal conjugate (PCV7), and diphtheria, tetanus, acellular pertussis (DTaP) vaccines according to standard childhood immunization schedules. Genotype analysis was performed on genomic DNA using Illumina Goldengate MHC panels (Mapping and Exon Centric). At the 1 year post vaccination check-up total, isotypic, and antigen-specific serum antibody levels were measured using multiplex immunoassays. A number of single nucleotide polymorphisms (SNPs) within MHC Class I and II genes were found to be associated with variations in the vaccine specific antibody responses and serum levels of immunoglobulins (IgG, IgM) and IgG isotypes (IgG1, IgG4) (all at $p < 0.001$). Linkage disequilibrium patterns and functional annotations showed that significant SNPs were strongly correlated with other functional regulatory SNPs. These SNPs were found to regulate the expression of a group of genes involved in antigen processing and

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presentation including HLA-A, HLA-C, HLA-G, HLA-H, HLA-DRA, HLA-DRB1, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOB, and TAP-2. The results suggest that genetic variations within particular MHC genes can influence immune response to common childhood vaccinations, which in turn may influence vaccine efficacy.

Keywords

Major histocompatibility complex; Genetic polymorphism; Childhood vaccine; Immune response

1. Introduction

Even with uniform administration schemes, large inter-individual variability exists in vaccine responsiveness among vaccine recipients. For example, 5–20% and 2–10% of healthy individuals experience either hypo- or non-responsiveness to hepatitis B (HBV) or measles vaccination, respectively [1–4]. A strong genetic component has been demonstrated in the regulation of immune responses to the vaccines. A number of polymorphisms have been reported to be associated with vaccine responsiveness including variants of the major histocompatibility complex (MHC) [5–7] and cytokine and cytokine receptor genes [8–11].

The MHC spans ~4Mb and comprises over 180 protein-coding genes, many of which determine immune function, susceptibility to complex diseases, and transplant rejection. MHC class II molecules are involved in the presentation of MHC-peptide complexes on the surface of antigen presenting cells to CD4+ T cells. These molecules are highly polymorphic and this diversity helps determine immune recognition. Along with the HLA genes, several functionally important genes are located in this region including those that code for complement proteins C4, C2 and Factor B, the cytokines tumor necrosis factor α and β and TAP (antigen peptide transporter) that function in antigen processing [12,13].

The contribution of the MHC variants to the vaccine immune response was first observed by a significant excess of HLA-DR7 and a total absence of HLA-DR1 in individuals that failed to respond to hepatitis B vaccine [14]. Subsequent studies revealed associations between certain HLA class II (HLA-DR, HLA-DQ) alleles and poor or non-immune response to HBV [6,9,15–19]. In addition, poor responsiveness to hepatitis B vaccine was associated with extended MHC haplotypes such as B8-DR3-SC01, B44-DR7-FC31 and B18-DRB1*0301-DQB1*0201 [6,15,18–20]. Several studies have also demonstrated the influence of HLA allelic variation on immune response to measles, mumps, rubella, and influenza vaccines [21–24]. For example, HLA class I B*8, B*13, and B*44 alleles were associated with IgG seronegativity after a single dose of measles vaccine whereas the A*29-C*16-B*44 haplotype was associated with low IgG antibody levels after two doses of the same vaccine [21,25]. The DPA1*0201 and DPB1*0401 alleles were associated with low and high levels of rubella-induced antibodies in two separate cohorts, respectively [26]. Furthermore, the DRB1*04-DQB1*03-DPB1*03 and DRB1*15/16-DQB1*06-DPB1*03 haplotypes were associated with low levels of rubella-specific antibodies [23].

Although the HLA complex is one of the most extensively studied regions in the human genome, the other genes in the MHC region have not yet been well investigated with regard

to vaccine responsiveness. In the present study, a focused approach has been taken to examine the association of SNPs within the MHC region with variation in childhood vaccine responses.

2. Materials and methods

2.1. Study population and vaccinations

Study procedures were approved by the Institutional Review Boards of all participating institutions. The subjects were infants seen in two University-affiliated general pediatrics clinics for routine 1 year old checkup examinations. These clinics routinely obtained blood by finger stick during the 1 year checkup to screen for anemia and lead poisoning. If parents gave informed consent, additional tubes of blood for genomic DNA were obtained. In addition, immunization records were reviewed to document history of immunization with HBV (Recombivax®, Merck&Co., Inc., White-house Station, NJ); DTaP (Daptacel®, Sanofi Pasteur, Ontario, CA); heptavalent pneumococcal conjugate vaccine (PCV7-serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) (Prevnar®, Wyeth, Philadelphia, PA); inactivated polio vaccine (IPV); and Haemophilus influenza type b (Hib) conjugate vaccine in accordance with then-current guidelines for childhood immunization [27]. A total of 135 healthy infants, aged 11.5–14 months of age (mean: 12.6 months), were recruited into the study. The majority of children were non-Hispanic whites (121) and male (77). The demographics and immunological variables of the participants that were included in the analysis are given in Table 1.

2.2. Genotyping

Genomic DNA was extracted from whole blood samples using the QIAamp blood kit (QIAGEN Inc., Chatsworth, CA). Genotyping was performed according to the standard protocol provided by Illumina using the MHC Panel Set and Golden Gate protocol (Illumina Inc., San Diego, CA). The MHC SNP set consisted of two oligonucleotide pools, MHC Mapping Panel and MHC Exon-Centric Panel for 1228 and 1293 SNP loci, respectively. Both panels cover 2360 independent loci spaced at an average of 2.08 kb (range: 0.005–71.05 kb). Genotyping was performed in a 16-well format using universal BeadChips. A total of 250 ng to 1 μg DNA was used for each assay depending on the source. Genotypes were auto called using GenomeStudio software (Illumina, Inc., San Diego, CA).

2.3. Microsphere coupling

Pneumococcal polysaccharides (PnPS) were obtained from ATCC, Manassas, VA. Pneumococcal cell wall polysaccharide (CPS) was obtained from Staten Serum Institute (Copenhagen, Denmark). Diphtheria and tetanus toxoids were obtained from University of Massachusetts Biologics Laboratories, Jamaica Plain, MA. The PnPSs were conjugated to spectrally distinguishable microspheres (Luminex, Austin, TX) using 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium [28]. Diphtheria and tetanus toxoids were conjugated to spectrally distinguishable microspheres using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and sulfo-N-hydroxysuccinimide [28].

2.4. Serum collection and analyses

Blood samples were collected at approximately 1 year of age (mean: 12.6 months). Serum was isolated and stored at −20 °C until analysis. Vaccine-specific antibody responses to PnPSs, diphtheria and tetanus toxoids; and total serum immunoglobulin levels (IgM, IgA, IgG and IgG subclasses) were measured by multiplex assay as previously described [29,30]. Briefly, microspheres coupled to PnPSs, diphtheria and tetanus toxoids were mixed and added to standards and samples diluted in PBS containing 1%BSA, 0.05%Tween 20, 10 μg/ml of CPS, and 100 μg/ml of PnPS 22. Measurement and data analysis were performed using the Bioplex multiplex testing platform (BioRad, Hercules, CA). Assays were performed in duplicate. Serum concentrations of IgG, IgA, IgM, and IgG subclasses were measured in duplicate using Beadlyte® assay according to the manufacturer's instructions (Upstate, Lake Placid, NY).

Levels of specific serum antibody to HBsAg were determined using a commercially-available enzyme immunoassay according to the manufacturer's instructions (ETI-AB-AUK PLUS, DiaSorin Inc., Stillwater, MN). All serum measurements were above the minimum detection limit of the assay.

2.5. Statistical analyses

SNP-specific deviations from the Hardy-Weinberg Equilibrium were tested using chi-squared goodness-of-fit tests. Antibody levels were transformed to their log values (base 2) before analysis to fit the normality assumptions. These variables were included in the analysis first as continuous variables then they were turned into binary variables at thresholds of 10% and 15% to examine the trend in data.

The genotype confidence score of the assay was set to 0.25 in GenomeStudio Genotyping module. Alleles that were not called in a sample were coded as missing in the analysis. For missing rates per individual and per SNP, a threshold of 2% was used. Datasets from exon-centric and mapping panels were merged using PLINK [31]. This dataset contained 1856 SNPs for 135 subjects including 77 males and 58 females. The initial two datasets had 111 markers in common and the concordance rate of these markers was 0.999. Initial datasets had 124 subjects in common thus, 11 subjects had set of markers either from exon-centric or mapping panels. The total genotype rate for the merged dataset was 0.96. Replicate sample comparisons within and across DNA genotyping plates also demonstrated high agreement (data not shown).

Statistical analysis was performed using PLINK version 1.07 [31]. Linear and logistic regression models, with adjustments for gender, age and ethnicity, were used to test for differences between antibody levels (as continuous and binary data) according to genotypes. Associations were tested individually and based on principal components determined from combinations of antibody levels. Linkage disequilibrium (LD) and haplotype blocks were assessed using default parameters in Haploview [32]. Pairwise LD was calculated only for SNPs within 200 kb. SNAP was used to find proxy SNPs within 500 kb based on LD and physical distance [33]. RegulomeDB was used to annotate SNPs with known and predicted regulatory elements [34].

3. Results

All analyses were conducted on both the quantitative antibody phenotype and the dichotomized serotype status (lowest 10% and 15%). Since discretization could result in arbitrary cut-off levels, the focus of this report will be on the results with the quantitative phenotypes. However, the results for both analyses were consistent with each other (overlaps with the binary analysis are marked in Tables 2 and 3).

3.1. Association between vaccine specific antibody responses and SNPs

1856 SNPs in 154 genes were studied for their influence on vaccine induced serum antibody levels. All genotype frequencies were in Hardy Weinberg Equilibrium. After adjusting for gender, age and ethnicity, several SNPs were significantly associated with vaccine specific antibody responses. Table 2 summarizes these associations and provides *p* values calculated in an additive manner. The RPP21 (rs3129820 and rs6936217), ZBTB12 (rs558702), BF (rs1270942), STK19 (rs389884), TNXB (rs1150758, 1150753), CREBL1 (rs1269852), NOTCH4 (rs3134942 and rs3131296), BTNL2 (rs3129950), HLA-DRA (rs984778, rs3135338, rs3135395 and rs2395178) and HLA-DQA1 (rs2187668) SNPs were significantly associated with variations in median anti-HBsAg antibody levels ($p< 0.001$). In addition, the GG genotype of the PSORS1C1 rs3130454 SNP was associated with a lower serum antibody levels to tetanus ($p< 0.001$). Regarding antibody response to PCV7, the HLA-DOB (rs2857130, rs2857127, rs6929716, rs7383433, rs5009557) and TAP2 (rs1015166) SNPs were associated with significant variations in PnPS4 ($p< 0.001$) serotype specific antibody titers. The HLA-DOB rs5009557 SNP was also associated with variation in PnPS9V serotype level. The GG genotype of LEMD2 rs755495 SNP was associated with higher serum antibody levels to serotype PnPS14 ($p<0.001$). SNPs in the COL11A2 (rs9368758, rs2269346), HSD17B8 (rs383711), RING1 (rs213210) were associated with variations in PnPS19F serotype-specific antibody titers ($p < 0.001$) while the NOTCH4 SNPs (rs2071280, rs2071287, rs2071277) were associated with altered response to PnPS23F serotype. Manhattan plots showing the association signals for each vaccine (and serotype) are provided in Supplementary Fig. 1. None of the other polymorphisms that were examined showed any significant association with immune responses to vaccine antigens.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.09.026.

3.2. Association between immunoglobulin levels and SNPs

Significant associations ($p < 0.001$) were observed between certain SNPs and serum concentrations of IgM, IgG, and IgG subclasses (Table 3). Total IgG and IgG1 subclass levels significantly effected in subjects with HLA-F and FLJ35429 SNPs while IgG4 levels varied significantly by the BTNL2 and C6orf10 SNPs (<0.001). The HLA-G, HLA-C, HLA-DRA, HCG9, FLJ35429, MAS1L, OR2W1, OR2J3, OR2J2, OR2H1, OR5U1, OR10C1, RFP, RFN39 and TRIM39 SNPs were significantly associated with variations in IgM levels ($p< 0.001$). Manhattan plots showing the association signals for each Ig class and subclass are given in Supplementary Fig. 2. There was no significant association between serum IgA levels and any of the tested SNPs.

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3.3. Association between antibody levels and principal components

In order to reduce data dimensionality, we performed principal component analysis (PCA). Immune responses were categorized into two groups. The first group consisted of vaccine specific antibody responses (10 variables) and the second group included Ig levels (7 variables). A number of significant associations were identified between individual principal components and genotypes (Supplementary Table 1). SNPs that were significant in both linear regression and PCA are marked in Tables 2 and 3.

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3.4. Association between antibody levels and haplotypes

A number of significant associations were identified between inferred haplotypes and vaccine-induced immune responses both in continuous and binary analyses. Table 4 shows haplotype frequencies and significant associations. Variation in median anti-HBsAg levels was significantly associated with ten haplotypes. The first haplotype included 11 SNPs that were mapped to the RPP21 gene. Two of these SNPs (rs3129820 and rs6936217) were also significant in linear regression analysis. Four of the other haplotypes also included SNPs that showed individual associations with immune response to hepatitis B vaccine. These haplotypes consisted of SNPs in the ZBTB12, BF, TNXB, and NOTCH4 genes. Variation in PnPS4 antibody level was associated with the haplotype that contained five SNPs also identified in the linear regression analysis. All these SNPs were mapped to the HLA-DOB gene. The haplotype correlated with variability in PnPS19F level was constructed by SNPs that were mapped to COL11A2, RXRB, SLC39A7, HSD17B8 and RING1 genes. However, only SNPs mapped to COL11A2, HSD17B8 and RING1 genes were individually associated with PnPS19F levels. Both NOTCH4 SNP and haplotype that included this SNP were associated with variation in PnPS23F serotype level. Haplotypes containing SNPs that showed individual associations are displayed in bold in Table 4.

Significant associations were also observed between certain haplotypes and serum immunoglobulin levels (Table 4). Many SNPs constructing these haplotypes were also identified in the linear regression analysis. Haplotypes consisting of FLJ35429 and BTNL2 SNPs were significantly associated with variation in IgG, IgG1 and IgG4 levels. Variability in serum IgM levels was associated with 15 haplotypes. Only four of them did not include SNPs that were identified in the linear regression analysis.

3.5. Regulatory information for significant associations

The 76 unique significant SNPs identified from initial analyses were used as inputs to the SNP Annotation and Proxy Search (SNAP) tool [33] to find additional SNPs in complete linkage disequilibrium (using an r^2 of 1). This led to the identification of an additional 149 perfectly correlated SNPs using data from the International HapMap Project [35]. The total set of 225 SNPs was then used as inputs to the RegulomeDB [34] web resource, which

integrates data from the ENCODE projects and other data sources regarding various types of functional assays including DNaseI-seq, ChIP-seq, RNAseq, and eQTL analyses. Coordinates of both significant and correlated SNPs were derived from hg19 to ensure that they match the locations of variants in RegulomeDB. SNPs with RegulomeDB scores between 1 and 3 (inclusive, where scoring refers to available datatypes supporting a functional role for the variant) and related genes are listed in Table 5.

4. Discussion

Consistent with previous studies, the present study demonstrates that MHC region variants significantly contribute to vaccine-specific antibody responses in a relatively specific and predictable manner. The MHC region exhibits high levels of allelic diversity and extensive patterns of LD encompassing multiple genes involved in immunity. The MHC class II molecules were extensively studied in relation to vaccine induced immune responses and their variations were found to be associated with altered antibody production against the presented antigen [36–38]. However, very little is published on vaccine-induced immunogenicity regarding genetic variation in other parts of the MHC region [7]. Since the significant association signals may tag nearby functional SNPs due to high levels of LD within the region, we identified highly correlated SNPs within 500 kb and assessed their regulatory potential. This analysis showed that significant signals from different regions of the MHC are correlated with SNPs that control the expression of a small number of genes involved in antigen processing and presentation.

Previous reports demonstrated that the immune response to hepatitis B vaccine is largely determined by HLA-DR and HLA-DQ alleles [9,17,39]. Certain HLA class II alleles were associated with high (DRB1*01, DRB1*11, DRB1*15, DQB1*0501, DPB1*0401) and poor or non-response to HBV (DRB1*03, DRB1*07, DQB1*02, DPB1*1101). In line with these observations, we found HLA-DR and HLA-DQ SNPs to be significantly associated with the variations in median anti-HBsAg antibody levels. HLA-DRA rs3135395, rs3135338, and rs984778 SNPs were in strong LD. Functional annotation of SNPs using RegulomeDB showed that these SNPs regulate the expression level of genes including HLA-DRA, HLA-DQA1, HLA-DRB1, HLA-DRB5 and ERG. Similarly, HLA-DQA1 rs2187668 SNP had a regulatory effect on HLA-DQA1, BTN3A2, HLA-A, HLA-DPB1, HLA-DQB1, HLA-DRB1 and HLA-DRB5 genes. Interestingly, the HLA-DQA1 gene was also regulated by NOTCH4 SNPs (rs3131296 and rs3134942) and two highly correlated TNXB (rs1150752, rs1150753) SNPs. We were not able to find regulatory information for RPP21 rs3129820 and rs6936217 SNPs, but both of them were highly correlated with two SNPs (rs3129822 and rs3094035) that affect the expression of HLA-A, BNTN3A2, HLA-C, HLA-DQA1, HLA-DQB1, HLA-DRB1, HLA-G and HLA-H genes. The role of other genes has not been extensively characterized in the context of vaccine immunity.

SNPs mapped to the HLA-DOB, TAP2, COL11A2, LEMD2, HSD17B8, RING1 and NOTCH4 genes were associated with the variations in immune response to PnPS serotypes. Five SNPs mapping to the HLA-DOB gene were associated with PnPS4 serotype and interestingly, they were correlated with the same SNP (rs2067577) that affects the regulation of HLA-DOB, HLA-DRB5 and TAP-2 genes. HLA-DR plays a central role in the

presentation of peptides on the cell surface for T-cell recognition. HLA-DOB is an important modulator in the HLA class II restricted antigen presentation pathway by interaction with the HLA-DM molecule in B-cells. The transporter associated with antigen processing (TAP) gene, a member of the ATP-binding cassette transporter super family, is involved in the processing of endogenous peptides that bind to MHC class I molecules [40]. It has been suggested that TAP polymorphisms may cause differential antigen processing and thereby influence antigen presentation by MHC molecules. TAP2 allelic variants have also been found associated with measles antibody response [41,42]. Based on their role in the process of the immunogenic peptides, it is plausible that genetic variability within HLA-DOB, HLA-DR and TAP-2 genes may affect the efficiency of antigen presentation and thereby vaccine immunogenicity. Two highly linked NOTCH4 SNPs (rs2071287 and rs2071277) showed significant association with PnPS23F serotype in linear regression analysis. Notably, these two SNPs were also associated with the third principal component of vaccine specific antibody group. The RegulomeDB database showed that the rs2071287 SNP has a regulatory effect on HLA-DQA1 and HLA-DQB1 genes. There are no previous studies reporting association between MHC SNPs and antibody levels to diphtheria. In line with this, we did not find any association between SNPs and diphtheria induced immune responses in our population. This could be related to the nature of the vaccine, as toxoid vaccines tend not to be highly immunogenic.

A large number of SNPs were associated with total serum Ig levels and mapped to BNTL2, RFP, OR2W1, OR2J3, OR2J2, OR5U1, OR10C1, OR2H1, MAS1L, FLJ35429, HLA-F, HLA-G, HCG9, RFN39, TRIM39, HLA-C and HLA-DRA genes. In the association analysis, the variations in median IgM, IgG and IgG1 levels were significantly associated with FLJ35429 and HLA-F linked SNPs. RegulomeDB showed that the FLJ35429 rs1611350 SNP affects the regulation of BTN3A2, HLA-A and ZFP57 genes. The function of the BTN3A2 and ZFP57 genes in immune response is yet unknown. Although no regulatory information exists for the HLA-F SNPs (rs1628578 and rs2517911), both variants were in strong LD with another HLA-F SNP rs1632967, which affects the expression of HLA-G. HLA-A and HLA-G are non-classical class I proteins that play a central role in antigen presentation and immunomodulation [43,44]. HLA-G and its polymorphic sites have been associated with susceptibility to viral infections and autoimmune diseases [45,46]. These three SNPs (rs1611350, rs1628578 and rs2517911) were associated with IgG1, IgG and IgM levels suggesting that genetic variability at these loci may play role in the quantitative regulation of other antibody responses and represent plausible candidate genetic modifiers of vaccine immunity.

Two SNPs mapped to the OR2J2 (rs3116830) and RFP (rs3118361) genes were associated with IgM levels and found to be correlated with the same five SNPs (rs3130893, rs3129791, rs3130837, rs3130845 and rs3131073) that affect HLA-A gene expression. The TRIM39 (rs3130380) and RNF39 (rs9261290) SNPs that were associated with IgM levels were also found to influence HLA-A gene regulation. Six IgM-associated SNPs mapped to the HLA-DRA gene and their correlated SNPs were found to be affecting the regulation of HLA-DRB5, HLA-DQB1 and HLA-DQA1 genes. Results from PCA analysis showed that the second principal component of Ig group was associated with SNPs mapped to the RFP, OR2W1, OR2J3 and OR2J2 genes. Some of the SNPs that overlapped with the SNPs

identified in the linear regression analysis (except rs381808) were also significant in logistic regression analysis with binary cut-off 15%. This might be explained by the fact that some SNPs were mapped to the same genes or haplotypes or they were physically close to each other to show association as a group.

The majority of the haplotype associations were related to the variations in median anti-HBsAg and IgM levels. Although some SNPs within the blocks mapped to genes involved in antigen presentation (HLA-A, HLA-G, HLA-DOB, TAP-2), most mapped to genes with unidentified immune functions (e.g., FLJ35429, C6orf15, RFP, OR5U1). Some of the haplotypes included SNPs that were also identified in the linear regression analysis. This might be due to a strong correlation between SNPs that construct the haplotype. Either high LD between SNPs caused the association to spread across the haplotype or strong association with the haplotypes let individual SNPs to be significant. SNPs identified only in linear regression analysis were possibly not included in any of the haplotypes or haplotypes including these SNPs did not reach statistical significance ($p < 0.001$). This is also true for the identified haplotypes that did not contain any significant SNPs from the regression analysis. Overall, strong association with certain haplotypes and individual SNPs that construct these haplotypes suggest that genetic variability in this region is strongly correlated with altered immune responses.

This is the first study reporting associations between SNPs within the entire MHC and immune response to childhood vaccines and suggests that this region is likely to contain a number of genes that affect vaccine responsiveness. The results were not corrected for multiple comparisons since our analysis was based on the well-defined role of the MHC in immune responses. Instead, we reported all tests that reached a $p < 0.001$ level of significance and focused on the functional relevance of SNPs associated with vaccine specific responses. Although functional annotation of SNPs was supported by experimental regulatory data, the significance of these findings requires further validation. Replication in independent samples, fine mapping and functional studies may reveal the genetic mechanisms underlying these associations. More importantly, pathways/allelic variants identified through genetic studies may help the development of more uniformly effective next-generation vaccine formulations that could improve vaccine immunogenicity and efficacy.

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Abbreviations

CPS	pneumococcal cell wall polysaccharide
DTaP	diphtheria, tetanus, and pertussis

HBsAg	surface antigen of hepatitis B virus
HBV	hepatitis B virus
Hib	haemophilus influenza type b
HLA	human leukocyte antigen
Ig	immunoglobulin
IPV	inactivated polio vaccine
LD	linkage disequilibrium
MHC	major histocompatibility complex
OR	odds ratio
PnPS	pneumococcal polysaccharides
PCV7	heptavalent pneumococcal conjugate
SNP	single nucleotide polymorphism

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Table 1

Demographic characteristics and immunological variables of the study population.

			***N*=135**
Demographics			
Age (mean months, range)			12.6 (11.5–14)
Gender (F/M)			58/77
Ethnicity (non-hispanic whites/others)			121/14
Antibody levels	Median	Mean	CI (95%)
HBV(mIU/ml)	153	499.93	290.34,709.51
Diphteria(IU/ml)	0.32	0.56	0.45,0.66
Tetanus (IU/ml)	0.2	4.1	−1.43,9.63
PnPS4 (μg/ml)	1.3	2.7	2.02,3.39
PnPS6B (μg/ml)	3.17	10.77	5.48,16.06
PnPS9V (μg/ml)	3.3	18.04	9.02,27.06
PnPS14 (μg/ml)	2.45	5.5	3.42,7.57
PnPS18C (μg/ml)	2.24	5.67	3.74,7.6
PnPS19F(μg/ml)	2.37	24.8	2.27,47.33
PnPS23F(μg/ml)	4.01	52.02	19.42,84.62
IgG (mg/dl)	639.1	701.89	648.48,755.31
IgG1 (mg/dl)	653.1	710.36	651.19,769.52
IgG2 (mg/dl)	66.4	105.27	82.83,127.71
IgG3 (mg/dl)	34	40.96	36.68,45.24
IgG4 (mg/dl)	2	24.01	6.41,41.61
IgM (mg/dl)	141.6	150.68	139.45,161.91
IgA (mg/dl)	6	6.93	6.17,7.69

Vaccine. Author manuscript; available in PMC 2015 November 10.

Table 2

Linear regression results for vaccine-specific antibody responses.

Vaccine	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
HBV	RPP21	−28937	**rs3129820**	AA	0	–	–	–	
				AG	24	63.5	281.97	84.74, 479.19	
				GG	101	191.3	587.49	316.12, 858.85	0.0004941
HBV	RPP21	−38015	**rs6939217**	AA	0	–	–	–	
				AG	26	63.5	270.33	88.94, 451.72	
				GG	99	191.3	596.6	319.97, 873.23	0.0001285
HBV	ZBTB12	−557	**rs558702**	AA	0	–	–	–	
				AG	18	43.5	151.11	−10.74, 312.95	
				GG	107	210	591.15	333.96, 848.33	0.0002137
HBV	BF	−61	**rs1270942**	AA	113	186.5	552.82	308.91, 796.72	
				AG	21	38.5	136.59	−7.98, 281.16	
				GG	0	–	–	–	7.88E–05
HBV	STK19	−363	rs389884	AA	113	186.5	552.82	308.91, 796.72	
				AG	21	38.5	136.59	−7.98, 281.16	
				GG	0	–	–	–	7.88E–05
HBV	TNXB	−1025	**rs1150758**	CC	1	140.7	140.7	–	
				CG	27	46.6	167.1	42.87, 291.32	
				GG	106	189.25	573.07	313.6, 832.54	0.0005311
HBV	TNXB	−2739	**rs1150753**	AA	107	210	591.15	333.96, 848.33	
				AG	18	43.5	151.11	−10.74, 312.95	
				GG	0	–	–	–	0.0002137
HBV	CREBL1	−2856	rs1269852	CC	0	–	–	–	
				CG	18	43.5	151.11	−10.74, 312.95	
				GG	107	210	591.15	333.96, 848.33	0.0002137
HBV	NOTCH4	[163/12]	**rs3134942**	AA	1	43.5	43.5	–	
				AC	25	74.84	275.66	92.79, 458.53	
				CC	99	212.44	597.6	320.82, 874.38	0.0009721
HBV	NOTCH4	−827	**rs313296**	AA	1	43.5	43.5	–	

Vaccine	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
				AG	25	74.84	275.66	92.79, 458.53	
				GG	99	212.44	597.6	320.82, 874.38	0.0009721
HBV	BTNL2	−4312	rs3129950	CC	4	19.25	25.5	−24.09, 75.09	
				CG	14	46.6	189.76	−23.89, 403.4	
				GG	107	210	591.15	333.96, 848.33	4.28E–05
HBV	HLA-DRA	−7559	rs984778	AA	62	106.2	209.42	133.79, 285.06	
				AG	55	183.1	540.01	317.94, 762.09	
				GG	17	583.54	1341.03	−166.48, 2848.55	0.0006534
HBV	HLA-DRA	−6430	rs3135338	AA	64	106.2	207.29	134.08, 280.5	
				AG	53	210	554.98	325.29, 784.67	
				GG	17	583.54	1341.03	−166.48, 2848.55	0.0005719
HBV	HLA-DRA	−2455	rs3135395	AA	17	583.54	1341.03	−166.48, 2848.55	
				AC	55	183.1	540.01	317.94,762.09	
				CC	62	106.2	209.42	133.79, 285.06	0.0006534
HBV	HLA-DRA	−2285	rs2395178	CC	62	106.2	209.42	133.79, 285.06	
				CG	55	183.1	540.01	317.94, 762.09	
				GG	17	583.54	1341.03	−166.48, 2848.55	0.0006534
HBV	HLA-DQA1	−567	rs2187668	AA	1	0	0	–	
				AG	24	45.05	135.61	11.91, 259.32	
				GG	109	187.2	569.54	317.13, 821.95	3.35E–05
Tet	PSORS1C1	−648	rs3130454	AA	56	0.24	3.43	−2.26, 9.11	
				AG	57	0.16	0.23	0.17,0.29	
				GG	12	0.13	0.2	0.05, 0.35	0.0005775
PnPS4	HLA-DOB	−4135	**rs2857130***	AA	17	3.79	5.5	2.16, 8.84	
				AT	54	1.5	3.11	1.88, 4.34	
				TT	63	0.98	1.59	1.14, 2.04	0.0003583
PnPS4	HLA-DOB	−3695	**rs2857127**	AA	16	3.9	5.66	2.1, 9.21	
				AG	55	1.55	3.11	1.9, 4.31	
				GG	63	0.98	1.59	1.14, 2.04	0.0004657
PnPS4	HLA-DOB	−3409	**rs6929716***	AA	63	0.98	1.59	1.14, 2.04	

Vaccine. Author manuscript; available in PMC 2015 November 10.

Vaccine	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
				AG	54	1.5	3.11	1.88, 4.34	
				GG	17	3.79	5.5	2.16, 8.84	0.0003583
PnPS4	HLA-DOB	2018	**rs7383433***	AA	17	3.79	5.5	2.16, 8.84	
				AG	54	1.5	3.11	1.88, 4.34	
				GG	63	0.98	1.59	1.14, 2.04	0.0003583
PnPS4	HLA-DOB	−553	**rs5009557***	AA	61	0.98	1.56	1.11, 2.01	
				AG	56	1.5	3.09	1.9, 4.28	
				GG	17	3.79	5.5	2.16, 8.84	0.0003038
PnPS4	TAP2	−148	rs1015166	AA	13	0.58	0.9	0.31,1.49	
				AG	59	1.07	2.07	1.25, 2.9	
				GG	62	2.19	3.67	2.43, 4.91	0.0007788
PnPS9V	HLA-DOB	−553	rs5009557	AA	61	2.74	8.24	0.04, 16.45	
				AG	56	3.38	26.03	8.12, 43.94	
				GG	17	7.1	27.59	−3.24, 58.42	0.0007529
PnPS14	LEMD2	−4863	rs755495	AA	4	1.62	2.7	−2.78, 8.19	
				AG	34	1.25	3.6	1.46,5.74	
				GG	87	2.79	5.92	3.18, 8.65	0.00089
PnPS19F	COL11A2	−82	rs9368758	AA	2	4.51	4.51	−34.68, 43.71	
				AG	16	7.94	140.74	−46.2, 327.68	
				GG	116	2.11	9.35	0.66, 18.05	0.0001841
PnPS19F	COL11A2	−1038	**rs2269346**	AA	2	4.51	4.51	−34.68, 43.71	
				AG	16	7.94	140.74	−46.2, 327.68	
				GG	116	2.11	9.35	0.66, 18.05	0.0001841
PnPS19F	HSD17B8	−45	**rs383711**	AA	2	4.51	4.51	−34.68, 43.71	
				AG	16	7.94	140.74	−46.2, 327.68	
				GG	116	2.11	9.35	0.66, 18.05	0.0001841
PnPS19F	RING1	−462	**rs213210**	AA	117	2.09	9.29	0.67, 17.91	
				AG	16	7.94	140.74	−46.2, 327.68	
				GG	2	4.51	4.51	−34.68, 43.71	0.0001718
PnPS23F	NOTCH4	−20	**rs2071280**	CC	67	6.15	99.82	35.38, 164.26	

Vaccine. Author manuscript; available in PMC 2015 November 10.

Vaccine	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
				CG	57	2.68	4.98	2.89, 7.07	
				GG	10	4.41	4.99	2.42, 7.55	0.0003241
PnPS23F	NOTCH4	−57	rs2071287	AA	28	2.81	3.63	2.56, 4.7	
				AG	72	4.36	28.48	0.29, 56.67	
				GG	34	7.87	143.22	29.57, 256.87	0.0006293
PnPS23F	NOTCH4	−24	rs2071277	AA	34	7.87	143.22	29.57, 256.87	
				AG	72	4.36	28.48	0.29, 56.67	
				GG	28	2.81	3.63	2.56, 4.7	0.0006293

* Markers that had significant *p*-values in logistic regression with binary cut-off 10%.

Underlined markers were significant in PCA.

Bold markers are significant in haplotype analysis.

Table 3

Linear regression results for immunoglobulin levels.

Variable	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
IgG	HLA-F	−6607	rs2517911	AA	82	711.75	779.3	704.2, 854.41	
				AG	48	565.7	593.31	528.77, 657.85	
				GG	4	467.05	422.65	239.3, 606	3.55E–05
IgG	HLA-F	−1219	rs1628578	AA	79	712.1	781.93	704.55, 859.3	
				AC	51	565.8	600.19	537.27, 663.1	
				CC	4	467.05	422.65	239.3, 606	5.59E–05
IgG	FLJ35429	−3984	rs1611350	AA	73	711.4	781.82	701.95, 861.69	
				AG	52	600.15	637.3	566.7, 707.91	
				GG	9	482.6	428.9	333.78, 524.02	2.18E–05
IgG	FLJ35429	−2622	**rs1610601**	AA	4	467.05	422.65	239.3, 606	
				AC	46	574.35	602.82	537.73, 667.92	
				CC	84	705.6	769.67	694.93, 844.4	0.0002386
IgG	FLJ35429	−76	**rs1633088**	AA	76	713	792.14	711.74, 872.53	
				AG	44	583.25	607.69	542.78, 672.59	
				GG	5	440.9	408.06	277.7, 538.42	5.69E–05
IgG1	HLA-F	−6607	rs2517911*	AA	82	698.85	771.48	692.1, 850.86	
				AG	48	595.9	639.54	550.28, 728.8	
				GG	4	331.2	317	240.95, 393.05	0.000619
IgG1	HLA-F	−1219	rs1628578*	AA	79	700.1	779.57	698.26, 860.87	
				AC	51	594	634.78	549.34, 720.22	
				CC	4	331.2	317	240.95, 393.05	0.0003055
IgG1	FLJ35429	−3984	rs1611350*	AA	73	700.1	780.94	697.15, 864.72	
				AG	52	626.2	663.82	573.44, 754.2	
				GG	9	357.4	411.21	276.69, 545.74	0.0001011
IgG1	FLJ35429	−76	**rs1633088***	AA	76	735.55	792.73	709.36, 876.1	
				AG	44	595.9	637.62	545.94, 729.31	
				GG	5	324.7	310.28	255.6,364.96	8.80E–05

Variable	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
IgG4	C6orf10	−395	rs2050190	AA	72	1.3	9.28	3.6,14.95	
				AG	53	2.5	15.55	3.27, 27.82	
				GG	9	44.4	188.8	−86.14, 463.74	0.0007056
IgG4	BTNL2	−14748	**rs3135363**	AA	66	0.85	7.45	2.12,12.78	
				AG	54	3.35	45.19	1.7, 88.67	
				GG	5	8.5	30.36	−15.21, 75.93	0.0008295
IgM	RFP	−8611	rs381808	AA	42	120.8	779.3	112.77,157.81	
				AT	56	144.25	593.31	133.46,166.53	
				TT	27	193.2	422.65	158.51, 204.52	0.0005431
IgM	RFP	−4252	rs3130838	AA	106	149.6	781.93	147.11,172.65	
				AG	19	96.5	600.19	84.14,130.14	
				GG	0	–	422.65	–	0.0005307
IgM	RFP	−2162	**rs2894066**	AA	29	101	781.82	93.9,130.27	
				AG	58	147	637.3	136.9,174.9	
				GG	47	164.1	428.9	150.05, 185.03	5.55E–05
IgM	RFP	−2579	**rs1237485**†	AA	44	117.35	422.65	110.86, 154.48	
				AG	53	145.2	602.82	135.54,169.56	
				GG	28	187.3	769.67	158.53, 202.91	0.0001627
IgM	RFP	−6522	**rs3118361**	AA	0	–	792.14	–	
				AG	19	96.5	607.69	84.14,130.14	
				GG	106	149.6	408.06	147.11,172.65	0.0005307
IgM	RFP	−13339	**rs3135329***,†	AA	27	98	779.3	92.26,131.5	
				AT	52	147	593.31	136.67,177.48	
				TT	46	161.95	422.65	152.26,186.62	4.12E–05
IgM	RFP	−16449	**rs3130843**	AA	29	108.1	781.93	95.38,149.25	
				AT	54	147	600.19	133.78,169.07	
				TT	42	171.5	422.65	154.52,191.14	6.55E–05
IgM	RFP	−23343	**rs763009**†	AA	27	193.2	781.82	161.51, 205.6	
				AG	54	145.1	637.3	134.81,168.5	
				GG	44	117.35	428.9	110.86, 154.48	9.52E–05

Variable	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
IgM	RFP	−55155	**rs3135322**†	AA	28	187.3	422.65	157.93, 202.51	
				AG	51	146.9	602.82	135.21,170.58	
				GG	46	120.8	769.67	112.59,154.33	0.0001742
IgM	OR2W1	−4129	**rs3130756**†	AA	42	117.35	792.14	108.58, 153.24	
				AG	54	147.75	607.69	137.05, 171.04	
				GG	29	181.4	408.06	156.26, 200.03	8.70E–05
IgM	OR2W1	−18170	**rs3117143**	AA	0	–	779.3	–	
				AC	19	96.5	593.31	84.14,130.14	
				CC	106	149.6	422.65	147.11,172.65	0.0005307
IgM	OR2J3	−6574	**rs3131091**†	AA	42	117.35	781.93	108.58, 153.24	
				AG	54	147.75	600.19	137.05, 171.04	
				GG	29	181.4	422.65	156.26, 200.03	8.70E–05
IgM	OR2J3	−6226	**rs3130766l**†	AA	42	117.35	781.82	108.58, 153.24	
				AG	54	147.75	637.3	137.05, 171.04	
				GG	29	181.4	428.9	156.26, 200.03	8.70E–05
IgM	OR2J2	−19782	**rs3129126l**†	AA	29	181.4	422.65	156.26, 200.03	
				AG	53	146.9	602.82	136.83,171.47	
				GG	43	117.7	769.67	109.52, 153.12	0.0001131
IgM	OR2J2	−17278	**rs3129173**	AA	106	149.6	792.14	147.11, 172.65	
				AC	19	96.5	607.69	84.14, 130.14	
				CC	0	–	408.06	–	0.0005307
IgM	OR2J2	−21483	**rs1977074**	AA	29	164.1	779.3	149.27, 194.49	
				AG	53	150.6	593.31	140.22, 174.93	
				GG	43	117.7	422.65	109.52, 153.12	0.0004792
IgM	OR2J2	−25224	**rs3116830**	AA	0	–	781.93	–	
				AG	19	96.5	600.19	84.14, 130.14	
				GG	106	149.6	422.65	147.11, 172.65	0.0005307
IgM	OR5U1	−51756	**rs12182511**†	CC	41	117.7	781.82	109.54, 155.15	
				CG	56	145.1	637.3	137.37, 171.18	
				GG	28	172.75	428.9	153.55, 197.7	0.000253

Variable	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
IgM	OR5U1	−34089	**rs3117326**	AA	0	–	422.65	–	
				AG	19	96.5	602.82	84.14, 130.14	
				GG	106	149.6	769.67	147.11, 172.65	0.0005307
IgM	OR5U1	−23067	**rs6456942**	AA	29	164.1	792.14	149.27, 194.49	
				AG	54	148.75	607.69	139.07, 173.5	
				GG	42	120.8	408.06	110.12, 154.6	0.000678
IgM	OR10C1	−2701	**rs1535039**	AA	106	149.6	779.3	147.11, 172.65	
				AG	19	96.5	593.31	84.14, 130.14	
				GG	0	–	422.65	–	0.0005307
IgM	OR2H1	−3258	**rs2746149**†	AA	104	149.6	781.93	147.33, 173.09	
				AG	21	96.5	600.19	87.28, 133.78	
				GG	0	–	422.65	–	0.0004298
IgM	OR2H1	−10604	**rs2746150**	AA	0	–	781.82	–	
				AG	19	96.5	637.3	84.14, 130.14	
				GG	106	149.6	428.9	147.11, 172.65	0.0005307
IgM	MAS1L	−7364	**rs1233489**	AA	0	–	422.65	–	
				AT	19	96.5	602.82	84.14, 130.14	
				TT	106	149.6	769.67	147.11, 172.65	0.0005307
IgM	MAS1L	−22142	**rs1233478**†	AA	6	112.3	792.14	48.49, 184.34	
				AC	44	115.05	607.69	112.46, 149.42	
				CC	75	156.1	408.06	151.76, 182.19	9.24E–05
IgM	FLJ35429	−3984	rs1611350	AA	73	159.3	779.3	148.59, 180.55	
				AG	52	134.3	593.31	124.18, 157.44	
				GG	9	77.5	422.65	60.74, 124.12	0.0003157
IgM	FLJ35429	−2622	**rs1610601**	AA	4	110.45	781.93	49.58, 155.02	
				AC	46	122.25	600.19	112.39, 150.36	
				CC	84	158.45	422.65	149.06, 177.48	0.0008349
IgM	HLA-G	−19782	**rs2734985**	AA	83	159.8	781.82	151.82, 182.1	
				AG	40	125.3	637.3	108.8, 139.77	
				GG	2	76.8	428.9	–192.57,346.17	0.0001734

Variable	Gene	Position	SNP	Genotype	*N*	Median antibody level (μg/ml)	Mean antibody level (μg/ml)	CI (95%)	*P*
IgM	HCG9	−33622	**rs356971**	AA	92	159.55	422.65	150.68,178.76	
				AC	33	117.7	602.82	99.27, 132.76	
				CC	0	–	769.67	–	0.0001766
IgM	RNF39	[86/195]	rs9261290	AA	105	150.6	792.14	147.63, 173.3	
				AG	20	97.25	607.69	84.92, 128.45	
				GG	0	–	408.06	–	0.0003493
IgM	TRIM39	−15910	**rs3130380**	AA	0	–	779.3	–	
				AG	18	97.25	593.31	82.48, 130.33	
				GG	107	148.6	422.65	146.82, 172.2	0.00063
IgM	HLA-C	−4934	rs2524069	AA	105	150.6	781.93	147.36, 173.05	
				AT	27	117	600.19	98.84, 140.4	
				TT	2	58.1	422.65	12.36, 103.84	0.0001513
IgM	HLA-DRA	−8386	rs3135339	CC	15	107	781.82	85.73, 134.13	
				CG	44	128.15	637.3	118.03, 150.29	
				GG	75	164.1	428.9	151.82, 184.59	0.0004524
IgM	HLA-DRA	−7805	rs2395172	AA	75	164.1	422.65	151.82, 184.59	
				AG	44	128.15	602.82	118.03, 150.29	
				GG	15	107	769.67	85.73, 134.13	0.0004524
IgM	HLA-DRA	−6708	rs3129859	CC	71	164.1	792.14	149.78, 182.94	
				CG	45	129.6	607.69	124.19, 159.98	
				GG	18	104	408.06	87.67, 130.35	0.0007321
IgM	HLA-DRA	−3992	rs983561	AA	75	164.1	779.3	151.82, 184.59	
				AC	44	128.15	593.31	118.03, 150.29	
				CC	15	107	422.65	85.73, 134.13	0.0004524
IgM	HLA-DRA	−2571	rs2395177	CC	14	108.85	781.93	87.77, 138.19	
				CG	40	126.75	600.19	114.85, 147.64	
				GG	71	165.7	422.65	154.28, 188.01	0.0003933
IgM	HLA-DRA	−494	rs3129872	AA	15	107	781.82	85.73, 134.13	
				AT	44	128.15	637.3	118.03, 150.29	
				TT	75	164.1	428.9	151.82, 184.59	0.0004524

*Markers that had significant *p*-values in logistic regression with binary cut-off 10%.

Vaccine. Author manuscript; available in PMC 2015 November 10.

† Markers that had significant *p*-values in logistic regression with binary cut-off 15.

Underlined markers were significant in PCA.

Bold markers are significant in haplotype analysis.

Table 4

Haplotype associations.

Variable	BP1	BP2	SNP1	SNP2	Haplotype	*F*	Beta	*T*	*P*	Genes
HBV	30443533	30471924	rs3129808	rs3130113	**AGCAAAGAAGA**	0.096	−1.32	12.8	5.00E–04	RPP21
HBV	31920017	31956199	rs9267576	rs589428	CGGTCGGGACA	0.0813	−1.66	20.3	1.00E–04	C6orf48|NEU1|C6orf29|BAT8
HBV	31959213	31978305	rs652888	rs558702	**GGAGA**	0.072	−1.55	14.6	5.00E–04	BAT8|ZBTB12
HBV	31980530	32002605	rs2763982	rs3020644	GAAGA	0.156	−1.06	14.4	5.00E–04	ZBTB12|C2
HBV	32024379	32036778	rs537160	rs419788	**AGGAGA**	0.0784	−1.54	16.7	3.00E–04	BF|RDBP|SKIV2L
HBV	32134085	32174155	rs1009382	rs17421624	**AGCGAGGA**	0.0761	−1.55	16.4	3.00E–04	TNXB
HBV	32274362	32290737	rs8192575	rs206015	**CAAGGACGTGG**	0.111	−1.1	11.8	0.0009999	NOTCH4
HBV	32298006	32302832	rs3132946	rs3096691	GGGGAG	0.216	−0.819	12.7	4.00E–04	NOTCH4
HBV	32303966	32306914	rs365053	rs2849015	AAAAA	0.116	−1.37	19.1	1.00E–04	NOTCH4
HBV	32315371	32319295	rs416352	rs411326	AAAAG	0.0728	−1.56	14.7	5.00E–04	NOTCH4
PnPS4	32871088	32888702	rs2621377	rs11244	**GCACGAAAGGAGG**	0.325	0.691	12.9	0.0007999	HLA-DOB
PnPS18C	29816201	29836188	rs1610603	rs1610628	GGAAAA	0.012	−3.22	13	0.0005999	FLJ35429
PnPS19F	33252221	33293896	rs1799908	rs213212	**AAGGGCAAACGCGAGAGAGGGGA**	0.0701	1.71	16.4	0.0006999	COL11A2|RXRB|SLC39A7|HSD17B8|RING1
PnPS23F	32263559	32272847	rs204993	rs2071280	**AAACGG**	0.287	−1.12	13.6	3.00E–04	PBX2|GPSM3|NOTCH4
IgG	29807135	29811241	rs2523402	rs2394160	**AGGAGGGAA**	0.201	−0.34	14.3	0.0005999	FLJ35429
IgG	29816201	29836188	rs1610603	rs1610628	**GGGGAA**	0.216	−0.376	17.4	2.00E–04	FLJ35429
IgG	31133030	31183094	rs2523849	rs1064190	AGGGAAGAGGGCAAGCA	0.164	0.343	11.7	0.0009999	C6orf15
IgG	31601867	31630648	rs2734574	rs6929796	TCACAAGAGGCAAGTGGCG	0.0224	0.84	12.3	0.0009999	BAT1|ATP6V1G2|NFKBIL1
IgG1	29816201	29836188	rs1610603	rs1610628	**GGGGAA**	0.216	−0.425	16.5	5.00E–04	FLJ35429
IgG2	30253719	30320795	rs7774730	rs3094635	CCGGGACAGGGGAAGGGACGAAGGGCGAT	0.0243	1.69	15.7	2.00E–04	TRIM15|TRIM26|FLJ45422
IgG4	31133030	31183094	rs2523849	rs1064190	AGGGAAGAGAGCGAGCA	0.068	2.2	12.7	4.00E–04	C6orf15
IgG4	32491419	32497626	rs3135382	rs3135363	**AAA**	0.736	−1.43	11.8	5.00E–04	BTNL2
IgM	28983481	29023087	rs209122	rs763009	**GGACAGGGGGTTGA**	0.426	0.333	17.3	1.00E–04	RFP
IgM	28983481	29023087	rs209122	rs763009	**AGGCTAGAAGAAGG**	0.0766	−0.581	12.5	0.0007999	RFP
IgM	29054899	29207558	rs3135322	rs3130778	**GGAGCAGAAACGAAAAAAGCAGGAAAACAAGATAGAGAAAAG**	0.076	−0.59	13.2	0.0007999	RFP|OR2W1|OR2B3|OR2J3
IgM	29216270	29364399	rs9393945	rs1884123	**GCCAAAGAACAGGAAGAGAAGGAACGGAGAGAGG**	0.419	0.294	12.4	4.00E–04	OR2J3|OR2J2|OR5U1
IgM	29216270	29364399	rs9393945	rs1884123	**GGAGGAACGGGAACGAAGGGAACCACAGGGAGGA**	0.076	−0.584	12.7	0.0007999	OR2J3|OR2J2|OR5U1
IgM	29390330	29443516	rs9393954	rs9380120	AATGAACAAGA	0.115	−0.436	11.2	0.0009999	OR5U1|OR5V1|OR12D3

Variable	BP1	BP2	SNP1	SNP2	Haplotype	*F*	Beta	*T*	*P*	Genes
IgM	29444033	29455023	rs4713211	rs238880	AAGGGA	0.444	0.274	11.7	0.0008999	OR12D3
IgM	29519411	29576788	rs1535039	rs1233487	**GAGGGGGAGAGGAAGACAAAG**	0.0717	−0.571	11.5	0.0009999	OR10C1|OR2H1|MAS1L
IgM	29580895	29591947	rs757256	rs1592410	**AAAGA**	0.212	−0.41	16	2.00E–04	MAS1L
IgM	29807135	29811241	rs2523402	rs2394160	**AGGAGGGAA**	0.201	−0.349	11.7	5.00E–04	FLJ35429
IgM	29926641	29931006	rs2734985	rs2428510	**GAAA**	0.175	−0.387	12.3	0.0005999	HLA-G
IgM	30017786	30028444	rs3094141	rs1655912	CGCAAC	0.194	−0.352	12.3	0.0007999	HLA-A
IgM	30059085	30119560	rs2735067	rs259939	**GCAGAAACCGCACG**	0.132	−0.498	15	2.00E–04	HCG9|ZNRD1
IgM	30344733	30424718	rs2844762	rs9380174	**GACAGGTGCAACGGTAATGGGAAAAAAATG**	0.072	−0.592	12.3	2.00E–04	FLJ45422|TRIM39|RPP21
IgM	31133030	31183094	rs2523849	rs1064190	AAGGGGAGAGAAAAGCA	0.092	−0.529	13.7	5.00E–04	C6orf15

BP1: physical position of left-most (5') SNP (base-pair). BP2: Physical position of right-most (3') SNP (base-pair). SNP1: left-most (5') SNP. SNP2: left-most (3') SNP. *F:* frequency. BETA (OR): regression coefficient (estimated odds ratio). *T*: test statistic (T from Wald test). *P*: empirical *p*-values from permutation procedures (10,000 permutations). Haplotypes containing SNPs that are individually associated with immune responses are shown in bold.

Table 5

Regulatory potential of associated/correlated SNPs and affected genes.

SNP	Gene	Vaccine/Ig	Correlated SNPs	Distance (bp)	Affected genes
rs3131296	NOTCH4	HBV	rs3131296	0	HLA-DQA1
rs3134942	NOTCH4	HBV	rs3134942	0	HLA-DQA1
rs3129820	RPP21	HBV	rs3129822	2639	HLA-A
			rs3094035	19567	HLA-A|BTN3A2|HLA-C|HLA-DQA1|HLA-DQB1|HLA-DRB1|HLA-G|HLA-H
rs6936217	RPP21	HBV	rs3129822	6439	HLA-A
			rs3094035	10489	HLA-A|BTN3A2|HLA-C|HLA-DQA1|HLA-DQB1|HLA-DRB1|HLA-G|HLA-H
rs1150753	TNXB	HBV	rs1150752	4859	HLA-DQA1
rs3135338	HLA-DRA	HBV	rs3135338	0	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA|ERG
			rs984778	1129	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA
			rs3135395	3975	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA
rs3135395	HLA-DRA	HBV	rs3135395	0	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA
			rs3135338	3975	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA|ERG
			rs984778	5104	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA
rs984778	HLA-DRA	HBV	rs984778	0	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA
			rs3135338	1129	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA|ERG
			rs3135395	5104	HLA-DQA1|HLA-DRB1|HLA-DRB5|HLA-DRA
rs2187668	HLA-DQA1	HBV	rs2187668	0	HLA-DQA1|BTN3A2|HLA-A|HLA-DPB1|HLA-DQB1|HLA-DRB1|HLA-DRB5
			rs9273327	17339	HLA-DQA1
			rs3129716	51552	HLA-DQA1|BTN3A2|HLA-A|HLA-DPB1|HLA-DQB1|HLA-DRB1|HLA-DRB5
rs5009557	HLA-DOB	PnPS4|PnPS9V	rs2067577	17512	HLA-DOB|HLA-DRB5|TAP2
rs2857127	HLA-DOB	PnPS4	rs2067577	14370	HLA-DOB|HLA-DRB5|TAP2
rs2857130	HLA-DOB	PnPS4	rs2067577	13930	HLA-DOB|HLA-DRB5|TAP2
rs6929716	HLA-DOB	PnPS4	rs2067577	14656	HLA-DOB|HLA-DRB5|TAP2
rs7383433	HLA-DOB	PnPS4	rs2067577	16047	HLA-DOB|HLA-DRB5|TAP2
rs2071287	NOTCH4	PnPS23F	rs2071287	0	HLA-DQA1|HLA-DQB1
rs2071277	NOTCH4	PnPS23F	rs2071287	1250	HLA-DQA1|HLA-DQB1
rs1611350	FLJ35429	IgG1|IgG|IgM	rs1611350	0	BTN3A2|HLA-A|ZFP57
rs1628578	HLA-F	IgG1|IgG	rs1632957	6835	HLA-G

SNP	Gene	Vaccine/Ig	Correlated SNPs	Distance (bp)	Affected genes
rs2517911	HLA-F	IgG1|IgG	rs1632957	4033	HLA-G
rs2734985	HLA-G	IgM	rs5013088	1727	HLA-A|Hs.519979|BTN3A2|HCG4|HLA-G|HLA-H|KIT|NDUFS1|TPD52L2|ZFP57
rs2395172	HLA-DRA	IgM	rs2395172	0	HLA-DRB5
			rs5000563	4293	HLA-DRB5|HLA-DQB1
rs983561	HLA-DRA	IgM	rs5000563	480	HLA-DRB5|HLA-DQB1
			rs2395172	3813	HLA-DRB5
rs3129872	HLA-DRA	IgM	rs3129872	0	HLA-DRB5|HLA-DQB1
			rs3129876	859	HLA-DRB5|HLA-DQA1
			rs3129877	1444	HLA-DRB5|HLA-DQB1
rs2395181	HLA-DRA	IgM	rs2395181	0	HLA-DRB5|HLA-DQA1
rs3129881	HLA-DRA	IgM	rs3129881	0	HLA-DRB5|HLA-DQA1
rs2395177	HLA-DRA	IgM	rs2395177	0	HLA-DRB5
rs3116830	OR2J2	IgM	rs3130893	186868	HLA-A
			rs3129791	213282	HLA-A
			rs3130837	219483	HLA-A
			rs3130845	244208	HLA-A
			rs3131073	246603	HLA-A
rs3118361	RFP	IgM	rs3130893	82420	HLA-A
			rs3129791	56006	HLA-A
			rs3130837	49805	HLA-A
			rs3130845	25080	HLA-A
			rs3131073	22685	HLA-A
rs9261290	RNF39	IgM	rs9261290	0	HLA-A
rs3130380	TRIM39	IgM	rs3130380	0	HLA-A
			rs3094064	17123	HLA-A
			rs3130377	44263	HLA-A
			rs3130350	48709	HLA-A|BTN3A2|HLA-C|HLA-DQA1|HLA-DQB1|HLA-DRB1|HLA-G|HLA-H|ZFP57