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HLA class II diversity in HIV-1 uninfected individuals from the placebo arm of the RV144 Thai vaccine efficacy trial

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Abstract

The RV144 HIV vaccine trial in Thailand elicited antibody responses to the envelope of HIV-1, which correlated significantly with the risk of HIV-1 acquisition. Human leukocyte antigen (HLA) class II molecules are essential in antigen presentation to CD4 T cells for activation of B cells to produce antibodies. We genotyped the classical HLA-DRB1, DQB1, and DPB1 genes in 450 individuals from the placebo arm of the RV144 study to determine the background allele and haplotype frequencies of these genes in this cohort. High-resolution 4 and 6-digit class II HLA typing data was generated using sequencing-based methods. The observed diversity for the HLA loci was 33 HLA-DRB1, 15 HLA-DQB1, and 26 HLA-DPB1 alleles. Common alleles with frequencies greater than 10% were *DRB1*07:01, DRB1*09:01, DRB1*12:02, DRB1*15:02, DQB1*02:01/02, DQB1*03:01, DQB1*03:03, DQB1*05:01, DQB1*05:02, DPB1*04:01:01, DPB1*05:01:01*, and *DPB1*13:01:01*. We identified 28 rare alleles with frequencies of less than 1% in the Thai individuals. Ambiguity for *HLA-DPB1*28:01* in exon 2 was resolved to *DPB1*296:01* by next-generation sequencing of all exons. Multi-locus haplotypes including HLA class I and II loci were reported in this study. This is the first comprehensive report of allele and haplotype frequencies of all three HLA class II genes from a Thai population. A high-resolution genotyping method such as next-generation sequencing avoids missing rare alleles and resolves ambiguous calls. The HLA class II genotyping data generated in this study will be beneficial not only for future disease association/vaccine efficacy studies related to the RV144 study, but also for similar studies in other diseases in the Thai population, as well as population genetics and transplantation studies.

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Keywords

HIV-1; Human leukocyte antigen; HLA-DPB1; HLA-DQB1; HLA-DRB1; RV144

Introduction

The first HIV-1 vaccine trial to show efficacy was the RV144 study conducted in Thailand (1). Two antibodies were identified to associate with HIV-1 infection in a follow-up study (2). Elucidating mechanisms for the antibody-mediated vaccine responses on HIV-1 acquisition in RV144 will be important in improving vaccine design and efficacy. Human leukocyte antigen (HLA) class II molecules are expressed on antigen-presenting cells and present peptides to CD4 T cells, which induce B cells to undergo affinity maturation and isotype switching. These B cells then produce antibody secreting plasma cells and memory cells. The classical HLA class II molecules DP, DQ, and DR are encoded by genes that are located in the major histocompatibility complex on chromosome 6. These genes are highly polymorphic and several HLA alleles have been shown to associate with humoral responses mediated by vaccines (3–6). It is therefore possible that the differences in vaccine-induced immune responses in the RV144 study could be because of variation in HLA class II genes between individuals.

Given the role of HLA class II in the generation of B cell responses and their ability to impact vaccine efficacy, it is essential to define the genetic background of a population and characterize allele frequencies of HLA class II before commencing disease association and genetic studies. No HLA class II genotypes have previously been characterized in the RV144 cohort, and HLA class II typing data from other Thai cohorts has mainly been generated by low-resolution DNA typing methods such as polymerase chain reaction (PCR) using sequence specific probes (PCR-SSOP) and sequence specific primers (PCR-SSP) (7–9). The majority of these reports also described HLA genotyping data from only the DRB1 and DQB1 loci but not the DPB1 locus (7, 8). The RV144 study conducted in Thailand consisted of 16,000 volunteers belonging to different regions of origin. This cohort was thus ideal for defining HLA genotypes in a sample that was large and was representative of the Thai population. We have previously characterized HLA class I diversity in a subset of the uninfected study participants from 450 individuals in the placebo arm of the RV144 vaccine trial in Thailand (10). We now present high-resolution DNA-based HLA class II genotypes for the same individuals. HLA-DRB1, DQB1, and DPB1 alleles were resolved to 4 or 6-digit resolution by sequence-based typing (SBT) and next-generation sequencing (NGS) on the Illumina MiSeq platform. HLA 4-digit allele frequencies for the DRB1 and DQB1 loci were compared with neighboring and other world populations. Multi-locus HLA haplotypes were also estimated for this cohort. The data generated will be important in understanding the underlying frequencies of HLA class II alleles in the RV144 study and inform genetic associations that may arise from immune responses induced by the HIV vaccine.

Materials and methods

Samples

We sampled 450 individuals from the RV144 Phase 3 prime-boost HIV vaccine trial as previously described (10). All individuals were from the placebo arm of the study and remained HIV-1 negative for the duration of the study. This study was approved by the local institutional review boards of the participating institutions and all individuals gave informed consent for participation in this study. All samples were classified into four groups based on geographic region of origin as described in an earlier study (10).

HLA class II genotyping

Genomic DNA (gDNA) samples were extracted and purified from peripheral blood mononuclear cells using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). HLA-DRB1 genotyping was performed with initial group-specific allele amplification using primers that were tagged with M13 sequences. PCR conditions to amplify the DRB1 locus were modified to use a SYBR Green assay as described previously (11). An amplified product was detected by dissociation curve analyses using a 7900HT Fast Real-Time PCR System with SDS v2.4 software (Life Technologies, Carlsbad, CA) and purified using Agencourt AMPure beads on a Biomek NXp instrument (Beckman Coulter, Miami, FL). HLA-DQB1 genotyping was performed by the SBT method as recommended by the 13th International Histocompatibility Workshop (12). Briefly, amplification was performed in 10 ul reaction volumes consisting of 50 ng gDNA, 50% GoTaq G2 Colorless Master Mix (Promega, Madison, WI), 5% DMSO (Sigma-Aldrich, St. Louis, MO), 200 nM of HLA-DQB1 specific forward primer with M13 tag (M5QB: 5′-TGT AAA ACG ACG GCC AGT GTC CTC GCA GAG GAT TTC G-3′), and 200nM of HLA-DQB1 specific reverse primer mix consisting of two primers with M13 tags (M3QBgeneric: 5′-CAG GAA ACA GCT ATG ACC ATG GGG CGA CGA CGC TCA CCT C-3′; M3QBgeneric_0601: 5′-CAG GAA ACA GCT ATG ACC ATG GGT CAA CCA CGC TCA CCT C-3′). Purified amplicons for both DRB1 and DQB1 were sequenced with M13 forward and reverse primers using BigDye Terminator cycle sequencing v3.1 kit and a 3730xl DNA analyzer (Life Technologies). HLA sequences were analyzed using Assign ATF software v1.0.2.45 (Conexio Genomics, Fremantle, Australia). To address allele ambiguity at the HLA-DQB1 locus, a subset of the samples was sequenced on a MiSeq platform (Illumina, San Diego, CA) by a multi-locus individual tagging NGS (MIT-NGS) method (11). Long-range PCR products of the full-length HLA-DQB1 gene were obtained using PrimeSTAR GXL DNA Polymerase (Clontech, Mountain View, CA) and primers modified from Hosomichi et al. (13). The primer located in the 5′UTR was modified to decrease allelic imbalance in the Thai population (5′-TAT GAC AGC AAT TTT CTC TCC CCT G-3′). Sample libraries were prepared using the Nextera XT DNA sample preparation kit, according to manufacturers' instructions, and sequenced on a MiSeq instrument using the paired-end 500 cycle MiSeq Reagent Kit (Illumina). HLA-DPB1 was also sequenced by the MIT-NGS method using previously described primers (13), with the forward primer replaced to enable amplification of all known DPB1 alleles in Thais (5′-GCC TAG TGA GCA ATG ACT CAT A-3′). HLA-DQB1 and DPB1 genotypes generated by MIT-NGS were assigned using Omixon Target software v1.8 (Omixon Biocomputing Kft, Budapest, Hungary).

Novel allele identification

Long-range amplicons from a potential novel allele were cloned using the TOPO XL PCR Cloning Kit (Life Technologies) according to manufacturers' suggestions. Colonies derived from One Shot chemical transformations were initially screened by direct sequencing of exon 2 following heat lysis (14). Plasmid DNA was obtained from positive transformants using the QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA). Sequencing was performed using HLA class II specific primers spanning the different exons with the BigDye Terminator cycle sequencing v3.1 kit and a 3730xl DNA analyzer (both Life Technologies) (primer sequences are available on request). HLA sequences were analyzed using Sequencher v5.0 (Gene Codes Corporation, Ann Arbor, MI) and Mutation Surveyor DNA Variant Analysis software v4.0.9 (SoftGenetics, State College, PA). Sequences from three clones from two individuals matched the sequence of a novel allele. The novel allele sequence was further confirmed by full-length NGS data from a sample homozygous for the allele. Sequences were submitted to GenBank.

Statistical analysis

Allele frequencies were counted first for the overall population and then according to region of origin. Fisher's exact test was used to test for differences in frequencies according to region of origin after excluding individuals belonging to the Southern region of Thailand because of small sample size (N=6). A *p*-value of less than 0.05 was considered statistically significant after Bonferroni correction for multiple comparisons. Expected and observed heterozygosities for HLA-DRB1, DQB1, and DPB1 were estimated using Arlequin v3.5.1.3 software (15). Deviation from Hardy-Weinberg Equilibrium (HWE) was also evaluated for the three HLA loci. Two and three-locus HLA haplotypes and haplotype frequencies were inferred using PROC HAPLO in SAS software (SAS Institute, Cary, NC). Because HLA class I data was available for the entire dataset (10), we computed five and six-locus haplotypes for the 450 samples. HLA class II frequencies were compared with other world populations having 4-digit HLA typing (7, 16–23).

Results

HLA class II alleles

HLA class II typing for HLA-DRB1, DQB1, and DPB1 was carried out on a total of 450 Thai individuals. The overall allelic diversity at each locus was 33 HLA-DRB1, 15 DQB1, and 26 DPB1 alleles. Observed heterozygosity at the HLA-DRB1, DQB1, and DPB1 loci was 90.0%, 86.7%, and 88.6%, respectively. There was no significant deviation from HWE for the three HLA loci. High-resolution, 4-digit HLA typing was obtained for HLA-DRB1 and DQB1 loci using Sanger-based sequencing. HLA-DPB1 typing data from the NGS platform was unambiguous and resolved to 4 or 6-digit HLA types. HLA-DRB1 alleles that occurred at a frequency greater than 5% were *HLA-DRB1*12:02* (16.7%), *DRB1*15:02* (14.4%), *DRB1*07:01* (10.4%), *DRB1*09:01* (10.1%), *DRB1*15:01* (8.6%), *DRB1*04:05* (5.2%), and *DRB1*03:01* (5.1%) (Table 1). The most prevalent HLA-DQB1 alleles, occurring at a frequency greater than 5%, were *DQB1*03:01* (18.7%), *DQB1*05:02* (17.3%), *DQB1*02:01/02* (13.9%), *DQB1*05:01* (13.6%), *DQB1*03:03* (12.0%), *DQB1*06:01* (7.9%), and *DQB1*05:03* (5.6%) (Table 2). The common HLA-DPB1 alleles

occurring at a frequency greater than 5% were *DPB1*05:01:01* (22.3%), *DPB1*13:01:01* (16.8%), *DPB1*04:01:01* (12.4%), *DPB1*02:01:02* (9.2%), and *DPB1*03:01:01* (5.6%) (Table 3). When stratified by region of origin, only *HLA-DRB1*01:01* ($p = 0.041$) was significantly different according to region.

Several rare alleles occurring at a frequency less than 1% were identified in the RV144 samples. These included *HLA-DRB1*14:05* (0.8%), *DRB1*15:04* (0.7%), *DRB1*12:01* (0.6%), *DRB1*04:04* (0.4%), *DRB1*14:10* (0.4%), *DRB1*01:01* (0.3%), *DRB1*13:01* (0.3%), *DRB1*14:22* (0.2%), *DRB1*15:03* (0.2%), *DRB1*15:06* (0.2%), *DRB1*04:10* (0.1%), *DRB1*04:87* (0.1%), *DRB1*08:19* (0.1%), *DRB1*13:03* (0.1%), *DRB1*14:18* (0.1%), *DQB1*06:09* (0.6%), *DQB1*06:04* (0.4%), *DQB1*06:03* (0.3%), *DQB1*02:07* (0.1%), *DPB1*135:01* (0.9%), *DPB1*10:01* (0.4%), *DPB1*19:01* (0.4%), *DPB1*26:01:02* (0.4%), *DPB1*23:01:01* (0.2%), *DPB1*30:01* (0.2%), *DPB1*27:01* (0.1%), *DPB1*48:01* (0.1%), and *DPB1*93:01* (0.1%). Owing to unresolved sequence ambiguities, one individual per locus was not assigned genotypes. Allele distributions of HLA-DRB1 and DQB1 in the RV144 Thai cohort compared with other Asian and major world populations of distinct ancestry are presented in Tables 4, 5.

We report the presence of a novel HLA-DPB1 allele in the RV144 Thai cohort (GenBank accession number KJ780721) with a frequency of 1.6%. We cloned the entire allele and show that sequences of exons 2–4 matched a newly identified allele in the IMGT/HLA database called *HLA-DPB1*296:01*. This allele is identical to *HLA-DPB1*28:01* in exon 2, but differs by six single nucleotide polymorphisms (SNPs) in exon 3. Full-length sequencing of HLA-DPB1 enabled us to further identify two SNPs in exon 1.

HLA class II haplotypes

Two and three-locus HLA haplotypes were imputed in the 450 RV144 individuals (Table 6). There were 17 DRB1-DQB1-DPB1 haplotypes having a frequency greater than 1%, with *DRB1*15:02-DQB1*05:01-DPB1*13:01:01* (6.9%) and *DRB1*09:01-DQB1*03:03-DPB1*05:01:01* (5.3%) being the most common with frequencies greater than 5%. For bi-locus DRB1-DQB1 haplotypes, five haplotypes occurred at a frequency greater than 5%, which were *DRB1*12:02-DQB1*03:01* (12.5%), *DRB1*15:02-DQB1*05:01* (10.9%), *DRB1*09:01-DQB1*03:03* (10.2%), *DRB1*07:01-DQB1*02:01/02* (8.8%) and *DRB1*15:01-DQB1*06:01* (5.2%). Common DRB1-DPB1 haplotypes occurring at a frequency greater than 5% were *DRB1*15:02-DPB1*13:01:01* (6.8%) and *DRB1*09:01-DPB1*05:01:01* (5.2%). Frequent DQB1-DPB1 haplotypes occurring at a frequency greater than 5% were *DQB1*05:01-DPB1*13:01:01* (6.9%), *DQB1*05:02-DPB1*05:01:01* (6.4%), and *DQB1*03:03-DPB1*05:01:01* (5.5%). The most frequent five and six-locus haplotypes are listed in Table 7.

Discussion

The RV144 vaccine produced different antibody responses that correlated with either decreased or increased risk of HIV-1 acquisition (2). HLA class II genes play an important role in generating antibody responses induced by vaccines. Population level variation in the polymorphic HLA genes is known to impact susceptibility to infections and diseases.

Therefore, defining HLA alleles by high-resolution genotyping in a cohort is key to understanding effects of HLA on disease outcome and vaccine responses. In this study, we report HLA class II allele and haplotype diversity in 450 uninfected individuals from the placebo arm of the RV144 study.

We identified 74 HLA class II alleles from the HLA-DRB1, DQB1, and DPB1 loci. HLA-DRB1 was the most polymorphic locus, followed closely by DPB1, with DQB1 showing less variation. In contrast, the IMGT/HLA database contains more HLA-DRB1 and DQB1 alleles than the DPB1 locus (http://www.ebi.ac.uk/ipd/imgt/hla/stats.html). Greater HLA-DPB1 polymorphism could be a feature unique to the Thai population, but is also likely the result of earlier indifference to the DPB1 locus. HLA-DP is not as well characterized as DR or DQ at either the protein or gene levels (24). Several HLA-DPB1 alleles are identical at exon 2, but differ in other exons. Sequencing by the traditional Sanger method precludes identification of several alleles that match at exon 2 but have variation in other coding regions. To address this drawback of the SBT method, we used an NGS-based sequencing method to characterize full-length sequences of HLA-DPB1 from the 5′UTR to the 3′UTR regions. The consequence of this more extensive sequence analysis is shown by a previous study reporting the presence of only 17 HLA-DPB1 alleles in Thais using PCR-SSOP (25). In comparison, we identified 26 HLA-DPB1 alleles using an NGS method that could generate unambiguous high-resolution HLA-DPB1 genotyping data from all exons. Allele ambiguities that were resolved included *HLA-DPB1*03:01:01/104:01, 04:02:01/105:01, 05:01:01/135:01, 13:01:01/107:01*, and *28:01/296:01*. Because all of these changes affect the HLA protein sequence, it is essential to define such allelic diversity of the HLA-DPB1 locus. Not all of these protein changes affect the peptide-binding groove, but other features of the HLA-DP protein can influence peptide presentation. For example, HLA-DP cell surface expression levels vary substantially between normal individuals and correlate with the ability to clear infectious disease (26). Further, SBT generates ambiguous HLA types for the frequent *HLA-DQB1*02:01:01G (02:01/02:02)* allele that has previously been reported at 2-digit resolution in the Thai population (7). Because the 8.1 ancestral haplotype containing the *HLA-DQB1*02* allele has been implicated with several autoimmune diseases (27), we sequenced a subset of 14 individuals by NGS to determine the *DQB1*02:01:01G* ambiguous HLA subtype in the Thai population. We observed that both *HLA-DQB1*02:01* and *DQB1*02:02* were present and were almost always linked with *DRB1*03:01* and *DRB1*07:01*, respectively.

Overall, the common alleles that matched previous studies from Thailand included *HLA-DRB1*12:02, DRB1*15:02, DRB1*07:01, DQB1*03:01, DQB1*05:02, DQB1*02:01/02:02, DQB1*05:01*, and *DQB1*03:03* (7, 28). Except for *HLA-DRB1*07:01* there were no common alleles with allele frequencies that were significantly different between our study and the Thai Northeastern population (7). However, this finding did not remain significant when compared with the subset of RV144 samples of Northeastern origin. We report the presence of several rare alleles with a frequency of less than 1% in the overall RV144 cohort that were not identified in other Thai samples, including *HLA-DRB1*04:10, DRB1*04:87, DRB1*08:19, DRB1*12:01, DRB1*14:10, DRB1*14:18, DRB1*14:22, DRB1*15:03, DRB1*15:04, DRB1*15:06, DQB1*02:07*, and *DQB1*06:09*. For the HLA-

DPB1 locus, all 17 DPB1 alleles previously identified in a Thai population primarily from Bangkok (25) were observed in our study, in addition to nine others detected only in the RV144 cohort. It was not possible to compare the HLA-DPB1 allele frequencies of our study with the previous report because the earlier study's genotyping method was restricted to exon 2 sequences, which resulted in missing several of the alleles that we now can discriminate. Additionally, *HLA-DPB1*28:01* was identified in the earlier report but is absent in the RV144 samples. We instead detected the presence of a novel allele *HLA-DPB1*296:01* that shares sequence homology with 28:01 in exon 2, but differs by several synonymous as well as non-synonymous SNPs in exons 1 and 3. Polymorphic residues in exons 1 and 3, coding for the leader peptide and beta-2 domain, could impact protein stability and binding of CD4 co-receptor, respectively (29, 30). *HLA-DPB1*296:01* is present at an overall frequency of greater than 1.5%, showing that even relatively frequent alleles can be missed due to inadequate sequence coverage.

Comparison of HLA-DRB1 and DQB1 allele frequencies of the RV144 samples with other world populations showed significant differences. Closely related populations determined by fewer numbers of significant differences at both loci included Vietnam, China, Taiwan, Mongolia, India and Asian American. Similarly, the most unrelated populations included European American, African American and Hispanic American. Except for one HLA-DRB1 allele, there were no significant differences in allele frequencies based on region of origin within Thailand for the RV144 cohort. Hence, haplotype frequencies were computed for the overall RV144 samples. The common bi-locus DRB1-DQB1 haplotypes were similar to previous findings (7, 28). The two most common three-locus haplotypes observed have previously been reported in the Han Chinese population (16). The most frequent five-locus haplotypes that included both HLA class I and II loci except HLA-DPB1 were comparable to previous data from Thailand and other Asian populations (7, 31). This is the first complete report of multi-locus haplotypes including HLA-DPB1 alleles from the Thai population. From the combined HLA data presented here and elsewhere (10) we can conclude that the RV144 cohort is representative of the Thai population. The current study provides comprehensive high-resolution HLA class II allele and haplotype frequency data and sets the stage for investigators interested in pursuing HLA disease association studies in both the RV144 and other Thai cohorts in the future.

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TABLE 1

HLA-DRB1 allele frequencies in the RV144 individuals[a]

				By region of origin[d]					
		Overall[c] (2*N*=900)		Central (2*N*=614)		Northeastern (2*N*=200)		Northern (2*N*=74)	
Allele	**G Grouping[b]**	**2*N***	**AF**	**2*N***	**AF**	**2*N***	**AF**	**2*N***	**AF**
*DRB1*01:01*	*DRB1*01:01:01G*	3	0.003	0	0.000	0	0.000	3	**0.041**
*DRB1*03:01*	*DRB1*03:01:01G*	46	0.051	30	0.049	12	0.060	4	0.054
*DRB1*04:03*		15	0.017	10	0.016	2	0.010	3	0.041
*DRB1*04:04*		4	0.004	4	0.007	0	0.000	0	0.000
*DRB1*04:05*		47	0.052	33	0.054	13	0.065	1	0.014
*DRB1*04:06*	*DRB1*04:06:01G*	9	0.010	5	0.008	2	0.010	1	0.014
*DRB1*04:10*		1	0.001	1	0.002	0	0.000	0	0.000
*DRB1*04:87*		1	0.001	0	0.000	1	0.005	0	0.000
*DRB1*07:01*	*DRB1*07:01:01G*	94	0.104	69	0.112	19	0.095	2	0.027
*DRB1*08:03*		13	0.014	10	0.016	3	0.015	0	0.000
*DRB1*08:19*		1	0.001	1	0.002	0	0.000	0	0.000
*DRB1*09:01*	*DRB1*09:01:02G*	91	0.101	66	0.108	16	0.080	9	0.122
*DRB1*10:01*		20	0.022	17	0.028	2	0.010	0	0.000
*DRB1*11:01*	*DRB1*11:01:01G*	23	0.026	16	0.026	6	0.030	1	0.014
*DRB1*11:06*	*DRB1*11:06:01G*	16	0.018	9	0.015	5	0.025	2	0.027
*DRB1*12:01*	*DRB1*12:01:01G*	5	0.006	5	0.008	0	0.000	0	0.000
*DRB1*12:02*		150	0.167	104	0.169	25	0.125	20	0.270
*DRB1*13:01*	*DRB1*13:01:01G*	3	0.003	2	0.003	1	0.005	0	0.000
*DRB1*13:02*		9	0.010	8	0.013	1	0.005	0	0.000
*DRB1*13:03*		1	0.001	1	0.002	0	0.000	0	0.000
*DRB1*13:12*		10	0.011	5	0.008	5	0.025	0	0.000
*DRB1*14:01*	*DRB1*14:01:01G*	27	0.030	22	0.036	4	0.020	1	0.014
*DRB1*14:04*		33	0.037	26	0.042	7	0.035	0	0.000
*DRB1*14:05*		7	0.008	3	0.005	2	0.010	2	0.027
*DRB1*14:10*		4	0.004	4	0.007	0	0.000	0	0.000
*DRB1*14:18*		1	0.001	1	0.002	0	0.000	0	0.000
*DRB1*14:22*		2	0.002	2	0.003	0	0.000	0	0.000
*DRB1*15:01*	*DRB1*15:01:01G*	77	0.086	49	0.080	18	0.090	7	0.095
*DRB1*15:02*		130	0.144	73	0.119	43	0.215	14	0.189
*DRB1*15:03*	*DRB1*15:03:01G*	2	0.002	2	0.003	0	0.000	0	0.000
*DRB1*15:04*		6	0.007	6	0.010	0	0.000	0	0.000
*DRB1*15:06*		2	0.002	1	0.002	1	0.005	0	0.000
*DRB1*16:02*		45	0.050	29	0.047	12	0.060	4	0.054

AF, allele frequency.

[a] Bold values: *p*-value threshold of <0.05 compared with the other two groups.

[b] Nomenclature used for alleles that were ambiguous for exon 2 by four-digit HLA typing.

[c] Based on 4-region classification.

[d] Southern region was excluded due to small sample size (2*N*=12).

TABLE 2

HLA-DQB1 allele frequencies in the RV144 individuals

				By region of origin[c]					
		Overall[b] (2*N*=900)		Central (2*N*=614)		Northeastern (2*N*=200)		Northern (2*N*=74)	
Allele	**G Grouping[a]**	**2*N***	**AF**	**2*N***	**AF**	**2*N***	**AF**	**2*N***	**AF**
*DQB1*02:01/02*	*DQB1*02:01:01G*	125	0.139	84	0.137	30	0.150	6	0.081
*DQB1*02:07*		1	0.001	1	0.002	0	0.000	0	0.000
*DQB1*03:01*	*DQB1*03:01:01G*	168	0.187	115	0.187	32	0.160	20	0.270
*DQB1*03:02*	*DQB1*03:02:01G*	32	0.036	22	0.036	6	0.030	4	0.054
*DQB1*03:03*	*DQB1*03:03:02G*	108	0.120	81	0.132	18	0.090	9	0.122
*DQB1*04:01*	*DQB1*04:01:01G*	33	0.037	24	0.039	7	0.035	1	0.014
*DQB1*04:02*	*DQB1*04:02:01G*	10	0.011	6	0.010	4	0.020	0	0.000
*DQB1*05:01*	*DQB1*05:01:01G*	122	0.136	74	0.121	33	0.165	14	0.189
*DQB1*05:02*	*DQB1*05:02:01G*	156	0.173	101	0.165	41	0.205	13	0.176
*DQB1*05:03*	*DQB1*05:03:01G*	50	0.056	38	0.062	9	0.045	2	0.027
*DQB1*06:01*	*DQB1*06:01:01G*	71	0.079	51	0.083	14	0.070	4	0.054
*DQB1*06:02*	*DQB1*06:02:01G*	10	0.011	7	0.011	2	0.010	1	0.014
*DQB1*06:03*	*DQB1*06:03:01G*	3	0.003	2	0.003	1	0.005	0	0.000
*DQB1*06:04*	*DQB1*06:04:01G*	4	0.004	3	0.005	1	0.005	0	0.000
*DQB1*06:09*	*DQB1*06:09:01G*	5	0.006	5	0.008	0	0.000	0	0.000

AF, allele frequency.

[a] Nomenclature used for alleles that were ambiguous for exon 2 by four-digit HLA typing.

[b] Based on 4-region classification.

[c] Southern region was excluded due to small sample size (2*N*=12).

TABLE 3

HLA-DPB1 allele frequencies in the RV144 individuals

			By region of origin[b]					
	Overall[a] (2*N*=900)		Central (2*N*=614)		Northeastern (2*N*=200)		Northern (2*N*=74)	
Allele	**2*N***	**AF**	**2*N***	**AF**	**2*N***	**AF**	**2*N***	**AF**
*DPB1*01:01:01*	22	0.024	16	0.026	5	0.025	1	0.014
*DPB1*02:01:02*	83	0.092	66	0.108	8	0.040	8	0.108
*DPB1*02:02*	42	0.047	29	0.047	10	0.050	3	0.041
*DPB1*03:01:01*	50	0.056	33	0.054	13	0.065	3	0.041
*DPB1*04:01:01*	112	0.124	76	0.124	24	0.120	11	0.149
*DPB1*04:02:01*	26	0.029	14	0.023	7	0.035	4	0.054
*DPB1*05:01:01*	201	0.223	142	0.231	36	0.180	21	0.284
*DPB1*09:01:01*	9	0.010	8	0.013	0	0.000	1	0.014
*DPB1*10:01*	4	0.004	2	0.003	0	0.000	1	0.014
*DPB1*13:01:01*	151	0.168	97	0.158	45	0.225	8	0.108
*DPB1*14:01*	42	0.047	27	0.044	12	0.060	2	0.027
*DPB1*17:01*	18	0.020	10	0.016	4	0.020	1	0.014
*DPB1*19:01*	4	0.004	2	0.003	2	0.010	0	0.000
*DPB1*21:01*	34	0.038	22	0.036	11	0.055	1	0.014
*DPB1*23:01:01*	2	0.002	2	0.003	0	0.000	0	0.000
*DPB1*26:01:02*	4	0.004	3	0.005	0	0.000	1	0.014
*DPB1*27:01*	1	0.001	1	0.002	0	0.000	0	0.000
*DPB1*30:01*	2	0.002	2	0.003	0	0.000	0	0.000
*DPB1*31:01*	15	0.017	9	0.015	5	0.025	1	0.014
*DPB1*48:01*	1	0.001	1	0.002	0	0.000	0	0.000
*DPB1*93:01*	1	0.001	0	0.000	1	0.005	0	0.000
*DPB1*104:01*	10	0.011	8	0.013	2	0.010	0	0.000
*DPB1*105:01*	13	0.014	10	0.016	2	0.010	1	0.014
*DPB1*107:01*	29	0.032	19	0.031	5	0.025	5	0.068
*DPB1*135:01*	8	0.009	5	0.008	3	0.015	0	0.000
*DPB1*296:01*	14	0.016	10	0.016	3	0.015	1	0.014

AF, allele frequency.

[a] Based on 4-region classification.

[b] Southern region was excluded due to small sample size (2*N*=12).

TABLE 4

Comparison of HLA-DRB1 allele frequencies between RV144 individuals and other populations[a]

Allele	G Grouping[b]	RV144 2*N*=900	Northeast Thais (7) 2*N*=800	Southern China (Han) (16) 2*N*=528	Taiwan (17) 2*N*=728	Mongolia (Khalkha) (18) 2*N*=200	Vietnam (19) 2*N*=340	Japan (20) 2*N*=742	South Korea (21) 2*N*=970	India (Delhi) (22) 2*N*=188	EUR (23) 2*N*=12792	AFA (23) 2*N*=3734	API (23) 2*N*=2454	HIS (23) 2*N*=2936
*DRB1*01:01*	*DRB1*01:01:01G*	0.003	0.001[c]	0.004	0.003	0.025		**0.065**	0.068	0.005	**0.091**	**0.026**	**0.027**	**0.039**
*DRB1*03:01*	*DRB1*03:01:01G*	0.051	0.061	0.066	0.067	0.090	0.047		0.029	**0.122**	**0.129**	0.071	0.054	0.073
*DRB1*04:03*		0.017	0.016	0.044	0.012	0.025	0.021	0.040	0.035	0.047	**0.006**	**0.002**	0.035	0.019
*DRB1*04:04*		0.004	0.003	0.006	0.005		0.003	0.001	**0.022**	0.026	**0.036**	0.007	0.009	**0.055**
*DRB1*04:05*		0.052	0.059	0.042	0.059	0.050	0.038	**0.115**	**0.092**	**0.005**	**0.004**	**0.010**	0.058	**0.019**
*DRB1*04:06*	*DRB1*04:06:01G*	0.010	0.004	0.021	0.029		0.006	**0.035**	**0.041**	0.005		**0.001**	0.021	0.002
*DRB1*04:10*		0.001		0.002				**0.018**	0.010			0.001	0.004	0.005
*DRB1*04:87*		0.001												
*DRB1*07:01*	*DRB1*07:01:01G*	0.104	**0.059**[c]	0.057	**0.045**	0.120	0.076	**0.003**	0.072	0.175	0.138	0.098	0.082	0.105
*DRB1*08:03/19*		0.015	0.014[c]	**0.059**	**0.098**	0.025	0.041	**0.081**	**0.080**		**0.001**		**0.052**	**0.001**
*DRB1*09:01*	*DRB1*09:01:02G*	0.101	0.071[c]	0.144	**0.194**	0.095	0.097	0.124	0.092	**0.011**	**0.008**	**0.032**	0.102	**0.010**
*DRB1*10:01*		0.022	0.024	0.011	0.011	0.040	0.056	0.009	0.017	0.048	**0.008**	0.019	0.031	0.015
*DRB1*11:01*	*DRB1*11:01:01G*	0.026	0.026	**0.070**	**0.099**	0.065	0.015	0.034	0.044	0.059	**0.057**	**0.087**	**0.051**	0.042
*DRB1*11:06*	*DRB1*11:06:01G*	0.018	0.026			0.005			**0.001**				**0.003**	
*DRB1*12:01*	*DRB1*12:01:01G*	0.006		**0.028**	**0.037**	0.025	0.009	**0.038**	**0.033**	0.005	0.015	**0.040**	**0.029**	0.012
*DRB1*12:02*		0.167	0.134	0.133	**0.098**	**0.045**	**0.353**	**0.015**	**0.033**	**0.011**	**0.000**	**0.003**	**0.074**	**0.001**
*DRB1*13:01*	*DRB1*13:01:01G*	0.003	0.004	0.009	0.001	**0.035**	0.006	0.007	0.009	**0.059**	**0.063**	**0.056**	**0.024**	**0.042**
*DRB1*13:02*		0.010	0.010	0.028	0.026	0.035	0.018	**0.077**	**0.087**	**0.059**	**0.040**	**0.064**	**0.036**	**0.039**
*DRB1*13:03*		0.001	**0.019**				0.018			0.016	0.010	**0.037**		**0.015**
*DRB1*13:12*		0.011		0.008	0.007								0.004	
*DRB1*14:01*	*DRB1*14:01:01G*	0.030	0.034	0.017	0.037	0.045	0.032	0.042	0.030	0.027	0.025	0.021	0.024	0.014
*DRB1*14:04*		0.037	0.028	**0.002**	**0.003**		0.018			0.074			0.020	
*DRB1*14:05*		0.008	0.004	0.019	0.008		0.012	0.011	**0.035**				0.018	
*DRB1*14:10*		0.004							0.002					
*DRB1*14:18*		0.001			0.001								0.001	
*DRB1*14:22*		0.002												

Allele	G Grouping[b]	RV144 2*N*=900	Northeast Thais (7) 2*N*=800	Southern China (Han) (16) 2*N*=528	Taiwan (17) 2*N*=728	Mongolia (Khalkha) (18) 2*N*=200	Vietnam (19) 2*N*=340	Japan (20) 2*N*=742	South Korea (21) 2*N*=970	India (Delhi) (22) 2*N*=188	EUR (23) 2*N*=12792	AFA (23) 2*N*=3734	API (23) 2*N*=2454	HIS (23) 2*N*=2936
*DRB1*15:01*	*DRB1*15:01:01G*	0.086	0.078	0.108	0.091	0.060	0.038	0.085	0.074	0.059	**0.144**	**0.029**	0.079	0.067
*DRB1*15:02*		0.144	0.183	**0.053**	**0.023**	**0.035**	**0.062**	0.100	**0.033**	**0.059**	**0.008**	**0.002**	**0.081**	**0.013**
*DRB1*15:03*	*DRB1*15:03:01G*	0.002										**0.117**	0.001	**0.011**
*DRB1*15:04*		0.007											0.001	
*DRB1*15:06*		0.002								0.021			0.004	
*DRB1*16:02*		0.050	0.075	0.053	0.025	**0.005**	0.018	**0.009**	**0.006**	0.011	**0.001**	**0.015**	**0.020**	**0.021**

AFA, African American; API, Asian American; EUR, European American; HIS, Hispanic American.

[a] Bold values: Significant at a *p*-value threshold of 0.05.

[b] Nomenclature used for alleles that were ambiguous for exon 2 by four-digit HLA typing.

[c] Reported as two-digit allele.

TABLE 5

Comparison of HLA-DQB1 allele frequencies between RV144 individuals and other populations[a]

Allele	G Grouping[b]	RV144 2*N*=900	Northeast Thais (7) 2*N*=800	Southern China (Han) (16) 2*N*=528	Taiwan (17) 2*N*=366	Mongolia (Khalkha) (18) 2*N*=200	Vietnam (19) 2*N*=340	Japan (20) 2*N*=742	South Korea (21) 2*N*=970	India (Delhi) (22) 2*N*=224	EUR (23) 2*N*=12792	AFA (23) 2*N*=1208	API (23) 2*N*=1172	HIS (23) 2*N*=1074
*DQB1*02:01 & 02:07*[c]	*DQB1*02:01:01G & 02:07*	0.140	0.109	0.121	0.126	0.175	0.091	**0.003**	**0.094**	**0.255**	**0.230**	**0.223**	0.109	0.185
*DQB1*03:01*	*DQB1*03:01:01G*	0.187	0.141	0.242	0.238	0.255	**0.400**	**0.113**	**0.135**	0.134	0.185	0.174	0.194	0.200
*DQB1*03:02*	*DQB1*03:02:01G*	0.036	0.025	0.070	0.036	0.040	0.024	**0.108**	**0.103**	0.046	**0.095**	0.037	**0.076**	**0.189**
*DQB1*03:03*	*DQB1*03:03:02G*	0.120	0.085	0.150	0.164	0.135	0.126	0.135	0.114	**0.032**	**0.045**	**0.016**	0.122	**0.021**
*DQB1*04:01*	*DQB1*04:01:01G*	0.037	0.045	0.040	0.060	0.050	0.035	**0.115**	**0.088**	0.005			0.045	
*DQB1*04:02*	*DQB1*04:02:01G*	0.011	0.010	0.008	0.003	**0.045**	0.006	**0.039**	**0.037**	0.005	0.025	**0.068**	0.023	**0.091**
*DQB1*05:01*	*DQB1*05:01:01G*	0.136	0.163	**0.034**	**0.038**	0.075	0.118	**0.075**	0.093	0.074	0.123	0.154	**0.074**	0.110
*DQB1*05:02*	*DQB1*05:02:01G*	0.173	0.203	**0.110**	**0.082**	**0.035**	**0.065**	**0.035**	**0.022**	**0.041**	**0.013**	**0.027**	**0.074**	**0.016**
*DQB1*05:03*	*DQB1*05:03:01G*	0.056	0.038	0.030	0.030	**0.010**	0.035	0.027	0.051	**0.134**	**0.025**	**0.015**	0.062	**0.013**
*DQB1*06:01*	*DQB1*06:01:01G*	0.079	0.075	0.112	**0.153**	0.055	0.065	**0.183**	0.096	0.094	**0.008**	**0.001**	**0.119**	**0.014**
*DQB1*06:02*	*DQB1*06:02:01G*	0.011	0.010	**0.038**	0.030	**0.050**	0.012	**0.082**	**0.071**	0.009	**0.143**	**0.198**	**0.044**	**0.081**
*DQB1*06:03*	*DQB1*06:03:01G*	0.003	0.004	0.009	0.003	**0.035**	0.006	0.007	0.009	**0.060**	**0.065**	**0.023**	**0.023**	**0.041**
*DQB1*06:04*	*DQB1*06:04:01G*	0.004	0.011[d]	0.008	0.003	0.025	0.003	**0.073**	**0.051**	0.018	**0.032**	**0.020**	0.014	**0.028**
*DQB1*06:09*	*DQB1*06:09:01G*	0.006		0.019	**0.033**	0.015		0.004	**0.037**		0.008	**0.035**	0.019	0.006

AFA, African American; API, Asian American; EUR, European American; HIS, Hispanic American.

[a] Bold values: Significant at a *p*-value threshold of 0.05.

[b] Nomenclature used for alleles that were ambiguous for exon 2 by four-digit HLA typing.

[c] Combined frequency of all *DQB1*02* subtypes.

[d] Reported as *DQB1*06(04-06)*.

TABLE 6

Estimated two[a] and three-locus haplotypes in the RV144 individuals with frequencies greater than 1%

DRB1-DQB1-DPB1	Frequency	DRB1-DQB1	Frequency	DRB1-DPB1	Frequency	DQB1-DPB1	Frequency
15:02-05:01:01G-13:01:01	0.069	12:02-03:01:01G	0.125	15:02-13:01:01	0.068	05:01:01G-13:01:01	0.069
09:01:02G-03:03:02G-05:01:01	0.053	15:02-05:01:01G	0.109	09:01:02G-05:01:01	0.052	05:02:01G-05:01:01	0.064
16:02-05:02:01G-05:01:01	0.030	09:01:02G-03:03:02G	0.102	16:02-05:01:01	0.030	03:03:02G-05:01:01	0.055
03:01:01G-02:01:01G-04:01:01	0.028	07:01:01G-02:01:01G	0.088	12:02-13:01:01	0.029	02:01:01G-04:01:01	0.048
12:02-03:01:01G-05:01:01	0.024	15:01:01G-06:01:01G	0.052	07:01:01G-04:01:01	0.028	04:01:01G-05:01:01	0.017
07:01:01G-02:01:01G-04:01:01	0.024	16:02-05:02:01G	0.050	03:01:01G-04:01:01	0.025	02:01:01G-17:01	0.015
04:05-04:01:01G-05:01:01	0.016	03:01:01G-02:01:01G	0.049	12:02-21:01	0.020	03:02:01G-02:01:02	0.014
12:02-03:01:01G-13:01:01	0.016	12:02-05:02:01G	0.041	15:02-14:01	0.019	03:01:01G-21:01	0.014
12:02-03:01:01G-02:01:02	0.016	04:05-04:01:01G	0.036	14:01:01G-05:01:01	0.019	05:03:01G-01:01:01	0.012
14:01:01G-05:02:01G-05:01:01	0.015	14:04-05:03:01G	0.034	07:01:01G-17:01	0.013	02:01:01G-04:02:01	0.011
15:02-05:01:01G-05:01:01	0.014	15:01:01G-05:02:01G	0.026	07:01:01G-04:02:01	0.011		
07:01:01G-02:01:01G-04:02:01	0.013	11:01:01G-03:01:01G	0.023				
12:02-03:01:01G-03:01:01	0.013	10:01-05:01:01G	0.022				
12:02-03:01:01G-21:01	0.012	14:01:01G-05:02:01G	0.022				
07:01:01G-02:01:01G-17:01	0.011	11:06:01G-03:01:01G	0.018				
12:02-05:02:01G-13:01:01	0.011	04:03-03:02:01G	0.017				
12:02-05:02:01G-21:01	0.011	08:03-06:01:01G	0.015				
		13:12-03:01:01G	0.011				

LD, Linkage disequilibrium.

[a]Haplotypes in significant LD.

TABLE 7

Estimated five and six-locus haplotypes in the RV144 individuals with frequencies greater than 1%

A-B-C-DRB1-DQB1	Frequency	A-B-C-DRB1-DQB1-DPB1	Frequency
02:07:01G-46:01:01G-01:02:01G-09:01:02G-03:03:02G	0.039	02:07-46:01:01G-01:02:01G-09:01:02G-03:03:02G-05:01:01	0.025
33:03:01G-44:03:01G-07:01:01G-07:01:01G-02:01:01G	0.037	33:03:01G-44:03:01G-07:01:01G-07:01:01G-02:01:01G-04:01:01	0.016
33:03:01G-58:01:01G-03:02:01G-03:01:01G-02:01:01G	0.026	33:03:01G-58:01:01G-03:02:01G-03:01:01G-02:01:01G-04:01:01	0.014
11:01:01G-15:02:01G-08:01:01G-12:02-03:01:01G	0.025	33:03:01G-44:03:01G-07:01:01G-07:01:01G-02:01:01G-13:01:01	0.011
11:01:01G-46:01:01G-01:02:01G-09:01:02G-03:03:02G	0.019	30:01:01G-13:02:01G-06:02:01G-07:01:01G-02:01:01G-17:01	0.011
30:01:01G-13:02:01G-06:02:01G-07:01:01G-02:01:01G	0.018		
24:07-15:02:01G-08:01:01G-15:01:01G-06:01:01G	0.012		