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ORIGINAL PAPER

Characterization of the major histocompatibility complex class II *DOB, DPB1*, and *DQB1* alleles in cynomolgus macaques of Vietnamese origin

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Abstract Major histocompatibility complex (MHC) molecules play an important role in the susceptibility and/or resistance to many diseases. To gain an insight into the MHC background and to facilitate the experimental use of cynomolgus macaques, the second exon of the *MhcMafa-DOB*, *-DPB1*, and *-DQB1* genes from 143 cynomolgus macaques were characterized by cloning to sequencing. A total of 16 *Mafa-DOB*, 16 *Mafa-DPB1*, and 34 *Mafa-DQB1* alleles were identified, which revealed limited, moderate, and marked allelic polymorphism at *DOB*, *DPB1*, and *DQB1*, respectively, in a cohort of cynomolgus macaques of Vietnamese origin. In addition, 16 *Mafa-DOB*, 5 *Mafa-DPB1*, and 8 *Mafa-DQB1* alleles represented novel sequences that had not been reported in earlier studies. Almost of the sequences detected at the DOB and DQB1 locus in the present study belonged to *DOB*01* (100%) and *DQB1*06* (62%) lineages, respectively. Interestingly, four, three, and one high-frequency alleles were detected at *Mafa-DOB*, *-DPB1*, and *-DQB1*, respectively, in this monkeys. The alleles with the highest frequency among these monkeys were *Mafa-DOB*010102*, *Mafa-DPB1*13*, and *Mafa-DQB1*0616*, and these were found in 33 (25.6%) of 129 monkeys, 32 (31.37%) of 102 monkeys, and 30 (31%) of 143 monkeys, respectively. The high-frequency alleles may represent high priority targets for additional characterization of immune function. We also carried out evolutionary and population analyses using these sequences to reveal population-specific alleles. This information will not only promote the understanding of MHC diversity and polymorphism in the cynomolgus macaque but will also increase the value of this species as a model for biomedical research.

Keywords High frequency · Major histocompatibility complex class II · *Macaca fascicularis*

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Introduction

Rhesus macaques have been used as animal models for various human diseases for a long time. With the 1978 ban on exportation of Rhesus macaques from India, researchers have become increasingly interested in an alternative macaque, the cynomolgus macaque, which has a shorter breeding cycle, a docile personality, and requires lower dosages of drugs. The cynomolgus macaque (*Macaca fascicularis*), also known as the crab-eating monkey or long-tailed macaque, is used mainly in animal models of diabetes, renal transplantation, virological research, SARS, tuberculosis, studies of the pathogenesis of simian immunodeficiency virus (SIV), and pharmacodynamic evaluation (O'Sullivan et al. 1997; Menninger et al. 2002; McAuliffe et al. 2004; Reed et al. 2009). Owing to the need for reliable data on experimental drug reactions provided by animal models, researchers have focused on genes of the immune system of cynomolgus monkeys, in particular, the genes of the major histocompatibility complex (MHC). Molecules of MHC class I and II play an important role in immune regulatory processes by presenting peptides of

intracellular or extracellular origin to $CD8^+$ or $CD4^+$ T cells, respectively.

The classical MHC genes of cynomolgus macaques can be divided into MHC-I and MHC-II genes. The MHC-I genes include mainly *-A* and *-B* alleles, and MHC-II genes include mainly *-DM*, *-DO*, *-DP*, *-DQ*, and *-DR* alleles. DO is a non-classical class II heterodimer that consists of α and β chains, which are encoded by the *DOA* and *DOB* genes located in the MHC class II region. The function of MHC-DO is poorly understood; it may act as a negative regulator by binding to HLA-DM and inhibiting the exchange reaction of class II-associated invariant chain peptides for antigenic peptides (Fernandez-Donoso et al. 1970; O'Sullivan et al. 1997). *HLA-DPB1* alleles have been demonstrated to be involved in corneal and renal transplantation, myasthenia gravis, multiple sclerosis, Hodgkin's disease, Beryllium disease, and sarcoidosis. In addition, an *MHC-DPB1* allele was found to be involved in the susceptibility to experimental autoimmune encephalomyelitis in rhesus macaques (Slierendregt et al. 1995a, b). Thus, the primate MHC-DPB1 plays a fundamental and important role in the peptide-binding selectivity of the DP antigen (Uda et al. 2005) and is a significant factor in the humoral response (Krebs et al. 2005). It has been reported that *HLA-DQB1* alleles have been associated as an increased risk of developing type 1 diabetes (Todd 1990; Todd 1997; Redondo et al. 2001), celiac disease (Murray et al. 2007), multiple sclerosis (Dyment et al. 1997; Schmidt et al. 2007, and narcolepsy (Kadotani et al. 1998).

It is not easy to elucidate the mechanism of naturally occurring immune protection in human immunodeficiency virus (HIV), and most direct supporting data are available from animal models in non-human primates. The SIVmac virus, which was isolated originally from cynomolgus monkeys, is now used frequently for research on vaccines against acquired immune deficiency syndrome (AIDS; Hu 2005). Many reports show that polymorphism of MHC genes in the cynomolgus monkey affects the results obtained with drugs significantly (Walsh et al. 1996; Mothe et al. 2003; Hao and Nei 2005) and is associated with control of viral diseases (Florese et al. 2008). Cynomolgus macaques from Mauritius may be particularly valuable because more than half of these animals share the MHC class I allele combination *Mafa-B*430101*, *Mafa-B*440101*, and *Mafa-B*460101*. The increased sharing of MHC-I allele in cynomolgus macaques of Mauritian origin may reduce the overall number of animals needed to study cellular immune responses in non-human primates dramatically, while simultaneously reducing the confounding effects of genetic heterogeneity in HIV/AIDS research (Krebs et al. 2005).

The MHC class II molecules of Rhesus macaques (*Macaca mulatta*) have been studied methodically, especially in animals of Indian origin. In contrast to Rhesus macaques, although alleles of the Mafa II class molecules, including *Mafa-DPB1* and *Mafa-DQB1*, have been identified by several groups (Otting et al. 2002; Doxiadis et al. 2006; Blancher et al. 2006; O'Connor et al. 2007), knowledge is limited of the MHC class II genes of the cynomolgus monkey and their degree of polymorphism. In addition, a more detailed study of the *Mafa-DPB1* sequence in three different geographical variants of cynomolgus monkeys from south-east Asia demonstrated that polymorphism of MHC-II genes is influenced by species and geography (Sano et al. 2006). It is very rare that cynomolgus macaques from different regions share MHC-II genes (Krebs et al. 2005).

Haplotype screening, which employs multiple markers rather than single genes, would be meaningful in MHC disease association studies because it is well-known that most of the MHC loci are tightly linked and exhibit very little recombination (Dukkipati et al. 2006). The high-frequency alleles may represent high priority targets for additional characterization of immune function (Wiseman et al. 2009). Therefore, to examine whether the MHC class II genes found in a cohort of Vietnamese cynomolgus macaques are common to for other geographical populations of cynomolgus macaques, the Mafa class II region was characterized in

Table 1 Primers used to amplify MHC class II alleles

Locus	Primer name	Sequence (5′ to 3′)	T_m (°C)	Product (bp)
DOB	DOB-F	GTAGCATTATTTCCCTTT	50	600
	DOB-R	ACAGACAACCGTTTATCC		
DPB1	DPB1-F1	CACAGAACTCGGTACTAGGAAA	52	700
	DPB1-R1	CTCAGGAACCTCAAACCC		
	DPB1-F2	CCTGAGTGGGAAGATTTG	55.5	588
	DPB1-R2	TCTCTCTGCTCCCATCCT		
DQB1	DQB1-F1	CACTGGTGAGCGGGAACT	52	700
	DQB1-R1	GGAGGCAAACGCATAAGG		
	DQB1-F2	CCCGCAGAGGATTTCGTG	62	250
	DQB1-R2	GGCGACGATGCTCACCTC		

Table 2 *MhcMafa-DOB* alleles identified in 129 randomly sampled cynomolgus macaques

Sequence no.	Accession no.	Allele name	No. of haplotypes	Gene frequency
O1	HM152983	*Mafa-DOB*010101*	11	0.085
O2	HM152984	*Mafa-DOB*010102*	33	0.256
O3	HM152985	*Mafa-DOB*010103*	17	0.132
O4	HM152986	*Mafa-DOB*010104*	14	0.109
O5	HM152987	*Mafa-DOB*010401*	20	0.155
O6	HM152988	*Mafa-DOB*010202*	12	0.093
O7	HM152989	*Mafa-DOB*010301*	6	0.047
O8	HM152990	*Mafa-DOB*010302*	1	0.008
O9	HM152991	*Mafa-DOB*010402*	4	0.031
O10	HM152992	*Mafa-DOB*010105*	3	0.023
O11	HM152993	*Mafa-DOB*010201*	1	0.008
O12	HM152994	*Mafa-DOB*010203*	2	0.016
O13	HM152995	*Mafa-DOB*010303*	1	0.008
O14	HM152996	*Mafa-DOB*0105*	1	0.008
O15	HM152997	*Mafa-DOB*010304*	2	0.016
O16	HM152998	*Mafa-DOB*010204*	1	0.008

the present study by sequencing of the polymorphic exon 2 of the *-DOB*, *-DPB1*, and *-DQB1* genes.

Materials and methods

Animals

Whole blood samples from 150 unrelated cynomolgus macaques, originally from Vietnam, were provided generously by South China Primates Research Central. Whole blood samples (3–5 ml) withdrawn from each monkey were collected into EDTA vacuum tubes. All the monkeys were clinically normal with no known diseases.

DNA isolation and sequencing of exon 2 of Mafa-DOB, -DPB1, and -DQB1

Genomic DNA was extracted from EDTA blood samples using an Animal Genomics DNA Mini Preparation Kit (NewProbe, China) as per the manufacturer's instructions. Sequences of exon 2 regions of *Mafa-DOB*, *-DPB1*, and

Table 3 *MhcMafa-DPB* alleles identified in 102 randomly sampled cynomolgus macaques

Sequence no.	Accession no.	Allele name	No. of haplotypes	Gene frequency
P1	HM153018	Mafa-DPB1*35	11	0.108
P2	HM153016	Mafa-DPB1*20	6	0.059
P3	HM153013	*Mafa-DPB1*51*	2	0.020
P4	HM153019	Mafa-DPB1*21	14	0.137
P5	HM153017	Mafa-DPB1*19	1	0.010
P6	HM153015	Mafa-DPB1*40	9	0.088
P7	HM153014	Mafa-DPB1*50	3	0.029
P8	HM371244	*Mafa-DPB1*52*	4	0.039
P9	HM371245	Mafa-DPB1*13	32	0.314
P10	HM371246	*Mafa-DPB1*53*	3	0.029
P11	HM371247	*Mafa-DPB1*54*	1	0.010
P12	HM371248	Mafa-DPB1*24	6	0.059
P13	HM371249	*Mafa-DPB1*55*	1	0.010
P14	HM371250	Mafa-DPB1*44	2	0.020
P15	HM371251	Mafa-DPB1*32	1	0.010
P16	HM371252	Mafa-DPB1*17	6	0.059

-DQB1 were obtained by direct sequencing of polymerase chain reaction (PCR) products according to the following procedures: the PCR amplification was performed in 50 μL reaction mixtures containing 25 μL of 2× Taq Plus PCR Master Mix, 1 μL (10 pm/μL) of each primer, 1 μL of template DNA, and 22 μL of ddH_2O. The sequences of the *DOB*, *DPB1*, and *DQB1* primers are shown in Table 1. In general, amplification was carried out for 3 min at 94°C, 32 cycles of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C, ending with 3 min at 72°C. The annealing temperature was adjusted on the basis of the T_m of the primers. The PCR products were subjected to agarose gel electrophoresis and ethidium bromide staining for visualization.

Phylogenetic analysis

The sequences of the exon 2 regions of *Mafa-DOB*, *-DPB1*, and *-DQB1* obtained in the present study were aligned, and the phylogenetic tree was generated, which was done using the neighbor-joining method (Saitou and Nei 1987) and Mega 4.0 software (Tamura et al. 2007). The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in fewer than 50% of bootstrap replicates are collapsed. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in

Table 4 *MhcMafa-DQB* alleles identified in 143 randomly sampled cynomolgus macaques

Sequence no.	Accession no.	Allele name	No. of haplotypes	Gene frequency
Q1	HM371224	*Mafa-DQB1*1603*	1	0.007
Q2	HM371225	Mafa-DQB1*1503	3	0.021
Q3	HM371226	Mafa-DQB1*1501	2	0.014
Q4	HM153006	Mafa-DQB1*0601	4	0.028
Q5	HM153000	Mafa-DQB1*2401	4	0.028
Q6	HM371227	Mafa-DQB1*1703	8	0.056
Q7	HM153008	Mafa-DQB1*0611	2	0.014
Q8	HM371228	Mafa-DQB1*0622	2	0.014
Q9	HM153009	Mafa-DQB1*1702	4	0.028
Q10	HM371229	*Mafa-DQB1*0627*	1	0.007
Q11	HM371230	Mafa-DQB1*0623	3	0.021
Q12	HM371231	Mafa-DQB1*1804	8	0.056
Q13	HM371232	Mafa-DQB1*0626	7	0.049
Q14	HM153001	Mafa-DQB1*0619	6	0.042
Q15	HM371233	*Mafa-DQB1*0628*	1	0.007
Q16	HM371234	Mafa-DQB1*0616	30	0.210
Q17	HM371235	Mafa-DQB1*0614	8	0.056
Q18	HM371236	*Mafa-DQB1*0629*	4	0.028
Q19	HM371237	*Mafa-DQB1*0630*	6	0.042
Q20	HM371238	*Mafa-DQB1*1818*	1	0.007
Q21	HM371239	Mafa-DQB1*1809	4	0.028
Q22	HM371240	*Mafa-DQB1*1819*	1	0.007
Q23	HM371241	Mafa-DQB1*1817	3	0.021
Q24	HM371242	Mafa-DQB1*1806	1	0.007
Q25	HM371243	Mafa-DQB1*1601	1	0.007
Q26	HM152999	Mafa-DQB1*1816	12	0.084
Q27	HM153002	Mafa-DQB1*0613	3	0.021
Q28	HM153003	Mafa-DQB1*1810	4	0.028
Q29	HM153004	Mafa-DQB1*0610	3	0.021
Q30	HM153012	Mafa-DQB1*170701	1	0.007
Q31	HM153005	Mafa-DQB1*1802	2	0.014
Q32	HM153007	Mafa-DQB1*060702	1	0.007
Q33	HM153010	*Mafa-DQB1*170802*	1	0.007
Q34	HM153011	Mafa-DQB1*1602	1	0.007

the units of the number of base substitutions per site. The rate of variation among sites was modeled with a gamma distribution (shape parameter = 1). New alleles were confirmed by three repeats sequencing. The names of new sequences were derived according to the published guidelines, and the Immuno Polymorphism Database of Major Histocompatibility Complex for Non-Human Primates was searched to avoid the same name(s) being assigned to different alleles (Klein et al. 1990; Robinson et al. 2005).

Results and discussion

Allele frequencies of Mafa-DOB, -DPB1, and -DQB1 in Vietnamese cynomolgus macaques

Limited polymorphism at the *MHC-DOB* locus and extensive polymorphism at the *MHC-DPB1* and *-DQB1* loci have been reported in the primates tested until now, and most of the variability is confined to exon 2, which encodes a major part of the peptide-binding site.

To analyze the genetic polymorphism and allelic variation in the *MhcMafa-DOB* gene, which may affect the efficiency of class II restricted antigen presentation and therefore be involved in the susceptibility to MHC associated diseases, 16 *Mafa-DOB* alleles were identified in this study, none of which had been described previously in rhesus and/or cynomolgus macaques, by direct sequencing of exon 2 of the *MhcMafa-DOB* gene using blood samples from 129 randomly sampled cynomolgus macaques. These novel sequences were submitted to GenBank and were assigned by the NHP Nomenclature Committee. Their accession numbers are listed in Table 2. All the new sequences are highlighted in italic and boldface type. The novel allele with the highest frequency among these cynomolgus macaques was *Mafa-DOB*010102*, and it was found in 33 (25.6%) of the 129 monkeys, followed by *Mafa-DOB*010401*, *Mafa-DOB*010103*, and *Mafa-DOB*010104*, which were detected in 20 (15.5%), 17 (13.2%), and 14 (10.9%) of the monkeys, respectively. Five alleles presented only once in these monkeys (Table 2). Only six allelic variations (*HLA-DOB*0101101*, *-*0101102*, *-*01012*, *-*01022*, *-*0104101*, and *-*0104102*) were identified until now. It has been demonstrated that strong linkage disequilibrium exists between *HLA-DOB*01022* and *HLA-DRB1*1502*, with no linkage disequilibrium between the *DOA* and the *DOB* genes (Naruse et al. 2002). A new allelic type of *DOB*010103* has been described in the Korean population (Gu et al. 2005). So, limited polymorphism in the *DOB* gene is profitable in the execution of the unique function of its product as a co-chaperone. Therefore, strong selection pressure operates to prevent generic variation in the DOB molecule in its interaction with the DM molecule thus maintaining the specified immunological function of regulating antigen presentation (Naruse et al. 2002; Lith et al. 2002). Ectopic expression of HLA-DO in mouse dendritic cells diminishes MHC class II antigen presentation (Fallas et al. 2004). It has also been demonstrated that a highly significant upregulation of *DOA* and *DOB* mRNA occurs in purified malignant cells, when compared with B cells from healthy donors (Souwer et al. 2009). The increased levels of mRNA were not translated into enhanced protein levels but could reflect aberrant transcriptional regulation, which forms a novel and additional prognostic indicator for survival in B cell chronic lymphocytic leukemia (Souwer et al. 2009).

By direct sequencing of the second exon of the *MhcMafa-DPB1* genes using blood samples from 102 randomly sampled cynomolgus macaques, 16 alleles of *MhcMafa-DPB1* were identified in this study, of which 11 were identical to alleles described formerly in cynomolgus macaques whose sequences could be retrieved from

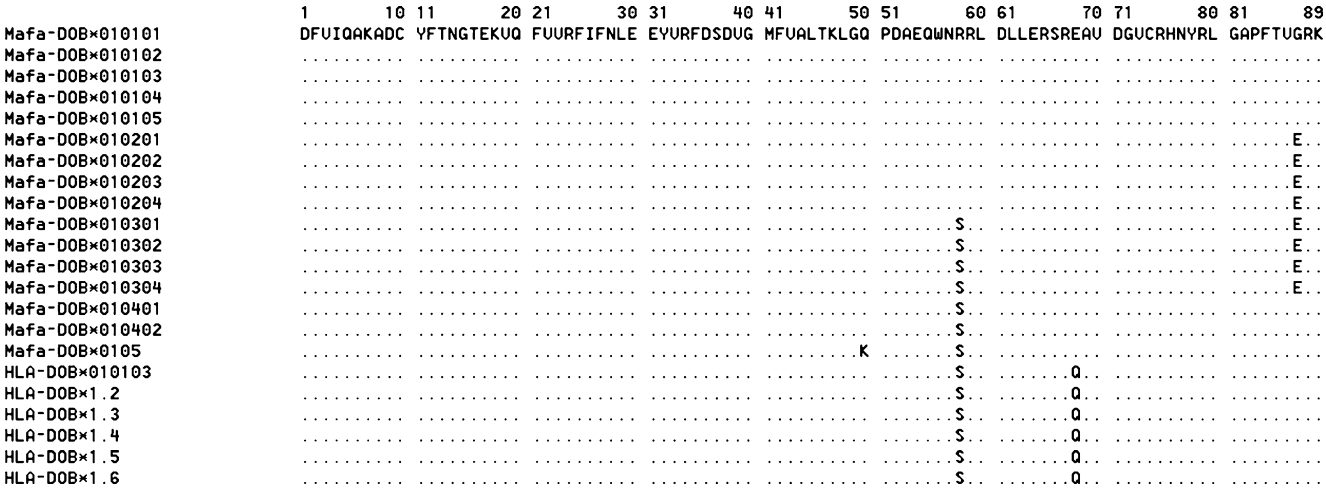

Fig. 1 Alignment of the deduced amino acid sequences of the second exon of 16 cynomolgus macaque *MhcMafa-DOB* alleles and 6 human *HLA-DOB* alleles

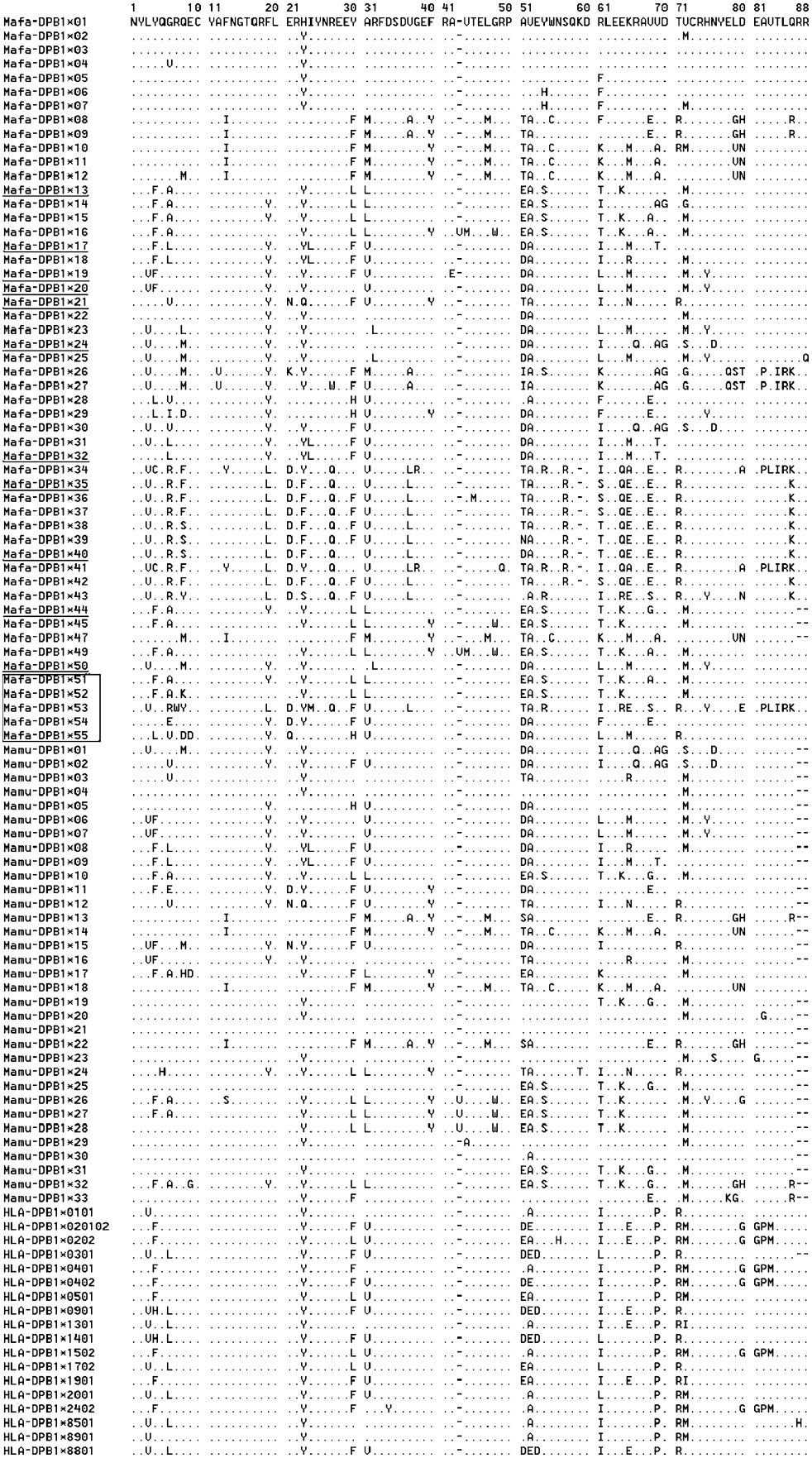

Fig. 2 Alignment of the deduced amino acid sequences of the second exon of 52 *Mafa-DPB1*, 33 *Mamu-DPB1*, and *18 HLA-DPB1* sequences. Five novel alleles were listed in the middle frame; previously reported alleles also detected in this study were indicated by an *underline*

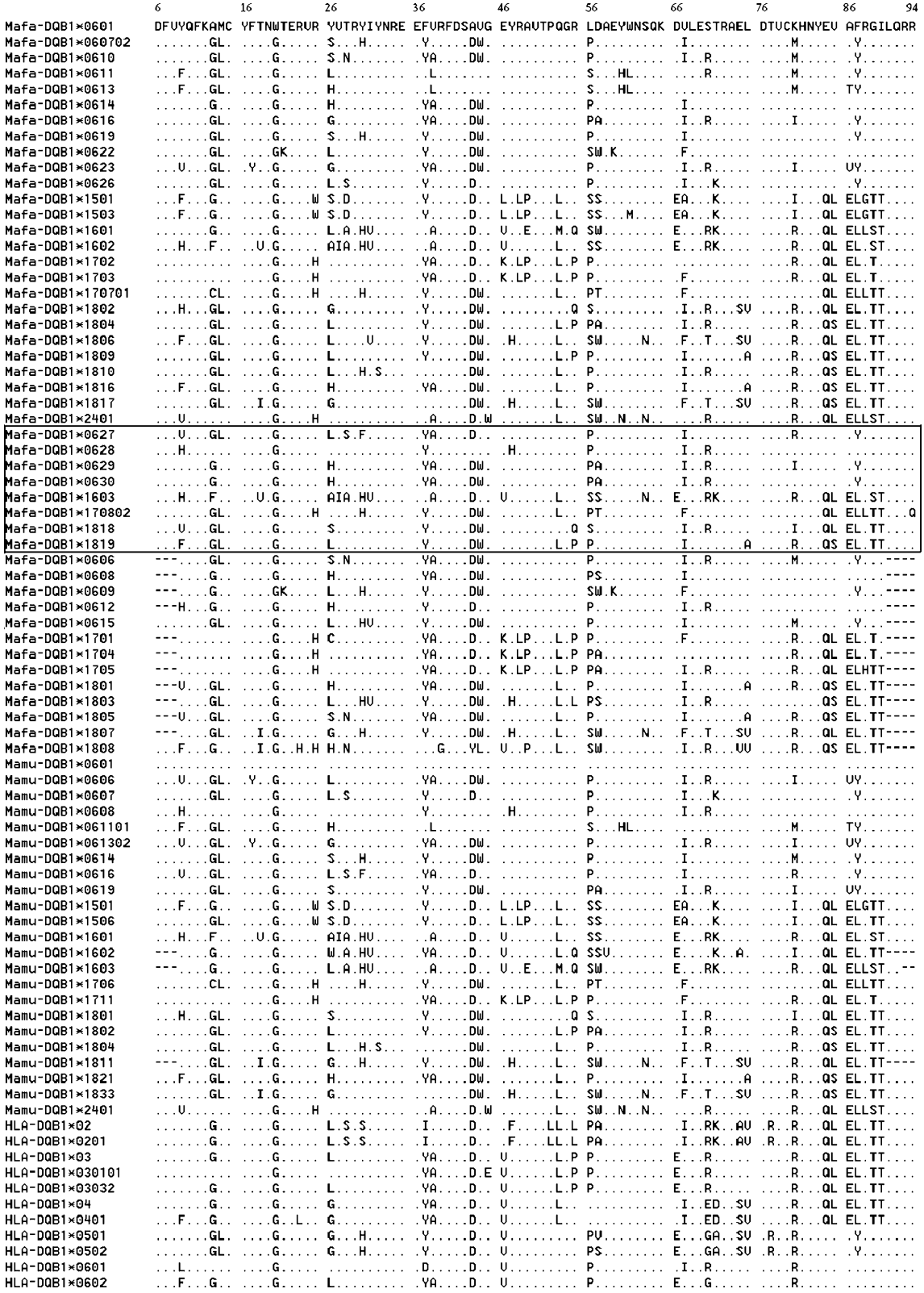

Fig. 3 Alignment of the deduced amino acid sequences of the second exon of 47 *Mafa-DQB1*, 23 *Mamu-DQB1*, and 11 *HLA-DQB1* sequences. Eight novel alleles were listed in the middle frame, 26 sequences common to the earlier studies were listed in the over frame, and other alleles not detected in this study were listed under the frame

GenBank. The other five alleles were not documented in the literatures or databases. These novel sequences were submitted to GenBank and were assigned by the NHP Nomenclature Committee. Their accession numbers are listed in Table 3, in which all the new sequences are highlighted in italic and boldface type. The allele with the highest frequency among these cynomolgus macaques was *DPB1*13*, which was found in 32 (31.37%) of the 102 monkeys. The next most frequent alleles were *Mafa-DPB1*21* and *Mafa-DPB1*35*, which were detected in 14 (13.72%) and 11 (10.77%) of the monkeys. Four alleles presented only once in these monkeys (Table 3). The frequency of the five novel alleles found in this study was less than 4%.

It has been shown that the most frequent alleles in Vietnam cynomolgus macaques are *Mafa-DPB1*13* and *-DPB1*35* (Sano et al. 2006), which supports our results above. In contrast to the result from Sano et al. (Sano et al. 2006), a high frequency of the *Mafa-DPB1*21* allele was detected in our study. The *Mafa-DPB1*21* allele was also observed in Mauritian cynomolgus macaques, but was not at a high frequency (O'Connor et al. 2007). Like *HLA-DPB1*, the *Mafa-DPB1* gene of the cynomolgus macaque also displays moderate polymorphism, and more than 50 alleles have been documented to date (Slierendregt et al. 1995a, b; Otting et al. 1998; Marsh et al. 2005; O'Connor et al. 2007). In cynomolgus macaques, point mutations might play crucial role in generating *DPB1* polymorphism, whereas in humans, much of the variability has been produced by frequent exchange of polymorphic sequence motifs (Zangenberg et al. 1995; Bontrop et al. 1999; Doxiadis et al. 2001).

By direct sequencing of the second exon of *MhcMafa-DQB1* genes using blood samples from 143 randomly sampled cynomolgus macaques, 34 *MhcMafa-DQB1* alleles were identified in this study, of which 26 were identical to alleles described formerly in cynomolgus macaques whose sequences could be retrieved from GenBank. The other eight alleles were not documented in the literatures or databases. These novel sequences were submitted to GenBank and were assigned by the NHP Nomenclature Committee. Their accession numbers are listed in Table 4, in which all the new sequences are highlighted in italic and boldface type. Most of the sequences (62%) observed in this study belong to *DQB1*06* lineages (15 alleles), the second most common (27%) belong to *DQB1*18* (9 alleles), and the rest (less than 1%) belong to the *DQB1*15*, *DQB1*16*, and *DQB1*17* lineages. The allele with the highest frequency among these cynomolgus macaques was *Mafa-DQB1*0616*, which was found in 30 (20.97%) of the 143 monkeys. Eleven alleles presented only once in these monkeys (Table 4). The frequency of the eight novel alleles found in this study was less than 4%, and most of them (seven of eight) were at less than 2%.

Although the *MhcMamu-DQB1*06111* (equivalent to the *-DQB1*061101*) allele was the most frequent (13%) in 105 randomly sampled Chinese rhesus macaques (Qiu et al. 2008), the allele, which corresponds to *MhcMafa-DQB1*0613* in the present study, was at a low frequency (2%) in the 143 monkeys tested. The *Mafa-DQB1* polymorphism has been studied earlier (Otting et al. 2002), and only eight of the 34 alleles detected in this study have not been reported previously. Given that the number of different *-DQ* alleles observed is nearly as high as the number of animals tested, it is likely that cynomolgus macaques display abundant *Mafa-DQB1* polymorphism and, when other populations are tested, the levels may reach or even exceed those reported for rhesus macaques (Robinson et al. 2003; Doxiadis et al. 2006).

Amino acid sequences encoded by the MhcMafa-DOB, -DPB1, and -DQB1 genes

Eighty-nine amino acid residues of *DOB* exon 2 that encode the DO antigen β domain of MHC class II molecules were blasted using 16 Mafa-DOB and six HLA-DOB sequences (Fig. 1). Almost all of these 22 *MHC-DOB* alleles were conserved except for four different amino acid sequences, located at amino acid positions 50, 58, 68, and 87, respectively. *Mafa-DOB*0101* and *Mafa-DOB*0104* were the most frequent in the Vietnamese population (Table 2), in which the amino acid positions 58

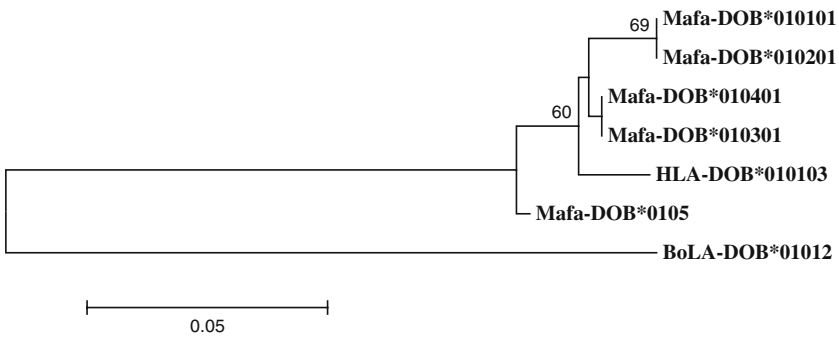

Fig. 4 Phylogenetic tree of 5 *Mafa-DOB*, 1 *HLA-DOB*, and 1 *BoLA-DOB* amino acid sequences

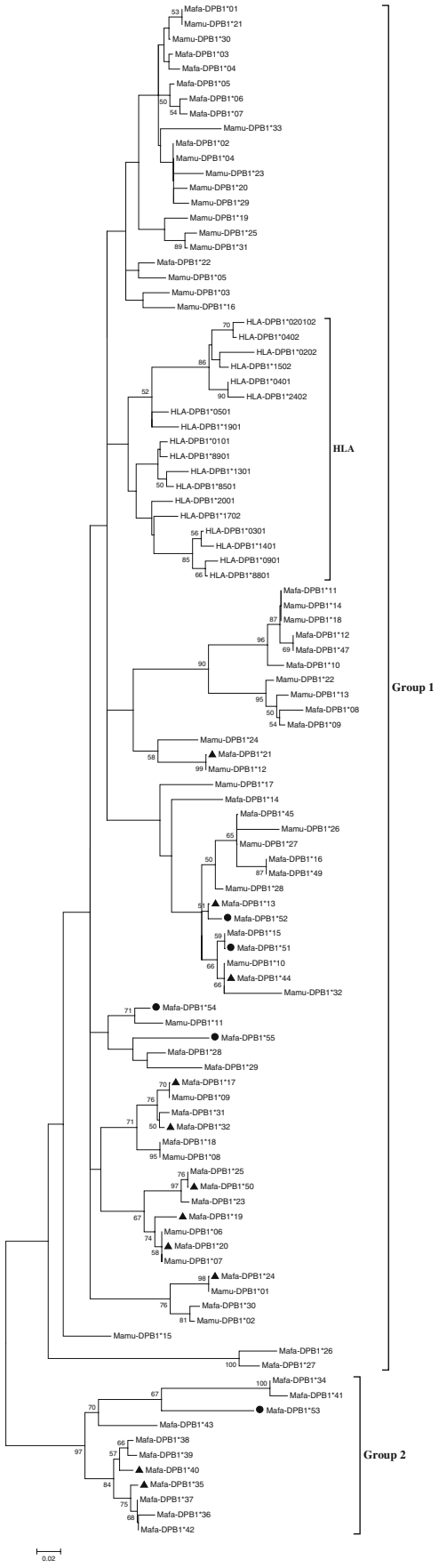

Fig. 5 Phylogenetic tree of 52 *Mafa-DPB1*, 33 *Mamu-DPB1*, and 18 *HLA-DPB1* amino acid sequences. Novel alleles identified in this study were shown with a *solid round spot*; previously reported alleles also detected in this study were shown with a *solid triangle*

and 87 were occupied by either Arg and Gly or Ser and Gly (Fig. 1). Of the 89 residue positions, three residues were polymorphic among the *Mafa-DOB* alleles in contrast to two residues among the *HLA-DOB* alleles. In addition, two species-specific amino acid residues were identified from the comparison between the *Mafa-* and *HLA-DOB* alleles, and of these, one was specific to *Mafa-DOB* (Fig. 2).

Eighty-seven amino acid residues of *DPB1* exon 2, which encodes the DP antigen β1 domain, were aligned using 52 Mafa-DPB1, 33 Mamu-DPB1, and 18 HLA-DPB1 sequences (Fig. 2). All 103 *MHC-DPB1* alleles had different amino acid sequences. Although all the *Mafa-DPB1* alleles were conserved at two cysteine sites (amino acid positions 10 and 72) and did not contain a terminator codon, an amino acid insertion or deletion was seen in nine *Mafa-DPB1 alleles* (Sano et al. 2006). *Mafa-DPB1*16* and *Mafa-DPB1*49* contained a methionine residue inserted between amino acid positions 43 and 44, whereas *Mafa-DPB1*35*, which was the most frequent in the Vietnamese population (Table 2), had a single amino acid residue deleted from position 58 (Fig. 2). Of the 87 residue positions, 54 residues were polymorphic among the *Mafa-DPB1* alleles, in contrast to 33 residues among the *Mamu-DPB1* alleles (Sano et al. 2006). In addition, 62 species-specific amino acid residues, located at 43 positions, were identified from the comparison between the *Mafa-* and *Mamu-DPB1* alleles, and of these, 59 were specific to *Mafa-DPB1* (Fig. 2).

The second exon in the MHC-DQB1 sequences encodes the β1 domain of MHC class II molecules, which contributes a major part to the peptide-binding domain that has a high degree of polymorphism. The amino acid sequence variations of MhcMafa-DQB1 identified so far (48 amino acid sequences) are shown in Fig. 3. The differences between the alleles and the consensus sequences range from 7 to 26 amino acid positions, averaging approximately 16 amino acid positions. There are 47 amino acid positions with codons for more than one amino acid residue, among which are three positions (26:7, 28:5, 57:5) with codons for five to seven amino acid residues. Similarly, there are 47 amino acid positions with codons for more than one amino acid residue, among which are four positions (26:8, 28:6, 57:6, 86:5) with codons for five to eight amino acid residues, in Mamu-MHC-DQB1 (Qiu et al. 2008). Among the amino acid positions classified as participating in pockets, based on HLA-DR and DQ structure (9, 11, 13, 28, 47, 57, 61, 67, 70, 71, 74, 74, 85, 86, 89, and 90; Diaz et al. 2000; Siebold et al. 2004;

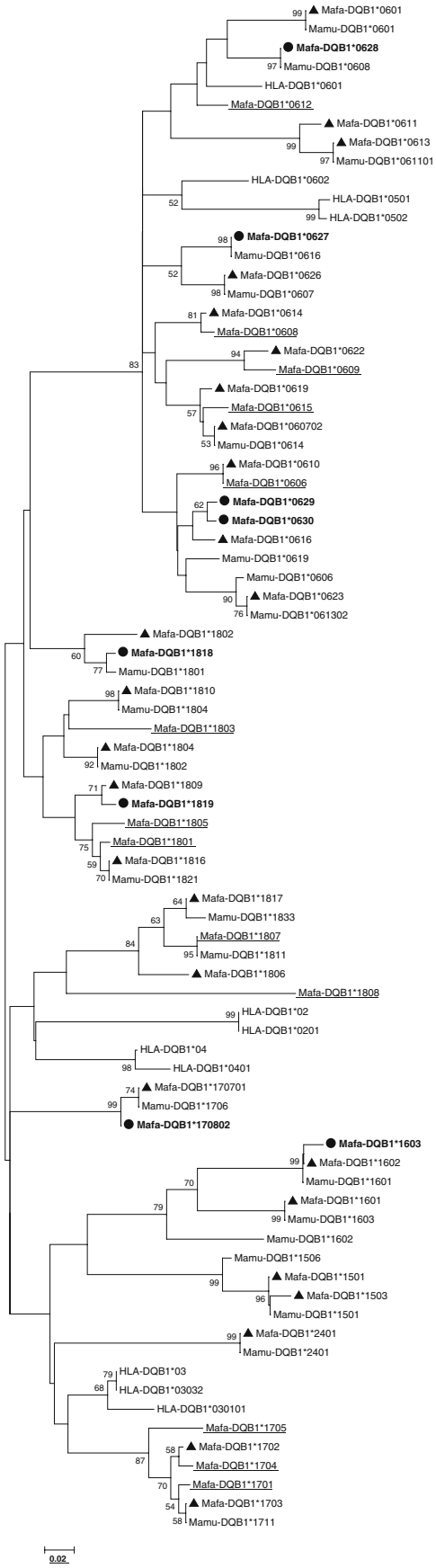

Fig. 6 Phylogenetic tree of 47 *Mafa-DQB1*, 23 *Mamu-DQB1*, and 11*HLA-DQB1* amino acid sequences. Novel alleles identified in this study were shown with a *solid round spot* and *boldface*, previously reported alleles also detected in this study were shown with a *solid triangle*; alleles not detected in this study were indicated by *underline*

Ettinger et al. 2006), most are with codons for two to five amino acid residues. Among the eight novel sequences (under the frame in Fig. 3), 36 amino acid positions with variable numbers of codons were found; for the 14 sequences not observed in this study (above the frame in Fig. 3), 34 such positions were found.

Phylogenetic tree of the MhcMafa-DOB, -DPB1, and -DQB1 genes

A phylogenetic tree was created using the novel *MhcMafa-DOB* gene sequences obtained in this study and those (1 *BoLA-DOB*, 1 *HLA-DOB* sequences) retrieved from GenBank. As evident in the phylogenetic tree (Fig. 4), all the novel sequences identified in this study grouped into three *DOB* allele lineages, including group 1 (*Mafa-DOB*0101* and *-DOB*0102*), group 2 (*Mafa-DOB*0103* and *-DOB*0104*), and one other (*Mafa-DOB*0105*), whereas the *HLA-DOB* alleles belonged to a separate group.

A phylogenetic tree constructed from a neighbor-joining analysis of 105 *MHC-DPB1* exon 2 sequences using 54 *Mafa-DPB1*, 33 *Mamu-DPB1*, and 18 *HLA-DPB1* allelic sequences is shown in Fig. 5. The general appearance of the tree is similar to a previously reported tree for the MHC class II polymorphisms in *Mafa-DPB1* and other primates (Bontrop et al. 1999; Sano et al. 2006). The 13 *Mafa-DPB1* alleles and all *HLA-DPB1* alleles were included in the separated group allele clusters. Of the 54 *Mafa-DPB1* alleles, 41 alleles were related closely to *Mamu-DPB1* alleles, suggesting the possibility of inter-species inheritance. In particular, in our present study *Mafa-DPB1*21*, *-DPB1*18*, and *-DPB1*24* were matched perfectly with *Mamu-DPB1*12*, *-DPB1*08*, and *-DPB1*01*, respectively, whereas *Mafa-DPB1*02*, *-DPB1*18*, *-DPB1*20*, and *-DPB1*24* were matched perfectly with *Mamu-DPB1*04*, *-DPB1*08*, *-DPB1*06*, and *-DPB1*01*, respectively (Sano et al. 2006).

A phylogenetic tree was created using 34 *MhcMafa-DQB1* gene sequences obtained in this study and those (14 *Mafa-DQB1*, 23 *Mamu-DQB1*, and 11 *HLA-DQB1* sequences) retrieved from GenBank. The relationships among the sequences of exon 2 from this study and those from other studies are shown in Fig. 6. As evident in the phylogenetic tree, novel sequences (shown with a solid round spot) identified in this study grouped into the *DQB1* allele lineages *DQB1*06* (4), **16* (1), **17* (1), and **18* (2). They tend to cluster with sequences that are common to the

earlier studies of *Mamu-DQB1* (shown with a solid triangle), rather than clustering with sequences detected only in the earlier reports on *Mafa-DQB1* (shown in black), with the exception of *DQB1*0629* and *DQB1*0630*, which clustered together. In addition, the high-frequency *DQB1*0616* allele identified in the present study clustered with *DQB1*0629* and *DQB1*0630*. All major *MhcMafa-DQB1* lineages that have been reported previously were detected (*DQB1*06*, **15*, **16*, **17*, **18*, **24*).

In conclusion, the *Mafa-DOB*, *-DPB1*, and *-DQB1* alleles detected in this manuscript are mostly specific for a given geographic area, and only a small number of alleles appears to be shared with other populations, providing an important addition to the limited immunogenetic information available for Vietnamese cynomolgus macaques. This suggests the fast evolution of *Mafa-DOB*, *-DPB1*, and *-DQB1* alleles due to adaptation to new environments. The high-frequency alleles among Vietnamese population, *Mafa-DOB*010102*, *Mafa-DPB1*13*, and *Mafa-DQB1*0616*, may represent high priority targets for additional characterization of immune function. Characterization of shared and unique MHC class II DNA sequences may be vital for disease research and may help better elucidate the biogeography of non-human primates.

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