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Comprehensive identification of high-frequency and co-occurring Mafa-B, Mafa-DQB1, and Mafa-DRB alleles in cynomolgus macaques of Vietnamese origin

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ARTICLE INFO

Article history:
Received 29 June 2011
Accepted 1 February 2012
Available online 9 February 2012

Keywords:
High-frequency alleles
Co-occurring alleles
Major histocompatibility complex
Macaca fascicularis

ABSTRACT

High-frequency alleles and/or co-occurring human leukocyte antigen (HLA) alleles across loci appear to be more important than individual alleles, because they might be markers of disease risk that have clinical value as biomarkers for targeted screening or the development of new therapies. To better elucidate the major histocompatibility complex background and to facilitate the experimental use of cynomolgus macaques, Mafa-B, Mafa-DQB1, and Mafa-DRB alleles were characterized and their combinations were investigated from 30 macaques of Vietnamese origin by cloning and sequencing. A total of 48 Mafa-B, 22 Mafa-DQB1, and 42 Mafa-DRB alleles were detected in this study, respectively. In addition, two Mafa-DQB1 and eight Mafa-DRB alleles represented novel sequences that had not been documented in earlier studies. Our results also showed that the macaque from Vietnam might be valuable because >30% of the test animals possessed Mafa-DRB w304(30%) and -DQB1*0616(30%). We report that the combination of major histocompatibility complex (MHC) class I and II alleles, including the combination of DRB3*0403-DRB w304, DRB1*1013-DRB w304, and Mafa-B*007:01-01-DRB w304, which was in 17%, 13%, and 13% of the animals, respectively. Interestingly, more than two Mafa-DQB1 alleles detected in one animal in this study suggest that Mafa-DQB1, like Mafa-DRB, might be a duplication in the chromosome, which have ever been documented in cynomolgus monkeys but has not yet been observed in rhesus macaques or other primates. Our results for the high frequency of commonly co-occurring MHC alleles across loci in a cohort of the Vietnamese cynomolgus macaque emphasized the value of this species as a model for biomedical research.

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1. Introduction

With the 1978 ban on exportation of rhesus macaques (Macaca mulatta) from India, the cynomolgus macaque (Macaca fascicularis) became an increasingly useful animal model for various diseases, including diabetes, severe acute respiratory syndrome (SARS), tuberculosis, simian immunodeficiency virus (SIV), renal transplantation, and pharmacodynamic evaluation [1–4]. Major histocompatibility complex (MHC) class I and class II molecules play key roles in immune regulatory processes by presenting peptides of intracellular or extracellular origin to CD8+ or CD4+ T cells, respectively. It has been suggested that certain co-occurring alleles might be markers of disease risk that have clinical value as biomarkers for targeted screening or the development of new therapies [5]. A number of research groups have suggested that HLA-DRB1/DQB1 and/or HLA class I alleles and haplotypes are associated with many diseases, including type 1 diabetes [6–10], pemphigus [11], pure red cell aplasia [12], allergies [13], low hepatitis activity [14], multiple sclerosis [15], primary Sjögren’s syndrome [16], Graves’ disease in Koreans [17], familial generalized vitiligo and early disease onset [18], lichen sclerosus [19], and rheumatoid arthritis [20,21]. It has been reported that the combination B*4402-DRB1*1101-DQB1*0301 was associated with an 11-fold increased risk of cervical cell cancer [5].

For a few diseases, in particular SIV infection, macaques have become the dominant preclinical model for human immunodeficiency virus (HIV) vaccine evaluation [22,23]. There are many reports that show polymorphism of MHC genes in the cynomolgus macaque affects the results obtained with drugs [24–26] and is associated with the control of viral diseases [27]. The cynomolgus macaque from Mauritius appears to be particularly valuable, because 88% of these animals have the MHC class I allele combination Mafa-A*25-A*29 [28] and more than half of these have the combination Mafa-B*43010-B*4401011013-DRB1*0402-DRB3*0101-DRB w304, which was in 17%, 13%, and 13% of the animals, respectively. Interestingly, more than two Mafa-DQB1 alleles detected in one animal in this study suggest that Mafa-DQB1, like Mafa-DRB, might be a duplication in the chromosome, which have ever been documented in cynomolgus monkeys but has not yet been observed in rhesus macaques or other primates. Our results for the high frequency of commonly co-occurring MHC alleles across loci in a cohort of the Vietnamese cynomolgus macaque emphasized the value of this species as a model for biomedical research.

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ously reduce the confounding effects of genetic heterogeneity in HIV/AIDS research [28–31]. The high-frequency alleles might be high-priority targets for additional characterization of the immune function [32]. In addition, co-occurring MHC alleles across loci appear to be more important than individual alleles. So, to examine the combination of MHC class I and class II alleles in a cohort of the Vietnamese cynomolgus macaque, the transcribed Mafa class I and II genes were characterized and analyzed by sequencing the polymorphic exon 2 of Mafa-DRB and Mafa-DQB1 genes and exons 2 and 3 of the Mafa-B gene.

### 2. Subjects and methods

#### 2.1. Animals

Whole blood samples from 33 unrelated test animals (M. fascicularis), 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 ethylenediaminetetraacetic acid (EDTA)-treated vacuum tubes. All of the monkeys were clinically normal with no known diseases.

#### 2.2. RNA isolation, cDNA synthesis, and cloning of MHC class i and ii cDNAs

For all of the animals used in this study, RNA was isolated from peripheral blood (Blood RNA kit, Omega Bio-Tek, Guangzhou, China) and subjected to One-Step reverse transcription–polymerase chain reaction (RT-PCR), as recommended by the supplier (Takara). To identify and investigate the presence and expression of Mafa-B, -DQB1 and -DRB, the first strand cDNA (1 μl) was amplified in a 25-μl reaction volume using coding region-specific forward and reverse primers to amplify Mafa-DQB1 and -DRB alleles for exon 2 and Mafa-B for exons 2 and 3 (Table 1). In general, amplification was carried out for 3 minutes

![Fig. 1. Distribution of Mafa-B alleles detected in a cohort of Vietnamese cynomolgus macaques. aNumber of animals sharing a certain allele. bNumber of alleles in one animal.](image)
at 94°C, 32 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 1 minute at 72°C, ending with 3 minutes at 72°C. The annealing temperature was adjusted based on the $T_m$ of the primers. The PCR products were subjected to agarose gel electrophoresis and ethidium bromide staining for visualization and were cloned using the PCR cloning kit (Qiagen). After transformation, for each animal, 40 to 50 colonies per locus were picked for plasmid isolation and were bidirectionally sequenced by service provider (Invitrogen, China).

2.3. Data analysis and nomenclature

Using Mega 4.0 software [33], the sequences of the exon 2 regions of Mafa-DRB, -DQB1, and the exons 2 and 3 of Mafa-B obtained in our study were aligned. New alleles were confirmed by sequencing three times. The names of new sequences were derived according to the published guidelines, and the Immuno Polymorphism Database of Major Histocompatibility Complex for Nonhuman Primates (IPD-MHC NHP) was searched to avoid the same name(s) being assigned to different alleles [34,35].

3. Results and discussion

3.1. Identification and allele frequencies of transcribed Mafa-B, Mafa-DRB, and -DQB1 alleles

A total of 48 Mafa-B alleles were identified in this study by cloning and sequencing of exon 2 and 3 of the Mafa-B gene using whole blood from 33 randomly chosen test animals. Their accession numbers are listed in the supplementary data file (Table S1). From one to eight Mafa-B alleles ranging were identified in the 33 individual animals, and from two to five in 29 (88%) of the test animals. The most frequent allele was Mafa-B*007:01:01, which was found in eight (24%) of the 33 macaques. The second most frequent alleles were Mafa-B*002:04, Mafa-B*039:01 and Mafa-B*085:01, all of which were detected in six (18%) individuals. The frequency of Mafa-B*030:13 was 15% and the frequency was 12% each for Mafa-B*007:05, Mafa-B*093:02, Mafa-B*037:01, Mafa-B*030:01:01 and Mafa-B*145:01. Another 15 alleles were present only once in these macaques (Fig. 2).

![Fig. 2. Distribution of Mafa-DRB alleles detected in a cohort of Vietnamese cynomolgus macaques. Number of animals sharing a certain allele. Number of alleles in one animal.](image-url)
It has been described that macaques from different origins have largely independent MHC I allele repertoires [29]. We found that some alleles including Mafa-B*018:01:01, Mafa-B*036:01:02, and Mafa-B*037:01 were shared by these animals in the previous study [36]. Moreover, some alleles, including Mafa-B*104:01:02 and Mafa-B*104:01:03, were highly similar to the Mafa-B*104:01:01 allele that was detected in the Mauritian macaques [36]. Importantly, Mafa-B*008:01 (HQ131700) and Mafa-B*017:02 (HQ131701), which were identified in Vietnamese macaques in our previous study, are highly similar to Mamu-B*08 and Mamu-B*17, respectively, which are associated with the control of viral replication in SIV-infected rhesus macaques. Surprisingly, we noted that one animal (macaque no. 65) expressed both Mafa-B*008:01 and Mafa-B*017:02 alleles, which were not expressed in the other macaques. However, these two populations might differ in their MHC class I allele repertoires [36]. The high-frequency MHC class I allele and its combination of Mafa-B*430101, Mafa-B*440101, and Mafa-B*460101 from Mauritian cynomolgus macaques [29] has not been detected in our study.

In this study, 22 Mafa-DQB1 alleles, two of which had not been reported in cynomolgus macaques, were identified by cloning and sequencing of exon 2 of the Mafa-DQB1 genes using blood samples from 30 randomly chosen animals. These novel sequences were submitted to GenBank and were assigned accession numbers by the NHP Nomenclature Committee. Their accession numbers are listed in additional file (Fig. 1). All the new sequences are highlighted in italic and boldface type. From 2 to 20 animals shared a certain allele and its number is shown in Fig. 1. All the new sequences are highlighted in italic and boldface type. From 2 to 20 animals shared a certain allele and its number is shown in Fig. 1. All the new sequences are highlighted in italic and boldface type.

The most common sequences (40%) observed in this study belong to DQB1*18 lineages (eight alleles), the second most common (24%) belong to DQB1*06 lineages (five alleles), the third most common belong to DQB1*17 lineages (three alleles), and the rest belong to the DQB1*15, DQB1*16, and DQB1*24 lineages. Mafa-DQB1*0616 were the most frequently detected alleles in nine (30%) of the macaques, which was coincident with our previous study based on DNA level [39]. Moreover, 12 alleles were found only once (Fig. 3). It has been reported that most of the sequences (73%) observed belong to DQB1*06 and DQB1*18 lineages and the rest (27%) belong to DQB1*15, DQB1*16, and DQB1*17 lineages in 105 randomly sampled Chinese rhesus macaques [40]. In addition, the MhcMamu-DQB1*1706 allele, which corresponds to MhcMafa-DQB1*170701 (100% similarity to MhcMamu-DQB1*1706) in the present study, was found in only three (2.86%) of 105 macaques in the previous study [39] and was also the low frequent (3.33%) in the 30 macaques tested in this study. Moreover, the MhcMamu-DQB1*1503 allele found in animals infected with SHIV (a virus combining parts of the HIV and SIV genomes) with a lower plasma viral load was the second most frequent (19%) in 105 randomly sampled Chinese rhesus macaques [40]. The MhcMamu-DQB1*1503 allele, which corresponds to MhcMafa-DQB1*1503 (100% similarity) in the present study, was the second most frequent (16.78%) in the 30 animals tested in this study. Mafa-DQB1*1503, one of the most frequent alleles in the present study, has been reported from different origins by many research groups, suggesting it is shared by cynomolgus macaques. The high frequency of the Mafa-DQB1*0616 allele in the present study, which was also found in rhesus macaques from different origins [41], suggest it might be shared by cynomolgus macaque populations of different origins. However, neither has been detected in Mauritian cynomolgus macaques.

A total of 42 Mafa-DRB alleles, eight of which had not been reported in cynomolgus macaques, were identified by cloning and sequencing of exon 2 of the MhcMafa-DRB gene using blood samples from 30 randomly chosen cynomolgus macaques in this study. These novel sequences were submitted to GenBank and were assigned accession numbers by the NHP Nomenclature Committee. Their accession numbers are listed in additional file (Fig. 2). All the new sequences are highlighted in italic and boldface type. From 2 to 5 Mafa-DRB alleles were identified in individual animals. The allele with the highest frequency among these cynomolgus macaques was Mafa-DRB1*w304, which was found in nine (30%) of the 30 macaques. The next most frequent alleles were Mafa-DRB1*1013, which was a novel allele that was detected in seven (23.33%) of the macaques, and the frequency of both Mafa-DRB1*w312 (a novel

![Fig. 3. Distribution of Mafa-DQB1 alleles detected in a cohort of Vietnamese cynomolgus macaques. *Number of animals sharing a certain allele. ‡Number of alleles in one animal.](image-url)
allele) and Mafa-DRB1*0101 was 16.67% and 18 alleles were found only once in these cynomolgus macaques (Fig. 2). Alleles MhcMafa-DRB3, MhcMafa-DRB4, and MhcMafa-DRB5, as well as MhcMafa-DRB, were detected in this study. Among them, Mafa-DRB3*0403 was highly frequent and was found in seven (23.33%) macaques. Mafa-DRB1*0101, the most frequent allele in this study, has been reported in cynomolgus macaques that originate from islands not far from Borneo and Malaysia [37], but it has not been detected in Mauritian cynomolgus macaques [42]. The highly frequent Mafa-DRB1*0101 allele in the present study had the second highest frequency in 40 Chinese cynomolgus macaques originating from the Guangxi Province in China [43]. However, the Mafa-DRB1*0103 allele reported as the most frequent [43] was not identified in our study. We hypothesize that this difference is the result of the small number of animals in the present study.

3.2. Identification of co-occurring alleles Mafa-B, Mafa-DRB, and -DQB1

It has been suggested that co-occurring HLA alleles across loci appear to be more important than individual alleles, which are closely correlated with disease [5]. Mauritian cynomolgus macaques might be particularly valuable because more than half of these animals have the MHC class I allele combination Mafa-B*430101, Mafa-B*44001, and Mafa-B*460101. Recognizing that MHC molecules are codominantly expressed, we focused on co-occurring alleles. We investigated the combination of MHC alleles across loci in Vietnamese cynomolgus macaques. Our result showed that both DRB3*0403 and DRB*w304 alleles were detected in the five monkeys (nos. 73, 75, and 78–80), both DRB1*0103 and DRB3*0403 were detected in the four monkeys (nos. 71, 75, 77, and 80), both DRB*w304 and B*007:01:01 were detected in the four monkeys (nos. 77–80), suggesting the combination of DRB3*0403-DRB*w304, DRB1*0103-DRB*w304 and DRB3*0403-B*007:01:01 was in 17%, 13%, and 13% of the animals, respectively (Fig. 2). In addition, some combinations were in 10% of the animals, including DRB1*1503-DRB*w304, DRB1*0106-DRB3*0403, DRB1*0106-B*008:05:01, and DRB1*0106-B*007:01:01 (Fig. 4). Therefore, we conclude that extraordinary MHC class I and II allele sharing is a characteristic feature of Vietnam cynomolgus macaques.

It has been reported that 20% of animals have the DRB*W2101-DRB*w501-DRB6*0101 haplotype [43], which is consistent with two earlier results demonstrating that more than 40% of macaques from Mauritius carried this haplotype [44,45]. Both Mafa-DRB1*0101 and Mafa-DRB3*0403 alleles were found at low frequency (3%) in the Vietnam cynomolgus macaques bred at Guangdong Province in China; however, the DRB6*0101 allele was not found in this study. It has been documented that MHC alleles/haplotypes were associated with sustained control of SIV-infected cynomolgus/rhesus macaques [22,23,30,46]. It has been shown that Mamu-B*17-positive SIV-infected rhesus macaques that also expressed these two MHC-II alleles had significantly lower viral loads than Mamu-B*17-positive animals that did not express Mamu-DRB1*1003 or -DRB3*0306 (p < 0.0001) [23]. The combination of Mamu-DRB1*0601-DQB1*1801 and Mamu-DRB1*0601-DRB1*0309-DRB3*W201 was found to be associated with rapid disease progression in SIV-infected rhesus macaques [22,46].

In conclusion, we suggest that Vietnamese cynomolgus macaques might be valuable because >30% of the test animals possessed Mafa-DRB1*0103 (30%) and Mafa-DQB1*007:01:01 (30%). The above high-frequency alleles among Vietnamese population may represent high-priority targets for additional characterization of immune function. Moreover, the combination of DRB3*0403-

![Fig. 4. Distribution and combination of the most frequent alleles in a cohort of Vietnamese cynomolgus macaques. Mafa-B/Mafa-DQB1/Mafa-DRB combinations in this cohort of animals. Mafa-B, Mafa-DQB1, and Mafa-DRB cDNA sequences identified in three or more clones in each animal are shown. Only the highly frequent alleles of Mafa-B, Mafa-DQB1, and Mafa-DRB are listed.](image-url)
DBR* w304, DBR1*1013-DBR* w304, and DRB* w304-B*007:01:01 was in 17%, 13%, and 13% of the animals, respectively. In addition, more than two Mafa-DQB1 alleles were found in one particular animal in the present study, suggesting that Mafa-DQB1, as well as Mafa-DRB, might be a duplication in the chromosome. This study is the first to reveal the high frequency of commonly co-occurring class I and class II MHC alleles across loci in a cohort of Vietnamese cynomolgus macaques, greatly enhancing the value of this species as a model for biomedical research.

Acknowledgments
We thank the Primate Research Center of South China for providing blood samples from animals. We thank Dr Natasja de Groot, Dr Nel Otting and IMGT Non-human Primate Nomenclature Committee for naming the Mafa-B, Mafa-DQB1 and Mafa-DRB sequences. This project was supported by the Natural Science Foundation of Guangdong, China (S2011040005261), the Fundamental Research Funds for the Central Universities of South China University of Technology (2012ZD0093 and 2011ZM0111), National Science and Technology Major Project of Key Drug Innovation and Development (2011ZX09307-303-03), and Science and Technology Planning Project of Guangdong Province, China (2010B0806020007).

Appendix. Supplementary data
Supplementary data associated with this article can be found, in the online version, at 10.1016/j.humimm.2012.02.003.

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