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BRIEF COMMUNICATION

# Comprehensive identification of MHC class II alleles in a cohort of Chinese rhesus macaques

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**Abstract** Rhesus macaque is a very important animal model for various human diseases, especially for AIDS and vaccine research. The susceptibility and/or resistance to some of these diseases are related to the major histocompatibility complex (MHC). To gain insight into the MHC background and to facilitate the experimental use of Chinese rhesus macaques, *Mamu-DPB1*, *Mamu-DQB1*, and *Mamu-DRB* alleles were investigated in 30 Chinese rhesus macaques through gene cloning and sequencing. A total of 66 alleles were identified in this study, including 14 *Mamu-DPB1*, 20 *Mamu-DQB1*, and 30 *Mamu-DRB* alleles as well as 2 high-frequency *Mamu-DPB1* alleles. Interestingly, one of the high-frequency *Mamu-DPB1* alleles had been undocumented in earlier studies. Eleven of the other alleles, including four *Mamu-DPB1*, three *Mamu-DQB1*, and four *Mamu-DRB* alleles were also novel. Importantly, like *MHC-DRB*, more than two *Mamu-DPB1* sequences per animal were detected in 13 monkeys, which suggested that they might represent gene duplication. Our data also indicated quite a few differences in the distribution of MHC class II alleles between the Chinese rhesus macaques and the previously reported Indian rhesus macaques. To our knowledge, our results revealed comprehensively the combination of MHC II alleles. This information will not only promote the understanding of Chinese rhesus macaque MHC polymorphism but will also facilitate the use of Chinese rhesus macaques in studies of human disease.

**Keywords** Major histocompatibility complex · Chinese rhesus macaques

Primates are the closest evolutionary relatives to humans and represent important animal models to study human biology and disease. The rhesus macaque, *Macaca mulatta*, is the most widely used primate organism for research. Their similarity to humans makes them invaluable models to study neuroscience, behavior, reproduction, AIDS, pharmacology, and more. Their response to infectious agents related to human pathogens has made macaques the preferred model for vaccine development. With the completion of whole-genome sequencing of the rhesus macaque, including Indian rhesus macaque and Chinese rhesus macaque, increasing attention has focused on identifying genetic factors contributing to the variability of disease susceptibility and resistance. Polymorphism of the major histocompatibility complex (MHC) gene is associated with a diverse group of immune defenses and for more than 100 diseases appears to be one of the important host factors related with susceptibility or resistance, resulting in different rates of disease progression in the rhesus macaques from India or those from China. MHC alleles are associated with resistance to diseases in rhesus macaques infected with simian immunodeficiency virus. Therefore, it will be useful to further understand the MHC background of rhesus macaques, helping a more precise elucidation of the experimental results from studies that use these animals.

Indian rhesus macaques have been studied almost exclusively for AIDS pathogenesis and vaccine studies for a couple of decades. However, increasing numbers of Chinese rhesus macaques have been used in corresponding studies in recent years because of the shortage of Indian rhesus macaques (Qiu et al. 2008). Until now, many publications have described the identification of the *Mamu-DPB1*, *Mamu-DQB1*, and *Mamu-DRB* allele in Chinese rhesus macaque, and reported that

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*Mamu-DPB1* genes display a medium degree of polymorphism and *Mamu-DQA1/DQB1* pairs are tightly linked as well as high level of *Mamu-DRB* region configuration polymorphism (Viray et al. 2001; Doxiadis et al. 2001; Doxiadis et al. 2003; Xu et al. 2010), but co-occurring MHC alleles across loci appear to be more important than individual alleles. To increase the understanding of the genetic differences and to expand our knowledge of the co-occurring MHC alleles across loci in Chinese rhesus macaques, we characterized the *Mamu-DPB1*, *Mamu-DQB1*, and *Mamu-DRB* alleles of 30 unrelated Chinese rhesus macaques. To accomplish this, RNA was isolated from peripheral blood samples obtained from each monkey using the E.Z.N.A Blood RNA kit (Omega Bio-Tek, Guangzhou, China) and subjected to PCR amplification using the PrimeScript RT reagent Kit with cDNA Eraser (Perfect Real Time; TaKaRa Bio, Dailin, China). To assess the expression of exon 2 and 3 of *Mamu-DPB1*, *Mamu-DQB1*, and *Mamu-DRB* alleles, first strand gDNA (1 μL) was amplified in a 20-μL reaction volume using coding region-specific forward and reverse primers (Table 1). In general, PCR amplification consisted of a denaturation cycle for 3 min at 94 °C followed by 32 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, with a final cycle at 72 °C for 8 min. The annealing temperature was adjusted based on the Tm of the primers. The PCR products were subjected to agarose gel electrophoresis and ethidium bromide staining for visualization and then cloned using a PCR cloning kit (Takara Bio). After transformation, for each animal, 20–30 colonies per locus were picked for plasmid isolation and unidirectional sequencing, which was performed by BGI Genomics Institute (Shenzhen, China). Sequences were analyzed using molecular Evolutionary Genetics Analysis software version 4.0 (Tamura et al. 2007), and revealed the distribution and frequency of MHC II alleles in the same macaque populations in the present study, including *Mamu-DPA1*, *Mamu-DQA1*, and *Mamu-DRA* alleles in our previous study. Novel allele sequences were confirmed by the sequencing three different clones and submitted to the GenBank database (www.ncbi.nlm.nih.gov/genbank/) and were assigned allele names by the Nonhuman Primate (NHP) Nomenclature Committee (www.ebi.ac.uk). All accession numbers of the alleles are listed in Fig. 1 and novel alleles were underlined.

In total, in the present study, 16 *Mamu-DPB1* alleles, including 5 novel alleles, were identified in Chinese rhesus macaques. Both *Mamu-DPB1*02:01* and *Mamu-DPB1*02:04:01* were the most frequent alleles and the latter was a novel allele, which was found in 14 of the 30 macaques. The second most frequent allele in the study population was *Mamu-DPB1*19:01:01*, which was detected in 11 individuals (Fig. 1). The high-frequency alleles may represent high-priority targets for additional characterization of immune function. Surprisingly, these high-frequency alleles identified in our population were not detected in 106 Chinese rhesus macaques (Xu et al. 2010) or in a cohort of Chinese rhesus macaque (Doxiadis et al. 2003). But, the *DPB1*02* lineages were reported in Chinese rhesus macaque (Doxiadis et al. 2001). This might be caused by using the different regional population. In this study, all 30 unrelated Chinese rhesus macaques were generously provided by the South China Primate Research & Developmental Center (Guangdong, China). We also found that in several cases, more than two *Mamu-DPB1* sequences were retrieved from a single individual due to *DPB1* gene duplications. However, this supposition requires investigation in a future study to enrich the polymorphism database of *Mamu-DPB1*.

As seen in Fig. 1, the most common sequences belonged to the *DPB1*01* and *DPB1*02* lineages. Reportedly, both *Mamu-DPB1*08:01* (old nomenclature was *DPB1*01*) and *Mamu-DPB1*06:01* (old nomenclature was *DPB1*02*) were origin-specific alleles in Indian rhesus macaque (Doxiadis et al. 2003). The former was also undetected but *Mamu-DPB1*06:05* was identified in the present (Fig. 1). Also, some origin-specific alleles in Burma rhesus macaque were not identified in our population, including *DPB1*05*, *DPB1*01*, and *DPB1*14*, which was coincident with the result of Doxiadis et al. (2003). In addition, up to now, the *DPB1*18* lineage has been undocumented in other original populations, and *Mamu-DPB1*18:01* was a novel allele in this study, so the lineage might be origin-specific allele in Chinese macaque. Moreover, a total of 15 *Mamu-DPA1* alleles were characterized by using the same population in our previous study (Deng et al. 2013) and can be seen from Fig. 1. Clearly, *Mamu-DP* genes displayed moderate polymorphism (Fig. 1).

**Table 1** Primers used to amplify *Mamu-DQB1*, *Mamu-DPB1*, and *Mamu-DRB* alleles

| Locus | Primer name | Primer sequence (5′ to 3′) | Temperature (°C) | Product size (bp) |
|---|---|---|---|---|
| DQB1 | DQB1-F | GAAGAAGGCTTTGCGGAT | 54.5 | 508 |
| | DQB1-R | GTCGCCGTTCCTAATAAG | | |
| DPB1 | DPB1-F | GACAGTGGCTCTGACGGCATTA | 57.7 | 357 |
| | DPB1-R | GACGAGCAGGTTGTGGTG | | |
| DRB | DRB-F | TGGCAGCTCTGACAGTGA | 55.2 | 394 |
| | DRB-R | CTGCCTGGATAGAAACCG | | |

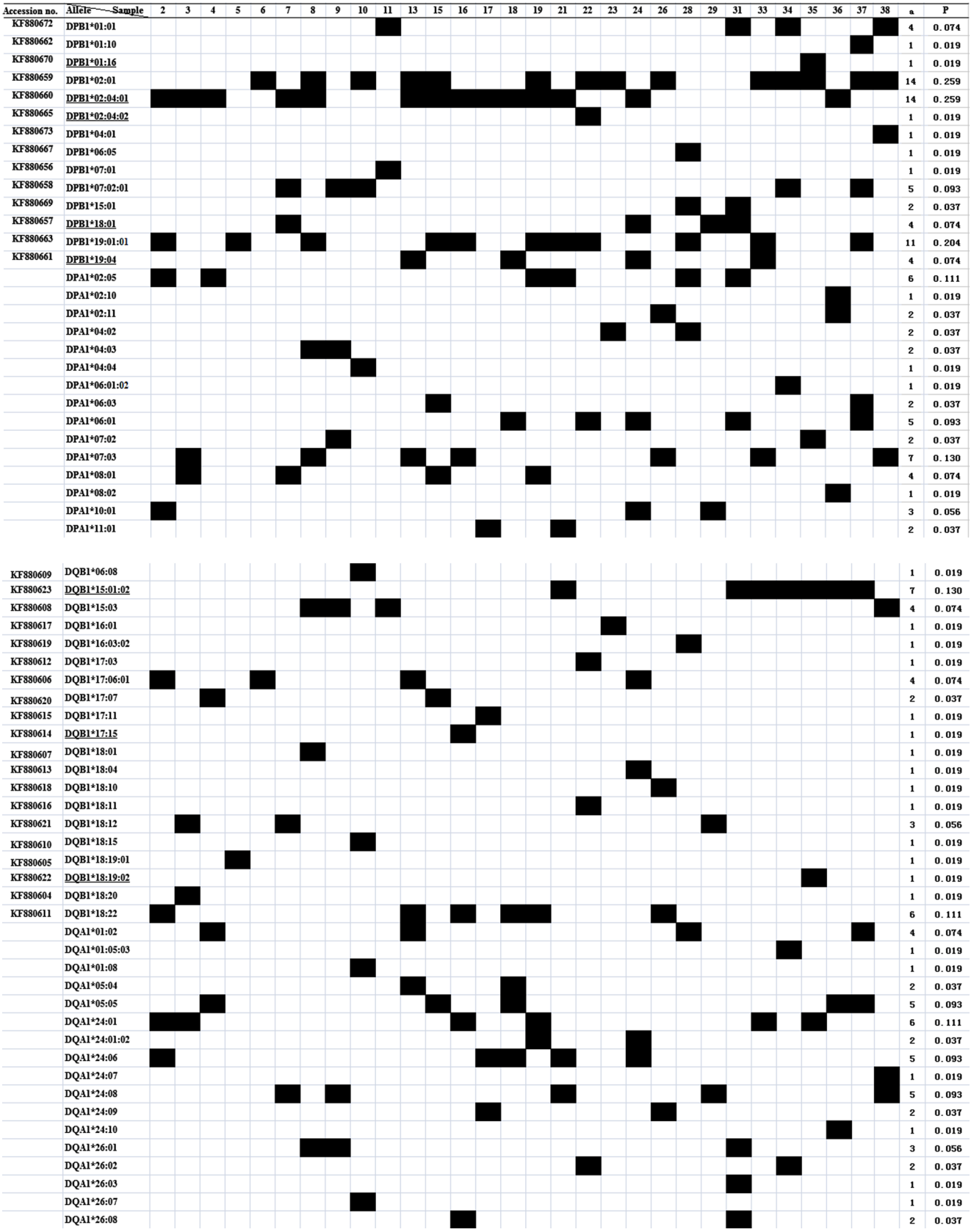


**Fig. 1** The distribution of *Mamu-DPB1*, *Mamu-DPA1*, *Mamu-DQB1*, *Mamu-DQA1*, *Mamu-DRB*, and *Mamu-DRA* alleles detected in a cohort of Chinese rhesus macaques. (*a*) Number of animals sharing a certain allele. Novel alleles identified in this study are indicated with an *underline*. All alleles with GenBank number were detected in this study and others were reported by our previous study in the same population

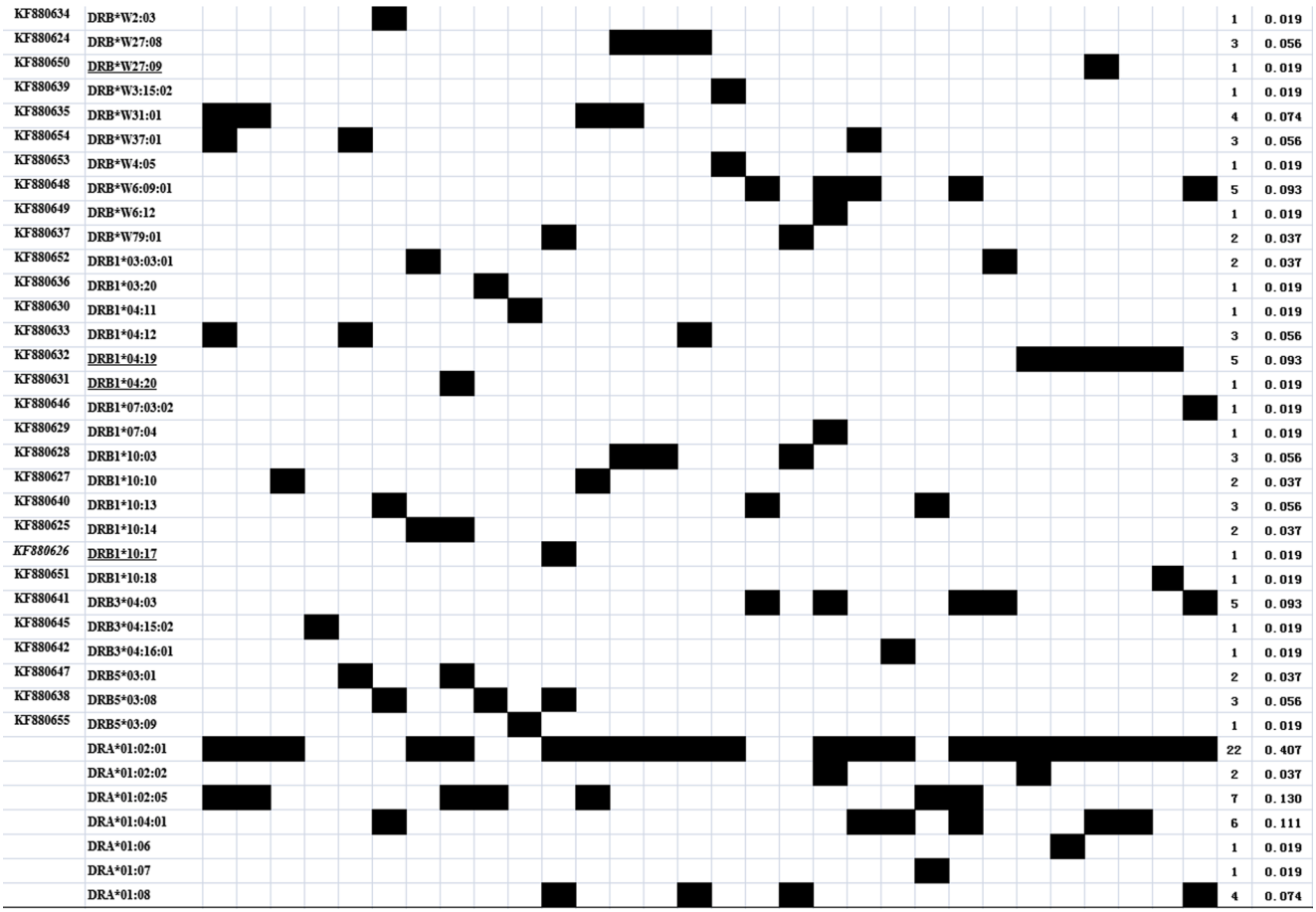


**Fig. 1** (continued)

With regard to *Mamu-DQB1*, we identified a total of 20 alleles, including 3 novel alleles, none of which were at a high frequency. In the present study, the most frequent alleles, *DQB1-*15:01:02*, were detected in seven individuals (13 %). These were also detected in seven out of 32 Chinese rhesus macaque previously (Doxiadis et al. 2003), but the frequency of *DQB1-*15:01* allele was only 7 % (Viray et al. 2001). However, in other studies, the most frequent allele was *Mamu-DQB1*06111* (22 %), followed by *DQB1*1503* (19 %) in 105 Chinese rhesus macaque (Qiu et al. 2008), whereas the latter also detected in this study was only 7 % (Fig. 1), the same as the result of Viray et al. (2001). A previous study found 24 *Mamu-DQB1* alleles in 150 rhesus macaques from three regions of these; *Mamu-DQB1*1801* and *Mamu-DQB1*0601* were the most prominent alleles in Indian rhesus macaques and Burmese origin macaques, respectively (Doxiadis et al. 2003). Importantly, the origin-specific alleles identified in Indian or Burmese macaque were not detected in our population except for *DQB1-*15:03*. However, four origin-specific alleles identified in Chinese macaque were not found in the present except for *DQB1-*17:06*, probably due to the different regional origin of our population or small sample size.

As seen in Fig. 1, the most common sequences belonged to the *DQB1*18* lineage (ten alleles), the second most common belonged to the *DQB1*17* lineage (five alleles). Although we only detected two alleles in the *DQB1*15* lineage, *DQB1-*15:01:02* and *DQB1-*15:03*, the former was the highest-frequency allele in this population. In our previous study, the most common sequences also belonged to the *DQB1*18* lineage (nine alleles) in cynomolgus macaque of Vietnamese origin, but the highest-frequency allele was *Mafa-DQB1*06:16* (Ling et al. 2012). Reportedly, most of the sequences (73 %) belong to *DQB1*06* (13 alleles) and *DQB1*18* (14 alleles) lineages, and the rest (27 %) belong to *DQB1*15*, *DQB1*16* and *DQB1*17* lineages in 105 Chinese rhesus macaque (Qiu et al. 2008). It was documented that alleles of the *DQA1*24* lineage can combine with members of the *DQB1*15*, *DQB1*17*, or *DQB1*18* lineage in Indian rhesus macaque (Doxiadis et al. 2001), which was consistent with our result in Chinese rhesus macaque (Fig. 1). However, the *Mamu-DQA1*01* lineage is not always coupled with members of the *DQB1*06* lineage and can also link with the other lineages, including *DQB1*15*, *DQB1*16*, or *DQB1*17* lineage in the present, which was different from the result of Doxiadis et al. in Indian macaque (2001). However, the

*DQB1*06* alleles are difficult to amplify, so this result must be interpreted with caution. In addition, we first showed that alleles of the *DQB1*18* lineage can combine with members of the *DQA1*5*, *DQA1*24*, or *DQA1*26* lineage in Chinese macaque (Fig. 1 and Supplemental file). Reportedly, *DQA1*24:01*/*DQB1*15:01* pairs were specific for rhesus macaques of Indian origin (Doxiadis et al. 2001). Although *DQA1*24:01:01-DQB1*15:01:02* was identified in this study population, in animals 34 and 36 which showed the *DQB1*24:01* allele, the second *DQA1* allele was not detected (Supplemental file). These results indicate that there were quite a few differences in the distribution and characterization of alleles between the two species.

A total of 30 *Mamu-DRB* alleles, including four novel alleles, were identified in this study. The most frequent alleles were *Mamu-DRB*W6:09:01*, *Mamu-DRB3*04:03*, and *Mamu-DRB1*04:19*, all of which were found in 5 of the 30 macaques and the latter was a novel allele (Fig. 1). In addition, all of these alleles belonged to four loci/lineages: *Mamu-DRB*W*, *Mamu-DRB1*, *Mamu-DRB3*, and *Mamu-DRB5*. Reportedly, the co-occurring HLA alleles across loci appear to be more important than individual alleles, which are closely correlated with disease (Madeleine et al. 2008). Our result showed that the combinations of MHC class II alleles, including the combination of *DPB1*02:01-DQB1*15:01:02-DRB1*04:19-DRA*01:02:01*, *DQB1*15:01:02-DRB1*04:19-DRA*01:02:01*, *DPB1*02:04:01-DRA*01:02:01*, *DPB1*02:01-DRA*01:02:01*, *DPB1*02:04:01-DQB1*18:22*, and *DPB1*02:04:01-*19:01:01*, were detected in 4, 5, 12, 11, 5, and 6 Chinese rhesus monkeys, respectively (Table 2). It was reported that Indian rhesus macaques that expressed *Mamu-B*17* and also expressed *Mamu-DRB1*1003* and *Mamu-DRB1*0306* had significantly lower viral loads than animals that expressed *Mamu-B*17* but did not express these alleles (Giraldo-Vela et al. 2008). In this study, *Mamu-DRB1*1003* was detected in three animals but *Mamu-DRB1*0306* was not detected in this population. This observation, too, may be due to *DRB* alleles, which have not been detected with the methods/primers used. Similarly, *Mamu-B*17* was also identified in other animals from this population but this allele had a low frequency (data unpublished). Therefore, identifying comprehensively MHC alleles in Chinese rhesus macaque will contribute to elucidate the genetic background of this species and promote its widely usefulness in the studies of human disease, especially for AIDS research.

**Table 2** Combination of alleles across loci in a cohort of the Chinese rhesus macaques

| Combination of alleles across loci | Number of animals sharing a combination of alleles (sample) |
|---|---|
| *DPB1*02:01-DQB1*15:01:02-DRB1*04:19-DRA*01:02:01* | 4 (33, 34, 35, 37) |
| *DQB1*15:01:02-DRB1*04:19-DRA*01:02:01* | 5 (33, 34, 35, 36, 37) |
| *DPB1*02:04:01-DRA*01:02:01* | 12 (2, 3, 4, 8, 13, 15, 16, 17, 18, 19, 24, 36) |
| *DPB1*02:01-DRA*01:02:01* | 11 (8, 13, 15, 19, 23, 26, 33, 34, 35, 37, 38) |
| *DPB1*02:04:01-DQB1*18:22* | 5 (2, 13, 16, 18, 19) |
| *DPB1*02:04:01-DPB1*19:01:01* | 6 (2, 8, 15, 16, 19, 21) |

In conclusion, we comprehensively identified MHC class II alleles, including *Mamu-DPB1*, *Mamu-DPA1*, *Mamu-DQB1*, *Mamu-DQA1*, *Mamu-DRB*, and *Mamu-DRA* alleles in 30 Chinese rhesus macaques and compared the frequency and distribution of alleles with those from Indian rhesus macaques and Vietnamese cynomolgus macaques by combining this study with our previous work. We found two high-frequency *Mamu-DPB1* alleles, an allele involving in lower viral loads, and several combinations of MHC class II alleles across loci, providing an important addition to the limited immunogenetic information available for Chinese rhesus macaques. We also found quite a few differences between Chinese rhesus macaques and Indian rhesus macaques. These and other intra-specific genetic differences among regional populations of rhesus macaques might influence the outcome of biomedical research in which they are used as subjects and illustrate the importance of completely genetically characterizing subjects used as animal models in biomedical research.

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