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BRIEF COMMUNICATION

Identification of *Mamu-DPA1*, *Mamu-DQA1*, and *Mamu-DRA* alleles in a cohort of Chinese rhesus macaques

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Abstract Rhesus macaques have long been used as animal models for various human diseases; 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-DPA1*, *Mamu-DQA1*, and *Mamu-DRA* alleles were investigated in 30 Chinese rhesus macaques by gene cloning and sequencing. A total of 14 *Mamu-DPA1*, 17 *Mamu-DQA1*, and 9 *Mamu-DRA* alleles were identified in this study. Of these alleles, 22 novel sequences have not been documented in earlier studies, including nine *Mamu-DPA1*, ten *Mamu-DQA1*, and three *Mamu-DRA* alleles. Interestingly, like *Mafa-DQA1* and *Mafa-DPA1*, more than two *Mamu-DQA1* and *Mamu-DPA1* alleles were detected in one animal in this study, which suggested that they might represent gene duplication. If our findings can be validated by other studies, it will further increase the number of known *Mamu-DPA1* and *Mamu-DQA1* polymorphisms. Our data also indicated significant differences in MHC class II allele distribution among the Chinese rhesus macaques, Vietnamese cynomolgus macaques, and the previously reported rhesus macaques, which were mostly of Indian origin. This information will not only promote the understanding of Chinese rhesus macaque MHC diversity and polymorphism but will also facilitate the use of Chinese rhesus macaques in studies of human disease.

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The rhesus macaque (*Macaca mulatta*) has long been used as an experimental model animal for biomedical research. Their response to infectious agents related to human pathogens has made macaques the preferred model for vaccine development. With the advancement of whole genome sequencing, increasing attention has focused on identifying genetic factors contributing to the variable expression of disease susceptibility and resistance. The MHC gene products play an essential role in immune regulatory processes, and polymorphism of the major histocompatibility complex (MHC) genes has been associated with the diversity of immune defenses and more than 100 diseases. MHC polymorphism appears to be one of the main host factors associated with susceptibility or resistance. This leads to an unprecedented variety of gene products and results in different rates of disease progression between the rhesus macaques from India and those from China. It has been demonstrated that MHC alleles are associated with disease progression in rhesus macaques infected with simian immunodeficiency virus. Therefore, knowledge about the MHC background of Chinese rhesus macaques will be useful in facilitating a more precise explanation of the experimental results obtained using these animals.

Until now, the identification of the *Mamu-DPA1* allele has been undocumented, except for the results of Otting and Bontrop (1995) and Giraldo-Vela et al. (2008). In contrast to *Mamu-DPA1*, several *Mamu-DQA1* alleles have been characterized (Rolfs et al. 2001a, 2001b; Viray et al. 2001; Doxiadis et al. 2003; Giraldo-Vela et al. 2008). Therefore, *Mamu-DQA1* and *Mamu-DPA1* genes display a high degree of polymorphism. So far, research in humans and nonhuman primates has shown that the DRA locus is fairly monomorphic in humans, whereas the rhesus macaques and cynomolgus

macaques are polymorphic at this locus (de Groot et al. 2004; O'Connor et al. 2007; Aarnink et al. 2010; Doxiadis et al. 2012). Like *Mamu-DPA1*, the identification of the *Mamu-DRA* allele is undocumented except for the results of de Groot et al. (2004).

Indian rhesus macaques have been used almost exclusively in AIDS pathogenesis and vaccine studies in recent years; however, increasing numbers of Chinese rhesus macaques and cynomolgus macaques have been used in corresponding studies in recent years because of the shortage of Indian rhesus macaques (Qiu et al. 2008). Until now, most reports on rhesus macaque MHC genotypes have been based on Indian rhesus macaques, and limited information is available on the MHC genotypes of Chinese rhesus macaques. To increase understanding of the genetic differences and to facilitate the further use of this macaque in future biomedical research, the genomes of both Chinese rhesus macaques and Vietnamese cynomolgus macaques were sequenced and analyzed. In our previous study, we found by analyzing the genetic divergence patterns that the cynomolgus macaque genome has been shaped by introgression after hybridization with the Chinese rhesus macaque (Yan et al. 2011). In addition, we documented a high degree of MHC polymorphism in Vietnamese cynomolgus macaques and detected a few alleles shared with other original cynomolgus macaques and rhesus macaques (Ling et al. 2011; Zhuo et al. 2011; Ling et al. 2012; Xiang et al. 2013). To elucidate further the genetic differences and possible relationships of MHC polymorphism in the two macaques and to expand our knowledge of the MHC genotypes in Chinese rhesus macaques, using the same primers and methods (Xiang et al. 2013), we characterized the *Mamu-DPA1*, *Mamu-DQA1*, and *Mamu-DR*A alleles of 30 unrelated Chinese rhesus macaques and compared with the distribution and frequency of MHC II alleles between the two macaque populations in the present study. Novel allele sequences were confirmed by the sequencing three different clones, were submitted to the GenBank database (www.ncbi.nlm.nih.gov/genbank/), and were assigned allele names by the Nonhuman Primate Nomenclature Committee (www.ebi.ac.uk). All accession numbers of the alleles are listed in Table 1, and the novel alleles were italicized.

In total, in the present study, 14 *Mamu-DPA1* alleles, including 9 novel alleles, were identified in Chinese rhesus macaques. Surprisingly, 26 *Mafa-DPA1* alleles were detected in the same number of Vietnamese cynomolgus macaques when the same primers were used (Xiang et al. 2013). The most frequent allele in Chinese rhesus macaques was *Mamu-DPA1*07:03*, which was found in 7 of the 28 macaques. Although the same primers were used, the most frequent allele in Vietnamese cynomolgus macaques was *Mafa-DPA1*02:09*, which was found in 7 of the 28 macaques (Xiang et al. 2013). The second most frequent allele in Chinese rhesus macaques was *Mamu-DPA1*02:05*, which was detected in six individuals (Table 1). We also found that one to two *Mamu-DPA1* alleles were expressed in most individual animals, except no. 36, in which three alleles were

Table 1 Distribution of *Mamu-DPA1*, *Mamu-DQA1*, and *Mamu-DRA* alleles detected in a cohort of Chinese rhesus macaques

Allele	Accession number	Total number of allele (individual number)[a]
Mamu-DPA1*02:05	KC428050	6 (2, 4, 19, 21, 28, 31)
*Mamu-DPA1*02:10*	KC428045	2 (20, 36)
*Mamu-DPA1*02:11*	KC428052	2 (26, 36)
Mamu-DPA1*04:02	KC428053	3 (23, 28, 30)
Mamu-DPA1*04:03	KC428047	2 (8, 9)
Mamu-DPA1*04:04	KC428042	1 (10)
*Mamu-DPA1*06:01:02*	KC428043	1 (34)
*Mamu-DPA1*06:03*	KC428046	2 (15, 37)
*Mamu-DPA1*07:02*	KC428044	2 (9, 35)
*Mamu-DPA1*07:03*	KC428041	7 (3, 8, 13, 16, 26, 33, 37)
Mamu-DPA1*08:01	KC428048	5 (3, 7, 15, 19, 30)
*Mamu-DPA1*08:02*	KC428054	1 (36)
*Mamu-DPA1*10:01*	KC428049	4 (2, 24, 27, 29)
*Mamu-DPA1*11:01*	KC428051	3 (17, 20, 21)
Mamu-DQA1*01:02	KC428065	5 (4, 13, 27, 28, 37)
*Mamu-DQA1*01:05:03*	KC428059	1 (34)
Mamu-DQA1*01:08	KC428067	1 (10)
Mamu-DQA1*05:04	KC428066	2 (13, 18)
*Mamu-DQA1*05:05*	KC428063	5 (4, 15, 18, 36, 37)
Mamu-DQA1*24:01	KC428061	6 (2, 3, 16, 19, 33, 35)
*Mamu-DQA1*24:01:02*	KC428055	3 (19, 20, 24)
*Mamu-DQA1*24:06*	KC428058	5 (2, 17, 18, 21, 24)
*Mamu-DQA1*24:07*	KC428060	1 (38)
*Mamu-DQA1*24:08*	KC428068	6 (7, 9, 21, 29, 30, 38)
*Mamu-DQA1*24:09*	KC428071	2 (17, 26)
*Mamu-DQA1*24:10*	KC428062	1 (36)
Mamu-DQA1*26:01	KC428056	5 (8, 9, 27, 30, 31)
Mamu-DQA1*26:02	KC428057	2 (22, 34)
Mamu-DQA1*26:03	KC428064	1 (31)
*Mamu-DQA1*26:07*	KC428069	1 (10)
*Mamu-DQA1*26:08*	KC428070	2 (16, 31)
Mamu-DRA*01:02:01	KC428082	24 (2, 3, 4, 8, 9, 13, 15, 16, 17, 18, 19, 20, 23, 24, 26, 29, 30, 31, 33, 34, 35, 36, 37, 38)
Mamu-DRA*01:02:02	KC428077	2 (23, 33)
Mamu-DRA*01:02:05	KC428081	8 (2, 3, 9, 10, 15, 20, 28, 29)
Mamu-DRA*01:04:01	KC428062	7 (7, 24, 26, 29, 30, 35, 36)
Mamu-DRA*01:06	KC428075	1 (34)
*Mamu-DRA*01:07*	KC428078	1 (28)
*Mamu-DRA*01:08*	KC428072	4 (13, 18, 22, 38)
*Mamu-DRA*01:09*	KC428076	1 (27)

[a] Number of animals sharing a certain allele. Novel alleles identified in this study are italicized

detected. More than two *Mamu-DPA1* alleles coexisted in one animal, suggesting that *Mamu-DPA1* might have been subject to a gene duplication. The above result was consistent with the findings related to *Mafa-DPA1* in Vietnamese cynomolgus macaques in our previous study (Xiang et al. 2013). However, this supposition requires investigation in a future study to enrich the polymorphism database of *Mamu-DPA1*.

As seen in Table 1, the most common sequences belonged to the DPA1*02 and DPA1*04 lineages (three alleles), the second most common belonged to the DPA1*06, DPA1*07, and DPA1*08 lineages (two alleles), and the rest belonged to the DPA1*10 and DPA1*11 lineage (one allele); DPA1*11 was identified as a new lineage. However, the most common sequences in Vietnamese cynomolgus macaques belonged to the DPA1*02 and DPA1*07 lineages (Xiang et al. 2013). The same primers were used to amplify *MHC-DPA1* alleles in the same number of Vietnamese cynomolgus macaques and Chinese rhesus macaques, but the numbers of the allele detected showed considerable differences between the two macaque species, as did the distribution of lineages.

With regard to *Mamu-DQA1*, a total of 17 alleles, including10 novel alleles, were identified in Chinese rhesus macaques. Interestingly, unlike *Mamu-DPA1*, the same numbers of *Mafa-DQA1* alleles were detected in the same number of Vietnamese cynomolgus macaques using the same primers (Xiang et al. 2013). Reportedly, 18 *Mamu-DQA1* alleles were detected in approximately 150 rhesus macaques (Doxiadis et al. 2003), and 12 *Mamu-DQA1* alleles were observed previously in 21 Chinese rhesus macaques (Viray et al. 2001). The most frequent alleles were *DQA1*24:01* and *DQA1*24:08*, both of which were detected in six individuals. The second frequent alleles were *Mamu-DQA1*26:01*, *Mamu-DQA1*05:05*, *Mamu-DQA1*24:06*, and *Mamu-DQA1*01:02*, all of which were detected in five individuals; *Mamu-DQA1*24:01:02* was found in three individuals (Table 1). Reportedly, the alleles *Mamu-DQA1*01:04*, *Mamu-DQA1*01:02*, and *Mamu-DQA1*25:01* were the most frequently observed in Indian rhesus macaques, at frequencies of 27.7, 16.9, and 19.2 %, respectively (Rolfs et al. 2001a). Importantly, the frequency of *Mamu-DQA1*01:02* in Indian rhesus macaques was approximately the same as that of the said allele in Chinese rhesus macaques, but the alleles *Mamu-DQA1*01:04* and *Mamu-*25:01* observed in Indian rhesus macaques were undetected in Chinese rhesus macaques in the present study. Moreover, the *Mamu-*240X* (21.4 %), *Mamu-*25:03* (11.9 %), and *Mamu-*01:08* (11.9 %) phenotypes, the most frequently observed *Mamu-DQA1* alleles in Chinese rhesus macaques, were either absent or less common in Indian and Burmese rhesus macaques, and alleles *24:01, *24:03, and *24:04 were combined into allele *240X (Viray et al. 2001). In the present study, the most frequent allele, *DQA1-*24:01*, was detected in six individuals, but *DQA1-*25:03* was undetected; the frequency of *DQA1-*01:08* was detected in one animal. At least one to two *Mamu-DQA1* alleles were expressed in most individual animals, except for two, in this study (Table 1). Interestingly, three alleles were detected in monkeys no. 18 and no. 31, which was consistent with previous findings in Vietnamese cynomolgus macaques (Xiang et al. 2013), suggesting that *Mamu-DQA1*, as well as *Mafa-DQA1*, might have been subject to a gene duplication. However, this supposition requires investigation in a future study designed to enrich further the polymorphism database of *Mamu-DQA1*.

As seen in Table 1, the most common sequences belonged to the DQA1*24 lineage (seven alleles), and the second most common belonged to the DQA1*26 lineage (five alleles). All sequences detected in this study belonged to four lineages, namely the DPA1*01, DQA1*05, DQA1*24, and DQA1*26 lineages, which is consistent with our previous results (Xiang et al. 2013) and earlier findings in Vietnamese cynomolgus macaques (Creager et al. 2011). The DPA1*23 lineage was not detected in our study, whereas it was identified in Chinese rhesus macaques (Doxiadis et al. 2003). The difference may be due to the small sample size and the different primers used in the present study. In summary, the same numbers of alleles were detected in the two macaque species, and the distribution of lineages was the same, when the same primers were used to amplify *MHC-DQA1* alleles in the same number of Vietnamese cynomolgus macaques and Chinese rhesus macaques.

A total of nine *Mamu-DRA* alleles, including three novel alleles, were identified in this study. The most frequent allele was *Mamu-DRA*01:02:01*, which was found in 24 of the 29 macaques. Using the same primers, our results showed that nine *Mafa-DRA* alleles were identified and that the most frequent allele was *Mafa-DRA*01:02:01* in Vietnamese cynomolgus macaques (Xiang et al. 2013), which was 100 % identical to *Mamu-DRA*01:02:01*. The second most frequent allele was *Mamu-DRA*01:02:05* (Table 1). In addition, all of these alleles belonged to one lineage: Mamu-DRA*01. Reportedly, the DRA locus is fairly monomorphic in humans, whereas rhesus macaques have 12 defined alleles in this locus (de Groot et al. 2004).

In conclusion, we successfully identified *Mamu-DPA1*, *Mamu-DQA1*, and *Mamu-DRA* alleles in 30 Chinese rhesus macaques and compared the frequency and distribution of alleles with those in Indian rhesus macaques and Vietnamese cynomolgus macaques by combining this study with our previous work. We found that only a small number of alleles appear to be shared with other populations, providing an important addition to the limited immunogenetic information available for Chinese rhesus macaques. This suggests the occurrence of rapid evolution of *Mamu-DRA*, *Mamu-DPA1*, and *Mamu-DQA1* alleles due to adaptation to new environments. The alleles found at high frequencies within the Chinese population may represent high-priority targets for additional characterization of immune function, may be vital

for disease research, and may help elucidate the biogeography of nonhuman primates.

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