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## Novel DRA alleles extracted from seven macaque cohorts

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In this document, we report the detection of 37 DRA alleles in macaque cohorts.

Non-human primates such as macaques are widely used as animal models in research on human diseases. Their

application in the study of immune-related disorders mandates the investigation of these primates' major histocompatibility complexes (MHC). For this purpose, various cohorts of rhesus (*Macaca mulatta*), cynomolgus (*Macaca fascicularis*), and pigtailed macaques (*Macaca nemestrina*) were subjected to the analyses of their Mhc-DR region, the equivalent of human leukocyte antigen (HLA)-DR in humans (1–4).

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**Table 1** New DRA alleles detected in seven cohorts of macaques. The primate centres that provided RNA samples are indicated in the third column. Animals with names containing Y are housed at Yulin, and those with names containing C are at the Hainan centre. The terms 'ext' and 'conf' are abbreviations for extension and confirmation of the respective alleles

Allele	Accession	Animal/primate centre	Remarks	Identical to
Indonesian cynomolgus		Alpha Genesis, US		
<i>Mafa-DRA*01:02:23</i>	HF938095	DX86, FC3F	also HF938091	
<i>Mafa-DRA*01:03:09</i>	HF938099	DM43, FC3L		
<i>Mafa-DRA*01:04:02</i>	HF938096	FC7B, FC7C	AB306651, EU877219 ext	
<i>Mafa-DRA*01:11</i>	HF938097	DV69, GB68		
<i>Mafa-DRA*01:12</i>	HF938098	FE8C, CG2G		
Cambodian cynomolgus		Jinggang and Hainan, China		
<i>Mafa-DRA*01:01:11</i>	LN624367	0501564		<i>Mamu-DRA*01:04:04</i>
<i>Mafa-DRA*01:02:22</i>	LN624368	0306143, 1105180	JQ698500 ext	<i>Mamu-DRA*01:02:02</i>
<i>Mafa-DRA*01:02:24</i>	LN626614	760, 802		<i>Mamu-DRA*01:02:09</i>
<i>Mafa-DRA*01:02:25</i>	LN624369	C1206043, C0602051		<i>Mane-DRA*01:02:01</i>
<i>Mafa-DRA*01:02:26</i>	LN624370	0511187		
<i>Mafa-DRA*01:10:03</i>	LN624371	1205008, 0707596		
Vietnamese cynomolgus		Yulin, China		
<i>Mafa-DRA*01:13</i>	LN624372	Y1203001, Y9407013		
Chinese rhesus		Chengdu and Yulin, China		
<i>Mamu-DRA*01:02:08</i>	HF938102	99027, 9904016Y		
<i>Mamu-DRA*01:02:09</i>	HF938103	09057, 09085		
<i>Mamu-DRA*01:03:01</i>	HF586896	02314, 97699	AJ586881 ext	<i>Mane-DRA*01:03:01, Mafa-DRA*01:03:01</i>
<i>Mamu-DRA*01:04:01</i>	HF586897	99088, 9706006Y	AJ586882, KC428062 conf	<i>Mafa-DRA*01:01:07</i>
<i>Mamu-DRA*01:04:02</i>	HF586898	99107, 9402005Y	AJ586883 conf	
<i>Mamu-DRA*01:04:04</i>	HF586899	9067		
<i>Mamu-DRA*01:04:05</i>	HF586901	99033		<i>Mane-DRA*01:04:02, Mafa-DRA*01:01:08</i>
<i>Mamu-DRA*01:04:06</i>	HF586902	9606001Y, 9706006Y		<i>Mafa-DRA*01:01:06</i>
<i>Mamu-DRA*01:04:07</i>	HF938101	9309009Y, 9606001Y		
<i>Mamu-DRA*01:06:01</i>	HF586900	99261, 9701010Y	KC428075 ext	
<i>Mamu-DRA*01:06:02</i>	HF938100	95512, 02818		
<i>Mamu-DRA*01:06:03</i>	HF938104	02874, 0905012Y		<i>Mafa-DRA*01:10:02</i>
<i>Mamu-DRA*01:07</i>	HF938105	94820, 99036	KC428078 ext	<i>Mafa-DRA*01:04:01, Mane-DRA*01:05</i>
<i>Mamu-DRA*02:01</i>	HF586903	99027, 09300	AM910165 genomic	<i>Mafa-DRA*02:01:01, Mane-DRA*02:01:03</i>
Pigtailed macaque		Johns Hopkins, US		
<i>Mane-DRA*01:01</i>	HF938114	15Y, 69Z		<i>Mafa-DRA*01:09</i>
<i>Mane-DRA*01:02:01</i>	HF938106	76Z, 88Z		
<i>Mane-DRA*01:02:02</i>	HF938107	71X, 42R		<i>Mafa-DRA*01:02:01</i>
<i>Mane-DRA*01:03:01</i>	HF938111	LJ2, 94Z	GQ214407 conf	<i>Mafa-DRA*01:03:01, Mamu-DRA*01:03:01</i>
<i>Mane-DRA*01:03:02</i>	HF938112	AT4B, 61Y		
<i>Mane-DRA*01:04:01</i>	HF938113	16V, 93W		
<i>Mane-DRA*01:05</i>	HF938108	JT1, 59Y		<i>Mafa-DRA*01:04:01, Mamu-DRA*01:07</i>
<i>Mane-DRA*01:06:01</i>	HF938109	5Y, 854		
<i>Mane-DRA*01:06:02</i>	HF938110	58Y, CT2C		
<i>Mane-DRA*02:01:01</i>	HF938115	74Z, UP1		<i>Mafa-DRA*02:01:04</i>
<i>Mane-DRA*02:01:02</i>	HF938116	12Y, 93Z		

The DRB region is characterised by allelic polymorphism, and by gene copy number variation or region configuration polymorphism. Whereas in humans only five DRB region configurations, with profound allelic polymorphisms, are known, the equivalent region in macaques is more complex. For example, in a study involving a pedigreed cohort of cynomolgus macaques, 28 different region configurations were described, in which the number of DRB genes ranges from two to five per haplotype (2).

In humans, the DRA gene is not duplicated and is virtually non-polymorphic. Only five transcribed HLA-DRA alleles are known, coding for two different  $\alpha$ -chains. In macaques, polymorphism for the DRA gene has been described, and many DRA alleles have been reported for cynomolgus, rhesus, and pigtailed macaques (<http://www.ebi.ac.uk/ipd/mhc/nhp/>) (5). Most base pair differences are synonymous, however, and the non-synonymous variation is mainly confined to the third codon of the leader peptide. This is in contrast to other MHC

class II genes, in which polymorphism is observed in the exons encoding the peptide-binding amino acids. Two lineages of DRA alleles are distinguished in macaques: *DRA\*01* and the less frequent *DRA\*02*. The proteins that are encoded by the second lineage differ for five amino acids, three of which are positioned in exon 2. These differences may have an influence on the binding of peptides.

Here, we report 37 additional Mhc-DRA alleles in cynomolgus macaques of various geographical origins, in Chinese rhesus monkeys, and in pigtailed macaques, detected by routinely conducted mRNA sequencing. The Indonesian cynomolgus macaques are housed at Alpha Genesis Inc. (Yemassee, SC). The Cambodian and Vietnamese cynomolgus samples, along with the Chinese rhesus macaque samples, were obtained from breeding centres in China (Table 1). The pigtailed macaque samples were provided by Johns Hopkins University (Baltimore, MD).

Using RNA samples derived from the animals, first-strand cDNA synthesis was performed with the RevertAid kit (Thermo Fisher Scientific, Landsmeer, The Netherlands), followed by polymerase chain reaction (PCR) with primers 5'DRA-F2 and 3'DRA-R2 (6). Cloning, Sanger-sequencing, and data-analyses were performed as previously described (2). The novel sequences, based on at least two PCR reactions, were submitted to the EMBL-EBI database, and – for official designations – to the non-human primate section of the IPD database. A total of 37 full-length mRNA sequences were submitted, respectively, 12 Mafa-DRA, 14 Mamu-DRA, and 11 Mane-DRA alleles, respectively (Table 1).

Five alleles extend known sequences, whereas three redundant submissions appeared to confirm already known full-length alleles. The other 29 sequences represent unreported DRA alleles. The rhesus macaque *Mamu-DRA\*02:01* allele was reported earlier as *Mamu-DRA\*0106*, based on genomic sequencing (1) and is identical to the alleles *Mafa-DRA\*02:01:01* and *Mane-DRA\*02:01:03* in

cynomolgus and pigtailed macaques. Other Mhc-DRA alleles are also shared by the macaque species, emphasising their common ancestry.

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#### Conflict of interest

The authors have declared no conflicting interests.

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