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MHC class I A region diversity and polymorphism in macaque species

Nel Otting · Annemiek J. M. de Vos-Rouweler ·
Corrine M. C. Heijmans · Natasja G. de Groot ·
Gaby G. M. Doxiadis · Ronald E. Bontrop

Abstract The *HLA-A* locus represents a single copy gene
that displays abundant allelic polymorphism in the human
population, whereas, in contrast, a nonhuman primate
species such as the rhesus macaque (*Macaca mulatta*)
possesses multiple *HLA-A*-like (*Mamu-A*) genes, which
parade varying degrees of polymorphism. The number and
combination of transcribed *Mamu-A* genes present per
chromosome display diversity in a population of Indian
animals. At present, it is not clearly understood whether
these different *A* region configurations are evolutionarily
stable entities. To shed light on this issue, rhesus macaques
from a Chinese population and a panel of cynomolgus
monkeys *(Macaca fascicularis)* were screened for various *A*
region-linked variations. Comparisons demonstrated that
most *A* region configurations are old entities predating
macaque speciation, whereas most allelic variation (>95%)
is of more recent origin. The latter situation contrasts the
observations of the major histocompatibility complex class
II genes in rhesus and cynomolgus macaques, which share
a high number of identical alleles (>30%) as defined by
exon 2 sequencing.

N. Otting (✉) · A. J. M. de Vos-Rouweler · C. M. C. Heijmans ·
N. G. de Groot · G. G. M. Doxiadis · R. E. Bontrop
Department of Comparative Genetics and Refinement,
Biomedical Primate Research Centre,
P.O. Box 3306, 2280 GH Rijswijk, The Netherlands
e-mail: otting@bprc.nl

Introduction

Major histocompatibility complex (MHC) represents a
multigene family that plays a crucial role in the generation
of adaptive immune responses in vertebrate species. A key
feature of the system is that some of its genes display
abundant polymorphism at the population level. In addition,
the number of *Mhc* class I or II genes may differ
significantly between species, as well as between individ-
uals of a species (Kelley et al. 2005). MHC polymorphisms
may have a profound impact on several features such as
disease susceptibility, organ transplantation, and reproduc-
tive success (Lagaaij et al. 1989; Goulder and Watkins
2004; Bontrop and Watkins 2005; Ziegler et al. 2005;
Smith et al. 2006). The MHC systems of various primate
species, including humans, have been studied extensively
(Watkins 1995; Antunes et al. 1998; Bontrop et al. 1999;
Adams and Parham 2001; de Groot et al. 2002; Lafont et al.
2004; Middleton et al. 2004; Marsh et al. 2005; Penedo et
al. 2005; Abbott et al. 2006; Huchard et al. 2006). For
example, the MHC of the rhesus macaque (*MhcMamu*), an
Old World primate species, has been shown to share many
similarities with the human leukocyte antigen (HLA)
system in humans. The evolutionary orthologs of the
HLA-DP, *-DQ*, and *-DR* genes are also present in rhesus
monkeys, and as in humans, these loci are polymorphic
(Bontrop et al. 1999). The number of *Mamu-DRB* region
configurations appears to be expanded in comparison with
humans, and some of these regions seem to harbor an
extended number of genes (Doxiadis et al. 2000). Subse-
quent cDNA studies have illustrated, however, that in

humans and rhesus macaques, comparable numbers of *DRB* genes are transcribed (de Groot et al. 2004).

Apart from *Mamu-E* (Knapp et al. 1998), -*F* (Otting and Bontrop 1993), -*G* (Boyson et al. 1996a; Castro et al. 1996), and -*AG* (Boyson et al. 1997), rhesus macaques also possess a *B*-like sequence, designated *Mamu-I*, which appears to be present in each haplotype (Urvater et al. 2000). As observed in humans, these nonclassical genes display low levels of polymorphism. An ortholog of the *HLA-C* gene appears to be absent in the rhesus macaque, whereas evolutionary equivalents of the classical *HLA-A* and -*B* genes have been described (Boyson et al. 1996b). The *Mamu-A* and -*B* genes have been subjected to several rounds of duplication as was shown by genomic sequencing (Daza-Vamenta et al. 2004; Kulski et al. 2004). Analysis of a panel of rhesus macaques, mainly originating from the Indian subcontinent, illustrated that the number and combination of *Mamu-A* and -*B* genes that are expressed per haplotype may differ at the population level (Otting et al. 2005). In the same study, marked differences in expression levels were shown. At this stage it is not known whether rhesus macaques originating from other geographic areas have unique *Mhc* class I alleles and/or region configurations. Comparative studies have illustrated that many *Mhc* loci and lineages predate speciation events. The sharing of *Mhc* alleles between two primate species seems to be a rare event, and only a few cases have been documented (Cooper et al. 1998; Evans et al. 1998). An exception is provided by rhesus and cynomolgus macaques, which seem to share a high number of *Mhc* class II alleles as was defined by exon 2 sequencing (Blancher et al. 2006; Doxiadis et al. 2006). Whether this sharing is because of introgression or purifying selection remains to be elucidated. Thus far, about 50 *Mafa-A* sequences have been published for the cynomolgus macaque (Uda et al. 2004; Krebs et al. 2005). The absence of pedigreed material did not allow us to define *Mafa-A* loci or region configurations. For that reason, a panel of pedigreed cynomolgus macaques was incorporated in the present study. Comparison with rhesus macaque *Mhc* class I sequences obtained from different populations enabled us to draw conclusions on the evolutionary stability of *A* region variations.

Materials and methods

Animals and cell lines

The Biomedical Primate Research Centre houses a self-sustaining colony of approximately 1,000 rhesus macaques, mainly of Indian origin, that have been pedigreed based on the segregation of serologically defined MHC allotypes (Bontrop et al. 1999; Penedo et al. 2005; Doxiadis et al.

2006). Furthermore, a large collection of DNA samples as well as B-cell lines is available. In this study, B-cells derived from rhesus macaques of Chinese origin and from cynomolgus monkeys were used to isolate RNA. Most cynomolgus monkey samples originate from a pedigreed group housed at the campus of the University of Utrecht.

cDNA cloning and sequencing

RNA was isolated from B cells (Rneasy kit, Qiagen) and subjected to One-step reverse transcriptase polymerase chain reaction (RT-PCR), as recommended by the supplier (Qiagen). The primers (5′MAS) AATTCATGGCGCCCCG AACCCTCCTCCTGG, and (3′MAS) CTAGACCACACA AGGCGGCTGTCTCAC were used, which are specific for class I *A* transcripts in macaques. The final elongation step was extended to 30 min to generate a 3′dA overhang. The RT-PCR products were cloned using the InsT/Aclone kit (Fermentas). After transformation, 16 to 32 colonies were picked for plasmid isolations. The separate *Mamu-A* loci show differences in expression levels, as indicated by the numbers of clones, picked from each animal. The alleles with high expression levels (majors) may be associated with serotypes and thus involved in classical antigen presentation, whereas those with low expression levels (minors) are considered nonclassicals and may exhibit more specialized types of functions (Otting et al. 2005).

Sequencing reactions were performed using the BigDye terminator cycle sequencing kit, and samples were run on an automated capillary sequencing system (ABI Genetic Analyzer 3100) as has been described previously (Otting et al. 2005).

Locus-specific PCR reactions

To test the presence of *Mamu*- and *Mafa-A4*14* alleles, 1 μl of the RT-PCR samples was taken for a single-specific-primer PCR (SSP-PCR) reaction in advance of the cloning step. The relevant primers, (5′A*14) GGGACCCGACG GGCGCCTCCAA and (3′A*14) GGCCCTCCAGGTAGA CTCTGTC have annealing sites in exon 3. Amplifications were carried out starting with 2 min at 94°C, followed by 25 cycles at 94°C, 65°C, and 72°C for 1 min each. The PCR products were subjected to direct sequencing, and the reactions were performed as described above.

Phylogenetic analysis and nomenclature

The sequences were analyzed with the Sequence Navigator Software version 1.0.1 (Applied Biosystems), and alleles are based on at least three clones with identical full-length sequences. To define loci and lineages, alignments of the sequences were made using the MacVector™ version 8.1.1

(Oxford Molecular Group), followed by manual adjustments. Phylogenetic analyses on the full-length (1,065 bp) sequences were also performed with the MacVector software. Neighbor-joining trees were constructed with the Kimura 2 parameter method. Bootstrap analyses were performed based on 1,000 replications.

In total, 130 unreported Mamu-A and Mafa-A sequences were submitted to the European Bioinformatics Institute and European Molecular Biology Laboratory (EBI-EMBL) database. Relevant information such as accession number and a reference cell-line are provided (Table 1). Moreover, all novel Mamu-A and Mafa-A sequences are named in accordance with a generally accepted nomenclature proposal (Klein et al. 1990; Robinson et al. 2003; Ellis et al. 2006). For example, Mamu-A1*0101 defines a Mhc allele in the rhesus macaque, which is encoded by one of the class I loci: namely, A1. The first two digits after the asterisk define the lineage, whereas the third and fourth digits define the allele number. These allele numbers are arbitrary, as they reflect the order in which the alleles were discovered. A fifth and sixth digit are used to mark a synonymous basepair difference between two sequences.

Results and discussion

Polymorphism and diversity of the Mamu-A region: comparison of Chinese and Indian rhesus macaques

In a previous communication, five different Mamu-A region configurations were defined in a population of Indian rhesus macaques (Otting et al. 2005). These configurations display diversity with regard to the number and combination of distinct Mamu-A genes present per chromosome. The loci have been designated Mamu-A1, -A2, -A3, and -A4, respectively (Fig. 1). As can be seen, each region configuration comprises a Mamu-A1 gene characterized by high transcription levels (major) combined with one or two other Mamu-A genes characterized by lower transcription levels (minors). The Mamu-A1 gene is probably responsible for executing the classical antigen presentation function (Evans et al. 1999; Sidney et al. 2000; Sette et al. 2005), whereas the others can be considered as nonclassicals and may interact, for instance, with the KIR gene family present on NK cells. Two complete MHC haplotypes in the rhesus macaque are sequenced (Daza-Vamenta et al. 2004; Kulski et al. 2004), and they represent two-region configurations. One haplotype contains the -A1/-A2 combination, whereas in the other, the -A1/-A4 pair is observed. Both region configurations are present in the animals of Indian origin (Fig. 1).

Within the present panel of 42 Chinese animals, 59 different full-length Mamu-A cDNAs were detected that can be grouped into various loci and lineages (Fig. 2). A

Table 1 Summary of Mamu-A and Mafa-A alleles detected in this study

Allele	Accession	Animals	Allele	Accession	Animals
Mamu-A1			Mafa-A1		
A1*0102	AM295885	EKK	A1*0101	AM295828	Yabaa
A1*0103	AM295886	Ri056	A1*0301	AM295829	Hoeba
A1*030102	AM295887	Ri185	A1*0302	AM295830	77
A1*0302	AM295888	r300	A1*1002	AM295831	k71
A1*0303	AM295889	8822	A1*1003	AM295832	Trufo
A1*0304	AM295890	Ri301	A1*1702	AM295833	Bufo
A1*0305	AM295891	Ri037	A1*1901	AM295834	77
A1*040102	AM295892	Ri165	A1*2202	AM295835	k94
A1*0402	AM295893	Ri366	A1*3101	AM295836	Gayo
A1*1001	AM295894	Ri002	A1*3102	AM295823	Bilboa
A1*1002	AM295895	Ri142	A1*3201	AM295824	80
A1*1102	AM295896	Ri136	A1*3801	AM295825	k66
A1*1701	AM295897	Ri145	A1*4002	AM295826	Pedro
A1*1801	AM295898	Ri011	A1*4102	AM295827	k65
A1*1802	AM295899	Ri018	A1*4302	AM295837	k65
A1*1803	AM295900	Ri260	A1*5201	AM295838	88
A1*1902	AM295901	Ri081	A1*5301	AM295839	k390
A1*1903	AM295902	BB10	A1*5501	AM295840	k127
A1*1904	AM295903	Ri197	A1*5601	AM295841	k766
A1*2201	AM295904	Ri009	A1*5701	AM295842	k2,
A1*2502	AM295905	Ri088	A1*5801	AM295843	Rastafa
A1*2602	AM295906	8765	A1*5901	AM295844	Roza
A1*2802	AM295907	Ri082	A1*6001	AM295845	Vivaa
A1*3201	AM295908	Ri094	A1*6101	AM295846	k135
A1*3301	AM295909	Ri253	A1*6201	AM295847	81
A1*4001	AM295910	Ri165	A1*6301	AM295848	Hippo
A1*4101	AM295911	Ri284	A1*6401	AM295849	Bufo
A1*4701	AM295912	Ri189	A1*6402	AM295850	k95
A1*4801	AM295913	8822	A1*650101	AM295851	81
A1*4901	AM295914	Ri088	A1*650102	AM295852	k390
A1*5001	AM295915	4053	A1*6601	AM295853	k73
A1*5101	AM295916	Ri281	A1*6602	AM295854	Milva
A1*5201	AM295917	Ri136	A1*6701	AM295855	83
A1*5301	AM295918	Ri056	A1*6801	AM295856	Juanita
A1*5401	AM295919	Ri260	A1*6901	AM295857	Zazaa
A1*5402	AM295920	Ri011	A1*7001	AM295858	Friko
A1*5601	AM295921	Ri146	A1*7101	AM295859	Zola
A1*5602	AM295922	Ri228	A1*7201	AM295860	Canada
A1*5701	AM295923	Ri006			
A1*5801	AM295924	Ri026			
Mamu-A2			Mafa-A2		
A2*050301	AM295925	Ri056	A2*0501	AM295861	Clint
A2*050302	AM295930	Ri226	A2*0502	AM295862	84
A2*050402	AM295926	Ri009	A2*0503	AM295863	k95
A2*0513	AM295927	Ri159	A2*0504	AM295864	Clint
A2*0514	AM295928	Ri095	A2*0505	AM295865	Bufo
A2*0515	AM295929	Ri009	A2*0506	AM295866	Trufo
A2*0517	AM295931	Ri284	A2*0507	AM295867	80,
A2*0518	AM295932	Ri094	A2*0508	AM295868	Zebra
A2*0519	AM295933	Ri137	A2*0509	AM295869	81
A2*0520	AM295934	Ri197	A2*0510	AM295870	Milva
A2*0521	AM295935	Ri126	A2*0511	AM295871	Hippo
A2*0522	AM295936	Ri026	A2*051201	AM295872	k73
A2*0523	AM295937	Ri028	A2*051202	AM295876	Dojo

Table 1 (continued)

Allele	Accession	Animals	Allele	Accession	Animals
A2*0524	AM295938	Ri301	A2*0513	AM295873	81
A2*0525	AM295939	Ri137	A2*0514	AM295874	k95
A2*0526	AM295940	Ri002	A2*0515	AM295875	Juanita
A2*2402	AM295941	BB67	A2*0516	AM295878	Jawa
A2*2403	AM295942	Ri366	A2*0517	AM295877	Laba
Mamu-A3			Mafa-A3		
A3*1307	AM295943	Ri136	A3*1301	AM295879	k2,
Mamu-A4			Mafa-A4		
A4*1404	AM295944	Ri137	A4*1401	AM295880	77
			A4*1402	AM295881	Bufo
Mamu-A5			Mafa-A5		
A5*300101	AM295945	BB67	A5*3001	AM295882	Gayo
A5*300102	AM295946	Ri081	A5*3002	AM295883	k766
A5*3002	AM295948	Ri088			
A5*3003	AM295947	Ri366			
Mamu-A6			Mafa-A6		
A6*0101	AM295949	Ri145	A6*0101	AM295884	k73
Mamu-A7					
A7*0101	AM295950	Ri082			
A7*0102	AM295951	Ri315			
A7*0103	AM295952	Ri009			

The Chinese rhesus macaques are indicated by Ri numbers. Nine alleles found in rhesus macaques of mixed breeding are also reported.

complete listing of the alleles detected in the Chinese macaques is provided as electronic supplementary material (Table 5). Because of codominant expression, rhesus macaques can be heterozygous for the *Mamu-A1* gene and thus can express up to two allotypes. Nine animals were found to express three different *Mamu-A1*-like sequences. These animals shared one of these sequences in separated clades in phylogenetic tree (Fig. 2; -A5, -A6, and -A7). The triplets of the three animals are indicated by asterisks. The clustering of the three alleles, obtained from one animal, in
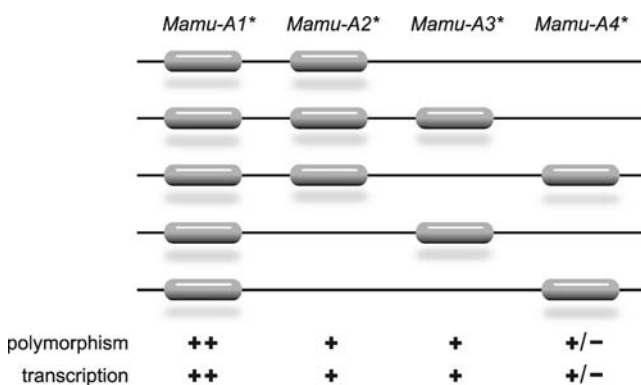

Fig. 1 Schematic representation of different *Mamu-A* region configurations observed in Indian rhesus macaques. The exact order and physical distances of the loci on the genome are still unknown. The relative levels of *polymorphism* and *transcription* of the loci are indicated. Two *Mamu-A* region configurations (1 and 5) are confirmed by the sequencing of the complete rhesus MHC region (Daza-Vamenta et al. 2004; Kulski et al. 2004)

clades distinct from -A2, -A3, and -A4 indicates that one allele belongs to a separate locus. The newly detected loci are named *Mamu-A5*, -A6, and -A7, according to the generally accepted nomenclature system (Klein et al. 1990; Robinson et al. 2003; Ellis et al. 2006). Representatives of two loci, A5 and A6, are also observed in cynomolgus macaques that express more than two A1-like sequences.

Thirty-three new *Mamu-A1* alleles were detected in the Chinese rhesus macaques. Although some of the alleles in this population group into lineages that are also present in Indian animals, sharing of alleles for the *Mamu-A1* locus is rare and was observed only once. The *Mamu-A1*26* allele is detected in both populations, whereas the *Mamu-A1*03* and for -A1*04, alleles are highly similar and differ for one synonymous basepair substitution. Hence, it is concluded that most allelic polymorphism observed for the *Mamu-A1* locus was probably generated after the rhesus macaque populations were separated. Sequence comparisons illustrated that most of the variations map at the contact residues of the peptide-binding site (data not shown). Thus, polymorphism at the highly divergent *Mamu-A1* locus, characterized by high expression levels, must have resulted from positive Darwinian selection (Hughes and Nei 1988; Borghans et al. 2004).

The *Mamu-A2* gene displays differential haplotype distribution in the Indian population of rhesus macaques (Fig. 1). This also appears to be the case for Chinese animals, as *Mamu-A2* cDNAs were detected in 39 out of 42 of them. In this panel, 16 alleles that differ from the ones earlier detected in Indian animals were defined. In the Indian population, low expression levels characterize the *Mamu-A2* gene, which appears also to be the case in Chinese animals.

Five Chinese macaques possess the *Mamu-A3*1307* sequence, which is always observed in combination with *Mamu-A1*1102*. In concordance with the Indian animals, the number of *Mamu-A3* clones detected also reflects low expression levels. A full-length *Mamu-A4*1404* cDNA was detected in only three animals. In contrast to the *Mamu-A4* allele in the Indian macaque, the Chinese allele has a stopcodon at the last triplet of exon 5 encoding the transmembrane part of the class I protein. This suggests that the corresponding gene product may settle in the membrane, but the signal transduction is impaired. As was observed earlier in Indian animals, this locus is characterized by extremely low expression levels (Fig. 1). For that reason, the presence of *Mamu-A4* cDNAs in the other animals was tested with SSP on the RT-PCR samples, and 17 out of 42 Chinese animals appeared to be positive for this locus. The *Mamu-A5*, -A6, and -A7 genes are also characterized by low expression levels and are considered to represent minors.

In contrast to the previously studied Indian population, the Chinese animals were selected randomly from a large

Fig. 2 Phylogenetic tree of *Mamu-A* and *Mafa-A* gene/alleles detected in this study. The *-A2* locus is represented by four alleles only. The tree is based on full-length sequences, although analyses on only exon 2 and 3 showed no significant differences. The nomenclature of the new alleles is based on a more extended tree (not shown) containing all the *Mamu-A* and *Mafa-A* alleles now available. *Asterisk*, three alleles of animal Ri081, of which one represents the locus *Mamu-A5*. *Double asterisks*, three alleles of animal Ri145, of which one represents the locus *Mamu-A6*. *Triple asterisks*, three alleles of animal Ri078, of which one represents the locus *Mamu-A7*
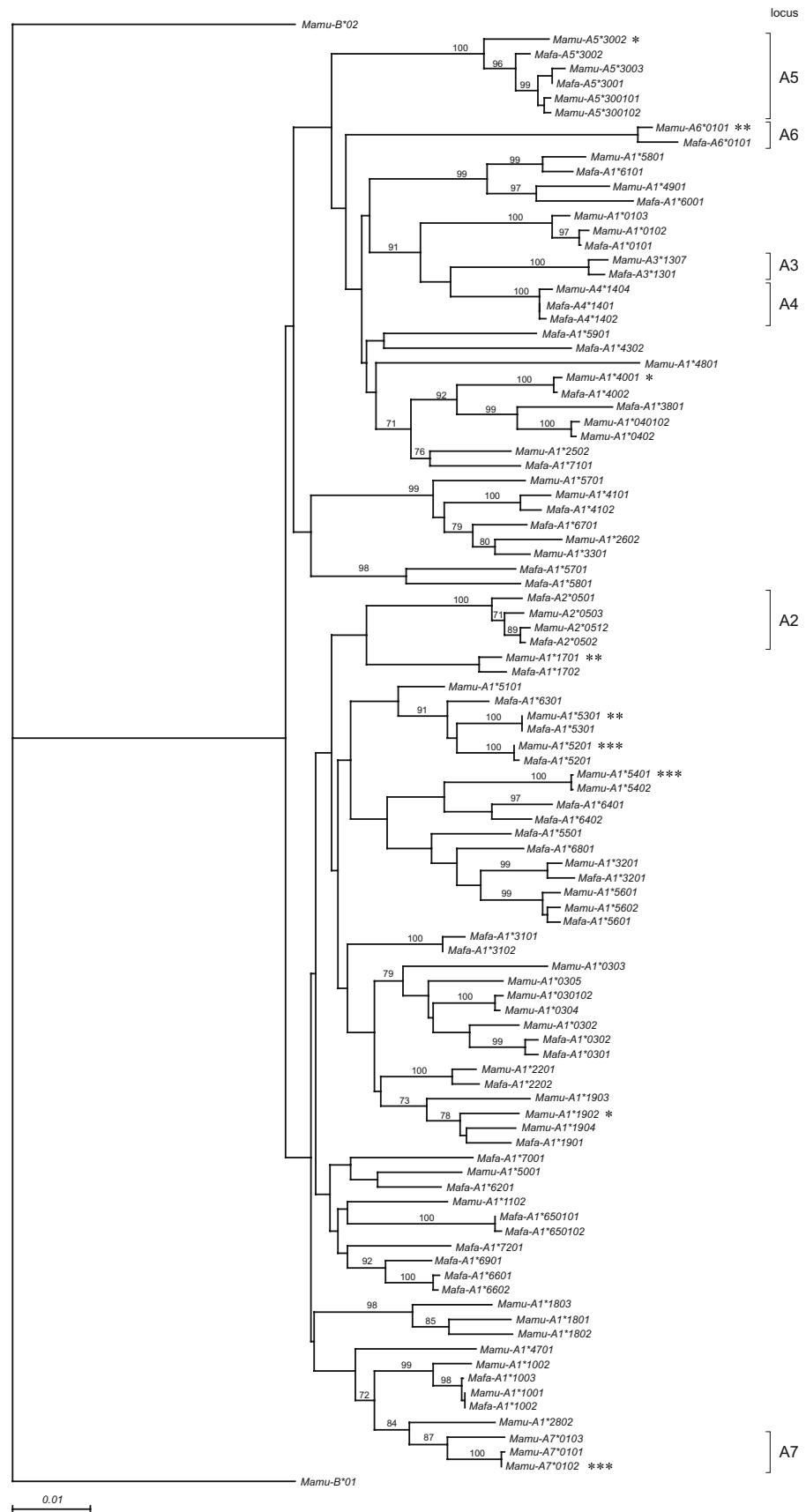
Table 2 Chinese *Mamu-A* haplotypes deduced by sharing of alleles grouped into two-region configurations

	Mamu-A1	*Mamu-A2*	*Mamu-A3*	Number
1	*1801	*1516		3
	*2201	*0515		6
	*5201	*0513		3
2	*1102		*1307	5

population. Because of the lack of pedigree data and segregation profiles, it was not possible to firmly establish the segregation of different *Mamu-A* genes present per chromosome. Haplotypes can only be deduced based on sharing of sequences between animals, and such haplotypes are listed in Table 2. At this stage, it is impossible to determine in which region configurations the *Mamu-A5, -A6,* and *-A7* genes are present.

Mafa-A region polymorphism and diversity in cynomolgus monkeys

Sixty-two alleles were detected in the 96 cynomolgus macaques analyzed. As observed in the rhesus macaques, most animals had two polymorphic *Mafa-A1* sequences, in combination with orthologs of the *Mamu-A2, -A3, -A4, -A5,* and/or *-A6* genes. In total, 38 *Mafa-A1* alleles were detected, illustrating the high level of polymorphism of this locus. These alleles were compared with sequences reported earlier by two other research groups (Uda et al. 2004; Krebs et al. 2005) that have used different nomenclature systems. Only four alleles *-A1*3101, -A1*3201, -A1*3801,* and *-A5*3001* detected in our panel are identical to the earlier described sequences *A*310101, A*320101, A*380101,* and *A*300101.*

A total of 79 out of 96 animals have at least one *Mafa-A2* sequence, and 18 alleles could be distinguished (Table 1). The oligomorphic *A2* locus is also present in the pigtailed macaque (*Macaca nemestrina*) as was recently described (Pratt et al. 2006; Lafont et al. 2007). In these studies, 90% of the animals possess the gene, and lower transcription levels were also observed in comparison to the other *Mane-A* alleles. The *Mafa-A3* gene is observed in only two animals, whereas the *Mafa-A4* locus is detected in 41 individuals. Two *Mafa-A4* alleles were found that differ in only one basepair (nonsynonymous) substitution (Fig. 2). As in the Chinese rhesus macaques, the *Mafa-A4* alleles have a stopcodon at the end of exon 5. The *Mafa-A5* and *-A6* alleles were detected in, respectively, eight and four animals and display low levels of polymorphism. Phylogenetic analyses illustrate that apart from loci rhesus and cynomolgus monkeys also share lineages (Fig. 2). As found in rhesus macaques, the *Mafa-A1* alleles are characterized by high transcription levels, whereas the other loci display moderate or low expression levels.

Because most of the cynomolgus macaques are pedigreed, the combination of sequences that are inherited on one chromosome could be defined. As an example, the kinship
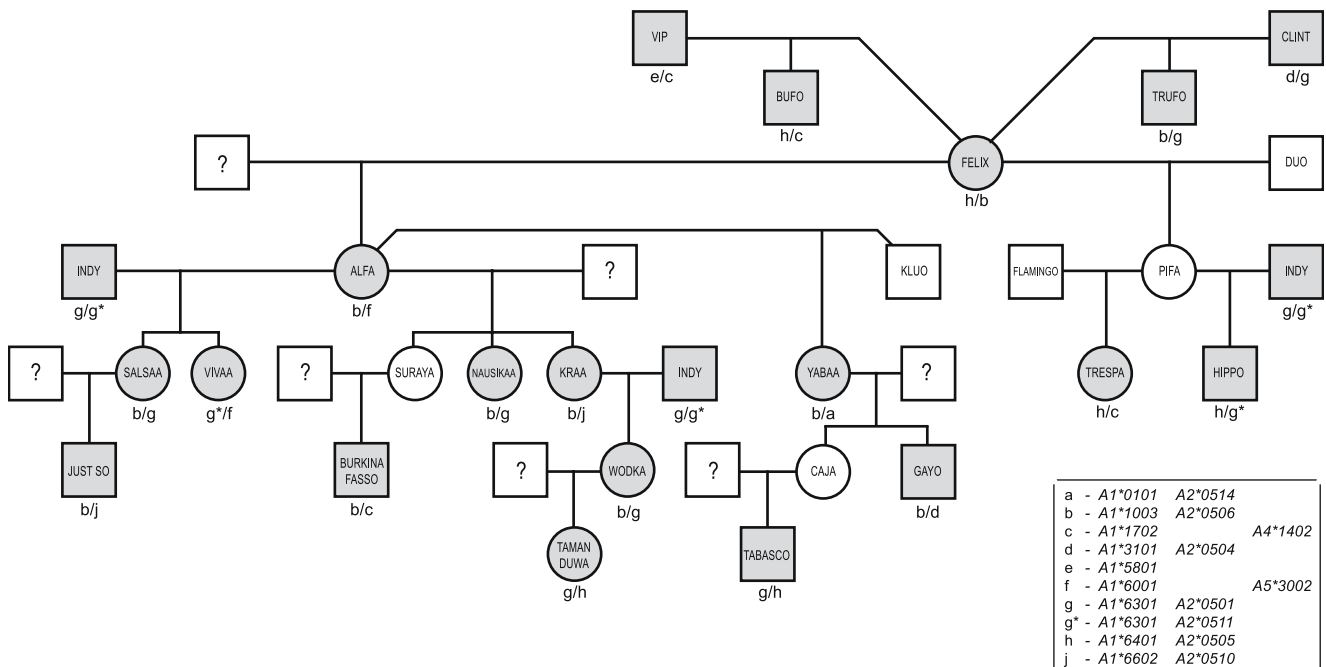

Fig. 3 Pedigree of a cynomolgus macaque family showing segregation of *Mafa-A* alleles. The animals analyzed are indicated by *shading*. A *question mark* indicates that the sire has not been identified

Table 3 *Mafa-A* haplotypes as defined by segregation grouped into seven-region configurations

		Mafa-A1	*Mafa-A2*	*Mafa-A3*	*Mafa-A4*	*Mafa-A5*	Number
1		*A1*4002*					6
		*A1*5801*					4
		*A1*7001*					3
2		*A1*0101*	*A2*0514*				4
		*A1*1003*	*A2*0506*				10
		*A1*1901*	*A2*0513*				3
		*A1*3101*	*A2*0504*				4
		*A1*5901*	*A2*0508*				12
		*A1*6301*	*A2*0501*				7
		*A1*6301*	*A2*0511*				3
		*A1*6401*	*A2*0505*				9
		*A1*6801*	*A2*0515*				3
		*A1*6601*	*A2*051201*				3
		*A1*6602*	*A2*0510*				10
		*A1*6801*	*A2*0515*				3
3		*A1*5701*		*A3*1301*			2
4		*A1*1702*			*A4*1402*		11
		*A1*7101*			*A4*14*		6
5		*A1*650102*	*A2*05*		*A4*14*		3
6			*A2*051202*		*A4*14*		9
7		*A1*6001*				*A5*3001*	2
		*A1*7201*				*A5*3001*	3

One region configuration appears to lack the *Mafa-A1* gene. At this stage, it is unknown whether the locus is really absent or was missed because of PCR failure, for example, because of a mutation in a primer site.

tree of one breeding group is provided (Fig. 3). The haplotypes are grouped based on the combination of loci (Table 3). Seven combinations of loci or region configurations are recognized (Fig. 4), and four of them (two to five) are also observed in the Indian rhesus macaques (Fig. 1), which indicates that they predate speciation. *Mafa-A5*3001* segregates in combination with *Mafa-A1*6001* and *-A1*7201* but not exclusively. The exact region configurations of *Mafa-A6* containing haplotypes are not yet known.
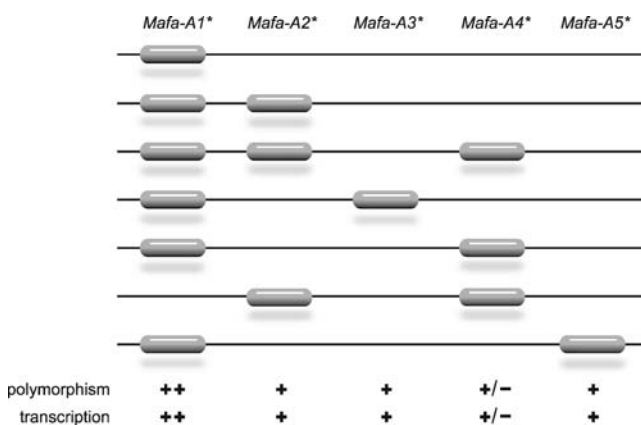

Fig. 4 Schematic representation of different *Mafa-A* region configurations in cynomolgus macaques. Four configurations (2–5) are shared with the Indian rhesus macaques (Fig. 1). One region configuration lacks the *Mafa-A1* gene. It is not yet known whether the locus is really absent or missed because of PCR failure. Should a *Mafa-A1* gene be found in extended studies than this region, configuration has to be deleted from the list

Sharing of *-A1* and *-A2* sequences between different macaque species

A comparison of all available *Mamu-A* to *Mafa-A* sequences showed that six full-length cDNA transcripts are shared between both species of macaque. This is the case for five *-A1* pairs and one *-A2* pair (Table 4). One of these cynomolgus monkey alleles is identical to an Indian rhesus allele, whereas the other five are shared with animals of Chinese origin. This observation was unexpected as the two rhesus macaque populations investigated share only one allele. The fact that cynomolgus macaques share more alleles with Chinese rhesus macaques than with Indian individuals may be explained by the fact that there is an overlap in the geographic areas inhabited by both species in Indochina (eastern Asia). It is known that rhesus macaques

Table 4 Identical Mhc class I sequences detected in the two species of macaque

Macaca mulatta	*Macaca fascicularis*
*Mamu-A1*0103*	*Mafa-A*260101*[a]
*Mamu-A1*4001*	*Mafa-A*400101*[a]
*Mamu-A1*1001*	*Mafa-A1*1002*
*Mamu-A*21*[b]	*Mafa-A1*0302*
*Mamu-A1*5301*	*Mafa-A1*5301*
*Mamu-A2*0519*	*Mafa-A2*051202*

[a] Published by Krebs et al. 2005.
[b] Published by Otting et al. 2005.

and cynomolgus monkeys can interbreed and produce offspring (Tosi et al. 2002). Sharing of alleles was not observed for the minors controlled by the *A3–A7* loci.

Different modes of selection operating on the *Mhc* class I and II genes in macaques

Although *Mamu-A* and *Mafa-A* sequences are interspersed in the phylogenetic tree, the vast majority of the alleles are species unique, and for rhesus macaque, most of them appear to be population specific. This is in sharp contrast to the situation observed for the macaque class II region, where sharing for different exon 2 sequences at the *Mhc-DR*, but especially the *-DQ* and *-DP* genes, is far more common (Blancher et al. 2006; Doxiadis et al. 2006). About half of the *Mafa-DPB1*, *-DQA1*, and *-DQB1* and one third of the *Mafa-DRB* sequences are identical to rhesus orthologs. As animals of the same populations have been used to study *Mhc* class I and II sequences, the present results exclude the possibility that this high sharing of *Mhc* class II sequences is because of introgression. A more likely explanation is that in macaques, the exon 2 sequences of the *Mhc* class II genes, which encode the peptide-binding site, have been subjected to purifying selection. As a consequence, many *Mhc* class II alleles in these two macaque species predate speciation processes. A databank search indicated that the phenomenon also extends to other macaque species (Robinson et al. 2003).

The *Mhc* class I alleles in macaques are largely unique, illustrating that selection has favored diversity. *Mhc* class I proteins are involved in the presentation of intracellular pathogens such as viruses and parasites that are known to evolve at high mutation rates. Recognition of Mhc class I molecules combined with a foreign peptide may result in the lysis of an infected cell. The Mhc class II molecules select peptides, originating from extracellular pathogens such as bacteria and fungi, for binding. Mhc class II-mediated activation may result in antibody production and/or providing help to cytotoxic T cells. The present results illustrate that, probably because of coevolution with intracellular pathogens, macaques have generated a highly complex and divergent Mhc class I repertoire. The fact that *Mhc* class I alleles evolve quickly, even within a species, has been documented in humans for instance (Belich et al. 1992; Watkins et al. 1992). The high degree of sharing of *Mhc* class II sequences seems to be unique for macaques and has not been observed in any other group of vertebrate species.

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