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Study of cynomolgus monkey (*Macaca fascicularis*) *DRA* polymorphism in four populations

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Abstract To describe the polymorphism of the *DRA* gene in *Macaca fascicularis*, we have studied 141 animals either at cDNA level (78 animals from Mauritius, the Philippines, and Vietnam) or genomic level (63 animals from the Philippines, Indonesia, and Vietnam). In total, we characterized 22 cDNA *DRA* alleles, 13 of which had not been described until now. In the Mauritius population, we confirmed the presence of three *DRA* alleles. In the Philippine and Vietnam populations, we observed 11 and 14 *DRA* alleles, respectively. Only two alleles were present in all three populations. All *DRA* alleles but one differ from the consensus sequence by one to three mutations, most being synonymous; so, only seven DR alpha proteins were deduced from the 22 cDNA alleles. One *DRA* cDNA allele, *Mafa-DRA*02010101*, differs from all other alleles by 11 to 14 mutations of which only four are non-synonymous. The two amino acid changes inside the peptide groove of *Mafa-DRA*02010101* are highly conservative. The very low proportion of non-synonymous/synonymous mutations is compatible with a purifying selection which is comparable to all previous observations concerning the evolution of the *DRA* gene in mammals. Homologues of the allele *Mafa-DRA*02010101* are also found in two other Asian macaques (*Macaca mulatta* and *Macaca nemestrina*). The forces able to maintain this highly divergent allele in three different macaque species remain hypothetical.

Keywords MHC · DRA · Macaque · Polymorphism

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Introduction

The cynomolgus macaque (*Macaca fascicularis*) is increasingly used as a nonhuman primate model for biomedical research in various domains such as tissue allograft rejection (Aoyama et al. 2009; Borie et al. 2002; Wieczorek et al. 2006), bone marrow graft (Derdouch et al. 2008; Lau et al. 2004), immune response against pathogens (Sato et al. 2008), or new vaccines (Kita et al. 2005; Morner et al. 2009; Turbant et al. 2009; Warfield et al. 2007). Recently, its use in simian immunodeficiency virus-induced AIDS (Burwitz et al. 2009; Florese et al. 2008; Mee et al. 2009b) or with other emergent pathogens (Baskin et al. 2009; Kuiken et al. 2003; Lawler et al. 2006; Reed et al. 2005) revealed the importance of the knowledge of the genetic background. Moreover, it was reported that the geographical origin of the animal can influence the response to drugs

or sensibility to the experimental disease (Menninger et al. 2002; Schmidt et al. 1977). The polymorphism of some loci involved in the physiology of the immune system has been extensively studied such as *DRB* (Blancher et al. 2006; Leuchte et al. 2004) MHC class I (Campbell et al. 2009; Pendley et al. 2008; Uda et al. 2005; Uda et al. 2004), the immunoglobulin locus (Calvas et al. 1999), the KIR locus (Bimber et al. 2008), chemokine and chemokine receptors (Puissant et al. 2003).

The map of the cynomolgus macaque MHC has recently been characterized (Watanabe et al. 2007). It demonstrates that the cynomolgus MHC shares the general organization of the rhesus monkey MHC characterized by numerous class I duplicated genes (Daza-Vamenta et al. 2004). This contrasts with the high homology in the class II region with humans as well as other primates. In the class II region, *DR*, *DQ*, and *DP* gene polymorphisms have been extensively characterized in *M. fascicularis* (Blancher et al. 2006; Leuchte et al. 2004; Otting et al. 2002; Sano et al. 2006). By contrast, the polymorphism of the *DRA* gene was studied only in the *Mafa* Mauritius population (O'Connor et al. 2007) which is well known to have a highly reduced polymorphism due to a founding effect (Blancher et al. 2008; Bonhomme et al. 2008; Lawler et al. 1995). We decided to study the *DRA* polymorphism in the coding region by the study of cDNA in animals from three of the four populations we have already studied (from Mauritius, the Philippines, and Vietnam). Vietnam was chosen as an example of the continental subpopulation. The Mauritius population was included because of its interest in preclinical models (Florese et al. 2008; Mee et al. 2009b). The Philippine population was studied because, as we have previously reported, this population probably experienced a strong selection in the class II region of the MHC (Bonhomme et al. 2007).

Materials and methods

Animals

In total, we studied 298 unrelated cynomolgus monkeys on Mauritius island (samples provided by Noveprim Co.), 212 from the Philippine archipelago (200 from Sicombrec Co. and 12 from INA Research Philippines INC), 88 from Vietnam (83 from Nafovanny Co. and five from the Shiga University of Medical Science, Shiga, Japan), and 13 from Indonesia (CV. Universal Fauna Breeder). Two unrelated *Macaca sylvanus* (*Masy*) (Ecole Nationale Vétérinaire, Toulouse, France) and one *Macaca nesmestrina* (*Mane*) (Ecole Nationale Vétérinaire, Toulouse, France) were studied. The blood collection and animal studies were conducted in accordance with the guidelines for animal experiments specific to each location.

DNA extraction

Genomic DNA was extracted from peripheral blood using either QIA amp Blood Kit (Qiagen, Courtaboeuf, France) or a standard phenol–chloroform method.

RNA extraction

Total RNA was extracted from buffy-coats by means of an RNeasy Mini Kit (Qiagen, Courtaboeuf, France).

Microsatellite analysis

In the three populations (on Mauritius island, the Philippines, and Vietnam), we studied *DRACA* microsatellite, as previously described (Bonhomme et al. 2005). For the Mauritius population, we have also studied microsatellites located in the MHC class II region (D6S2892, D6S2876, D6S2747, D6S2745, and D6S2771) by means of primers described by Wiseman et al. (2007). Genotypes were determined using DNA Size Standard-kit-600 (Beckman Coulter, Villepinte, France) after denaturation and separation of the amplification products on a capillary electrophoresis using CEQ8000 analyzer and scored with the software CEQ8000 Genetic Analysis System v8.0 (Beckman Coulter, Villepinte, France).

Genetic diversity

Genetic diversity was measured using the number of alleles (nA) and the expected and observed heterozygosity (He and Ho), calculated with Arlequin v.3.1 software (Excoffier et al. 2005). To test departures from Hardy–Weinberg equilibrium, the *Fis* statistics within samples, and the corresponding exact *P* values were computed by a Markov chain method implemented in Genetix v4.05.2 (Belkhir et al. 2004). The linkage disequilibrium (LD) between the microsatellite *DRACA*, *DRA* and the *DRB* region was tested using ratio test, implemented in Arlequin v.3.1 which gives the significance level of LD by the calculation of an exact *P* value obtained from multiple permutations.

Characterization of *DRB* alleles by denaturing gradient gel electrophoresis and sequencing

Genomic *DRB* exon 2 sequences of animals from Mauritius (N=298), the Philippines (N=200), and Vietnam (N=50) were determined by denaturing gradient gel electrophoresis

and sequencing (DGGE-sequencing) as described previously (Blancher et al. 2006).

Characterization of *DRA* cDNA sequences (*cDRA*)

A *DRA* cDNA fragment of 734 base pairs (bp) was obtained by using the one-step reverse transcriptase (RT)-PCR kit from Qiagen (Qiagen, Courtaboeuf, France), with primers DRAmac1Forward 5′-ATG GCC GAA AGT GGA GTC C-3′and DRAmac774Reverse 5′-GCA TAT GTC TCA CAG AGG CCC-3′. The cycling parameters were 30 min at 50°C to obtain cDNA (reverse transcription reaction and denaturation of the cDNA template), followed by 15 min at 95°C (activation of Hot Star Taq polymerase and inactivation of reverse transcriptase), followed by 35 cycles of 30 s at 94°C denaturation step, an annealing step of 30 s at 60°C, an extension step of 1 min at 72°C, and 10 min at 72°C (final extension). When compared with the full-length coding region, the resulting amplified fragments lack 19 and 12 nucleotides at the beginning and the end, respectively. The resulting amplified products were separated on agarose gels, purified using QIAquick Gel Extraction Kit (Qiagen, Courtaboeuf, France) and directly sequenced on both strands by using both DRAmac1Forward, DRAmac 774Reverse, DRAmac421Forward 5′-GTG GTC AAA GTT ACA TGG C-3′, and DRAmac475Reverse 5′-CTG ACA CTC CTG TGG TGA C-3′ primers with the fluorescent Dye Terminator Cycle Sequencing method on a CEQ 8000 automated sequencer (Beckman Coulter, Villepinte, France). When direct sequencing revealed no ambiguities, the animal was considered to be homozygous and the sequence was validated after having been found in at least two unrelated animals. When direct sequencing revealed ambiguities, the *DRA* cDNA amplified fragments were cloned into the pCR2.1 Topo plasmid vector (Invitrogen, Leek, The Netherlands) and sequenced to allow characterization of both *DRA* cDNA alleles.

Characterization of *DRA* gDNA sequences

A *DRA* DNA fragment of 1,198 to 1,318 bp from partial intron 1 to partial intron 3 was obtained by PCR amplification, using Gold Taq polymerase (ABI), with sense primers DRA-F1 5′-AGCATGTCCTTCACCCAGA ACT-3′ or DRA-F2 5′-ACTTCCTGCCTACATGTATG-3′, and the anti-sense primers DRA-R1 5′-TAAAGGAGATT GAGTGATGGTCCA-3′ or DRA-R2 5′-AAGCTGGGTAG TAGGAGAAG-3′. The cycling parameters were 10 min at 95°C, followed by 30 cycles of 30 s at 94°C denaturation step, an annealing step of 20 s at 65°C, and an extension step of 2 min at 72°C and 5 min at 72°C (final extension). PCR products were cloned into the PGEM-T Easy vector with the TA cloning kit according to the protocol provided by the manufacturer (Promega, Madison, WI) and sequenced by using the ABI3130 genetic analyzer (Applied Biosystems, CA) in accordance with the protocol of the Big Dye terminator method. To avoid PCR and sequencing artifacts generated by polymerase errors, 16 clones were sequenced per individual. The nucleotide sequences of all individuals were also determined by direct sequencing of the PCR products using PCR primers as sequencing primers.

Sequence analysis and phylogenic trees

All *DRA* sequences were deposited in the Genbank database (accession references are given in the tables) and submitted to the IPD-MHC NHP database (Robinson et al. 2005) for allele nomenclature. Cynomolgus monkey allele names were assigned to *DRA* sequences by the IPD-MHC NPH database following classical rules (Klein et al. 1990). Multiple sequence alignments were obtained by using ClustalW 1.83 (Thompson et al. 1994). Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007). The phylogenetic trees were built using the neighbor-joining method (Saitou and Nei 1987) with evolutionary distances computed by using the Maximum Composite Likelihood method (Tamura et al. 2004). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). A phylogenetic network was built to describe all possible phylogenetic pathways of the *DRA* coding sequences of *Mafa*, *Mamu* (*Macaca mulatta*), *Mane*, and *Masy*. The network was rooted by using the human *HLA–DRA* sequence as outgroup.

Study of EST databases

We extracted 154 *DRA* expressed sequence tag (EST) from three libraries (thymus [QthA, Lib.24021, n=9,566], spleen [QspA, Lib.23009, n=9,783] and bone marrow [QbmA, Lib.22708, n=9472], available in the Unigene database (http://www.ncbi.nlm.nih.gov/UniGene/lbrowse2.cgi), obtained from a 4-year-old male Malaysian *M. fascicularis* (Osada et al. 2009). For each library, we built *DRA* cDNA consensus sequence by using Macvector software (Macvector Inc, Cary, NC, USA).

Results

Polymorphism of microsatellite *DRACA* and linkage disequilibrium

We studied the polymorphism of the *DRA*-associated *DRACA* microsatellite in three cynomolgus macaque populations from Mauritius island (N=298, Noveprim),

the Philippines archipelago (N=200, Sicombrec), and Vietnam (N=50, Nafovanny). All the animals were genotyped for the *DRB* genes by DGGE and sequencing, and the *DRB* haplotypes were deduced from the Mauritius and the Philippine reference haplotypes already described (Blancher et al. 2006). In the Vietnamese population, the high degree of polymorphism of the *DRB* region did not allow us to deduce the *DRB* haplotypes (to be published elsewhere).

In total, we found 21 alleles of microsatellite *DRACA* among which only three are in common in the three populations (Table 1). The Mauritius sample is the least polymorphic with only five alleles which are common with the Vietnamese sample. The latter is the most polymorphic with 17 alleles, eight of which are common with the Philippines sample, in which we found 12 alleles.

Statistical tests did not reveal Hardy–Weinberg equilibrium departure in the Mauritius or the Philippine samples. In contrast, in the Vietnamese population sample, we observed an excess of homozygous animals (Table 2).

The linkage disequilibrium between *DRACA* and *DRB* haplotypes is complete in the Mauritius population sample ($p<10^{-6}$). The *DRACA–DRB* haplotypes are shown in Fig. 1. In the Philippines population sample, the *DRACA–DRB* LD was almost complete ($p<10^{-6}$), and the most frequent haplotypes are shown in Fig. 2 which also describes one recombinant *DRACA–DRB* haplotype.

There is no direct, specific concordance between microsatellite allele sizes and cDNA allele sequences. For instance, in the Mauritian cynomolgus macaques, there are five microsatellite allele sizes but only three cDNA allele sequences (Fig. 1). In the Mauritius population, the LD between *DRACA* microsatellite and *cDRA* allele cannot be calculated on the basis of the eight animals studied here. The compilation of our results with those from O'Connor is not possible because insufficient details are provided in the publication from this group. In the Philippine population, the LD between *DRACA* microsatellite and *cDRA* is highly probable ($p<10^{-6}$; N=20 animals). In the Vietnamese population, on the basis on the 50 animals studied here, the LD between the *DRACA* microsatellite and *cDRA* is highly probable ($p<10^{-6}$) although the description of Vietnamese *DRACA–DRA* haplotypes remains elusive.

Study of cDNA *DRA* sequences

We studied 78 unrelated cynomolgus monkeys from three different populations: Mauritius island (N=8, Noveprim), the Philippines archipelago (N=20, Sicombrec), and Vietnam (N=50, Nafovanny). In the Mauritius and Philippine populations, the animals were selected for their *DRB* genotype with the aim to explore the *DRA* alleles associated to each *DRACA–DRB* haplotype of both populations. For the most frequent haplotypes, we studied one homozygous animal, while for the least frequent haplotypes, we studied heterozygous animals having one of the rare haplotypes and one of the most frequent haplotypes. In total, we deduced the cDNA *DRA* sequences associated with the most frequent and the rarest *DRACA–DRB* haplotypes.

In the Mauritius population, seven frequent *DRB* haplotypes are found (Blancher et al. 2006; Leuchte et al. 2004; O'Connor et al. 2007). Two rare recombinant *DRB* haplotypes (#4c and #4d) were also described (Blancher et al. 2006). The *cDRA* alleles associated with these two rare recombinant haplotypes were not studied because no RNA samples from appropriate animals were available. In total, we describe here seven *DRACA–DRA–DRB* haplotypes deduced from the study of eight animals by cloning and sequencing of *DRA* cDNA (Fig. 1). We confirm the results reported by O'Connor et al. for six *DRACA–DRA–DRB* haplotypes (O'Connor et al. 2007), and we report here the first the association of *Mafa–DRA*01020101* with the haplotype *DRB* #3 (Leuchte et al. 2004; Blancher et al. 2006) and the microsatellite-defined haplotype H7 (Mee et al. 2009a; see Fig. 1). In total, in the Mauritius sample, only three *DRA* cDNA alleles, previously described by O'Connor and colleagues, are observed (Table 3; O'Connor et al. 2007), out of which (*Mafa-DRA*01020101* and *Mafa-DRA*02010101*) were also found in Vietnam and the Philippine animals (Table 3), while the third (*Mafa-DRA*010101*) was found only in Mauritius monkeys (Table 3). Even by the study of genomic sequences, this Mauritius allele was absent in the samples from Vietnam, the Philippines, and Java (see further section).

In the Philippine population, twelve *DRB* haplotypes have previously been described (Blancher et al. 2006). Two of them (#1b and #13b) were not studied for the *cDRA*-associated sequences because no RNA samples were available (see foot note for haplotype #1b[1]). We describe here for the first time one more Philippine *DRB* haplotype, referred to as *DRB* #2b, which displays two *DRB* loci (Fig. 2b). At one locus, haplotype #2b shares the allele (*DRB*W501*) with the Mauritius *DRB* haplotype #2a, while at the second locus, the Philippine haplotype #2b displays the allele *DRB*W7201* (Genbank access number FJ655856) which differs by six mutations in exon 2 from allele *DRB*W2101* found in the Mauritius *DRB* haplotype #2a. In total, we studied 20 animals here representative of

[1] Writing the present article, we detected an error concerning *DRB* haplotype #1b that we reported in the article Blancher et al. 2006 (Fig. 2). The latter haplotype is absent from the Mauritius population but was observed in a single animal from The Philippines, while haplotype #1a was not observed in The Philippine sample. We apologize for this error.

Table 1 Allelic frequencies (in %) of *DRACA* microsatellite in three cynomolgus macaque populations

Microsatellite length (bp)	Mauritius (*N*=596)	The Philippines (*N*=400)	Vietnam (*N*=100)
237	24.8	19.5	1.0
239	0	42.3	5.0
241	0	0	3.0
247	0	0	8.0
249	0	0	11.0
251	0	0.5	0
253	0	0	4.0
255	0	0	3.0
257	0	7.8	2.0
259	0	0	1.0
261	0	0.5	0
265	0	12.5	6.0
266	21.1	0	2.0
268	29.0	0	5.0
270	2.0	0.5	13.0
272	23.0	0.8	27.0
274	0	5.5	4.0
276	0	5.3	1.0
278	0	0	4.0
283	0	4.5	0
286	0	0.5	0
allele number	5	12	17

Legend: *N* is the haplotype number studied for each population

11 Philippine *DRB* haplotypes and have evidenced 11 *cDRA* alleles (Table 3). Figure 2b describes the *DRACA–cDRA–DRB* haplotypes deduced from the study of 20 animals (Fig. 2a). Two *DRB* haplotypes (#5 and #10) are associated with several *cDRA* (Fig. 2) and symmetrically, three out of 11 *cDRA* alleles (*Mafa-DRA*01020101*, *Mafa-DRA*01020701*, and *Mafa-DRA*01010401*) are associated with more than one *DRB* haplotype. Interestingly, one class II Philippine haplotype is compatible with a recombination between two other class II Philippine haplotypes (see Fig. 2b).

As for the Vietnamese population, because the great diversity of the *DRB* region did not allow us to determine the *DRB* haplotypes, we decided to study *DRA* cDNA of 50 randomly chosen unrelated animals. In this sample, we found 14 *cDRA* sequences (Table 3). Only four of these Vietnamese *cDRA* alleles are found in the two other populations (Table 3). The most frequent Vietnamese allele, *Mafa-DRA*01020101*, occurred in 39 out of the 50 animals, out of which 29 appeared homozygous by direct sequencing of their amplified *DRA* cDNA. We found here that the observed frequencies of *cDRA* homozygous animals exceed largely the theoretical frequency (*Ho*=0.38, *He*=0.53, $p<10^{-6}$). In this 50-animal Vietnamese sample, an excess of homozygous animals was also observed for *DRACA* (Table 2). These observations led us to conclude that the sample we studied most probably presented an excess of homozygous animals resulting from a sampling effect and that the putative difficulty to reveal some heterozygous animals by RT-PCR direct sequencing can reasonably be eliminated.

Comparison of *DRA* cDNA sequences

In total, we describe 22 *DRA* cDNA sequences in *M. fascicularis* out of which four have already been described

Table 2 Genetic diversity at *DRACA* microsatellite in Mauritius, Philippine, and Vietnam populations

Population	n_A	H_o	H_e	*P* value	F_{is}	*P* value
Mauritius (298)	5	0.79	0.76	0.100	−0.04	0.0893
the Philippines (200)	12	0.73	0.75	0.749	0.03	0.1844
Vietnam (50)	17	0.72	0.88	0.005	0.19	<0.0001

Legend: population (number of animals studied), n_A number of alleles, H_e expected heterozygosity; H_o observed heterozygosity

a

Reference animal	*DRACA*	c*DRA*	*DRB* haplotype	Microsatellite haplotype in class II
121518	272 / 272	*DRA*01020101 / DRA*01020101*	#1a / #1a	H3 / H3
42-23	237 / 237	*DRA*02010101 / DRA*02010101*	#2 / #2	H1 / H1
OBDR5	270 / 270	*DRA*01020101 / DRA*01020101*	#3 / #3	H7 / H7
OBDK5	266 / 266	*DRA*01020101 / DRA*01020101*	#4a / #4a	H4 / H4
13788	266 / 266	*DRA*010101 / DRA*010101*	#4b / #4b	H5 / H5
OBAN5	268 / 272	*DRA*01020101 / DRA*01020101*	#5 / #1a	H6 / H3
13744	268 / 268	*DRA*01020101 / DRA*01020101*	#5 / #6	H6 / H2
OBCN5	268 / 268	*DRA*01020101 / DRA*01020101*	#6 / #6	H2 / H2

b

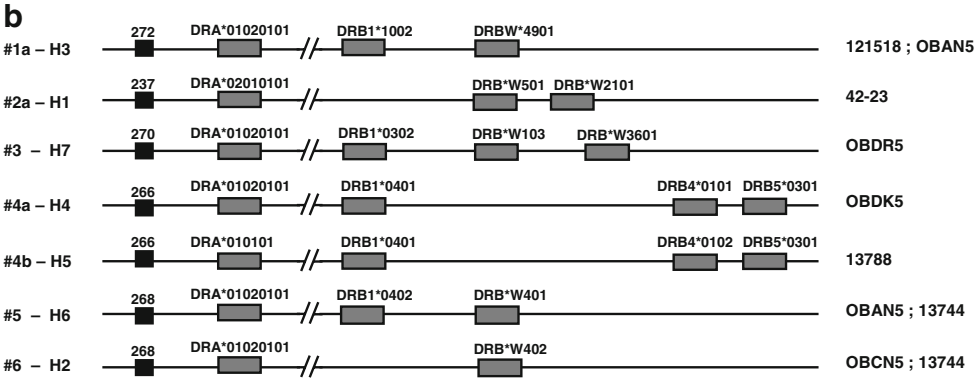

Fig. 1 *DRACA–DRA–DRB* haplotypes in Mauritius population. **a** For each animal studied in Mauritius population (n=8), we describe the *DRACA* microsatellite alleles, *cDRA* alleles, and *DRB* haplotypes. **b** Schematic representation of the *DRACA–DRA–DRB* haplotype in the Mauritius population (the order of *DRB* genes on each haplotype is arbitrary). Haplotypes *DRACA–DRA–DRB* are identified by numbers preceded by "*number sign*" (these numbers correspond to the *DRB* haplotype numbers described previously by Blancher et al. 2006) and by numbers following the letter "*H*" corresponding to the nomenclature of Wiseman et al. (2007). Animal identification codes are given *on the right* of each haplotype

in *M. fascicularis* in Mauritius (O'Connor et al. 2007) and Indonesia (Mee E.T. and Rose N.J., unpublished data) (Table 3). One *Mafa DRA* cDNA sequence found in Genbank (*Mafa-DRA*01020301*, AM943637), revealed in an Indonesian cynomolgus macaque, was not found in our animal sample. The comparison of all *Mafa* c*DRA* aligned sequences revealed 26 variable positions, 17 of which are synonymous by reference to the consensus sequence which is identical to the *Mafa-DRA*01020101* allele (Fig. 3). Among the nine variable amino acid positions, two are located in the leader peptide, three are in the alpha1 domain, one in the alpha2 domain, one in the connecting peptide, and two in the trans-membrane domain (Fig. 3). By and large, the dN/dS ratio (non-synonymous substitutions per non-synonymous site divided by synonymous substitutions per synonymous site) was much lower than one in all pairwise allele comparisons, suggesting a strong purifying selection (Hughes and Nei 1989) (Electronic Supplementary Material 1).

All *Mafa DRA* cDNA sequences but one (*Mafa-DRA*02010101*) differ only by one to three mutations from the consensus sequence (Fig. 3). The *Mafa-DRA*02010101* sequence excluded, pairwise comparisons of all other *M. fascicularis* sequences revealed from one to six mutations with a ratio of non-synonymous/synonymous mutations varying from 0/5 to 3/1 (see Fig. 3 for more details). The allele *Mafa-DRA*02010101* which is present in the three populations of *M. fascicularis* studied here (Table 3), displays 11 to 14 mutations when compared to other *Mafa-DRA* alleles with a dN/dS ratio varying between 0.40 and 0.75. By comparison with other *Mafa* c*DRA* alleles, *Mafa-DRA*02010101* displays eight specific mutations (not shared with any other allele). The deduced *Mafa-DRA*02010101* protein differs from the six other deduced *Mafa DRA* proteins by four specific (not shared with any other allele) amino acid substitutions (Fig. 3). The substitution in the leader peptide is not conservative (Ile-Thr); the three substitutions located in the alpha 1 domain are highly conservative (Glu-4-Asp, Phe-22-Tyr and Ile-31-Leu, Fig. 3). Only two of them (Phe-22-Tyr and Ile-31-Leu) involve amino acid residues in contact with the antigenic peptides presented by the DR groove (Reche and Reinherz 2003).

The comparison of the 22 *Mafa* cDNA sequences with homologous sequences from other macaque species revealed that eight of the *Mafa cDRA* sequences described here are identical to the *DRA* cDNA sequences described previously in *M. mulatta* (Fig. 3 and Table 3). Among the 19 SNPs (single nucleotide polymorphisms) found in the coding region of *Mamu*, almost all (17 out of 19) are present in *M. fascicularis*.

From the alignment of all the macaque *DRA* cDNA sequences (Fig. 3), we produced a phylogenetic network from the polymorphic sites of *Mafa*-, *Mamu*-, *Masy*-, *Mane-DRA* cDNA alleles (Fig. 4). The network displays several reticulations which suggest inter-allelic exchanges. In the neighbor-joining tree (Fig. 5), most of the macaque *DRA* sequences form a star-like phylogeny which includes the two *Masy* alleles (*Masy-DRA*01*, EU877221 and *Masy-DRA*02*, EU877222) described for the first time. A second group including *Mafa-DRA*02010101*, *Mamu-DRA*010601* cDNA sequence and two *Mane cDRA* (see Figs. 5,

a

Reference animal	*DRACA*	c*DRA*	*DRB* haplotype
Cyn-239	239 / 239	*DRA*010302 / DRA*010302*	#15 / #15
Cyn-314	237 / 237	*DRA*010301 / DRA*010301*	#11 / #11
Cyn-321	239 / 239	*DRA*010303 / DRA*010302*	#7 / #15
Cyn-355	239 / 239	*DRA*010303 / DRA*010303*	#7 / #7
Cyn-376	239 / 276	*DRA*010303 / DRA*0105*	#7 / #5
Cyn-381	265 /265	*DRA*01010401 / DRA*01010401*	#13a / #13a
Cyn-388	251 / 239	*DRA*02010101 / DRA*01020501*	#2b / #7
Cyn-391	239 / 286	*DRA*010303 / DRA*01020101*	#7 / #10
Cyn-475	237 / 272	*DRA*010301 / DRA*0109*	#11 / #12
Cyn-483	257 / 261	*DRA*01020701 / DRA*01020701*	#9 / #9
Cyn-491	265 /265	*DRA*01010401 / DRA*01010401*	#13a / #13a
Cyn-600	257 /257	*DRA*01020701 / DRA*01020701*	#9 / #9
Cyn-610	274 / 265	*DRA*01020101 / DRA*01010401*	#8 / #14
Cyn-611	237 / 265	*DRA*010301 / DRA*01010401*	#11 / #14
Cyn-633	239 / 272	*DRA*010303 / DRA*0109*	#7 / #12
Cyn-695	239 / 274	*DRA*010303 / DRA*01020101*	#7 / #8
Cyn-697	276 / 265	*DRA*01040201 / DRA*01010401*	#5 / #13a
Cyn-705	239 / 283	*DRA*010303 / DRA*01020201*	#7 / #10
Cyn-721	251 / 237	*DRA*02010101 / DRA*010301*	#2b / #11
Cyn-810	251 / 257	*DRA*02010101 / DRA*01020701*	#2b / #10

b

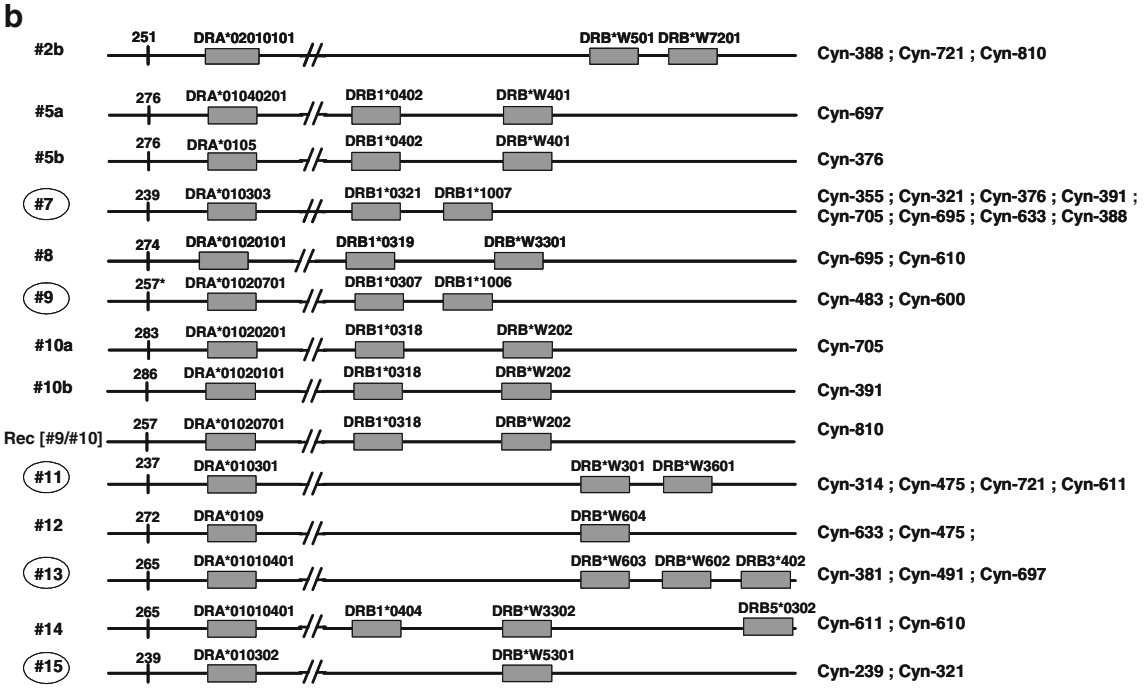

Fig. 2 *DRACA–DRA–DRB* haplotypes in the Philippine population. **a** For each animal studied in the Philippine population (n=20), we describe the *DRACA* microsatellite alleles, *cDRA* alleles and *DRB* haplotypes. **b** Schematic representation of the *DRACA–DRA–DRB* haplotypes in the Philippine population (the order of *DRB* genes on each haplotype is arbitrary). Haplotypes *DRACA–DRA–DRB* are identified by *numbers* (*given on the left*) which correspond to the *DRB* haplotype numbers described previously (Blancher et al. 2006). Animal identification codes are given *on the right of each haplotype*. *Numbers with a ring around them* correspond to *DRACA–DRA–DRB* haplotypes found in homozygous animals. The other haplotypes were deduced by the study of heterozygous animals. As for haplotype #9, the microsatellite length mostly encountered is 257, but one animal homozygous for *cDRA* and haplotype *DRB* (Cyn-483) displayed two lengths (257 and 261) for the *DRACA* microsatellite. All *DRACA* alleles found in the sample of 200 animals described in Table 1, except a rare one (*DRACA* allele 270 present only in two animals), belong to the *DRACA–DRA–DRB* haplotypes defined here

3 for more details) is highly divergent. The allele *Mafa-DRA*02010101* differs by 13 mutations from *Mafa-DRA*01020101* which most probably corresponds to the macaque *cDRA* ancestral sequence (Fig. 4). This gives a 12-million-year estimate for the time of divergence between the two alleles (by calibration with the number of mutations between human and macaque *cDRA*).

Study of *DRA* genomic DNA sequences

In order to enlarge our dataset, we also studied *DRA* partial genomic sequences from animals for which no mRNA samples were available. The animals studied were from three different populations [the Philippines (N=12, INA Research Philippines INC), Indonesia (N=13, CV Univer-

Table 3 Cynomolgus monkey *DRA* allele distribution in the three populations studied and identical alleles described previously

IMGT/NPH *Mafa-DRA* allele names	Genbank access codes	Presence of allele in populations			Previously described identical alleles, Genbank access codes	
		Mauritius	Philippines	Vietnam		
*DRA*010101*	EU877215	√			*Mafa-DRA*0101*	EF208826(1)
*DRA*01010401*	EU877218		√		nf	
*DRA*010105*	EU921295			√	*Mamu-DRA*010204*	AJ586877(2)
*DRA*01020101*	EU877216	√	√	√	*Mafa-DRA*01020101*	EF208827(1)
					*Mamu-DRA*010201*	AJ586874(2)
*DRA*01020201*	EU877210		√		nf	
*DRA*01020501*	EU877204			√	nf	
*DRA*01020701*	EU877217		√		nf	
*DRA*01020801*	EU877203			√	nf	
*DRA*01020901*	EU877205			√	*Mamu-DRA*010401*	AJ586882(2)
*DRA*010212*	EU877202			√	*Mamu-DRA*010205*	AJ586878(2)
*DRA*010216*	EU921296			√	nf	
*DRA*010218*	EU921294			√	*Mamu-DRA*010207*	AJ586880(2)
*DRA*010301*	EU877212		√	√	*Mafa-DRA*0103*	AM943638(3)
					*Mamu-DRA*010301*	AJ586881(2)
					*Mane-DRA*01*	GQ214407(4)
*DRA*010302*	EU877211		√		nf	
*DRA*010303*	EU877209		√		nf	
*DRA*010304*	EU877207			√	nf	
*DRA*010305*	EU877206			√	nf	
*DRA*01040201*	EU877219		√		nf	
*DRA*0105*	EU877214		√		nf	
*DRA*0106*	EU877208			√	*Mamu-DRA*0105*	AJ586884(2)
*DRA*0109*	EU877213		√	√	nf	
*DRA*02010101*	EU877220	√	√	√	*Mafa-DRA*02010101*	EF208828(1)
					*Mamu-DRA*010601*[a]	AM910165(5)

Legend:

[a] Expressed sequence tag (EST) of *M. nemestrina* available in Genbank (EB520956, EB521652) similar to *Mafa-DRA*02010101*. Reference quoted in the tables: (1; O'Connor et al. 2007), (2; de Groot et al. 2004), (3; unpublished, deposited by Mee and Rose, found in Indonesian cynomolgus macaque), (4; an *M. nemestrina* animal, studied in this study, homozygous for an allele strictly identical with *Mafa–DRA*010301*), (5; full-length cDNA deduced from *Mamu-DRA* genomic DNA sequence published by Doxiadis et al. 2008), *nf* not found

sal Fauna Breeder), and Vietnam (N=38)]. From these 63 cynomolgus macaques, we found a total of 41 *Mafa-DRA* genomic sequences (fragment of 1,198 to 1,318 base pairs from partial intron 1 to partial intron 3) from which only 18 partial cDNA alleles (exons 2 and 3 only) are deduced. The geographic origins of the animals that have led to the description of the alleles are given in Electronic Supplementary Material 2 where we also provide the Genbank access codes and identity with c*DRA* allele described above. Among these 41 alleles, only two are in common in the three populations (*Mafa-DRA*01020101* and *Mafa-DRA*01020401*). The neighbor-joining phylogenetic tree of these genomic sequences (Electronic Supplementary Material 4a) revealed that most describe a star phylogeny while four sequences highly homologous to *Mafa-DRA*02010101* are grouped on a highly divergent branch. In the tree built with intronic sequences, this group is also clearly apart from all of others (Electronic Supplementary Material 4b) because the proportion of mutated sites accumulated in the non-coding parts of these alleles is equivalent to that found in the coding region (Electronic Supplementary Materials 3 and 4).

Comparison of *Mafa DRA* cDNA alleles with EST libraries

We have produced *DRA* cDNA consensus sequences from three libraries obtained from one animal originating from Malaysia (Osada et al. 2009; Table 4). In three EST

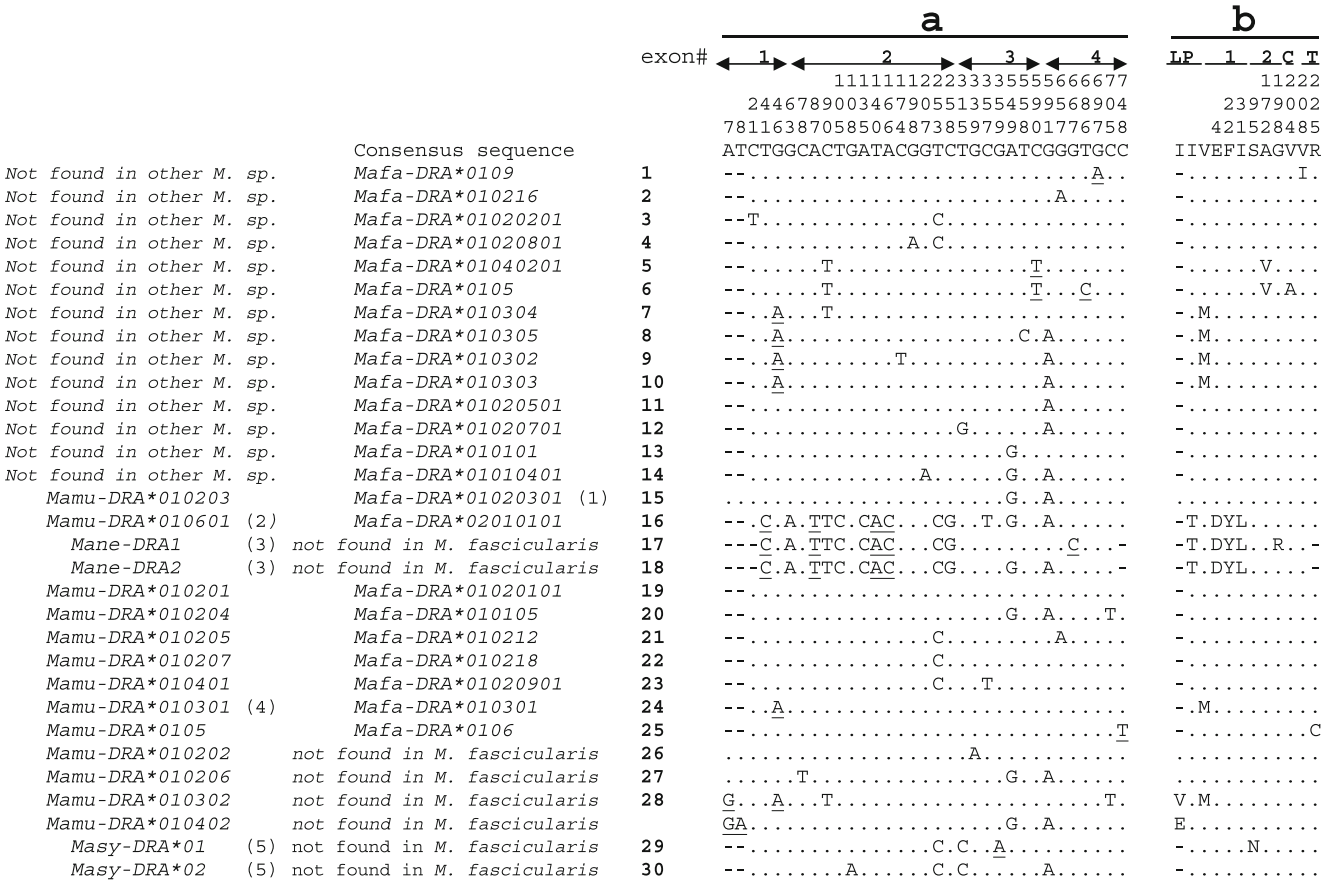

Fig. 3 Polymorphic sites of the full-length coding *DRA* cDNA in macaques. Legend: The figure describes the *Mafa-*, *Mamu-*, *Mane-*, and *Masy-DRA* polymorphic sites (**a**) and corresponding amino acid changes (**b**).The *Mafa-DRA*01020101* sequence is used as reference. *Numbers* attributed to each sequence correspond to the c*DRA* allele numbering in Fig. 4 (phylogenetic network). The *number 1* nucleotide position corresponds to the first nucleotide of the start codon and amino acid numeration is based on IMGT numbering. Only polymorphic sites are shown. Identity to the consensus is illustrated by a *dot*. Non-synonymous polymorphic sites are *underlined*. *LP*, leader peptide, $\alpha 1$ alpha 1 domain, $\alpha 2$ alpha 2 domain, *C* connecting peptide, *T* trans-membrane domain. (*1*) Sequence not found in our study, revealed in an Indonesian cynomolgus macaque [AM943637 by Mee E.T. and Rose N.J. (unpublished)], (*2*) Full-length cDNA deduced from *Mamu-DRA* genomic DNA sequence (AM910165, Doxiadis et al. 2008). (*3*) Two expressed sequence tags (EST) of *M. nemestrina* [*Mane-DRA1* (EB520956) and *Mane-DRA2* (EB521652)] available in Genbank. (*4*) c*DRA* sequence of a homozygous *M. nemestrina* (*Mane-DRA01*, GQ214407; this study).(*5*) *Masy-DRA*01* (EU877221) and *Masy-DRA*02* (EU877222) sequences deduced from two *M. sylvanus* animals (this study)

libraries, we obtained two consensuses strictly identical with *Mafa-DRA*010212* and *Mafa-DRA*02010101*. The two alleles are expressed at comparable levels. The *DRB* cDNA sequences found in the three EST libraries differed from those of *Mafa-DRB* alleles (*Mafa-DRB*W501*, or *Mafa-DRB*W2101*, or *Mafa-DRB*W7201*) belonging to haplotypes #2a or #2b associated with *Mafa-DRA* 02010101* in Mauritius and the Philippines, respectively.

Discussion

The heterodimeric DR membrane proteins consist of a couple of DR alpha and DR beta proteins encoded by the *DRA* and the *DRB* genes, respectively. In all mammal species studied so far, the DR beta protein is highly polymorphic, especially in the amino acid positions which are in contact with the peptide, while the DR alpha protein is much less polymorphic. The number of *DRB* genes per haploid genome is polymorphic in all mammal species studied so far while the *DRA* gene is never duplicated. The maintenance of the *DRB* gene polymorphism results from a balancing selection (Satta 1993). Although the *DRA* gene polymorphism retained less attention than that of *DRB*, its study is required to delineate the selective constraints which influence the evolution of the *DRA* gene.

The aim of our study was to describe, in *M. fascicularis* the polymorphism of the *DRA* gene. We studied 141 animals representative of four populations either by characterization of the *DRA* cDNA (78 animals) or partial *DRA* genomic sequences (63 animals). In total, we characterized 22 *Mafa-DRA* cDNA alleles (18 of which had not been described

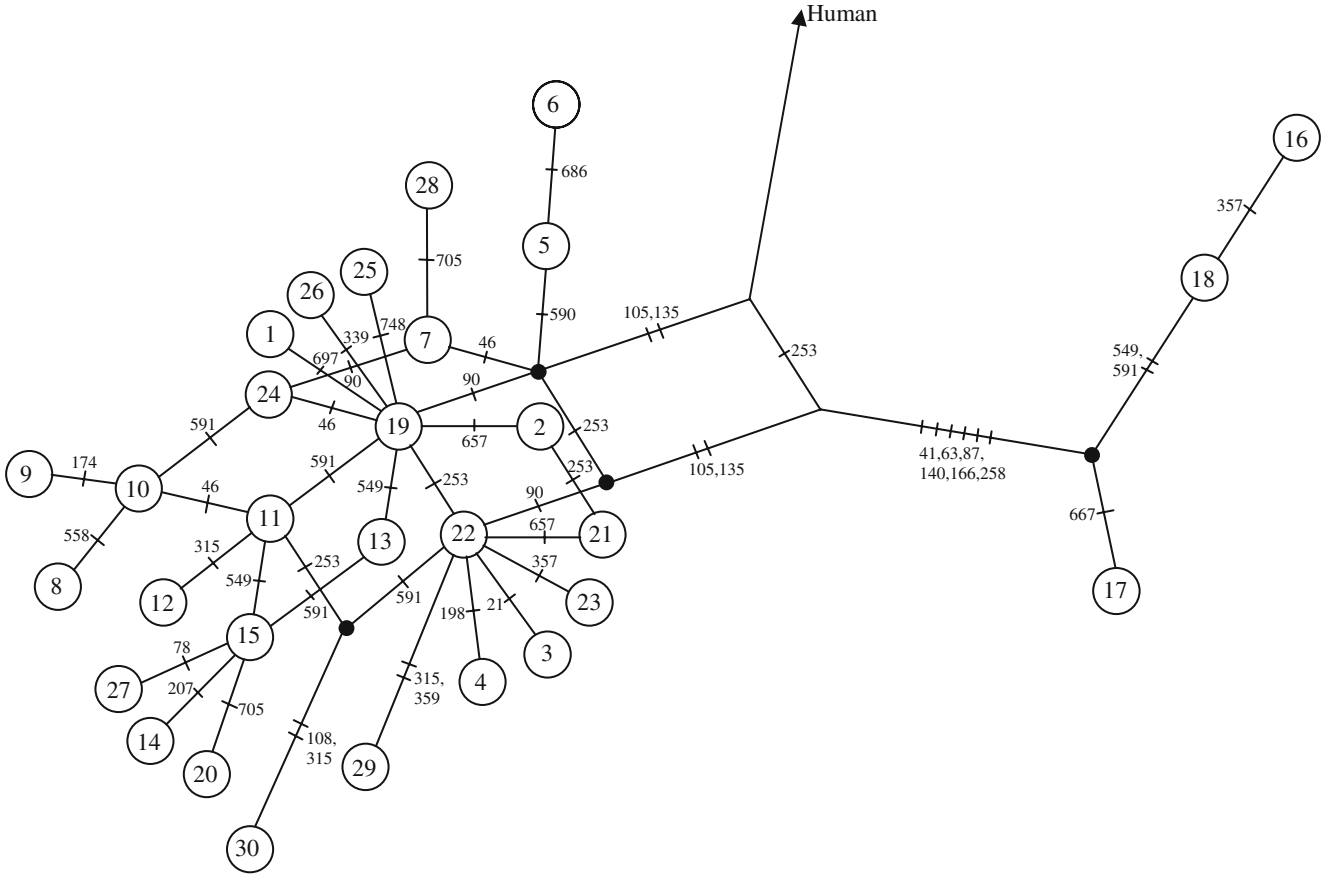

Fig. 4 Phylogenetic network of the macaque cDNA sequences. The network includes the *Mafa*-, *Mamu*-, *Mane*-, *Masy*-*DRA* nucleotide sequences and was rooted by using the human *HLA–DRA* sequence as outgroup. *Each number noted in a circle* represents the *cDRA* allele and the *other numbers* correspond to the position of the polymorphic sites on the full-length coding nucleotide sequence (*position number 1* corresponds to the first nucleotide of the start codon). The correspondence between numbers in *circles* and the *DRA* alleles is given in Fig. 3. Two reticulations (polymorphic sites situated in positions 549 and 591), involving sequence numbers 28 (*Mane-DRA*2, EST) and 29 (*Mafa-DRA*02010101*) are not shown in the phylogenetic network. The *Mafa-DRA*01020101* (noted 19 in the figure) probably corresponds to the macaque *cDRA* ancestral sequence

until now in *M. fascicularis*) and 41 partial (partial intron1 to partial intron3) *Mafa-DRA* genomic alleles. *Mafa-DRA* polymorphism is higher than already reported in *M. mulatta* (the other macaque species which has been extensively studied for *cDRA* polymorphism). Only 13 *Mamu cDRA* alleles were described in the latter species (de Groot et al. 2004; Doxiadis et al. 2008), eight of which are identical to 22 *Mafa-DRA* described in this study.

Despite the large number of animals and diversity of populations studied here, *DRA* gene polymorphism in *M. fascicularis* is not completely defined by this study. Indeed, the comparison of our c*DRA* sequence data set to the *M. fascicularis* c*DRA* sequences available in databanks (including the EST databanks), revealed one Indonesian c*DRA* allele (*Mafa-DRA*01020301*, Genbank access code AM943637) which is missing in our full-length c*DRA* allele collection. Moreover, the *Mafa-DRA*010101* cDNA allele was found only in the Mauritius population, which was founded only 100 generations ago (Lawler et al. 1995). Hence, the probability that *Mafa-DRA*010101* derived from another allele by mutations is very faint. It is more likely that Mauritius *Mafa-DRA*010101* could have been brought by a founder animal from a population incompletely studied here, such as Java or Sumatra (Blancher et al. 2008; Tosi and Coke 2007). Indeed, a systematic study of a larger range of population samples including other *M. fascicularis* populations (various continental populations and other island populations such as from Sumatra, Borneo) would be required for an exhaustive description of *DRA* polymorphism in this species.

In most pairwise comparisons between *Mafa* c*DRA* alleles, mutations are synonymous with dN/dS ratios less than 1, confirming that, in *M. fascicularis*, the *DRA* gene has been submitted to a strong purifying selection (Hughes and Nei 1989). In the *Mafa* c*DRA* alleles neighbor-joining tree, the *Mafa-DRA*02010101* allele lies alone on a branch remote from all other *Mafa* c*DRA* alleles which are grouped with a star-like phylogeny. We estimated at about 12 million years the time of divergence of the *Mafa-DRA*02010101* allele

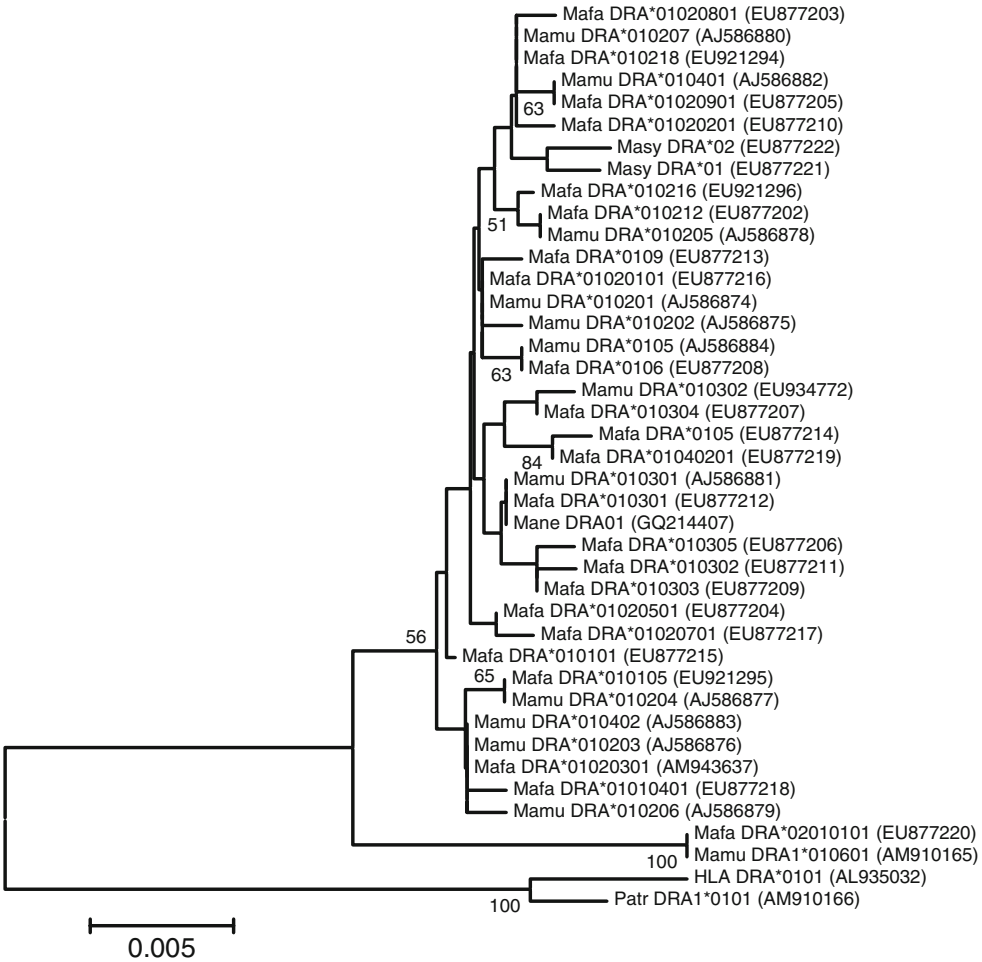

Fig. 5 Phylogenetic tree of macaque cDNA DRA sequences. The tree of *Mafa*-, *Mamu*-, *Mane*-, and *Masy*-c*DRA* alleles (734 bp) was obtained by means of the neighbor-joining method with complete deletion option. The tree was rooted with two outgroups (*HLA-DRA*0101* and *Patr-DRA*10101*). The percentage of replicate trees greater than 50% in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown *next to the branches*. The evolutionary distances are in the units of the number of base substitutions per site. The two Mane EST derived from ovary mRNA [*Mane-DRA1* (EB520956) and *Mane-DRA2* (EB521652)] were not included in the neighbor-joining tree because they are not full-length. However, they are clearly homologues of the *Mafa-DRA*02010101* (see Fig. 3, which describes the *DRA* cDNA variable sites)

from the most recent common ancestor of all *Mafa* c*DRA* alleles. This divergence time largely precedes macaque species radiation in Asia which could explain that homologues of *Mafa-DRA*02010101* are found in two other macaque species: *M. mulatta* (*Mamu-DRA1*010601*) and *Macaca nemestrina* (Fig. 3). This allele sharing is most probably the result of trans-species evolution (Klein 1987), although introgression could be considered as an alternative hypothesis (Bonhomme et al. 2009; Street et al. 2007).

It remains to be explained how this allele could persist for so long in three different species. One of the most probable causes of this persistence is balancing selection which might result from functional difference between the allele *Mafa-DRA*02010101* and the other *Mafa-DRA* alleles. *Mafa-DRA*02010101* differs from all other *Mafa-DRA* alleles by eight specific mutations among which four are non-synonymous. One amino acid change, present in the leader peptide is not conservative (Ile->Thr) and could have an impact on the exportation of the DR alpha protein influencing the density of peptide-presenting *DRA–DRB* heterodimers at the surface of the presenting cells. The three other amino acid changes, Glu-4-Asp, Phe-22-Tyr, and Ile-31-Leu, are conservative, located in the alpha-1 domain, and only the two latter involve amino acid residues which are in contact with the peptide presented by the *DRA–DRB* heterodimer (Reche and Reinherz 2003). Although these substitutions are conservative, they could have an impact on the functional properties of the DR peptide groove.

Table 4 Number of EST corresponding to the two *DRA* alleles of a Malaysian cynomolgus monkey in EST libraries derived from three lymphoid organs

EST libraries		*Mafa-DRA*	
		**010212*	**02010101*
Spleen	QspA,Lib.23009, *n*=9,783	40	32
Thymus	QthA,Lib.24021, *n*=9,566	26	25
Bone marrow	QbmA,Lib.22708, *n*=9,472	17	14
	Total EST	83	71

On the other hand, the mutations described here in the coding region of *Mafa-DRA*02010101* (as well as intronic mutations) could have accumulated through a hitchhiking effect related to distant mutations in the *DRA* gene promoter region or in the *DRB* gene, for example. Indeed, mutation in the promoter region could affect the transcription level of the gene. To explore this eventuality, we studied the representation of two *DRA* alleles in three EST libraries deriving from mRNA of three lymphoid organs of one Malaysian *DRA*-heterozygous animal (*Mafa-DRA*02010101*/*Mafa-DRA*010212*). The numbers of EST on the two alleles do not differ in the three organs analyzed (Table 4), and we concluded that the level of transcription of the allele *Mafa-DRA*02010101* does not differ significantly from that of *Mafa-DRA*010212*.

In total, in *M. fascicularis*, from the results of the present study and those available in the literature, 30 c*DRA* alleles are characterized and defined by 29 polymorphic positions. This polymorphism is much higher than in humans. For example, in the Philippine population, we found 11 *Mafa-DRA* alleles and 13 *Mafa-DRB* haplotypes. In humans, only five *HLA–DRA* alleles are known [two, named *HLA-DRA*0101* and *HLA-DRA*010202*, and three revealed by the study of 58 unrelated individuals (Voorter and Van den Berg-Loonen 2006)] while more than 700 *HLA–DRB1* alleles (defined only on exon 2) have been described. Although, the *HLA–DRA* gene has not been studied in some large samples in diverse populations, it is unlikely that *HLA–DRA* gene polymorphism reaches a level comparable to that of the *Mafa-DRA* gene.

Although the polymorphism of the *Mafa-DRA* gene is higher than that observed in humans, it is clearly lower than that observed in *Mafa-DRB* genes. Further studies are needed to clarify the functional constraints that result in the predominance of purifying selection on the *DRA* gene while the *DRB* genes are under diversifying selection.

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References

Aoyama A, Ng CY, Millington TM, Boskovic S, Murakami T, Wain JC, Houser SL, Madsen JC, Kawai T, Allan JS (2009) Comparison of lung and kidney allografts in induction of tolerance by a mixed-chimerism approach in cynomolgus monkeys. Transplant Proc 41:429–430

Baskin CR, Bielefeldt-Ohmann H, Tumpey TM, Sabourin PJ, Long JP, Garcia-Sastre A, Tolnay AE, Albrecht R, Pyles JA, Olson PH, Aicher LD, Rosenzweig ER, Murali-Krishna K, Clark EA, Kotur MS, Fornek JL, Proll S, Palermo RE, Sabourin CL, Katze MG (2009) Early and sustained innate immune response defines pathology and death in nonhuman primates infected by highly pathogenic influenza virus. Proc Natl Acad Sci USA 106:3455–3460

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, a software for population genetics. http://www.genetix.univ-montp2.fr/genetix/genetix.htm

Bimber BN, Moreland AJ, Wiseman RW, Hughes AL, O'Connor DH (2008) Complete characterization of killer Ig-like receptor (KIR) haplotypes in Mauritian cynomolgus macaques: novel insights into nonhuman primate KIR gene content and organization. J Immunol 181:6301–6308

Blancher A, Tisseyre P, Dutaur M, Apoil PA, Maurer C, Quesniaux V, Raulf F, Bigaud M, Abbal M (2006) Study of cynomolgus monkey (*Macaca fascicularis*) MhcDRB (Mafa-DRB) polymorphism in two populations. Immunogenetics 58:269–282

Blancher A, Bonhomme M, Crouau-Roy B, Terao K, Kitano T, Saitou N (2008) Mitochondrial DNA sequence phylogeny of 4 populations of the widely distributed cynomolgus macaque (*Macaca fascicularis fascicularis*). J Hered 99:254–264

Bonhomme M, Blancher A, Crouau-Roy B (2005) Multiplexed microsatellites for rapid identification and characterization of individuals and populations of Cercopithecidae. Am J Primatol 67:385–391

Bonhomme M, Blancher A, Jalil MF, Crouau-Roy B (2007) Factors shaping genetic variation in the MHC of natural non-human primate populations. Tissue Antigens 70:398–411

Bonhomme M, Blancher A, Cuartero S, Chikhi L, Crouau-Roy B (2008) Origin and number of founders in an introduced insular primate: estimation from nuclear genetic data. Mol Ecol 17:1009–1019

Bonhomme M, Cuartero S, Blancher A, Crouau-Roy B (2009) Assessing natural introgression in 2 biomedical model species, the rhesus macaque (*Macaca mulatta*) and the long-tailed macaque (*Macaca fascicularis*). J Hered 100:158–169

Borie D, Hausen B, Larson M, Klupp J, Stalder M, Birsan T, Morris R (2002) A life-supporting technique of renal allotransplantation in *Macaca fascicularis* to evaluate novel immunosuppressive drugs in nonhuman primates. J Surg Res 107:64–74

Burwitz BJ, Pendley CJ, Greene JM, Detmer AM, Lhost JJ, Karl JA, Piaskowski SM, Rudersdorf RA, Wallace LT, Bimber BN, Loffredo JT, Cox DG, Bardet W, Hildebrand W, Wiseman RW, O'Connor SL, O'Connor DH (2009) Mauritian cynomolgus macaques share two exceptionally common major histocompatibility complex class I alleles that restrict simian immunodeficiency virus-specific CD8+ T cells. J Virol 83:6011–6019

Calvas P, Apoil P, Fortenfant F, Roubinet F, Andris J, Capra D, Blancher A (1999) Characterization of the three immunoglobulin G subclasses of macaques. Scand J Immunol 49:595–610

Campbell KJ, Detmer AM, Karl JA, Wiseman RW, Blasky AJ, Hughes AL, Bimber BN, O'Connor SL, O'Connor DH (2009) Characterization of 47 MHC class I sequences in Filipino cynomolgus macaques. Immunogenetics 61:177–187

Daza-Vamenta R, Glusman G, Rowen L, Guthrie B, Geraghty DE (2004) Genetic divergence of the rhesus macaque major histocompatibility complex. Genome Res 14:1501–1515

de Groot N, Doxiadis GG, De Groot NG, Otting N, Heijmans C, Rouweler AJ, Bontrop RE (2004) Genetic makeup of the DR region in rhesus macaques: gene content, transcripts, and pseudogenes. J Immunol 172:6152–6157

Derdouch S, Gay W, Negre D, Prost S, Le Dantec M, Delache B, Auregan G, Andrieu T, Leplat JJ, Cosset FL, Le Grand R (2008) Reconstitution of the myeloid and lymphoid compartments after the transplantation of autologous and genetically modified CD34 + bone marrow cells, following gamma irradiation in cynomolgus macaques. Retrovirology 5:50

Doxiadis GG, de Groot N, de Groot NG, Doxiadis II, Bontrop RE (2008) Reshuffling of ancient peptide binding motifs between HLA-DRB multigene family members: old wine served in new skins. Mol Immunol 45:2743–2751

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Florese RH, Wiseman RW, Venzon D, Karl JA, Demberg T, Larsen K, Flanary L, Kalyanaraman VS, Pal R, Titti F, Patterson LJ, Heath MJ, O'Connor DH, Cafaro A, Ensoli B, Robert-Guroff M (2008) Comparative study of Tat vaccine regimens in Mauritian cynomolgus and Indian rhesus macaques: influence of Mauritian MHC haplotypes on susceptibility/resistance to SHIV (89.6P) infection. Vaccine 26:3312–3321

Hughes AL, Nei M (1989) Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. Proc Natl Acad Sci USA 86:958–962

Kita Y, Tanaka T, Yoshida S, Ohara N, Kaneda Y, Kuwayama S, Muraki Y, Kanamaru N, Hashimoto S, Takai H, Okada C, Fukunaga Y, Sakaguchi Y, Furukawa I, Yamada K, Inoue Y, Takemoto Y, Naito M, Yamada T, Matsumoto M, McMurray DN, Cruz EC, Tan EV, Abalos RM, Burgos JA, Gelber R, Skeiky Y, Reed S, Sakatani M, Okada M (2005) Novel recombinant BCG and DNA-vaccination against tuberculosis in a cynomolgus monkey model. Vaccine 23:2132–2135

Klein J (1987) Origin of major histocompatibility complex polymorphism: the trans-species hypothesis. Hum Immunol 19:155–162

Klein J, Bontrop RE, Dawkins RL, Erlich HA, Gyllensten UB, Heise ER, Jones PP, Parham P, Wakeland EK, Watkins DI (1990) Nomenclature for the major histocompatibility complexes of different species: a proposal. Immunogenetics 31:217–219

Kuiken T, Rimmelzwaan GF, Van Amerongen G, Osterhaus AD (2003) Pathology of human influenza A (H5N1) virus infection in cynomolgus macaques (*Macaca fascicularis*). Vet Pathol 40:304–310

Lau M, Vayntrub T, Grumet FC, Lowsky R, Strober S, Hoppe R, Larson M, Holm B, Reitz B, Borie D (2004) Short tandem repeat analysis to monitor chimerism in *Macaca fascicularis*. Am J Transplant 4:1543–1548

Lawler SH, Sussman RW, Taylor LL (1995) Mitochondrial DNA of the Mauritian macaques (*Macaca fascicularis*): an example of the founder effect. Am J Phys Anthropol 96:133–141

Lawler JV, Endy TP, Hensley LE, Garrison A, Fritz EA, Lesar M, Baric RS, Kulesh DA, Norwood DA, Wasieloski LP, Ulrich MP, Slezak TR, Vitalis E, Huggins JW, Jahrling PB, Paragas J (2006) Cynomolgus macaque as an animal model for severe acute respiratory syndrome. PLoS Med 3:e149

Leuchte N, Berry N, Kohler B, Almond N, LeGrand R, Thorstensson R, Titti F, Sauermann U (2004) MhcDRB-sequences from cynomolgus macaques (*Macaca fascicularis*) of different origin. Tissue Antigens 63:529–537

Mee ET, Badhan A, Karl JA, Wiseman RW, Cutler K, Knapp LA, Almond N, O'Connor DH, Rose NJ (2009a) MHC haplotype frequencies in a UK breeding colony of Mauritian cynomolgus macaques mirror those found in a distinct population from the same geographic origin. J Med Primatol 38:1–14

Mee ET, Berry N, Ham C, Sauermann U, Maggiorella MT, Martinon F, Verschoor EJ, Heeney JL, Le Grand R, Titti F, Almond N, Rose NJ (2009b) Mhc haplotype H6 is associated with sustained control of SIVmac251 infection in Mauritian cynomolgus macaques. Immunogenetics 61:327–339

Menninger K, Wieczorek G, Riesen S, Kunkler A, Audet M, Blancher A, Schuurman HJ, Quesniaux V, Bigaud M (2002) The origin of cynomolgus monkey affects the outcome of kidney allografts under Neoral immunosuppression. Transplant Proc 34:2887–2888

Morner A, Douagi I, Forsell MN, Sundling C, Dosenovic P, O'Dell S, Dey B, Kwong PD, Voss G, Thorstensson R, Mascola JR, Wyatt RT, Karlsson Hedestam GB (2009) Human immunodeficiency virus type 1 env trimer immunization of macaques and impact of priming with viral vector or stabilized core protein. J Virol 83:540–551

O'Connor SL, Blasky AJ, Pendley CJ, Becker EA, Wiseman RW, Karl JA, Hughes AL, O'Connor DH (2007) Comprehensive characterization of MHC class II haplotypes in Mauritian cynomolgus macaques. Immunogenetics 59:449–462

Otting N, de Groot NG, Doxiadis GG, Bontrop RE (2002) Extensive Mhc-DQB variation in humans and non-human primate species. Immunogenetics 54:230–239

Osada N, Hirata M, Tanuma R, Suzuki Y, Sugano S, Terao K, Kusuda J, Kameoka Y, Hashimoto K, Takahashi I (2009) Collection of *Macaca fascicularis* cDNAs derived from bone marrow, kidney, liver, pancreas, spleen, and thymus. BMC Res Notes 2:199

Pendley CJ, Becker EA, Karl JA, Blasky AJ, Wiseman RW, Hughes AL, O'Connor SL, O'Connor DH (2008) MHC class I characterization of Indonesian cynomolgus macaques. Immunogenetics 60:339–351

Puissant B, Abbal M, Blancher A (2003) Polymorphism of human and primate RANTES, CX3CR1, CCR2 and CXCR4 genes with regard to HIV/SIV infection. Immunogenetics 55:275–283

Reche P, Reinherz E (2003) Sequence variability analysis of human class I and class II MHC molecules: functional and structural correlates of amino acid polymorphisms. J Mol Biol 331:623–641

Reed DS, Larsen T, Sullivan LJ, Lind CM, Lackemeyer MG, Pratt WD, Parker MD (2005) Aerosol exposure to western equine encephalitis virus causes fever and encephalitis in cynomolgus macaques. J Infect Dis 192:1173–1182

Robinson J, Waller MJ, Stoehr P, Marsh SG (2005) IPD—the Immuno Polymorphism Database. Nucleic Acids Res 33:D523–D526

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sano K, Shiina T, Kohara S, Yanagiya K, Hosomichi K, Shimizu S, Anzai T, Watanabe A, Ogasawara K, Torii R, Kulski JK, Inoko H (2006) Novel cynomolgus macaque MHC-DPB1 polymorphisms in three South-East Asian populations. Tissue Antigens 67:297–306

Sato H, Kobune F, Ami Y, Yoneda M, Kai C (2008) Immune responses against measles virus in cynomolgus monkeys. Comp Immunol Microbiol Infect Dis 31:25–35

Satta Y (1993) Balancing selection at HLA loci. In: Takahata N, Clark AG (eds) Mechanism of molecular evolution. Japan Scientific Societies Press Sinauer Associated Inc., Tokyo, Japan

Schmidt LH, Fradkin R, Harrison J, Rossan RN (1977) Differences in the virulence of *Plasmodium knowlesi* for *Macaca irus* (*fascicularis*) of Philippine and Malayan origins. Am J Trop Med Hyg 26:612–622

Street SL, Kyes RC, Grant R, Ferguson B (2007) Single nucleotide polymorphisms (SNPs) are highly conserved in rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. BMC Genomics 8:480

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA 101:11030–11035

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tosi AJ, Coke CS (2007) Comparative phylogenetics offer new insights into the biogeographic history of *Macaca fascicularis* and the origin of the Mauritian macaques. Mol Phylogenet Evol 42:498–504

Turbant S, Martinon F, Moine G, Le Grand R, Leonetti M (2009) Cynomolgus macaques immunized with two HIV-1 Tat stabilized proteins raise strong and long-lasting immune responses with a pattern of Th1/Th2 response differing from that in mice. Vaccine 27:5349–5356

Uda A, Tanabayashi K, Yamada YK, Akari H, Lee YJ, Mukai R, Terao K, Yamada A (2004) Detection of 14 alleles derived from the MHC class I A locus in cynomolgus monkeys. Immunogenetics 56:155–163

Uda A, Tanabayashi K, Fujita O, Hotta A, Terao K, Yamada A (2005) Identification of the MHC class I B locus in cynomolgus monkeys. Immunogenetics 57:189–197

Voorter CBR, Van den Berg-Loonen E (2006) The *HLA–DRA* gene and its extending polymorphism (abstract, 32nd Annual ASHI Meeting). Human Immunology 67:S106

Warfield KL, Swenson DL, Olinger GG, Kalina WV, Aman MJ, Bavari S (2007) Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. J Infect Dis 196(Suppl 2):S430–S437

Watanabe A, Shiina T, Shimizu S, Hosomichi K, Yanagiya K, Kita YF, Kimura T, Soeda E, Torii R, Ogasawara K, Kulski JK, Inoko H (2007) A BAC-based contig map of the cynomolgus macaque (*Macaca fascicularis*) major histocompatibility complex genomic region. Genomics 89:402–412

Wieczorek G, Bigaud M, Menninger K, Riesen S, Quesniaux V, Schuurman HJ, Audet M, Blancher A, Mihatsch MJ, Nickeleit V (2006) Acute and chronic vascular rejection in nonhuman primate kidney transplantation. Am J Transplant 6:1285–1296

Wiseman RW, Wojcechowskyj JA, Greene JM, Blasky AJ, Gopon T, Soma T, Friedrich TC, O'Connor SL, O'Connor DH (2007) Simian immunodeficiency virus SIVmac239 infection of major histocompatibility complex-identical cynomolgus macaques from Mauritius. J Virol 81:349–361