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Characterisation and comparative analysis of MHC-DPA1 exon 2 in the owl monkey (*Aotus nancymaae*)

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ABSTRACT

The *Aotus nancymaae* (owl monkey) is an important animal model in biomedical research, particularly for the preclinical evaluation of vaccine candidates against *Plasmodium falciparum* and *Plasmodium vivax*, which require a precisely typed major histocompatibility complex. The exon 2 from *A. nancymaae* MHC-DPA1 gene was characterised in order to infer its allelic diversity and evolutionary history. Aona-DPA1 shows no polymorphism and is related to other primate DPA alleles (including Catarrhini and Platyrrhini), constituting an ancient trans-specific and strongly supported lineage with different variability and selective patterns when compared to other primate–MHC-DPA1 lineages. *A. nancymaae* monkeys have thus a smaller MHC-DP polymorphism than MHC-DQ or MHC-DR.

1. Introduction

Major histocompatibility complex (MHC) class II molecules display peptides on the surface of antigen-presenting cells (APC) for subsequent recognition by T cells, thereby performing a key defence role against pathogens. MHC class II molecules are heterodimers assembled from an α and a β glycopeptide chains encoded by the MHC class II A and B genes, respectively. Three main MHC class II loci, named HLA-DR, -DQ, and -DP, encode functional antigen-presenting molecules in primates. Genetic polymorphism and diversifying selection tied to functional and structural restrictions are common characteristics of these main loci. Such polymorphism is mainly restricted to the second exon of MHC class II A and B genes, constituting the molecule's peptide binding region (PBR) (Klein et al., 1993b).

MHC-DP is an ancient locus shared by divergent mammalian orders (Takahashi et al., 2000; Yuhki et al., 2003). However, its polymorphism and functionality vary. For example, MHC-DP acquires a pseudo-genic nature in felines, as also occurs in murinae (mouse-like rodents), even though MHC-DP is the most polymorphic MHC class II locus in other rodents, such as the mole rat (*Spalax* genus) (Klein et al., 1993a; Yuhki et al., 2003; Kelley et al., 2005).

MHC-DP is the most centromeric locus within the primate MHC gene cluster region, being constituted by four genes: DPA1 and DPB1 genes and DPA2 and DPB2 pseudogenes. This arrangement (position and number) is apparently the same in all primates and was established before the split between Platyrrhini and Catarrhini ~43 million years ago (MY) (Klein et al., 1993a; Steiper and Young, 2006).

MHC-DPA1 variability in primates varies amongst nonexistent and low polymorphism, whilst for MHC-DPB1, it fluctuates from moderate to high polymorphism (Otting and Bontrop, 1995; Slierendregt et al., 1995; Bontrop et al., 1999; Doxiadis et al., 2001). HLA-DPA1 exhibits low polymorphism in humans, where 28 alleles have been reported to date, compared to the 138 alleles described for HLA-DPB1 (Robinson, et al., 2003). In contrast, *Callithrix jacchus* (the common marmoset, a neo-tropical primate), has the MHC-DP region inactive, not expressing any MHC-DP molecule (Antunes et al., 1998). In spite of such low polymorphism, MHC-DPA1 can be important in modulating an immune response, since HLA-DPA1*0301 appears to be involved in the genetic susceptibility to *Schistosoma haematobium* and several chronic inflammatory diseases (May et al., 1998; Dai et al., 2010).

Previous studies have characterised *Aotus* MHC class II genes and molecules: MHC DQA-DQB (Diaz et al., 2000), MHC-DRB1 (Niño-Vasquez et al., 2000; Suarez et al., 2006), and MHC-DPB1 (Diaz et al., 2002). These neo-tropical primates have been shown to be susceptible to various human infectious diseases (Lujan et al., 1986; Polotsky

Abbreviations: MHC, Major histocompatibility complex; APC, antigen-presenting cells; PBR, peptide binding region; NWM, new world monkeys; OWM, old world monkeys; NJ, neighbour joining; ME, minimum-evolution; ML, maximum likelihood; LRSH, local rearrangements of tree topology around an edge; Pars, parsimony; GRMD, global rate minimum deformation method; MY, million years; SLAC, single likelihood ancestor counting; FEL, fixed effects likelihood; REL, random effects likelihood; Sub/S/MY, substitution per site per million years; TSP, trans-specific polymorphism.

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et al., 1994; Noya et al., 1998). They can develop human malaria, particularly *Plasmodium falciparum* (Gysin, 1988; Rodriguez et al., 1990; Collins, 1994) and *Plasmodium vivax* asexual/blood stage infections (Pico de Coana et al., 2003). This makes the owl monkey a highly valuable animal model for biomedical research. To complete this landmark, the study of MHC-DPA1 might play a key role in understanding the immune response against *Plasmodium* (Diaz et al., 2002) and contributes towards gaining a deeper knowledge about the immune system of owl monkeys. The exon 2 from *Aotus nancymaae* MHC-DPA1 gene was characterised to infer its allelic diversity, variability patterns, the amount and kind of its variation, the type of changes involved, as well as the extent of natural selection and evolutionary relationships within the primate context.

2. Materials and methods

2.1. Animals

Six *A. nancymaae* monkeys (4 males and 2 females) were randomly caught from different familiar groups in Lagos de Leticia and Atacuari River, two widely separated zones (80 km) in the Colombian Amazon. The monkeys were captured with the authorisation of the official environmental authority of Colombia in this region, CORPOAMAZONIA, which granted the Fundación Instituto de Inmunología de Colombia (FIDIC) permission for the capture, study, and scientific research with these primates in the Colombian Amazon (Resolutions #1966/2006 and 0028/2010 and previous authorisations beginning in 1982). This research has been performed following the guidelines approved by FIDIC's ethics committee. The studied animals have been always under the supervision of expert veterinarians and biologists, and after experimental procedures, they are released back into the Amazon jungle in optimal health conditions in the presence of a representative from CORPOAMAZONIA.

2.2. RNA extraction, cDNA synthesis, PCR, cloning, and sequencing

Leukocytes were obtained from six healthy *A. nancymaae* monkeys by density gradient separation of peripheral blood obtained by venous puncture. Total cellular RNA was isolated from peripheral blood mononuclear cells using the TRIzol one-step procedure (Invitrogen Life Technologies, CA, USA). Moloney murine leukaemia virus reverse transcriptase (Promega, Madison, WI, USA) was used for cDNA synthesis, according to the manufacturer's instructions.

Two PCR of MHC-DPA1 exon 2 were independently performed for each monkey; PCR primers used were GH98 (5′-CGCGGATCCTGTGTCAACTTATGCCGCG-3′) and GH99 (5′-CTGGCTGCAGTGTGGTTGGAACGCTG-3′) (Otting and Bontrop, 1995) at a final 0.8 μM concentration. The PCR mixture contained 1.5 μM $MgCl_2$, 50 mM Tris (pH 8.3) and 2.5 U Taq DNA polymerase (Promega). Five microlitres of cDNA was added to each reaction for a 25 μl final volume. These reactions were heated to 95 °C for 5 min and then amplified for 40 cycles as follows: denaturing for 30 s at 94 °C, annealing for 1 min at 65 °C, and extension for 2 min at 68 °C. A final extension cycle was run at 65 °C for 1 min and 68 °C for 5 min.

A WIZARD PCR Preps Purification kit (Promega) was used for purifying PCR products which were then ligated into pGEM T vector (Promega). MiniPreps Purification Kit (Mo Bio, Carlsbad, CA, USA) was used for isolating double-strand plasmid DNA. Three clones from each PCR were randomly chosen and sequenced using fluorescent dye-labelled dideoxy terminators (Applied Biosystems, Foster City, CA, USA) in an ABI Prism 310 genetic analyser (Applied Biosystems).

2.3. MHC-DPA1 sequences

A total of 64 exon 2 MHC-DPA1 gene sequences from 11 primates (suborder Anthropoidea) were used. **Platyrrhini** (new world monkeys (NWM)): *A. nancymaae*—owl monkey (*Aona*, 1 sequence, reported here) and *Saimiri sciureus*—squirrel monkey (Sasc, 3 sequences); **Catarrhini**: **Cercopithecidae** (old world monkeys—OWM): *Macaca arctoides*—stump-tailed macaque (Maar, 1 sequence), *Macaca fascicularis*—crab-eating macaque (Mafa, 6 sequences), *Macaca mulatta*—rhesus monkey (Mamu, 17 sequences), and *Papio hamadryas*—hamadryas baboon (Paha, 1 sequence); **Hominoidea** (humans and apes): *Homo sapiens*—human (HLA, 25 sequences), *Pan troglodytes*—chimpanzee (Patr, 3 sequences), *Gorilla gorilla*—gorilla (Gogo, 3 sequences), *Pongo pygmaeus*—Bornean orangutan (Popy, 3 sequences), and *Pongo abelii*—Sumatran orangutan (Poab, 1 sequence).

The following are the GenBank accession numbers of the studied sequences: Aona-DPA1*01—AF529200, Gogo-DPA1*0401—AF026701, Gogo-DPA1*0402—AF026702, Gogo-DPA1—CU104655, HLA-DPA1*010302—AF074848, HLA-DPA1*010304—DQ274060, HLA-DPA1*0104—X78198, HLA-DPA1*0105—X96984, HLA-DPA1*010601—U87556, HLA-DPA1*010602—EU729350, HLA-DPA1*0107—AF076284, HLA-DPA1*0108—AF346471, HLA-DPA1*0109—AY650051, HLA-DPA1*0110—DQ274061, HLA-DPA1*020101—X78199, HLA-DPA1*020102—L31624, HLA-DPA1*020103—AF015295, HLA-DPA1*020104—AF074847, HLA-DPA1*020105—AF098794, HLA-DPA1*020106—AF165160, HLA-DPA1*020203—AF092049, HLA-DPA1*02021—X79475, HLA-DPA1*02022—X79476, HLA-DPA1*0203—Z48473, HLA-DPA1*0204—EU304462, HLA-DPA1*0301—M83908, HLA-DPA1*0302—AF013767, HLA-DPA1*0303—AY618553, HLA-DPA1*0401—L11643, Maar-DPA1*0201—AF026703, Mafa-DPA1*0201—AF026704, Mafa-DPA1*0202—EF208806, Mafa-DPA1*0204—AM943632, Mafa-DPA1*0401—EF208808, Mafa-DPA1*0701—EF208809, Mafa-DPA1*0702—EF208810, Mamu-DPA1*0101—Z32411, Mamu-DPA1*0201—EF204945, Mamu-DPA1*0203—EF204950, Mamu-DPA1*0208—FJ544416, Mamu-DPA1*0401—FJ544417, Mamu-DPA1*0402—FJ544415, Mamu-DPA1*0403—GQ471885, Mamu-DPA1*0601—EF204949, Mamu-DPA1*0701—EF204946, Mamu-DPA1*0801—EU305663, Mamu-DPA1—AB219099, Mamu-DPA1—AB219100, Mamu-DPA1—AB219101, Mamu-DPA1—AB250754, Mamu-DPA1—AB250756, Mamu-DPA1—AB219102, Mamu-DPA1—AB250757, Paha-DPA1*0201—AF026706, Patr-DPA1*0201—AF026707, Patr-DPA1*0202—AF026693, Patr-DPA1*0301—AF026694, Poab-DPA1—AC207096, Popy-DPA1*0201—AF026695, Popy-DPA1*0202—AF026696, Popy-DPA1*0401—AF026697, Sasc-DPA1*0501—AF026698, Sasc-DPA1*0502—AF026699, Sasc-DPA1*0601—AF026700.

2.4. Sequence analysis

Clustal X (Thompson et al., 1997) was used for aligning the MHC-DPA1 exon 2 sequences. The *A. nancymaae* sequence was included and an amino acid alignment was also performed. HLA-DRA1*010101 and HLA-DQA1*010101 were used as outgroups. The resulting alignment had a total of 189/63 nucleotide/amino acid positions (Supplementary materials 1 and 2).

GENEDOC (Nicholas et al., 1997) was used for calculating the percent of identity (i.e., equal positions between sequences) and similarity (i.e., positions with conservative substitutions between sequences, in this case, assessed by the PAM 250 substitution matrix) in the considered alignments. Means and standard deviations of pairwise nucleotide and amino acid identity and similarity (this last one for amino acid sequences only) inside each group of sequences were analytically calculated.

Each position's variation for MHC-DPA1 exon 2 amino acid aligned sequences was represented by using WebLogo (Crooks et al., 2004). All amino acids occupying each position were indicated, in which the height of every amino acid letter represented its relative frequency in that position. The logo also allowed conservative and nonconservative substitutions for each position to be determined, where the variation in an amino acid symbol's colour indicated nonconservative changes and its preservation represented conservative changes based on PAM