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Sequence and diversity of MHC *DQA* and *DQB* genes of the owl monkey *Aotus nancymaae*

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Abstract The New World primate *Aotus nancymaae* has been recommended by the World Health Organization (WHO) as a model for evaluation of malaria vaccine candidates, given its susceptibility to experimental infection with the human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*. We present here the nucleotide sequences of the complete cDNA of MHC-*DQA1* and of the polymorphic exon 2 segments of MHC-*DQB1/DQB2*. In a group of three nonrelated animals captured in the wild, five alleles of MHC-*DQA1* could be identified. They all belong to one lineage, namely *Aona-DQA1*27*. This lineage has not been described in any other New World monkey species studied. In a group of 19 unrelated animals, 14 *Aona-DQB1* alleles could be identified which are grouped into the two lineages *Aona-DQB1*22* and *Aona-DQB1*23*. These lineages have been described previously in the common marmoset and cotton-top tamarin. In addition, two *Aona-DQB2* sequences could be identified which are highly similar to *HLA-DQB2* sequences. Essential amino acid residues contributing to MHC *DQ* peptide binding pockets number 1 and 4 are conserved or semi-conserved between HLA-DQ and Aona-DQ molecules, indicating a capacity to bind similar peptide repertoires. These results fully support the use of *Aotus* monkeys as an animal model for evaluation of future subunit vaccine candidates.

Key words *Aotus nancymaae* · MHC class II *DQ* genes · Allelic lineages · Polymorphism · Peptide binding

Introduction

The function of major histocompatibility complex (MHC) class I and class II molecules is to collect peptide fragments inside the cell and transport them to the cell surface, where the peptide-MHC complex is surveyed by the immune system (Germain and Margulies 1993). In humans, three MHC class II loci, called *HLA-DR,* -DQ and *-DP*, produce functional antigen-presenting heterodimers. Each class II heterodimer is made up of the non-covalent association of two glycopeptide chains: the α chain and the β chain, encoded by, for example, for *DQ*, *DQA* and *DQB* genes. MHC *DQA* and *DQB* genes are described in various nonhuman primate species (Bontrop 1994). Some MHC *DQB* lineages are at least 30 million years old and predate the divergence of hominoid and Old World primate species (Otting et al. 1992). Two sets of closely related gene *DQ* pairs have been identified in humans: *DQA1-DQB1* and *DQA2-DQB2*. The polymorphic *DQA1-DQB1* genes encode the MHC *DQ* molecules, whereas a product of the *DQA2-DQB2* genes has not been identified in humans (Bontrop et al. 1999). Polymorphism of MHC *DQ* molecules appears to be responsible for variations in the immune responses of individuals to antigens and may contribute to susceptibility or resistance against infectious diseases and autoimmune disorders (Hill 1998; Todd et al. 1987).

The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank nucleotide sequence databases and have been assigned the accession numbers AF201293 – AF201297 and AF213629 – AF213644

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The contemporary living primates can be classified into New World monkeys (Platyrrhini) and Old World simians (Catarrhini). The New World monkeys and the Old World simians radiated about 58 million years ago (Ciochon and Chiarelli 1980). One of several species of New World monkeys employed in biomedical research over the past several decades is *Aotus spp,* which can sustain in a predictable way the development of asexual forms of the two major human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax* from different geographic areas (Gysin 1998). In 1988, the WHO recommended *Aotus spp*. as an experimental model for *P. falciparum* blood-stage infections and for the evaluation of candidate malaria vaccines (Gysin 1998).

The immunogenetic background of *Aotus* monkeys has been investigated to confirm the suitability of this model for the evaluation of potential peptide vaccine candidates (Patarroyo et al. 1987). After the characterization of the *TCRAV* and *TCRBV* repertoire (Favre et al. 1998; Vecino et al. 1999) and the exon 2 of MHC *DRB* genes (Nino-Vasquez et al. 2000), we present here results of an analysis of the *DQA1* (*MhcAona-DQA1*) and *DQB1*/2 (*MhcAona-DQB1* and *MhcAona-DQB2*) genes of *A. nancymaae.*

Materials and methods

Animals

The animals analyzed in this study were caught in the Colombian Amazon area close to Leticia and were kept at the monkey colony of the Instituto de Inmunologia. Mononuclear cells from 19 healthy monkeys were obtained by femoral venous puncture and density gradient separation using Ficoll hypaque, or by spleenectomy followed by density gradient separation as described (Garraud et al. 1994). One B lymphoblastoid cell line was established from monkey 3026 by transformation with Epstein-Barr virus.

Amplification of Aona-DQA1 genes by polymerase chain reaction

Total RNA was isolated from spleen cells of monkeys 11190, 11192 and 11145 using a RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. After reverse transcription using Superscript (Gibco-BRL, Oensingen, Switzerland) and oligo $dT_{12–18}$ primer (Gibco-BRL), the complete cDNA of MHC-*DQA1* was amplified by PCR with primers DQA-Amp5 (5'-AAAAAGCTAGCACAGCTCAGARCAG-CAACTG-3′) and DQA-Amp3 (5′-GGGGCTCGAGATTCA-CAAKGGCCCTTGGTG-3′) as described (Yasunaga et al. 1996). The following temperature profile was employed: 5 min 94°C; 30 cycles of 1 min 94°C, 1 min 59°C, 1 min 72°C, and the reaction was completed by a final extension step of 7 min at 72°C. The same cDNAs were amplified in a second amplification with primers DQA-Amp5 and DQA-Amp3.2 (5′-GATGGCGATGCACCTTCCCTTCC-3′). This set of primers was employed to amplify the complete cDNA, since they are located outside the coding regions. The PCR reaction was run under the same conditions as described above.

Amplification of exon 2 sequences of Aona-DQB genes

Genomic DNA was extracted either from PBMC, spleen cells or the lymphoblastoid cell line from monkey 3026 using the Nucleospin C+T kit (Macherey-Nagel, Oensingen, Switzerland) according to the manufacturer's protocol. DNA samples were amplified by PCR with primers DQB-DB130 (5′-AGG-GATCCCCGCAGAGGATTTCGTGTACC-3′) and DQB-DB131 (5′-TCCTGCAGGGCGACGACGCTCACCTCCCC-3′) as described (Bugawan and Ehrlich 1991). PCR cycling conditions were as follows: 5 min 96°C; 35 cycles of 1 min 96°C, 1 min 60°C, 1 min 72°C; 7 min 72°C; soak at 4°C. From monkeys 11190, 11192 and 11145, the second exon of MHC *DQB* was amplified from cDNA using the same primer pair and conditions as described for genomic DNA. The amplicon was visualized on a 3% agarose gel (Gibco-BRL).

Cloning and DNA sequencing reaction

The PCR products were purified using a High Pure PCR product purification kit (Boehringer Mannheim, Indianapolis, Ind.) according to the manufacturer's protocol and were cloned into pGEM5 T vector (Promega, Madison, Wis.). Plasmid double-stranded DNA was isolated using the Nucleospin kit (Macherey-Nagel). Plasmid inserts were sequenced in both directions using ABI Prism 310 Genetic Analyzer (Perkin Elmer, Foster City, Calif.) and analyzed using the provided software.

Nomenclature

Official designations were obtained from R. E. Bontrop and N. G. de Groot (Biomedical Primate Research Centre, Rijswijk, The Netherlands) based upon shared sequence motifs, phylogenetic analysis and comparison with sequences found in other New World monkeys (Antunes et al. 1998). The reported alleles represent at least three identical clones that were obtained after independent amplifications from the same animal or in different animals.

Phylogenetic analysis

Phylogenetic analysis was performed employing the PHYLIP 3.572 package available under http://bioweb.pasteur.fr. A neighbor-joining phylogenetic tree (Saitou and Nei 1987) was constructed from genetic distance values (Kimura 1980).

Results

Polymorphism of Aona-DQA1 alleles

The cDNA from three randomly chosen *A. nancymaae* monkeys was used to amplify the full-length MHC *DQA1* gene. The resulting PCR products were cloned and 28 inserts were sequenced. Five different alleles were identified. These *Aona-DQA1* nucleotide sequences are given in Fig. 1A and have been assigned the GenBank accession numbers AF201293 – AF201297. The deduced amino acid sequences are shown in Fig. 1B. None of the sequences display features that would suggest they are pseudogenes. From every monkey, two different alleles could be amplified (Table 1). All five identified *Aona-DQA1* alleles belong to the same lineage, namely *Aona-DQA1*27.*

A

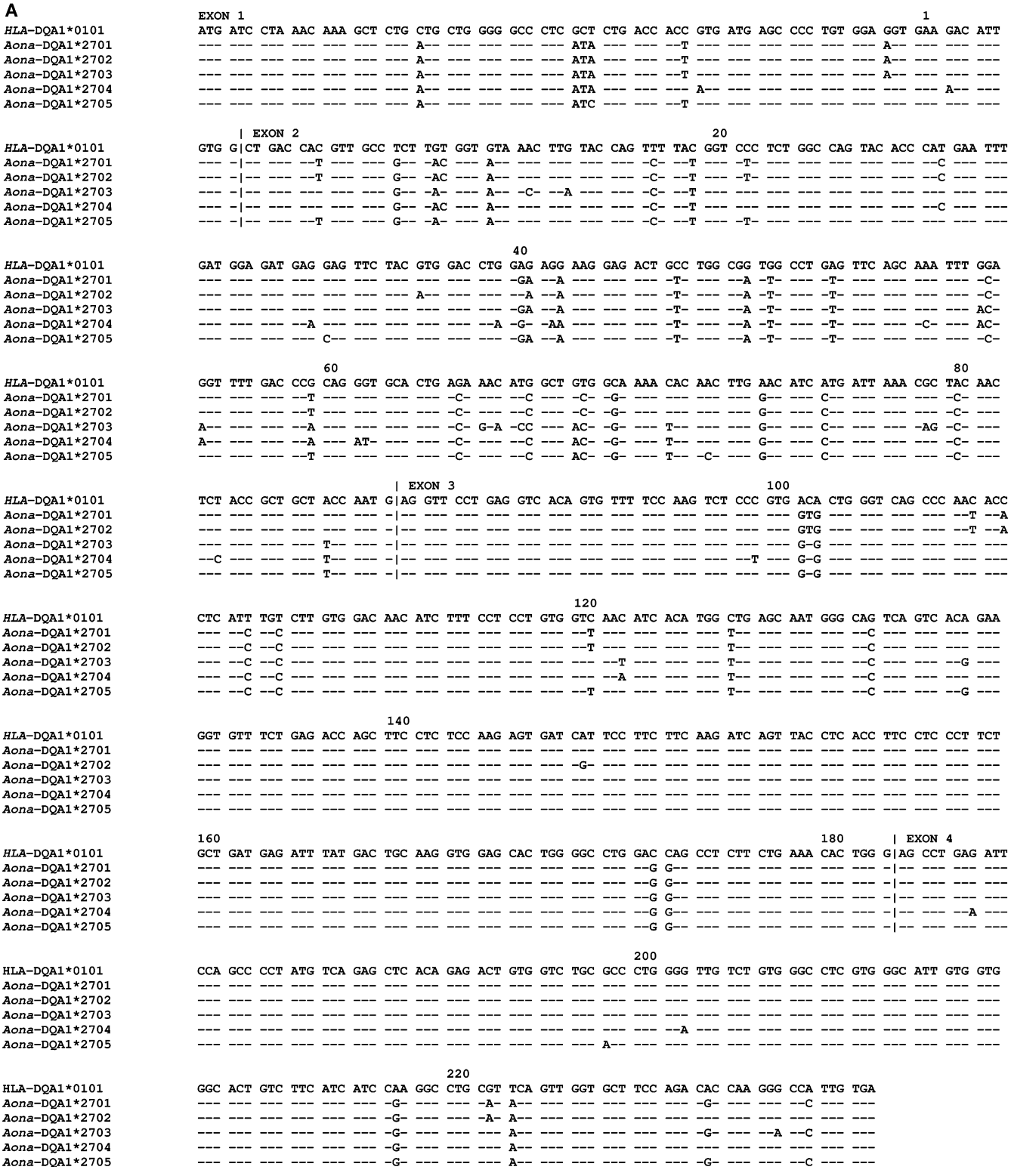

Fig. 1. A Nucleotide sequence of *Aona-DQA1* alleles. In the numbering system used, codon 1 specifies the first amino acid residue of the mature protein. The *top line* represents the sequence of *HLA-DQA1**0101. Identity with the top sequence is indicated by *dashes* (–-). The boundaries of the exons 1–4 are marked. **B** Alignment of *Aona-DQA1* amino acid sequences obtained by the translation of the nucleotide sequences given in **A**. The *top line* gives the amino acid sequence of *HLA-DQA1*0101*. Identity with the top sequence is indicated by *dashes* (–-). A *back-slash* (/) marks gaps inserted to maximize the alignment

B

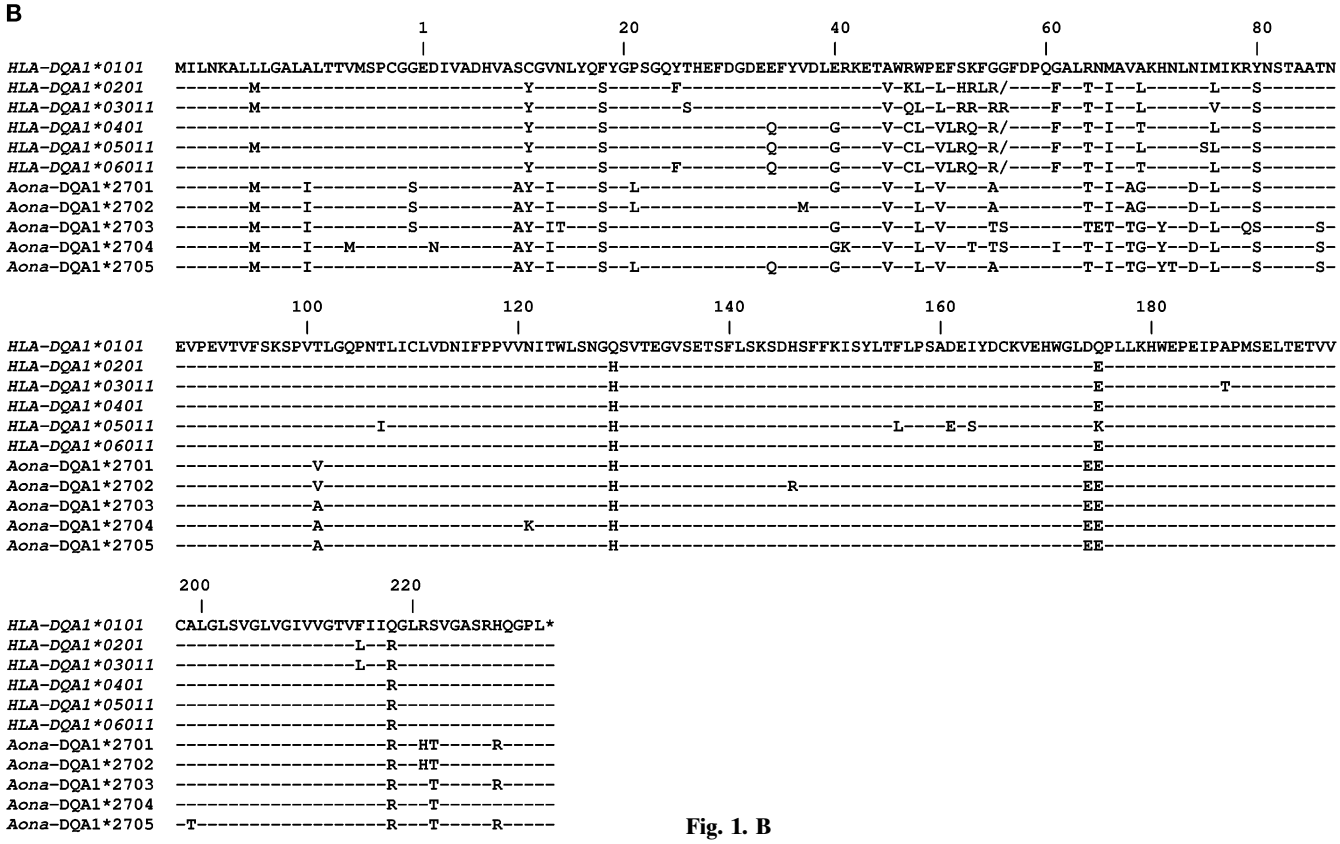

Fig. 1. B

Table 1. Distribution of the *Aona-DQA1* and *-DQB1/2* alleles found in 19 *A. nancymaae* monkeys. Alleles were detected by nucleotide sequence analysis after PCR amplification. *Aona-DQB1*22012*, *Aona-DQB1*2301*, *Aona-DQB1*2302* and *Aona-DQB1*2312* have been amplified from one animal. These sequences are confirmed by two independent PCR reactions with 4–10 independent clones sequenced from each allele. The other *Aona-DQB1/2* alleles were derived from more than one animal and therefore from independent PCR reactions and up to 19 clones were sequenced per allele. *ND* Not determined

Monkey code number	*Aona-DQA1*	*Aona-DQB1*	*Aona-DQB2*
3026	ND	*2308	
7208	ND	*2306	*0101; 0102
8078	ND	*2308	*0101
8105	ND	*2311	
8138	ND	*2305; *2308; *2309; *2310; *2311	
8183	ND	*2310	
8149	ND	*2307; *2308	
8222	ND	*2305	
8230	ND	*2311	
8232	ND	*2308; *2311	
8290	ND	*2307; *2308	*0101
8294	ND	*22011; *2312	
9191	ND	*22012; *2301; *2307, *2308	
9200	ND	*2302; *2304	
9452	ND	*22011	*0102
9472	ND	*22011; *2303; *2308; *2309	
11145a	*2701; *2702	*2306	
11190a	*2701 ; *2703	*2303; *2304; *2308; *2309	
11192a	*2704 ; *2705	*2308	*0102

[a]Alleles identified in these animals were derived from cDNA

A

```
                    10                                  20                                  30
Consensus           CAG TTT AAG GGC ATG TGC TAC TTC ACC AAC GGG ACG GAG CGC GTG CGG GGT GTG ACC AGA TAC ATC TAT AAC CGA GAG GAG
Aona-DQB1*22011      -- G-- --- --- --- --- --- --- -GG --- --- --- --- --- --- --C CT- --- --- C-- -T- --- --- --- --- --- ---
Aona-DQB1*22012      -- G-- --- --- --- --- --- --- -GG --- --- --- --- --- --- --C CT- --- --- C-- -T- --- --- --- --- --- ---

Aona-DQB1*2301       -- --- --- --T C-C --- --- --- --- --- --- --- --- --- --- --- A-- --- -A- CAT --- G-- --- --- --- --- ---
Aona-DQB1*2302       -- --- --- --T C-C --- --- --- --- --- --- --- --- --- --- --- A-- --- -A- CAT --- G-- --- --- --- --- ---
Aona-DQB1*2303       -- --- --- --T C-C --- --- --- --- --- --- --- --- --- --- --T CT- --- --- -A- --- G-- --- --- --- --- ---
Aona-DQB1*2304       -- --- --- --T C-C --- --- --- --- --- --- --- --- --- --- --T CA- --- --- -A- --- G-- --- --- --- --- ---
Aona-DQB1*2305       -- --- --- --T C-C --- --- --- --- --T --- --- --- --- --- --T CT- --- --A GA- C-- G-- --- --- --- --- ---
Aona-DQB1*2306       -- --- --- --T C-C --- --- --- --- --- --- --A --- --- --- --T CT- --- --A GA- C-- G-- --- --- --- --- ---
Aona-DQB1*2307       -- --- --- TTT C-C --- --- --- --- --- --- --A --- --- --- --T CT- --- --- -A- --- G-- --- --- --- --- ---
Aona-DQB1*2308       -- --- --- --T C-C --- --- --- --- --- --- --A --- --- --- --T CA- --- --- -A- --- G-- --- --- --- --- ---
Aona-DQB1*2309       -- --- --- --T C-C --- --- --- --- --- --- --A --- --- --- --T CA- --- --- -A- --- G-- --- --- --- --- ---
Aona-DQB1*2310       -- --- --- TTT C-C --- --- --- --- --- --- --A --- --- --- --T CT- --- --- -A- --- G-- --- --- --- --- ---
Aona-DQB1*2311       -- --- --- TTT C-C --- --- --- --- --- --- --A --- --- --- --T CT- --- --- -A- --- G-- --- --- --- --- ---
Aona-DQB1*2312       -- --- --- AAT T-C --- --- --- --- --- --- --A --- --- --- --T CA- T-- -A- -A- --- G-- --- --- --- --- ---

Aona-DQB2*0101       -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- G-- --- --- --- --- --- --C --- ---
Aona-DQB2*0102       -- --- --- --- --- --- --- A-- --- --- --- --- --- --- --- --- --- --- --- G-- --- --- --- --- --- --C --- ---


                            40                                  50                                  60
Consensus           TAC GTG CGC TTC GAC AGC GAC GTG GGG GAG TAC CGG GCG GTG ACG CCG CTG GGG CGG CCT GTC GCC GAG TAC TGG AAC AGC
Aona-DQB1*22011     --- C-- --- --- --- --- --- --- --- --- --- -T- C-C --- --- --- --- --- -C- --C -A- --- --- --- -T- --- G--
Aona-DQB1*22012     --- C-- --- --- --- --- --- --- --- --- --- -T- C-C --- --- --- --- --- -C- --C -A- --- --- --- -T- --- G--

Aona-DQB1*2301      --- --- --- --- --- --- --- C-- --- --- --T --- --- --- --- --- --- --- --- --- -A- --- --- --- -AC --- ---
Aona-DQB1*2302      -T- --- --- --- --- --- --- --- --- --- C-T --- --- --- --- --- --- --- --- --- -A- --- --- --- --- --- --
Aona-DQB1*2303      --- --- --- --- --- --- --- C-- --- --- --T --- --- --- --- --- --- --- --- --- -A- --- --- C-- -AC --- ---
Aona-DQB1*2304      -T- A-- --T --- --- --- --- --- --- --- --T --- --- --- --- --- --- --- --- --- CA- --- --- --- -TC --- G--
Aona-DQB1*2305      -T- A-- --T --- --- --- --- --- --- --- --T --- --- --- --- --- --- --- --- -A- A-T --- --- --- -TC --- G--
Aona-DQB1*2306      --- --- --- --- --- --- --- --- --- --- -T- T-- --- --- --- --- --T --- --- -A- AC- --- --- --- --- --- ---
Aona-DQB1*2307      --- --- --- --- --- --- --- C-- --- --- --T --- --- --- --- --- --- --- --- --- -A- --- --- --- -TC --- G--
Aona-DQB1*2308      --- --- --- --- --- --- --- C-- --- --- --T --- --- --- --- --- --- --- --- --- -A- --- --- --- -TC --- G--
Aona-DQB1*2309      --- --- --- --- --- --- --- C-- --- --- --T --- --- --- --- --- --- --- --- --- -A- --- --- --- -TC --- G--
Aona-DQB1*2310      -T- --- --- --- --- --- --- C-- --- --- --T --- --- --- --- --- --- --- --- --- -A- --- --- --- --- --- ---
Aona-DQB1*2311      --- --- --- --- --- --- --- C-- --- --- --T --- --- --- --- --- --- --- --- -C- --- -A- --- --- --- --- --- ---
Aona-DQB1*2312      -T- --- A-- --- --- --- --- --- C-- --- --T --- --- --- --- --- --- --- --- --- TA- --- --- --- --- --- ---

Aona-DQB2*0101      --T -C- --- --- --- --- --- --- --- --- --- -T- --- --- --- --C GA- --- --- --- /// AG- A-- --- G-- --- --- -A-
Aona-DQB2*0102      --- -C- --- --- --- --- --- --- --- --- --- -T- --- --- --- --C GA- --- --- --- /// AG- A-- --- G-- --- --- -A-


                                    70                                  80                                  90
Consensus           CAG AAG GAC GTC CTG GAG AGG ACC CGG GCG GAG TGG GAC ACG GTG TGC AGA CAC AAC TAC GAG GTG GCG TAC CGC GGG ATC
Aona-DQB1*22011     --- --- --- T-- --- --- --- --- --- --- --- CT- --- --- --- --- --G --- --- --- C-- C-- -A- -T- -C- -C- ---
Aona-DQB1*22012     --- --- --- T-- --- --- --- --- --- --- --- CT- --- --- --- --- --- --- --- --- C-- C-- -A- -T- -C- -C- ---

Aona-DQB1*2301      --- --- --- A-- --- --- --C --- --- --- --- ACA --- --A --- --- --- --- --- --- C-- T-- -A- CT- -T- AC- -C-
Aona-DQB1*2302      --- /// --- A-- --- --- --- GT- --- --- --- ATA --- --- --- --- --- --- --- --- C-- C-- -A- CT- -T- AC- -C-
Aona-DQB1*2303      --- --- --- A-- --- --- --C --- --- --- --- CT- --- --- --- --- --- --- --- --- C-- T-- -A- CT- -T- AC- -C-
Aona-DQB1*2304      --- --- --- A-- --- --- --- --- --- --- --- ATA --- --- --- --- --- --- --- --- C-- -G- -A- CT- -T- AC- -C-
Aona-DQB1*2305      --- --- --- A-- --- --- --C --- --- --- -C- GT- --- --- --- --- --- --- --- --- C-- C-- -A- CT- -T- AC- -C-
Aona-DQB1*2306      --- --- --- A-- --- --- --- GT- --- --- --- ATA --- --- --- --- --- --- --- --- C-- T-- -A- CT- -T- AC- -C-
Aona-DQB1*2307      --- --- --- A-- --- --- --C --- --- --- --- CT- --- --- --- --- --- --- --- --- C-- C-- -A- CT- -C- AC- -C-
Aona-DQB1*2308      --- --- --- A-- --- --- --C --- --- --- --- CT- --- --- --- --- --- --- --- --- C-- C-- -A- CT- -T- AC- -C-
Aona-DQB1*2309      --- --- --- A-- --- --- --C --- --- --- --- CT- --- --- --- --- --- --- --- --- C-- C-- -A- CT- -C- AC- -C-
Aona-DQB1*2310      --- --- --- A-- --- --- --C --- --- --- --- CT- --- --- --- --- --- --- --- --- C-- C-- -A- CT- -T- AC- -C-
Aona-DQB1*2311      --- /// --- A-- --- --- --- --- --- --- --- CT- --- --- --- --- --- --- --- --- C-- C-- -A- CT- -T- AC- -C-
Aona-DQB1*2312      --- --- --- A-- --- --- --- GT- --- --- -T- GT- --- --- --- --- --- --- --- --- C-- -C- -A- CT- -T- AC- -C-

Aona-DQB2*0101      T-T --- --- T-- T-- --- CA- CAG --- --C -C- GT- T-- --- --- --- --- --- --- --- --- -C- -A- CT- --- AC- -C-
Aona-DQB2*0102      T-T --- --- T-- T-- --- CA- CAG --- --C -C- GT- T-- --- --- --- --- --- --- --- --- -C- -A- CT- --- AC- -C-


Consensus           CTG CAG A
Aona-DQB1*22011     TC- --- C
Aona-DQB1*22012     TC- --- C

Aona-DQB1*2301      T-- --- C
Aona-DQB1*2302      T-- --- C
Aona-DQB1*2303      T-- --- C
Aona-DQB1*2304      T-- --- C
Aona-DQB1*2305      T-- --- C
Aona-DQB1*2306      T-- --- C
Aona-DQB1*2307      T-- --- C
Aona-DQB1*2308      T-- --- C
Aona-DQB1*2309      T-- --- C
Aona-DQB1*2310      T-- --- C
Aona-DQB1*2311      T-- --- C
Aona-DQB1*2312      T-- --- C

Aona-DQB2*0101      T-- --- C
Aona-DQB2*102       T-- --- C
```

Fig. 2. A Nucleotide sequence of *Aona-DQB1/2* alleles. In the numbering system used, codon 1 specifies the first amino acid residue of the mature protein. The *top line* represents the consensus sequence of primate *MHC DQB* genes. Identity with the top sequence is indicated by *dashes* (–-). A *back-slash* (/) marks gaps inserted to maximize the alignment. **B** Alignment of *Aona-DQB*1/2 amino acid sequences obtained by the translation of the nucleotide sequences given in **A**. The *top line* gives the consensus amino acid sequence of primate *MHC-DQB* alleles. Identity with the top sequence is indicated by *dashes* (–-). A *back-slash* (/) marks gaps inserted to maximize the alignment. The amino acid positions contributing to pockets 4 and 9 are shaded in *gray*

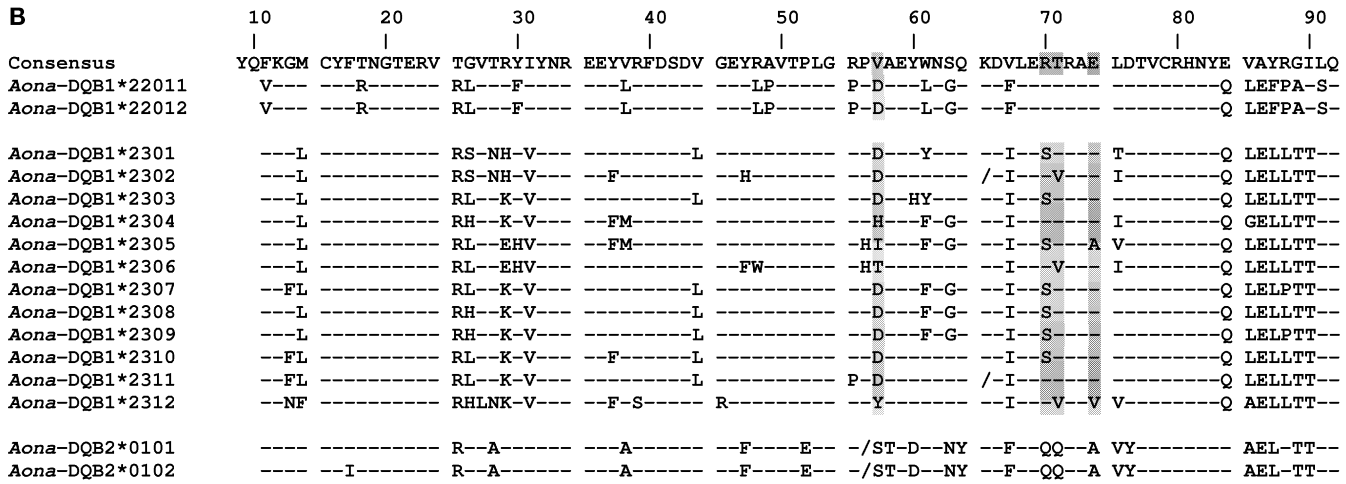

Fig. 2. B

Monkey 11190 shared the *Aona-DQA1*2701* allele with animal 11145 (Table 1). For comparison, several human alleles are also depicted in Fig. 1B. Amino acid polymorphism observed in *Aotus* can in most cases also be found in *HLA-DQA1* alleles. *DQA2*-like genes were not identified.

Polymorphism of Aona-DQB1 alleles

The second exon of *Aona-DQB* genes was amplified from cDNA or genomic DNA of 19 monkeys. A total of 122 sequences were sequenced which identified 14 *Aona-DQB1* alleles. These alleles belong to two lineages, namely *Aona-DQB1*22* and *Aona-DQB1*23* (Fig. 2A). The deduced amino acid sequences are given in Fig. 2B. Five of these alleles were amplified from both genomic DNA and cDNA, namely *Aona-DQB1*2303*, *Aona-DQB1*2304*, *Aona-DQB1*2306*, *Aona-DQB1*2308* and *Aona-DQB1*2309*.

A phylogenetic tree constructed for the exon 2 sequences by the neighbor-joining method (Saitou and Nei 1987) shows that the majority of *Aotus* alleles are closely related to each other. The *Aona-DQB1*22011* allele forms a clade with the *Caja-DQB1*2201* allele. Sequences of representative *HLA-DQB1* and *Caja-DQB1* alleles are located on different branches than the *Aona-DQB1*23* alleles (Fig. 3).

Presence of Aona-DQB2 sequences

The evolutionary equivalents of *HLA-DQB2* alleles were detected in *Aotus* (Fig. 2A, B). The two alleles *Aona-DQB2*0101* and *Aona-DQB2*0102* showed 96% and 94% identity to *Caja-DQB2* (AF004746) and 91% and 92% identity to *HLA-DQB2* (X87344), respectively.

Amino acids contributing to Aona-DQB1 peptide binding pockets

Using the *HLA-DR* structure as a model for class II peptide binding, 15 amino acid positions in *DQB1* were classified as participating in pockets: 11, 13, 28, 47, 57, 61, 67, 70, 71, 74, 78, 85, 86, 89 and 90 (Brown et al. 1993; Stern et al. 1994). The variability at these positions was assessed from the identified *DQB1* alleles. As demonstrated in the variability plot of Fig. 4, the vast majority of amino acid positions participating in the peptide binding region are polymorphic. Position 57 of the *DQ* β chain is particularly interesting in humans because it is associated with autoimmune diabetes (Todd et al. 1987). In different *HLA-DQB1* alleles, this position is occupied either by Asp or non-Asp (Ala, Val or Ser) residues. In *Aona-DQB1* sequences, position β57 is occupied predominantly by Asp and alternatively by Ile, Thr, Tyr and Ser.

Discussion

T cells recognize complexes of MHC class II molecules and bound peptide. The understanding of the specificity of peptide-MHC class II interactions was greatly facilitated by the elucidation of the three-dimensional structure of MHC class II molecules (Madden 1995). The crystal structures of *HLA-DR1* suggest that the general principles of peptide binding to class II proteins are very similar for different species and different class II molecules (Brown et al. 1993; Stern et al. 1994). Indeed, residues forming hydrogen bonds to the peptide backbone in *HLA-DR1* are conserved in all class II proteins, implying similar polyproline type II helical conformation, N-to-C terminal orientation, and spacing of anchor residues in the bound peptides (Stern et al. 1994). The *HLA-DR1* structure revealed pockets in the peptide binding grooves accommodating several anchor residues of a peptide.

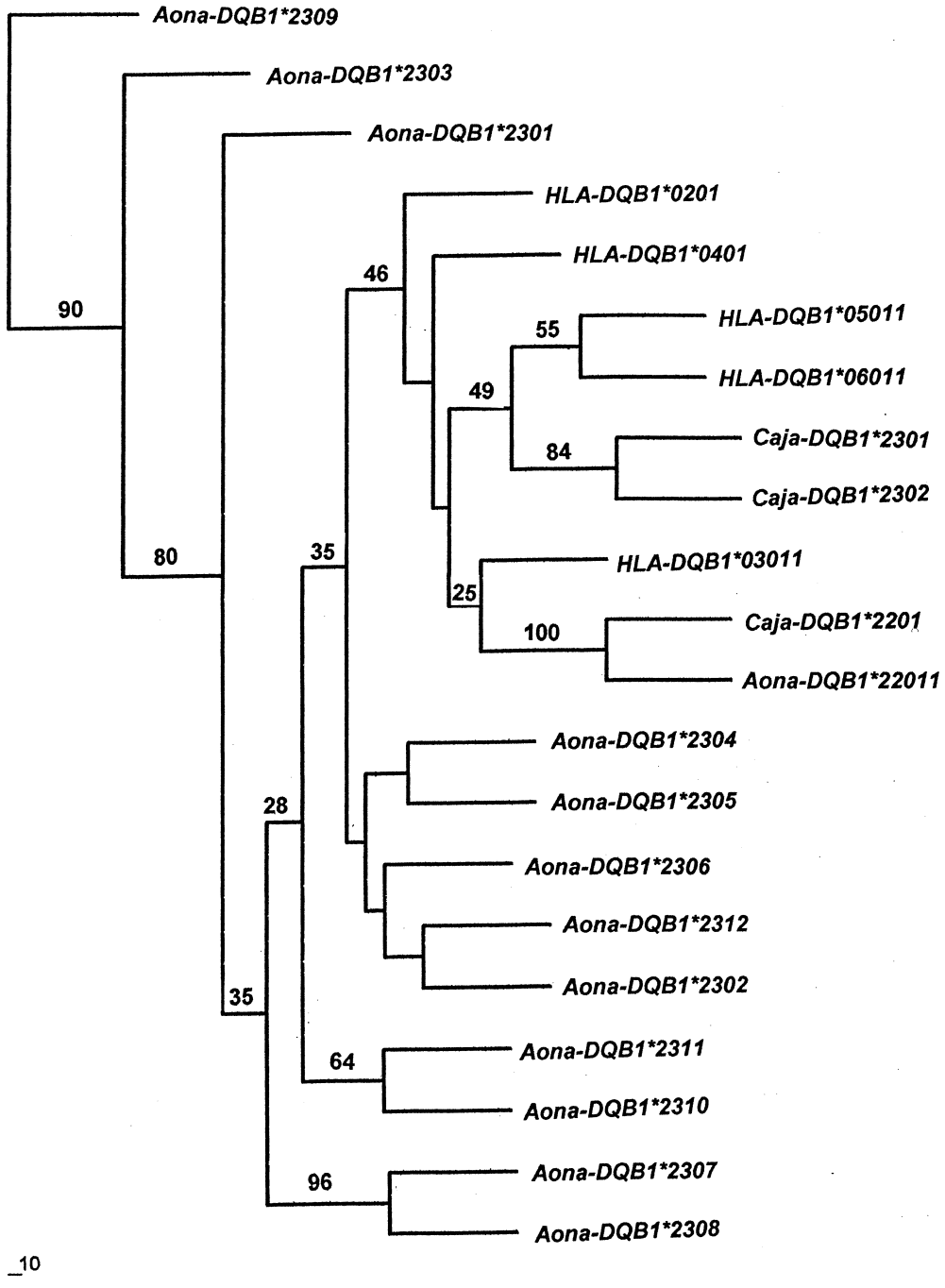

Fig. 3. Phylogenetic tree constructed according to the neighbor-joining method (Saitou and Nei 1987). The relationship between the newly described *Aona-DQB1* alleles and selected alleles of *Callithrix jacchus* and human is shown. Bootstrap values are indicated at the *branches*. The sequences included: *HLA-DQB1*0201* (L40179), *HLA-DQB1*03011* (L34096), *HLA-DQB1*0401* (L34099), *HLA-DQB1*05011* (L34101), *HLA-DQB1*06011* (L34104), *Caja-DQB1*2301* (AF004744), *Caja-DQB1*2302* (AF004745) and *Caja-DQB1*2201* (AF004743)

The specificity of the peptide-binding pockets is influenced by polymorphic amino acid residues of the MHC molecules resulting in allele-specific class II binding motifs.

In the present study, we analyzed the nucleotide sequences of the *DQA1* and *DQB1*/*DQB2* genes of *A. nancymaae.* The *Aona-DQA1* alleles sequenced have been characterized as cDNAs. Five alleles, all belonging to the same lineage of *DQA1*, namely *Aona-DQA1*27*, were identified after sequencing of several independent clones. This lineage has been assigned according to the second exon sequences and it has not been described in other Catarrhini and Platyrrhini so far (Bontrop et al. 1999). Because of the limited numbers of animals analyzed, we cannot draw conclusions on how many other *DQA* lineages might be present in the *A. nancymaae* population. Polymorphic amino acid residues in the *Aona-DQA1* alleles are also present in *HLA-DQA1* alleles (Fig. 1B). Most variation seen within the *Aona-DQA1*27* lineage can currently be explained by point mutations, whereas intra-allelic exchanges of motifs were not evident.

Functional expression of *DQB2* instead of *DQB1* has been described in a cell line of *A. trivirgatus* (Gaur et al. 1992). However, using a different set of primers from those used by Gaur and collegues

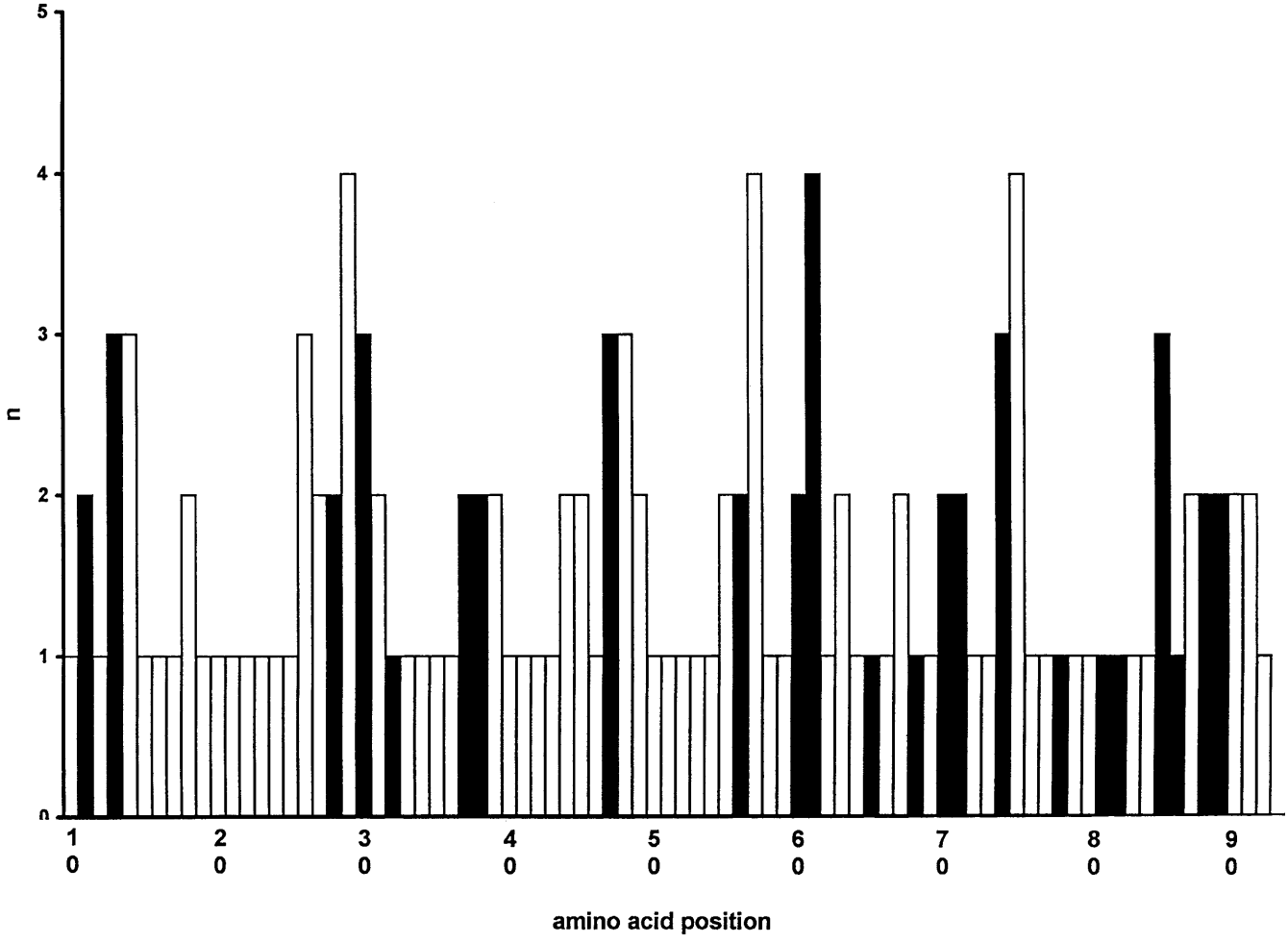

Fig. 4. Amino acid variability plot (Wu and Kabat 1970) for derived amino acid sequences of *Aona-DQB1* exon 2. *Filled* and *empty bars* indicate the amino acid residues involved in the peptide binding region (PBR) and non-PBR, respectively (Brown et al. 1993). *Numbers* along the *X axis* denote amino acid position based on a complete exon 2 sequence and "*n*" at the *Y axis* denotes the number of different amino acids found at the corresponding positions

(1992), we could amplify *DQB1* alleles from both genomic DNA and cDNA. The number of different amino acids occurring at the same position in the identified *DQB1* alleles is highest at positions that are likely to be part of the peptide binding region (PBR) of Aona-DQB1 molecules (Fig. 4) (Brown et al. 1993). Hence, positive selection appears to promote variability at the PBR sites. This feature is characteristic for genes that encode proteins with antigen-presenting function and indicates that *DQB1* alleles are functional in *A. nancymaae* (Bergstrom and Gyllensten 1995). Variation seen within *Aona-DQB1* lineages could be explained by point mutations, whereas intra-allelic exchanges of motifs seem to play a marginal role. This is in contrast to our findings with *Aona-DRB* alleles where intra-allelic exchange of motifs is common (Nino-Vasquez et al. 2000). Family data will be required to assign genes to haplotypes.

One monkey (animal 8138) was investigated in greater detail than the others. Sequence analysis of a great number of cloned PCR products from genomic DNA of this monkey showed that the *Aona-DQB1*2305*, *Aona-DQB1*2308*, *Aona-DQB1*2309*, *Aona-DQB1*2310,* and *Aona-DQB1*2311* alleles could be identified. Four different *Aona-DQB1* alleles could be identified in the monkeys 9191, 9472 and 11190. These results suggest that more than two *Aona-DQB1*23* alleles are present at the genomic DNA and cDNA level. Duplication events like at the *Aona-DRB* locus may thus also have occurred at the *Aona-DQB1* locus. A detailed contig mapping study would be needed to confirm how many *Aona-DRB* and *Aona-DQB* loci are present in the *Aotus MHC*.

In *Aotus*, as well as in the cotton-top tamarin and common marmoset, the evolutionary equivalents of *HLA-DQB2* were detected (Bontrop et al. 1999). This locus is regarded as a pseudogene locus in humans. The high homology of the *Aona-DQB2*0102* and *Aona-DQB2*0202* to *HLA-DQB2* sequences underlines the old age of this locus and may reflect the lack of positive selection also in *Aotus*.

While peptide-binding motifs have been elucidated for many *HLA-DR* alleles, the peptide binding specificity of human *HLA-DQ* is still controversial (Reizis et al. 1998). Based on the crystallographic structure of murine I-A, the equivalent of *HLA-DQ*, four essential pockets named P1, P4, P6 and P9 could interact with the amino acid side chains of bound peptides (Fremont et al. 1998). Pocket P4 is described as one major determinant of peptide binding (Fu et al. 1995). It could be shown that, in particular, the polymorphic residues at positions β70, β71 and β74 contributing to pocket P4 strongly influence the peptide binding specificity (Stern et al. 1994; Ou et al. 1998). Peptides carrying a negative charge at relative position 4 favorably bind to class II molecules with a positively charged pocket P4, but not to class II molecules with a negatively charged pocket P4 (Djoulah et al. 1999). The positions β70, β71 and β74 are occupied in humans by motifs RKA (positive), RTE (neutral), EDS (negative), GTE (negative) and RTA (positive), with the resulting overall charges given in brackets. *Aona-DQB1* alleles carry the motifs RTE (neutral), STE (negative), STA (neutral), RVE (neutral) and RVV (positive). The motif RTE is present in humans and *Aotus*. Hence, both species have P4 pockets which are charged (positive and negative) or neutral.

The Asp/non-Asp dimorphism found at position β57 of *HLA-DQB1* alleles contributes to the peptide binding specificity to HLA-DQ molecules as demonstrated by peptide binding studies (Nepom et al. 1996). According to protein crystal structure analysis, residue β57 contributes to the ninth peptide binding pocket and forms an ion pair with the Arg present at position 79 on the $DQ\alpha$ chain (Brown et al. 1993; Stern et al. 1994). The amino acid positions β57 and α79 are also occupied by Asp and Arg, respectively, in the *Aotus* monkeys. Therefore, similar rules might govern the binding of certain amino acid side chains in pocket 9 in *Aotus* and humans.

In humans, the combination of *DQA1* and *DQB1* alleles is highly restricted by haplotype and there is evidence for structural restriction on the α and β chains forming functional heterodimers (Bergstrom and Gyllensten 1995). This phenomenon could indicate that the narrow repertoire of *DQA1* lineages in *Aotus* is imposed by the limited repertoire of *DQB1* alleles or vice versa.

The *Saoe-DQA1* and *Saoe-DQB1* loci of the cottontop tamarin appear to be essentially monomorphic (Gyllensten et al. 1994). Furthermore, in the common marmoset the diversity of *DQA* and *DQB* alleles is also very limited (Antunes et al. 1998). The results of the analysis of MHC *DQB1* sequences in a randomly selected population of monkeys indicates that *A. nancymaae* is the first example of a New World monkey species displaying a polymorphic *Aona-DQB1* repertoire.

In summary, we present sequences of five *Aona-DQA*1 alleles present in three randomly selected animals. Fourteen alleles of *Aona-DQB1* and two alleles of *Aona-DQB2* could be identified in 19 animals. Taken together, the *DQA1* and *DQB1* polymorphism in the New World monkey *A. nancymaae* seems to be more limited than in Old World Simians (Bergstrom and Gyllensten 1995), but functionally important residues for peptide binding are conserved between *Aotus* and humans.

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