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Restricted MHC class I A locus diversity in olive and hybrid olive/yellow baboons from the Southwest National Primate Research Center

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Abstract

Baboons are valuable models for complex human diseases due to their genetic and physiologic similarities to humans. Deep sequencing methods to characterize full-length major histocompatibility complex (MHC) class I (MHC-I) alleles in different nonhuman primate populations were used to identify novel MHC-I alleles in baboons. We combined data from Illumina MiSeq sequencing and Roche/454 sequencing to characterize novel full-length MHC-I transcripts in a cohort of olive and hybrid olive/yellow baboons from the Southwest National Primate Research Center (SNPRC). We characterized 57 novel full-length alleles from 24 baboons and found limited genetic diversity at the MHC-I A locus, with significant sharing of two MHC-I A lineages between 22 out of the 24 animals characterized. These shared alleles provide the basis for development of tools such as MHC:peptide tetramers for studying cellular immune responses in this important animal model.

Keywords

Major histocompatibility complex I; *Papio anubis*; *Papio anubis cynocephalus*; *Papio hamadryas*; Roche/454 pyrosequencing; Illumina MiSeq sequencing; baboon

Introduction

Baboons are important animal models because of their physiologic and genetic similarities to humans (Harding 2013, 2017). Developmental, reproductive, and metabolic processes of baboons mirror those in humans (Comuzzie et al. 2003; Cox et al. 2013; Havel et al. 2017). These similarities make baboons a useful model for studying complex human diseases including hypertension, obesity, and diabetes as well as infectious diseases such as pertussis

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(Cox et al. 2013; Comuzzie et al. 2003; Pinto and Merkel 2017; Warfel and Merkel 2014; Mahaney et al. 2017). The genetic similarities allow the use of gene arrays and DNA methylation arrays developed for human samples on baboons for high throughput studies (Cox et al. 2013). In addition, these animal's tissues and organs are similar in size and function to their human counterparts, making baboons particularly attractive for preclinical organ transplantation research (Cox et al. 2013) (Bauer et al. 2010) (Michel et al. 2015). Beyond the biological similarities between baboons and humans, there are several specialized resources available for baboons, including a pedigreed breeding colony at Southwest National Primate Research Center (SNPRC) and the first nonhuman primate whole-genome linkage map (Cox et al. 2013). Consequently, there are many historical studies using baboons to study complex human disease to use as the basis for new studies.

Despite the genetic characterization of baboons, little is known about sequence variants in classical major histocompatibility complex class I (MHC-I) genes. Based on sequencing MHC-I variants in many other African and Asian nonhuman primate populations, we predict baboons have multiple polymorphic MHC-I A and B genes. Defining the exact sequences of these closely related MHC-I allelic variants is challenging. For some nonhuman primate populations, such as Asian origin rhesus and cynomolgus macaques, thousands of allelic variants have been defined. Comparatively little attention has been paid to defining allelic variants in African nonhuman primates. 144 and 131 MHC-I alleles have been sequenced from sooty mangabeys and African green monkeys, respectively. Baboons, in contrast, had only 15 characterized full-length MHC-I transcripts in July 2015, when we added the 57 full-length MHC-I alleles characterized here to the Immuno Polymorphism Database (IPD) (Robinson et al. 2015). Recently, an additional ~90 new olive baboon full-length MHC-I A or B alleles have been added to IPD (van der Wiel et al. 2018).

In this study, we describe the use of multiple sequencing techniques to characterize 57 full-length MHC-I transcripts from 15 olive baboons, 8 olive/yellow baboon hybrids, and 1 Ethiopian sacred (hamadryas) baboon from the SNPRC. Samples were amplified with primers previously developed by our laboratory that anneal to the UTR or within the MHC-I gene transcript that are conserved not only between MHC-I alleles, but also between nonhuman primate species. These primers had been previously used to provide full-length MHC-I genotyping data for several nonhuman primate species from a variety of origins including Indian rhesus macaques, Chinese rhesus macaques, pig-tailed macaques, Mauritian cynomolgus macaques, Indonesian cynomolgus macaques, Filipino cynomolgus macaques, and sooty mangabeys (Wiseman et al. 2013, 2009; Karl et al. 2013, 2008, 2014; O'Leary et al. 2009; Campbell et al. 2009; Budde et al. 2010; Pendley et al. 2008; Heimbruch et al. 2015; Dudley et al. 2014). Given the high genetic similarity between macaques, sooty mangabeys and baboons, we expected that the primers we have previously used to amplify the full-length MHC-I transcript in these animals would amplify baboon MHC-I transcripts (Heimbruch et al. 2015; Dudley et al. 2014; Rogers and Gibbs 2014). Using these primers, our findings were in agreement with previous baboon studies and similar to other well-characterized nonhuman primates, in that individual baboons possessed more than two MHC class I A (MHC-I A) and class I B (MHC-I B) transcripts, while lacking transcripts homologous to HLA-C (Sidebottom et al. 2001; Prilliman et al. 1996). Overall, this study significantly increases the knowledge of MHC-I transcripts expressed in

baboons substantially improving the baboon as a model for both transplantation research and studies of complex human diseases.

Materials and methods

Animals

Peripheral blood mononuclear cells (PBMC) were obtained from fifteen captive olive baboons (*Papio anubis* (PCA)), eight hybrid olive/yellow baboons (*Papio anubis cynocephalus* (PCX)), and one Ethiopian sacred baboon (*Papio hamadryas* (HAN)) from the Southwest National Primate Research Center (Table 1). The baboon population at SNPRC was initiated in the 1960s with a founder population of 384 animals (Cox et al. 2013). The animals used in this experiment were bred in captivity. The pedigree of these animals was provided by the SNPRC. Two familial trio sets consisting of dam, sire and offspring were included within the 24 animals, while none of the other animals share a dam or sire (Table 1). The Texas Biomedical Research Institute Animal Care and Use Committee approved all research involving SNPRC baboons.

cDNA synthesis and PCR amplification

RNA was isolated from frozen PBMCs using the Roche MagNA Pure instrument and RNA high-performance kit (Roche, Indianapolis, IN, USA) following manufacturer's protocols, as adapted for use with a Tecan Liquid Handler. RNA from each animal was normalized to 10 ng/ul and cDNA was synthesized using the Superscript III cDNA first-strand synthesis kit with oligo (dT) primers (Invitrogen, Carlsbad, CA, USA). cDNA was used as a template to generate four different amplicons from each MHC-I transcript ranging in size from 195 bp to 1.2 kb (full-length transcript). A schematic representation of these amplicons relative to the MHC-I gene is shown in Figure 1. The primer sequences, the length, and the sequencing method used to sequence each amplicon are shown in Table 2. The FL-UTR alt forward primer was designed to amplify MHC-I A alleles more robustly by incorporating nucleotide changes specific to MHC-I A alleles based on cDNA and genomic sequence data that was available in GenBank for macaques at the time the primer was designed. This primer contains a degenerate nucleotide (V), which represents A, C or G, as well as additional nucleotide changes relative to FL-UTR that better match known MHC-I A alleles. The cycling conditions for all amplicons were 98°C for 3 min, 24 cycles 98°C for 5 s, 60°C for 10 s, 72°C for 20 s followed by a 5-min period of final elongation at 72°C. To confirm PCR products, all samples were run on a FlashGel (Lonza Group Ltd., Basel, Switzerland). It should be noted that we combined the FL-UTR forward primer, FL-Leader forward primer and the three full length reverse primers together in a single tube for PCR amplification (Table 2). Also, the FL-UTR-alt forward primer was combined with the same three reverse primers in a single tube (Table 2). All other amplicons with primer sets shown in Table 2 were amplified independently from each other. We used four different primer sets to generate our genotyping amplicon (195 bp product) (Table 2). Each primer set contains the same sequence-specific sequence but introduces a 0-3 nucleotide insertion (small letters in the genotyping amplicon primer sets in Table 2) resulting in amplicons that are slightly different lengths. This insertion is necessary to optimize amplicon sequencing on the Illumina MiSeq. The genotyping primers also contain the CS1 or CS2 universal tag used in

the Fluidigm system for Illumina sequencing. These tags are used to prime the addition of Illumina indices to the PCR product to barcode the sequences from each animal. The genotyping primers shown in Table 2 contain the CS2 tag on the 5' primer sequence, an additional 0-3 nucleotides (lowercase) followed by the sequence-specific primer sequence. The 3' primer sequence contains the CS1 tag, 0-3 added nucleotides (lowercase) followed by the sequence-specific primer sequence.

Roche/454 pyrosequencing (530 bp)

The 530 bp scaffold PCR products with molecular identifier (MID) sequences, added to differentiate samples when multiplexing, were purified twice using Agencourt AMPure XP beads with a bead to sample volume ratio of 1:1 (Beckman Coulter, Brea, CA, USA). The concentration of DNA in each sample was then quantified using the Quant-iT dsDNA HS assay kit and Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). Samples were pooled in equimolar ratios for sequencing. The pools were further prepared for sequencing following the manufacturer's protocol and as described previously (Dudley et al. 2014). Briefly, samples were amplified using emulsion PCR, processed through breaking and enriching, and were then sequenced on the Roche/454 GS Junior using Titanium technology.

Illumina MiSeq sequencing (1.2 kb)

The FL-UTR and FL-Leader PCR products were amplified in a single tube for each animal. The forward full-length primers (MHC-sequence-specific region shown in Table 2) had a 454 adaptor and MID tag on the 5' end to provide a buffer between the start sequence or leader sequence in the transcript and the end of the cDNA strand. Increasing the distance between the start codon or leader sequence and the end of the transcript provides more opportunity for the transposase used to prepare MiSeq libraries to encompass the start codon or leader sequence in cleaved cDNA, providing better sequence coverage of these regions of the transcript. Each sample amplified with the FL-UTR-alt primer set was prepared alone. The products were purified using Agilent AMPure XP (Beckman Coulter, Brea, CA, USA) beads at a bead to sample volume ratio of 1:1. Next, the 1.2 kb full-length MHC-I PCR products were fragmented and simultaneously indexed using Nextera XT tagmentation (Illumina, San Diego, CA, USA). The average length in base pairs of the fragmented libraries was then determined using bioanalysis with a high-sensitivity chip (Agilent Technologies, Santa Clara, CA, USA). Libraries for each sample were purified again using Agilent AMPure XP beads in a bead to sample volume ratio of 1:1. The samples were then quantitated and normalized to generate a 1 nM pool for sequencing. The pool was diluted according to the Illumina MiSeq sample preparation protocol and was run on an Illumina MiSeq using a 600-cycle MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA).

Illumina MiSeq genotyping (195 bp)

Samples were prepared for MHC-I genotyping as described previously (Wiseman et al. 2013). Briefly, ~195 bp PCR amplicons were amplified using high-fidelity Phusion polymerase (New England Biolabs, Ipswich, MA, USA) using primers described in Table 2. PCR cycling conditions and primers were used as described above for all reactions presented here. Briefly, primers binding to a region in exon 2 of the MHC-I gene and containing the universal Fluidigm CS1 and CS2 tags were used to generate a 195 bp amplicon. These

primers were combined with primers containing the complement sequences to the CS1 and CS2 tags attached to the Illumina MiSeq adaptors and indices in the same tube so that the final PCR products contained the adaptors necessary for sequencing and the indices necessary to differentiate sequences from each animal. The amplicons were then purified using Agilent AMPure XP beads at a bead to sample volume ratio of 1.3:1. Samples were pooled at equimolar ratios and diluted for sequencing according to the Illumina MiSeq sample preparation protocol (Illumina, San Diego, CA, USA).

Data Analysis: Roche/454 pyrosequencing

FASTQ data files from our Roche/454 run were imported into Geneious Pro software (v7.1.5) (http://www.geneious.com, (Kearse et al. 2012)). These files were separated by MID tag and trimmed on both the 5' and 3' ends with an error probability limit of 0.01. Sequences longer than 300 bp in length were extracted for each MID tag and de novo assembled using the built-in assembly algorithm with a minimum overlap identity of 100% and 0% maximum mismatches per read. Primers were trimmed with high stringency from the generated contigs with an error probability of 0.0001, a maximum low-quality limit of 0, and a maximum ambiguities threshold of 0. These trimmed consensus sequences were then de novo assembled with a 99% minimum overlap identity and with 1% maximum mismatches per read. The consensus sequences were trimmed with high stringency as described above before being saved to individual folders labeled with the animal names and the primer set used. These consensus sequences act as the scaffolds onto which MiSeq reads were assembled to build full-length novel MHC-I transcripts.

Data Analysis: MiSeq sequencing

Data from the MiSeq and analyzed data from the Roche/454 were assembled as previously described (Dudley et al. 2014). Briefly, FASTQ files were parsed by index number, removed from the Illumina MiSeq and imported into Geneious Pro for analysis. To begin, the forward and reverse reads for each sample were paired together with an expected distance of 600 bp. Next, the 5' and 3' ends of the paired reads were trimmed with a quality of 0.01. Out of the remaining sequences, those with a minimum length of 100 bp and a maximum length of 250 bp were extracted. Primers were then trimmed from the extracted sequences removing the three reverse primers, the forward MID tag, and the leader primer. During primer trimming two mismatches were allowed and the minimum match length was set to five. After primer trimming, sequences over 100 bp in length from the primer sequences were extracted.

Next the MiSeq reads and Roche/454 consensus sequences from a given animal generated as described above were de novo assembled together, creating iteration 1. During this de novo assembly no gaps were allowed, no mismatches were allowed and the minimum overlap was set to 100%. The 530 bp scaffold on its own distinguishes most sequence variants, particularly at the lineage level, since it spans the region of the MHC-I gene with the most variation between alleles. By requiring 100% matching between this scaffold and the MiSeq reads, correct full-length transcripts are built out from the scaffold using the MiSeq sequencing reads. Iteration 1 was then trimmed with an error probability limit of 0.0001 on both the 5' and 3' ends. No low-quality bases or ambiguities were allowed. This consensus sequence for iteration 1 was then used as the scaffold and all the MiSeq reads for a given

animal were de novo assembled again to it using the same parameters, creating iteration 2. This process was repeated until seven iterations per animal were completed and the MHC-I transcripts were full-length (~1.2 kb) and could no longer be extended.

Analysis of SBT195 genotyping

Sequence reads were demultiplexed and the primer and molecular identifer sequences used to generate the genotyping sequences were removed. Quality-based consensus sequences from FASTQ files for each paired-end read were merged. These consensus sequences for each animal were then aligned to a previously created database containing all known baboon and rhesus macaque MHC-I sequences trimmed to the same 195 bp region as our genotyping sequence. The baboon sequences characterized by either the Roche/454 sequencing or by the full-length method incorporating the fragmented full-length MHC-I transcripts identified in our cohort were also included in this database.

Determining novel alleles

MHC-I transcripts from all animals found to be full-length (~1.2 kb) were trimmed from the putative methionine that initiates the open reading frame to the stop codon and de novo assembled with a minimum overlap set to 100% with 0 mismatches allowed. MHC-I transcripts found in more than one animal or in both full-length primer sets within a single animal (FL-UTR and FL-UTR-alt) were searched against a combined database containing all known rhesus macaque and baboon MHC-I alleles. Alleles were then characterized as novel (one or more sequence differences relative to previously identified sequences) or known (exact match to previously identified sequence).

Conservative criteria were used to exclude potentially inaccurate sequence variants. Transcripts only identified in one animal and detected in only one of the two PCR primer sets (for FL-UTR or FL-UTR alt amplicons) were disregarded. Sequences from each animal were sorted by abundance and low-abundance sequences that could result from the chimeric combination of two more abundant sequences were discarded; however, chimeric assemblies of transcripts were rare. MHC-I transcripts without sequence coverage across the entire transcript or with uneven distribution of sequences across the transcript were also disregarded. These stringent criteria almost certainly led to authentic sequences being discarded, systematically underreporting the total number of sequence variants per animal. Since we were primarily interested in surveying for novel MHC-I alleles, we prioritized finding authentic transcripts over identifying every allele in each animal. For this study, MHC-I alleles found in hybrid animals cross-bred between the olive and yellow baboons were given the Paan (*Papio anubis cynocephalus*) designation by the Immuno Polymorphism Database (IPD), which is typically given to the olive baboon population (*Papio anubis*).

Data availability

The sequences from the novel alleles found in this study have been deposited in the NCBI nucleotide database GenBank (www.ncbi.nlm.nih.gov/nuccore/) and with the Immuno Polymorphism database (http://www.ebi.ac.uk/ipd/) (Robinson et al. 2015). GenBank and IPD accession numbers for each sequence are provided within Tables 3 and 4 of this

manuscript. Data that did not generate full-length alleles are available from the corresponding author upon request.

Results and Discussion

Amplification of full-length baboon MHC class I

All twenty-four samples representing 15 olive baboons, 8 olive/yellow baboon hybrids, and 1 Ethiopian sacred (hamadryas) baboon were amplified with the primer sets that we previously established, as well as with an additional primer developed to amplify MHC-I A transcripts more efficiently as described in the materials and methods (FL-UTR alt, Table 2). (Heimbruch et al. 2015; Dudley et al. 2014; Rogers and Gibbs 2014). This data suggests that we can characterize full-length MHC-I transcripts in baboon species using our established primer sets.

Sequencing and assembling of baboon MHC class I alleles

The average number of Roche/454 reads acquired for each animal in this cohort was <1000 reads. Previous work suggests that we should obtain >2,000 reads/animal in order to characterize some of the less transcriptionally abundant alleles (Dudley et al. 2014). Therefore, our scaffolds with low read numbers likely limited the characterization of alleles in this dataset to the most transcriptionally abundant alleles. While this means that less transcriptionally abundant alleles may be missed, the purpose of this work was to survey for new MHC-I alleles in baboons rather than fully characterize each animal. Previous work has shown that transcriptionally abundant alleles may be more important than less transcribed MHC-I alleles for restricting immune responses, at least in some diseases such as SIV (Budde et al. 2011). Since we do not have many trio sets, this dataset is not powered to define haplotypes.

MHC class I allele discovery in baboons

The results from this study significantly expand the number of full-length MHC-I alleles known in baboons. Altogether, we characterized 57 full-length nameable novel alleles from 24 animals (Tables 3 and 4) plus 3 alleles previously published (Prilliman et al. 1996; Sidebottom et al. 2001) (Figure 2). We characterized a total of 11 novel alleles from the MHC-I A loci and 46 novel alleles from the MHC-I B loci. We characterized between 1-3 MHC-I A alleles and 0-7 MHC-I B full-length alleles per animal (Figure 2). This is concordant with a previous study that showed duplication of the MHC-I A and B loci within Old World primates and more diversified alleles at the MHC-I B loci than at the A loci (Sidebottom et al. 2001). No homologue of HLA-C was identified, supporting the previous hypothesis of a differing pathway of class I evolution between Old World monkeys and humans following divergence of a common ancestor (Sidebottom et al. 2001). Interestingly, the single Ethiopian sacred baboon that was characterized shared eight of nine of the MHC-I alleles found with the olive and hybrid olive/yellow animals. These data were collected prior to the advent of accurate, long-read Pacific Biosciences transcript sequencing that our group and others are now using to directly sequence full-length transcripts (Karl et al. 2017; Prall et al. 2017; Semler et al. 2017; Westbrook et al. 2015). Future work will undoubtedly expand the number of known baboon MHC-I sequence variants using such long-read technology

(van der Wiel et al. 2018). However, it is likely that a dual approach using a more accurate sequencing technique will still need to be used in combination with the long-read technology to correct errors in a similar manner to what was used here.

To estimate the number of sequence variants that may have been missed by our scaffold-building approach, which was limited by low Roche/454 sequencing read numbers, we also sequenced a short region of exon 2 using primers that we typically use for genotyping macaques and sooty mangabeys. This genotyping approach, which likely picks up more of the less transcriptionally abundant transcripts due to the depth of sequence coverage (average = 25,736 reads mapped to the database/animal), identified 2-4 (average= 3) MHC-I A alleles and 5-11 (average= 8) MHC-I B alleles per animal (Figure 2). This indicates that on average we missed one MHC-I A allele and two MHC-I B alleles per animal with our full-length approach. This is similar to the number of alleles found within each of the two MHC loci in rhesus macaques, cynomolgus macaques, and sooty mangabeys. Because these short genotyping sequences do not meet the standard for naming by the Immunopolymorphism Database, we used this data exclusively to quantify the underestimation of the full-length sequence data.

Allele diversity in the baboon population

Interestingly, 22 of the 24 animals contained the same two MHC-I A lineage groups (Paan-A*01 and Paan-A*02). These two most common lineages appear to make up a single inferred haplotype (Table 2), which was also found in a recently published paper by van der Wiel et al. in a baboon colony housed in France (van der Wiel et al. 2018). There is not sufficient trio information to define additional haplotypes from the dataset described here nor a sufficient number of animals sequenced. It should be noted that the alleles found in the trio sets are not complete enough to infer full haplotypes as some alleles were likely missed. Furthermore, since some of the alleles shown in Figure 2 were only found with the genotyping amplicon (presumably because they are less abundant), alleles within a single lineage may not be distinguishable. For example, the single nucleotide polymorphism that differentiates Paan-A*01:01 and Paan-A*01:02 lies outside of the genotyping amplicon. Therefore, animals where this allele was only found with the genotyping amplicon can’t be distinguished as having one or the other allele (shown in Figure 2), further making haplotyping difficult from this dataset. Interestingly, these lineages were also found in the few baboon MHC alleles previously characterized and published (Sidebottom et al. 2001; Prilliman et al. 1996).

The baboon colony at SNPRC was established in the late 1950s and early 1960s with a founder population of 384 baboons trapped in Darajani, Kenya. The cohort used for this experiment contains two trio sets and one pair of half-siblings (Table 1) with the remaining animals not sharing a dam or sire. Therefore, these animals do not all share a common immediate ancestor and the relatively large founder population prevents limited diversity due to inbreeding. This supports the hypothesis that some of the limited diversity was created in the wild before the founder population was brought to SNPRC. Between 1963-1979 Amboseli National Park in Kenya underwent a decrease in its yellow baboon population from 2,500 to 123 individuals (Aoyama et al. 2009). The rate of decline was greatest

between 1964-1969. This distinct bottleneck occurred due to an elevation of the soil salinity zone, which impacted food and shelter sources for the baboon population along with other species. The Amboseli National Park is within 62 miles of Darajani, Kenya, where the baboons from the SNPRC cohort were initially collected. The exact years the founder population was collected and whether or not these baboons were impacted by a similar bottleneck as in Amboseli National Park is unknown. This biogeographic event in close proximity and time period to the founding population of the SNPRC presents a possible explanation for a bottleneck leading to the limited diversity seen within the MHC-I A region in this baboon cohort. Additional cohorts will need to be evaluated to ensure that this unusual allele sharing is not due to the colony structure at the SNPRC or the small number of animals characterized in this manuscript. Alternatively, it is possible that despite specifically designing a primer set that should bind better to MHC-I A alleles, some alleles are missing due to primer inconsistencies, although missing alleles does not negate the fact that A*01 and A*02 lineages are found in a high percentage of the small number of animals tested at SNPRC. Finally, the recently published paper by van der Wiel et al. used the same primer sets and were able to sequence a variety of baboon MHC-I A alleles, suggesting that the primer design is sufficient to amplify these transcripts (van der Wiel et al. 2018).

The limited diversity of the MHC-I A region in baboons from SNPRC is similar to that of the well-studied Mauritian cynomolgus macaques. In this population, thought to be founded by only a handful of animals, the three most common haplotypes share two common *Mafa-A1*063* and *Mafa-A4*01* lineages (88% of the total island population) (Lawler et al. 1995; Burwitz et al. 2009). While these *Mafa-A* lineages are very common, the *Mafa-B* alleles in this population show much greater diversity, similar to the baboon population described here. The occurrence of these two lineages in 88% of MCM have made these animals an advantageous model for studying HIV vaccines designed to elicit CD8 T-cell immune responses restricted by these two lineages as well as for transplant research (Burwitz et al. 2017).

Baboons are especially useful for studying the relationship between immunogenetics and chronic disease. For example, comprehensive understanding of the MHC in baboons could lead to more effective prevention strategies and therapies for type-1 diabetes. Activation of CD8 T cells that target and destroy pancreatic β-cells leads to type-1 diabetes and specific MHC-I alleles have been associated with susceptibility/resistance to disease (Szablewski 2014). In addition, individuals with MHC alleles that increase the risk of type-1 diabetes upregulate MHC-I molecules on islets of Langerhans cells where insulin is produced. This heightens sensitization of CD8 T cells to these targets, facilitating their destruction (Sarikonda et al. 2014). An improved understanding of MHC-I polymorphism in baboons could identify analogous MHC-I alleles in baboons that increase susceptibility to type-1 diabetes.

While the limited information obtained thus far about baboons does not suggest the same overall MHC-I limited diversity as Mauritian cynomolgus macaques, knowledge that a few common alleles are shared among many of the pedigreed animals from SNPRC provides important benefits for the use of baboons from this colony in immunogenetic studies. For example, given our data, in almost any SNPRC baboon present in a study one could test the

CD8 T-cell responses restricted by one of the two shared MHC-I A alleles by creating a MHC:peptide tetramer and characterizing an epitope restricted by that allele. By characterizing full-length alleles, tools such as MHC:peptide tetramers and cell lines expressing specific MHC-I molecules can be generated. These sequences may also form the basis for identifying allele-specific monoclonal antibodies that can evaluate transplant chimerism. Such animals could be pivotal for testing preventative interventions. Overall, the 57 full-length alleles characterized in this study provide the foundation to extend infectious disease and transplant immunobiology in baboons.

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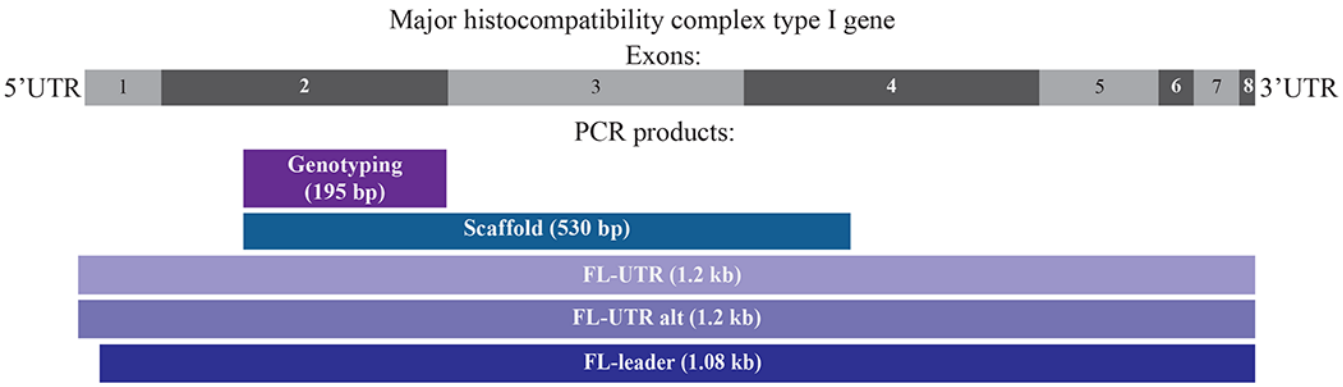
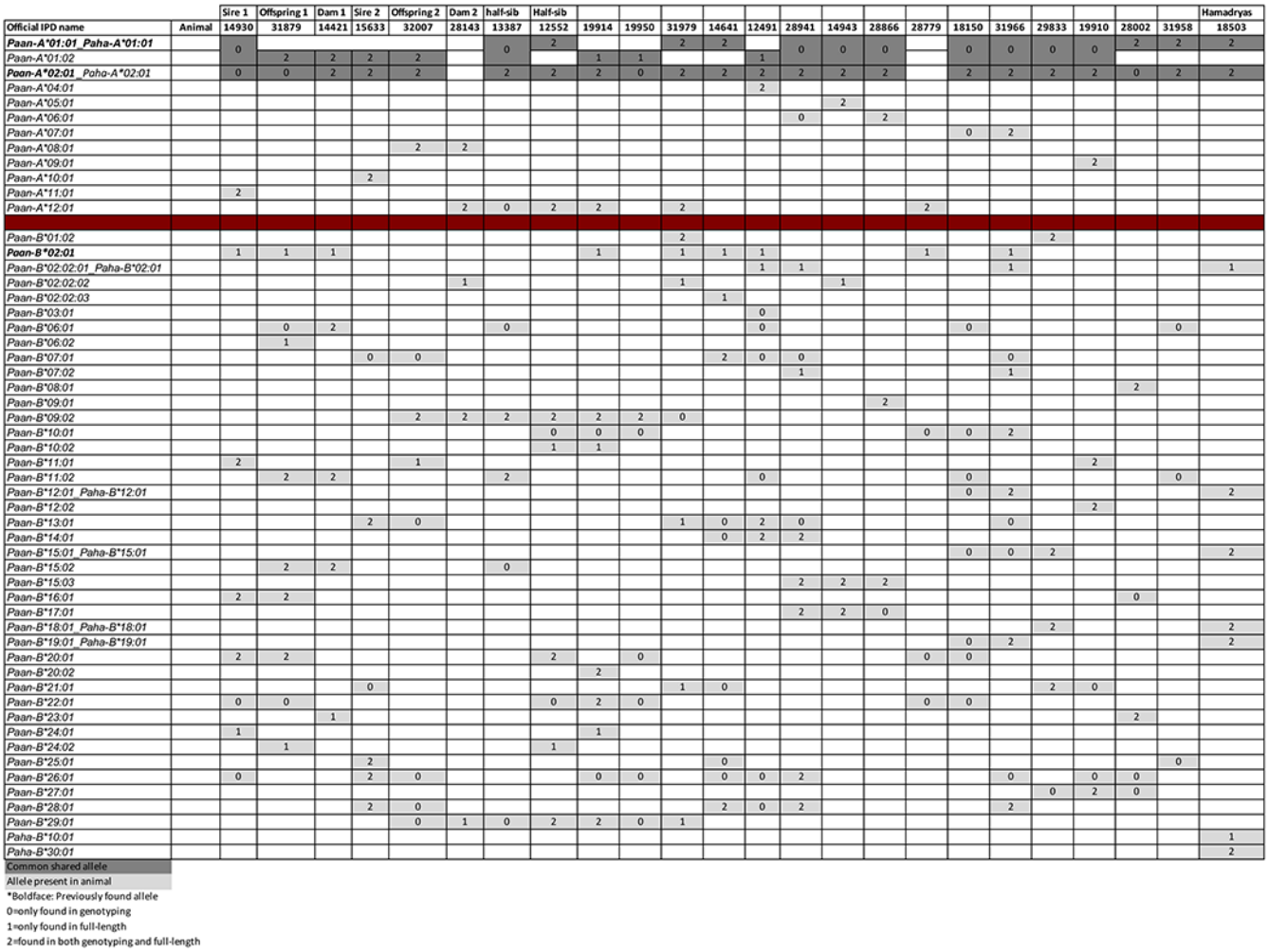


Fig. 1.

The exon structure of the classical MHC class I exon along with the regions amplified using the five different primer sets used in this study. The FL-UTR and FL-UTR alt primers amplify the entire 1.2 kb region of the exon. The FL-leader primer amplifies just downstream of the leader peptide region to the end of the gene (1.08 kb) and the scaffold primer amplifies the 530 bp region in exons 2-4. The genotyping primer amplifies a 195 bp portion of exon 2. This representation is not necessarily drawn to scale.



		Sire 1	Offspring 1	Dam 1	Sire 2	Offspring 2	Dam 2	half-sib	Half-sib																Hamadryas
Official IPD name	Animal	14930	31879	14421	15633	32007	28143	13387	12552	19914	19950	31979	14641	12491	28941	14943	28866	28779	18150	31966	29833	19910	28002	31958	18503
Paan-A*01:01_Paha-A*01:01		0						0	2			2	2		0	0	0		0	0	0	0	2	2	2
*Paan-A*01:02*			2	2	2	2				1	1			1											
Paan-A*02:01_Paha-A*02:01		0	0	2	2	2		2	2	2	0	2	2	2	2	2	2		2	2	2	2	0	2	2
*Paan-A*04:01*														2											
*Paan-A*05:01*																2									
*Paan-A*06:01*															0		2								
*Paan-A*07:01*																			0	2					
*Paan-A*08:01*						2	2																		
*Paan-A*09:01*																						2			
*Paan-A*10:01*					2																				
*Paan-A*11:01*		2																							
*Paan-A*12:01*							2	0	2	2		2						2							
*Paan-B*01:02*												2									2				
Paan-B*02:01		1	1	1						1		1	1	1				1		1					
*Paan-B*02:02:01_Paha-B*02:01*														1	1					1					1
*Paan-B*02:02:02*							1					1				1									
*Paan-B*02:02:03*													1												
*Paan-B*03:01*														0											
*Paan-B*06:01*			0	2				0						0					0					0	
*Paan-B*06:02*			1																						
*Paan-B*07:01*					0	0							2	0	0					0					
*Paan-B*07:02*															1					1					
*Paan-B*08:01*																							2		
*Paan-B*09:01*																	2								
*Paan-B*09:02*						2	2	2	2	2	2	0													
*Paan-B*10:01*									0	0	0							0	0	2					
*Paan-B*10:02*									1	1															
*Paan-B*11:01*		2				1																2			
*Paan-B*11:02*			2	2				2						0					0					0	
*Paan-B*12:01_Paha-B*12:01*																			0	2					2
*Paan-B*12:02*																						2			
*Paan-B*13:01*					2	0						1	0	2	0					0					
*Paan-B*14:01*													0	2	2										
*Paan-B*15:01_Paha-B*15:01*																			0	0	2				2
*Paan-B*15:02*			2	2				0																	
*Paan-B*15:03*															2	2	2								
*Paan-B*16:01*		2	2																				0		
*Paan-B*17:01*															2	2	0								
*Paan-B*18:01_Paha-B*18:01*																					2				2
*Paan-B*19:01_Paha-B*19:01*																			0	2					2
*Paan-B*20:01*		2	2						2		0							0	0						
*Paan-B*20:02*										2															
*Paan-B*21:01*					0							1	0								2	0			
*Paan-B*22:01*		0	0						0	2	0							0	0						
*Paan-B*23:01*				1																			2		
*Paan-B*24:01*		1								1															
*Paan-B*24:02*			1						1																
*Paan-B*25:01*					2								0											0	
*Paan-B*26:01*		0			2	0				0	0		0	0	2					0		0	0		
*Paan-B*27:01*																					0	2	0		
*Paan-B*28:01*					2	0							2	0	2					2					
*Paan-B*29:01*						0	1	0	2	2	0	1													
*Paha-B*10:01*																									1
*Paha-B*30:01*																									2

Common shared allele
Allele present in animal
*Boldface: Previously found allele
0=only found in genotyping
1=only found in full-length
2=found in both genotyping and full-length

Fig. 2.

MHC class I transcripts found in each animal from this study. Transcripts were identified by either full-length sequencing (1.2 kb; designated with 1), by genotyping (195 bp; designated with a 0) or by both methods (designated with a 2). Highly shared lineages are indicated in dark gray. Light gray boxes indicate each allele present in each animal.

Table 1:

Description of the animals used for this study.

Animal ID	Species[a]	Familial Info
14930	PCA	Trio 1-Sire
14421	PCX	Trio 1-Dam
31879	PCX	Trio 1-Offspring
15633	PCA	Trio 2-Sire
28143	PCA	Trio 2-Dam
32007	PCA	Trio 2-Offspring
12552	PCA	Half- Sibling Set
13387	PCX	Half- Sibling Set
12491	PCX	
28002	PCA	
28779	PCA	
28866	PCX	
31958	PCX	
31966	PCX	
31979	PCA	
14641	PCA	
14943	PCX	
18150	PCA	
18503	HAN	
19910	PCA	
19914	PCA	
19950	PCA	
28941	PCA	
29833	PCA	

[a]PCA, olive baboon *Papio anubis*; PCX olive/yellow hybrid baboon (*Papio anubis* x *Papio anubis cynocephalus*); HAN, Ethiopian sacred baboon (*Papio hamadryas*) (Bailey et al. 2014)

Table 2

Primer oligonucleotides used for the various sequencing methods in this study

Amplicon Name	Forward Primer	Reverse Primer	Leng of ampl
FL-UTR[a] **FL-Leader**[a]	5’AGAGTCTCCTCAGACGCCGAG 5’CCCCGAACCCTCCTCCTG	5’CCTCGCAGTCCCACACAAG 5’CCTGCTTCTCAGTTCCACACAAG 5’CTGCATCTCAGTCCCACACAAG	1.2 kb 1.08 k
FL-UTR alt[b]	5’ATTCTCCGCAGACGCCVAG	5’CCTCGCAGTCCCACACAAG 5’CCTGCTTCTCAGTTCCACACAAG 5’CTGCATCTCAGTCCCACACAAG	1.2 kb
Scaffold	5’GTGGGCTACGTGGACGAC	5’TGATCTCCGCAGGGTAGAAG	530 b
Genotyping	5’TACGGTAGCAGAGACTTGGTCTtcaGGGCTACGTGGACGACAC 5’TACGGTAGCAGAGACTTGGTCTcaGGGCTACGTGGACGACAC 5’TACGGTAGCAGAGACTTGGTCTaGGGCTACGTGGACGACAC 5’TACGGTAGCAGAGACTTGGTCTGGGCTACGTGGACGACAC	5’ACACTGACGACATGGTTCTACAactGCCTCGCTCTGGTTGTAGTAG 5’ACACTGACGACATGGTTCTACActGCCTCGCTCTGGTTGTAGTAG 5’ACACTGACGACATGGTTCTACAtGCCTCGCTCTGGTTGTAGTAG 5’ACACTGACGACATGGTTCTACAGCCTCGCTCTGGTTGTAGTAG	195 b

[a]In addition to the gene-specific sequence shown, the forward and reverse primers here contain GS FLX Titanium adaptor A or B respectively, and a multiplex identifier (MID) sequence to uniquely tag sequences from each animal (not shown).

[b]Primer designed to amplify more MHC-I A alleles.

Table 3

Novel transcripts found in 15 *Papio anubis* and 8 hybrid *Papio anubis* x *Papio cynocephalus* baboons using our combined Roche/454 and Illumina sequencing approach

Novel *Paan* transcripts	GenBank ID	Immuno Polymorphism Database ID	Total # of baboons expressing transcript
*Paan-A*01:02*	KR229932	0006911	7
*Paan-A*04:01*	KP893569	0006783	1
*Paan-A*05:01*	KP893572	0006786	1
*Paan-A*06:01*	KP893575	0006789	2
*Paan-A*07:01*	KP893577	0006791	2
*Paan-A*08:01*	KP893589	0006797	2
*Paan-A*09:01*	KP893601	0006809	1
*Paan-A*10:01*	KP893603	0006811	1
*Paan-A*11:01*	KP893604	0006812	1
*Paan-A*12:01*	KP893581	0006826	6
*Paan-B*01:02*	KP893590	0006798	2
*Paan-B*02:02:01*	KP893587	0006795	3
*Paan-B*02:02:02*	KP893593	0006801	3
*Paan-B*02:02:03*	KP893617	0006823	1
*Paan-B*03:01*	KP893568	0006782	1
*Paan-B*06:01*	KP893570	0006784	6
*Paan-B*06:02*	KP893576	0006790	1
*Paan-B*07:01*	KP893571	0006785	6
*Paan-B*07:02*	KP893586	0006794	2
*Paan-B*08:01*	KP893573	0006787	1
*Paan-B*09:01*	KP893574	0006788	1
*Paan-B*09:02*	KP893580	0006825	7
*Paan-B*10:01:01*	KP893578	0008785	6
*Paan-B*10:02*	KP893613	0006820	2
*Paan-B*11:01*	KP893585	0006793	3
*Paan-B*11:02:01*	KP893600	0008786	6
*Paan-B*12:01*	KP893588	0006796	2
*Paan-B*12:02:01*	KP893602	0008787	1
*Paan-B*13:01*	KP893591	0006799	7
*Paan-B*14:01*	KP893592	0006800	3
*Paan-B*15:01*	KP893606	0006814	3
*Paan-B*15:02*	KP893594	0006802	3
*Paan-B*15:03*	KP893584	0006829	3
*Paan-B*16:01*	KP893595	0006803	3
*Paan-B*17:01*	KP893596	0006804	3
*Paan-B*18:01*	KP893597	0006805	1

Novel *Paan* transcripts	GenBank ID	Immuno Polymorphism Database ID	Total # of baboons expressing transcript
*Paan-B*19:01*	KP893598	0006806	2
*Paan-B*20:01*	KP893599	0006807	6
*Paan-B*20:02*	KP893616	0006822	1
*Paan-B*21:01*	KP893605	0006813	5
*Paan-B*22:01*	KP893607	0006815	7
*Paan-B*23:01*	KP893609	0006816	2
*Paan-B*24:01*	KP893610	0006817	2
*Paan-B*24:02*	KP893618	0006824	2
*Paan-B*25:01*	KP893611	0006818	3
*Paan-B*26:01*	KP893612	0006819	11
*Paan-B*27:01*	KP893615	0006821	3
*Paan-B*28:01*	KP893582	0006827	6
*Paan-B*29:01*	KP893583	0006828	7

Table 4

Novel transcripts found in a *Papio hamadryas* baboon using our combined Roche/454 and Illumina sequencing approach. All transcripts were found in animal 18503.

Novel *Paha* transcripts	GenBank ID	Immuno Polymorphism ID
*Paha-A*02:01*	MG774886	70018617
*Paha-B*02:01*	KR150750	0006899
*Paha-B*10:01*	KP893608	70011126
*Paha-B*12:01*	KR150751	0006903
*Paha-B*15:01*	KR150754	0006908
*Paha-B*18:01*	KR150752	0006904
*Paha-B*19:01*	KR150753	0006906
*Paha-B*30:01*	KP893614	70011128