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Mamu-DQA1 Allele and Genotype Frequencies in a Randomly Sampled Breeding Colony of Rhesus Macaques (*Macaca mulatta*)

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We studied the allelic and genotypic distribution of the major histocompatibility class-II locus DQA1 observed in a random sample of Indian rhesus macaques (*Macaca mulatta*) from a major breeding facility in the United States. The DNA was isolated from whole blood samples collected between 1991 and 1994 from 65 Indian rhesus monkeys. Polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP), which involves use of specific amplification of DQA1 exon 2 and subsequent restriction digestion of the 242-base pair fragment, was used to genotype the animals for the 20 known macaque (*Mamu*)-DQA1 alleles. Frequencies for four alleles (DQA1*240x, *2502, *2503 and *0102) differed significantly from those reported in a smaller sample of rhesus macaques from the German Primate Center. The modest genetic survey of *Mamu*-DQA1 genotypes presented here will be particularly useful in designing epidemiologic studies that investigate associations between immunogenetic background and disease susceptibility in macaque models of human disease.

To unravel the complex etiopathogenesis of disease, epidemiologists consider host, agent, and environmental factors that interact and contribute to disease incidence. In traditional epidemiology, attention was disproportionately focused on disease-causing agents or environmental factors. Host factors, although viewed as important in influencing development and severity of disease, were often considered broadly in the catch-all categories of age, sex, and breed. Advances in the theory of genetic epidemiology and the technology of molecular genetics, however, have made possible more powerful statistical analysis and greater definition of genetically determined biological markers that influence susceptibility or disease patterns in populations. The most well-documented example of highly polymorphic host genetic systems associated with etiopathogenesis of disease is that of the major histocompatibility complex (MHC).

The MHC class-II molecules are highly polymorphic dimeric membrane proteins whose function is to bind and present peptide fragments from processed antigens. The class-II region is subdivided into subregions, two of which, DR and DQ, have been studied thoroughly, especially in humans (1). Each subregion contains multiple alpha and beta genes, with most of the polymorphic residues observed in the second exons of the beta genes (2, 3). Apart from DQA1, the class-II alpha genes are invariant or oligomorphic (4). Unlike other primates, including some New World monkeys, Old World monkeys do not have DQA2 and DQB2 genes (5, 6). In the past five years, molecular data have been generated for class-II loci of nonhuman primates (6, 7).

Numerous human populations have been genotyped for the DQA1 locus (8). Consequently, many associations between human *Mhc*-DQ loci and various diseases, including the human immunodeficiency virus (HIV), insulin-dependent diabetes mellitus (IDDM), melanoma, narcolepsy, celiac disease, lepromatous leprosy (LL), and early-onset pauciarticular juvenile chronic arthritis (EOPA-JCA), have been well documented (9-17). Strong sequence homologies between human and non-human primate MHC class-II gene sequences (18, 19) support the hypothesis that macaque class-II loci might also influence host response to autoimmune or infectious diseases, thereby allowing development of nonhuman primate models of these diseases in humans. However, this potential has been documented in only one study, in which significant associations between *Mamu*-DQB1 alleles and disease progression in simian immunodeficiency virus (SIV)-infected rhesus macaques were observed (20). Little is known about population frequencies of *Mamu*-DQA1 alleles at the DNA level in Indian rhesus macaques bred in captivity in the United States. To date, only one study of Indian rhesus macaque DQA1 population characteristics has been published (21), that of a group (n = 38) of Indian rhesus monkeys at the German Primate Center (DPZ) in Gottingen, Germany.

Here we present *Mamu*-DQA1 frequencies for a modestly larger (n = 65) randomly sampled group of Indian *Macaca mulatta* maintained at a major breeding colony in the United States, LABS of Virginia (formerly Laboratory Animal Breeders and Services, Inc., Yemassee, S.C.). These data, along with the data presented in the novel research of Christ and co-workers (21) can provide the baseline for assessing the representativeness of these alleles in the gene pools of other, smaller groups sampled from the general population of captive macaques. In addition, these data presented here and in future surveys should facilitate experiments designed to address more specifically whether rhesus homologues of human alleles that have been associated with susceptibility and/or disease progression are present in the breeding stock of rhesus macaques available in the United States.

Materials and Methods

Animals. Approximately 3 ml of whole blood, drawn into vacutainer tubes with EDTA as anticoagulant, was obtained from

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65 randomly chosen Indian rhesus macaques housed at LABS of Virginia. These rhesus macaques were originally purchased from Cayo Santiago, and they descend from ancestral stock captured near Lucknow in northern India. The DNA was isolated from the buffy coat fraction by use of salt extraction (22), and was stored frozen in TE buffer (10 mm Tris HCl, 1 mm EDTA, ph 8.0). All experiments were conducted according to guidelines set forth in the *Guide for the Care and Use of Laboratory Animals* (23).

Amplification and restriction enzyme digestion. Primers GH26 (5'-GTGCTGCAGGTGTAAACTTGTACCAG-3') and GH27 (5'-CACGGATCCGGTAGCAGCGGTAGAGTT-3') were used to amplify primate *Mhc*-DQA1 exon 2 (24) under the following conditions: 3 U of *Taq* DNA polymerase (Perkin Elmer, Foster City, Calif.), 1 to 3 μl of sample (250 nmol of DNA/μl), 25 pmol each of primers GH26 and GH27, 200 μ*M* dATP, dCTP, dGTP, and dTTP, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, and 10 m*M* Tris-HCl, pH 8.3, in a total volume of 120 μl of polymerase chain reaction (PCR) mixture. After 3 min at 95°C, the reaction mixture was subjected to 40 cycles of 94°C for one minute, 65°C for one minute, and 73°C for one minute. Nine minutes at 72°C completed extension. An aliquot (8 l) of each amplified sample was run on 1.5% TBE (Tris-borate-EDTA) agarose gels (Agarose MP, Boehringer Mannheim, Indianapolis, Ind.) with a molecular weight marker of 100-base pair (bp) increments (GIBCO BRL, Grand Island, N.Y.) to confirm amplification of the 242-bp fragment of the DQA1 second exon. The remaining PCR reaction volume was purified (High Pure PCR Purification Kit; Boehringer Mannheim) to remove amplification reagents.

Restriction enzymes and genotyping methods. Table 1 presents the approximate fragment sizes expected following digestion with the restriction enzyme series *Dde* I, *Mse* I, *Scr*F I, *Fnu* 4HI, *Bsm*A I, *Mnl* I, *Nla* III, *Bsp*H I, and *Sfc* I (New England Biolabs, Beverly, Mass.). Fragment patterns were compiled from Christ and co-workers (21), Sauermann and co-workers (25), and Sauermann (26). A 6.0- to 7.5-μl aliquot of purified PCR product was added to the restriction digest mixture, which contained 2 to 3 U of restriction enzyme per reaction. Digestion was carried out at either 37 or 55°C, depending on the enzyme used. Minor modifications of previous protocols were made, including the use of 10% TBE polyacrylamide (instead of agarose) gels run at 205 V for 75 to 90 min and replacement of ethidium bromide by the SYBR Green-I stain (gels were stained for at least 45 min with 100 ml of a 1:10,000 dilution of SYBR Green I concentrated stock; Molecular Probes, Eugene, Oreg.) to improve resolution of restriction digest fragments smaller than 50 bp (27). Gels were viewed at a wavelength of 254 nm on a transilluminator

Statistical methods. The random selection of animals was conducted by use of a table of random numbers (28), and was based on simple random sampling from an estimated population size of approximately 4,500 macaques, according to Scheaffer (29) for sample size required to estimate the population proportion p with a bound on the error of estimation (0.05). A priori estimation of p was obtained from the frequency of *Mamu*-DQA1*0102 (2.6%) given in Christ and co-workers (21). The calculated sample size required for estimation of p was increased to accommodate potential exclusion of samples known to be of poor quality (n = 16) or samples that might be represented more than once. Actual exclusion of samples involved only a single sample that was represented twice.

The Hardy-Weinberg (HW) principle was used to estimate an expected genotype distribution, and the χ^2-statistic was used to determine whether the sample observed here departed from HW equilibrium (30, 31).

Since the animals obtained from LABS are not a large unified breeding population but rather are subdivided into smaller breeding groups, the expected level of heterozygosity (H), or gene diversity, 84.0%, was calculated [according to Nei (32) where $H = 1 - \Sigma X_i^2$; where X_i = allele frequencies]. The expected level of H describes the distribution of genotypes in a population that is considered as a random-mating entity (33). The difference between expected H (84.0%) and observed H (79.0%) was calculated, using a χ^2 goodness of fit test statistic. The difference between expected and observed H indicates a deficiency of heterozygotes or excess of homozygotes. Weir's global homozygosity test statistic was used to compare observed frequency of homozygotes with that expected under HW, and several local single degree-of-freedom tests were applied to particular pairs of alleles that were suspected contributors to disequilibruim (34).

To examine the probability that two samples (i.e., LABS versus DPZ rhesus macaques) were drawn from a single population, comparisons of the multiple allele frequencies at the DQA1 locus between the two groups were carried out by estimating a series of 95% confidence intervals from binomial standard errors (33, 35). Non-overlapping limits were interpreted as evi-

Table 1. Approximate restriction fragment sizes expected from restriction enzyme digestion of the 242-base pair amplification product, *Mamu*-DQA1 exon 2

DQA1 allele	*Dde* I	*Bsm*A I	*Nla* III	*Bsp*H I	*Sfc* I	*Scr*F I	*Mnl* I	*Fnu* 4HI	*Mse* I
*2301	162;80	167;72	185;57	No cut	no cut	152;90	111;66;48	222;16;4	205;37
*2302	162;80	171;71	185;57	No cut	no cut	152;90	112;47;43;23;17	149;73;16;4	205;37
*240x	162;80	136;71;35	185;57	No cut	no cut	152;90	112;47;43;23;17	222;16;4	205;37
*2402	162;80	135;107	185;57	No cut	no cut	152;90	112;47;43;23;17	222;16;4	205;37
*2501	162;80	135;38;35;34	146;58;38	200;39	no cut	152;90	110;88;43	222;16;4	205;37
*2502	162;80	135;38;35;34	No cut	No cut	No cut	152;90	110;88;43	222;16;4	205;37
*2503	162;80	135;38;35;34	185;57	185;54	No cut	152;90	110;88;43	222;16;4	205;37
*0101	122;79;41	135;72;35	113;91	200;39	No cut	152;90	151;88	222;16;4	126;113
*0102	91;79;41;31	135;69;38	185;57	No cut	No cut	129;111	110;85;44	222;16;4	126;59;54
*0103	122;79;41	135;72;35	146;58;38	200;39	No cut	132;90;20	110;48;44;40	222;16;4	126;113
*0104	122;79;41	135;72;35	146;58;38	200;39	176;66	132;90;20	110;48;44;40	222;16;4	126;113
*01051	121;77;41	133;71;35	112;91;36	199;40	No cut	152;90	108;87;44	222;16;4	126;113
*01052	121;80;41	136;71;35	112;91;36	199;43	No cut	152;90	111;87;44	222;16;4	126;116
*0106	121;77;41	133;71;35	No cut	No cut	No cut	132;90;20	108;87;44	222;16;4	126;59;54
*0107	121;80;41	136;71;35	203;39	199;43	No cut	132;90;20	111;87;44	171;48;16;4;3	126;116
*0108	121;80;41	136;106	185;57	No cut	No cut	132;90;20	111;47;44;40	222;16;4	126;116
*0501	127;112	135;72;35	185;57	No cut	No cut	127;112	151;88	222;16;4	No cut
*0502	127;112	132;107	185;57	No cut	No cut	127;112	151;88	222;16;4	No cut
*0503	127;112	132;107	185;57	No cut	No cut	Not 127;112	151;88	222;16;4	No cut

Expected fragment patterns were compiled from Christ and co-workers (21), Sauermann and co-workers (25), and Sauermann (26).

dence that the two sample frequencies were not drawn from a single inclusive population.

Results

The allele frequencies that characterize the sample studied here are given in Table 2. At present, *Mamu*-DQA1*2401 and *2403 are not distinguishable by any known restriction site; therefore, these two alleles, which differ from each other at two nucleotide positions (positions 57 and 133 of the amplified exon) (36), were considered as one allele, "DQA1*240x", in this and other tables. *Mamu*-DQA1*2401 and *2403 are distinguishable by use of DNA sequencing. *Mamu*-DQA1*0501 and *0502, which have been reported in Indian, Chinese, Burmese, and Vietnamese rhesus macaques, were not observed in this group of animals (21, 27). For three animals, one or both alleles were not resolvable by use of restriction fragment length polymorphism (RFLP) typing methods; this contributed to the "unresolved" category, which comprised 2.3% of the total sample of alleles. Gene frequencies ranged from 1.2% (DQA1*0101) to 27.7% (DQA1*0104). Both DQA1*2501 and *0102 were also high in frequency in this group, at 19.2 and 16.9%, respectively.

The numbers of genotypes observed are presented in Table 3, and are compared with the calculated number of genotypes expected under HW equilibrium. Of the 65 animals genotyped, 17 were homozygous and 48 were heterozygous for *Mamu*-DQA1 alleles. The observed distribution of genotypes did not differ significantly ($P > 0.50$) from that expected on the basis of HW equilibrium. The expected level of H, 84%, exceeded the observed level of H (number of heterozygous individuals/total individuals) of 73.8%, but the difference was not significant ($\chi^2 = 0.316$, $\pi > 0.10$). One or more factors can influence gene diversity: stratification of subgroups considered erroneously as a unified breeding group (Wahlund's effect), inbreeding, or typing errors leading to pseudohomozygosity (34, 37, 38). Weir's global homozygosity single degree-of-freedom test statistic was significant (χ^2 =15.19, $P < 0.001$). Local single degree-of-freedom test statistics were calculated to examine whether specific pairs of four alleles (*Mamu*-DQA1*0102, *0104, *2501 and *2502) had genotypic frequencies that departed from HW expectation (34). No particular pair of alleles had genotypic frequencies inconsistent with HW frequencies, which might indicate that the significance observed in the global homozygosity test is the result of homozygote frequencies contributing to the departure from expected values. The excess of homozygotes may be an artifact of "preferential amplification" of the aforementioned four alleles (25, 26), thus giving truly heterozygous samples the appearance of homozygosity when subjected to the series of restriction digests. This phenomenon, called "pseudohomozygosity" has been discussed by Weir (34); Ely and co-workers (38) identified this as a source of typing error in typing of short tandem repeat (STR) loci in rhesus macaques. Although pseudo-homozygosity or other typing errors might have influenced the results of this study, selective retesting of rhesus with the alleles showing an excess of homozygosity gave the same results. Since these samples were selected from a population that is genetically subdivided into a dozen or more (Mendelian) breeding groups, an excess of homozygosity is to be expected due to Wahlund's effect when several different populations are artificially pooled into a single population that is not panmictic (37, 39). Stratified sampling of the multiple breeding groups was not done since these breeding groups were recently artificially composed from a larger free-range population and information was not available on the composition of breeding groups in that ancestral free-range population. Animals purchased for subsequent biomedical research are often chosen in non-systematic manner from all breeding groups. Without exact knowledge of the underlying breeding structure of the sample observed here, it is not possible to distinguish the source of excess homozygosity.

To our knowledge, *Mamu*-DQA1 population data have been reported for only one other group of Indian rhesus macaques (21). A comparison of the allele frequencies observed here with

Table 2. Number observed and distribution (%) of *Mamu*-DQA1 alleles in a sample (n = 65) of rhesus macaques from LABS of Virginia

Allele	No. Observed[a]	Frequency	(%)
2301	2	0.015	(1.5)
240x	14	0.108	(10.8)
2402	2	0.015	(1.5)
2501	25	0.192	(19.2)
2502	8	0.062	(6.2)
2503	6	0.046	(4.6)
0101	1.5	0.012	(1.2)
0103	2.5	0.019	(1.9)
0104	36	0.277	(27.7)
0105	4	0.031	(3.1)
0106	4	0.031	(3.1)
0102	22	0.169	(16.9)
Unresolved[b]	3	0.023	(2.3)

[a]Number of times counted on haplotypes; total haplotypes = 130.
[b]Not possible to discriminate all alleles in some heterozygous combinations. Polymerase chain reaction-restricted fragment length polymorphism analysis (PCR-RFLP) does not distinguish between *Mamu*-DQA1*2401 and *2403, so these alleles are collectively designated as DQA1*240x (21).

Table 3. Hardy-Weinberg fit for *Mamu*-DQA1 data

Genotype	Observed	Expected
2301/2301	1	0
2301/0104	0	1
2401,3/2401,3	0	1
2401,3/2501	4	3
2401,3/2502	2	1
2401,3/2503	1	1
2401,3/0101	0.5	0
2401,3/0103	0.5	0
2401,3/0104	2	4
2401,3/0102	4	2
2402/2402	1	0
2402/0104	0	1
2501/2501	3	2
2501/2502	1	2
2501/2503	2	1
2501/0101	1	0
2501/0103	1	0
2501/0104	7	7
2501/0105	0	1
2501/0106	2	1
2501/0102	1	4
2502/0104	3	2
2502/0106	1	0
2502/0102	1	1
2503/0104	1	2
2503/0102	2	1
0103/0104	1	1
0104/0104	8	5
0104/0105	2	1
0104/0106	0	1
0104/0102	3	6
0105/0105	1	0
0105/0102	0	1
0106/0102	1	1
0102/0102	4	2

H: Sample data reveal no departures from Hardy-Weinberg equilibrium.
$\chi^2 = 23.88$, $\chi^2_{crit} = 76.15$.
Degrees of freedom = number of possible genotypes - number of alleles.
= 78 - 12
= 66

those observed in the smaller (n = 38) group of Indian rhesus macaques from the DPZ is presented in Table 4. Like the group studied here, the DPZ rhesus descend from the founders of Cayo Santiago. Both groups had comparable frequencies of DQA1*2501 and *0104, but differed significantly in the frequencies of *240x, *2502, *2503, and *0102. Alleles DQA1*240x, *2503, and *0102 were significantly higher in frequency in our sample, whereas the DPZ colony had a significantly higher frequency of the DQA1*2502 allele. Other allele comparisons did not differ significantly. Although it was not clear how, or from what population structure, the DPZ animals were selected, it was noted that they were "mainly inbred" (21) and that an unspecified number of animals were included if previous serologic typing of the DQA locus indicated homozygosity (U. Sauermann, personal communication). However, of the 38 animals genotyped, 21.1% were homozygous and 78.9% were heterozygous, values that are similar to those observed in the LABS group (26.2 and 73.8%, respectively). The close agreement between observed (78.9% in DPZ study and 73.8% in this study) and expected (84% this study) level of H is not inconsistent with the hypothesis that the samples were drawn from a single random mating population that has not yet achieved a level of inbreeding sufficient to significantly reduce heterozygosity.

Table 5 lists the known macaque and human DQA1 alleles as well as the placement of these alleles in evolutionary lineages proposed by Kenter and co-workers (18), Christ and co-workers (21), Sauermann and co-workers (25), and Sauermann (26). Alleles are assigned to one of the four allelic lineages on the basis of amino acid motifs and sequence specific patterns. Identity between alleles from different, but closely related species is rare, and all but a few allelic sequences differ from each other by several point mutations. It should not be construed that similar nomenclature (i.e., HLA-DQA1*0501 and *Mamu*-DQA1*0501) is a sign of identity of sequence. Macaque and human alleles in lineages I and II are closely related, but not identical, in amino acid sequence, whereas alleles from *Mhc*-DQA1 lineages III and IV (DQA1*2501, *2502, *2503, *2401, *2402, and *2403) have distinct sequence motifs that have not been observed in humans.

Discussion

One goal of the study reported here was to contribute to the knowledge of the DQA1 genetics of rhesus macaques, as a step toward a better understanding of the role of genetic risk factors in infectious disease susceptibility. We sought to expand on what little is known about population characteristics of the *Mamu*-DQA1 locus in a group of Indian rhesus macaques commonly used as animal models in biomedical research. Our results illustrate the increased range of allelic variability observed in a sample of rhesus macaques in contrast to that observed in smaller samples. Macaques are costly to purchase, and difficult and expensive to maintain; by necessity, many studies using macaques have small sample sizes (n < 20) (40, 41). However, the estimation of risk in a multiple allelic system requires a large number of subjects (42). The low power of statistical tests in studies with small sample sizes can be increased by adopting a more liberal value of alpha, or by ensuring selection of a more homogeneous group of study subjects (43). Since it has been suggested by Svejgaard and Ryder (42) that alpha levels in genetic association studies be set at a more stringent level (i.e., $P \leq 0.001$), we suggest that it would be more practical to increase power by adopting measures that increase the effective size of samples. For example, now that the genotypes of macaques at the *Mhc*-DQA1 locus can be easily and readily characterized, it is possible to design prospective studies involving animals that have two or three genotypes that are suspected risk or protective factors, and thereby reduce the within-group variability that reduces our ability to detect statistically significant effects (43).

Table 4. Comparison of *Mamu*-DQA1 LABS allele frequencies with DPZ sample (n=38) frequencies using estimated confidence limits[a]

Allele	LABS frequency	DPZ frequency[b]
2301	0.015 ± 0.0209	0
2401,3	0.108 ± 0.0533*	0
2402	0.015 ± 0.0209	0
2501	0.192 ± 0.0676	0.263 ± 0.099
2502	0.062 ± 0.0414*	0.197 ± 0.0894
2503	0.046 ± 0.036*	0
0101	0.012 ± 0.0187	0
0103	0.019 ± 0.0235	0.040 ± 0.0435
0104	0.277 ± 0.0768	0.316 ± 0.1031
0102	0.169 ± 0.0644*	0.013 ± 0.0255

[a]Confidence limits were constructed, using the binomial standard error, where $p = 1 - q$ = allele frequency, N = sample size, variance (V) = $pq/2N$, and standard error (se) = $\sqrt{V}$.
[b]*Mamu*-DQA1 allele frequencies observed in Indian rhesus macaques from the German Primate Center (DPZ), Gottingen, Germany (21).
*Confidence limits not overlapping.

We expected that the allele frequencies observed in the LABS sample and the DPZ sample would not differ significantly, since the breeding stock of both primate facilities originated from the Cayo Santiago colony, Puerto Rico (Alecia Lilly, personal communication; 21). The DPZ sample was similar to the LABS population with regard to half of the allele frequencies, but differed in that alleles DQA1*2301, *240x, *2402, *2503, *0101, and *0105 were not observed. It is possible that the small size of the DPZ sample resulted in sampling errors, whereas the larger randomly sampled (albeit, genetically subdivided) group from LABS more accurately reflected the genetic structure of the population from which it was sampled. This illustrates one value of the maintenance of captive animals for biomedical research in genetically subdivided populations ("metapopulations" [44]). Since allele extinction is random in each sub-population, it is unlikely that any given allele, even one of low frequency, will be lost in all randomly mating subdivisions of the larger population. Thus, a subdivided population can be a more valuable reservoir of genetic heterogeneity, compared with maintaining a single panmictic population. In addition, the appearance of rare alleles, especially in groups or individual animals whose origin is known to differ from that of other animals sampled, could reveal the otherwise unrecognized presence of more than a single geographic subspecies within a given study group that is assumed to be genetically homogeneous.

Sequence analyses of primate DNA indicate that the MHC class-II polymorphisms are old and stable entities (4, 45). For example, on the basis of polymorphic DNA sequence motifs shared between humans and macaques, the DQA1 alleles are estimated to be 22 to 28 million years old (18). Phylogenetic analyses of amino acid sequence of primate alleles (using the method of maximal parsimony) have indicated that there is more similarity between homologous alleles of various species than between non-homologous alleles of the same species, a phenomenon that forms the basis of the "transpecies" hypothesis of MHC evolution (4, 46).

Certain lineages, comprising DQA1 alleles, are common to humans, chimpanzees, and monkeys (47); Table 5 shows lineages that are specifically common to humans and macaques (18). It is worth

Table 5. Compilation of data for Mamu-DQA1 allelic lineages (18, 21, 25, 26), with emphasis on known macaque and human alleles. Nomenclature has been updated according to Bontrop (36) and Klein (66)

Lineage	*Mamu*-DQA1 allele	HLA-DQA1 allele
Mhc-DQA1 I	DQA1*0501	HLA-DQA1*0501
	DQA1*0502	HLA-DQA1*0401
		HLA-DQA1*0601
Mhc-DQA1 II	DQA1*0101	HLA-DQA1*0101
	DQA1*0102	HLA-DQA1*0102
	DQA1*0103	HLA-DQA1*0103
	DQA1*0104	HLA-DQA1*0301
	DQA1*0105	
	DQA1*0106	
	DQA1*0107	
	DQA1*0108	
Mhc-DQA1 III	DQA1*2501	No human orthologues
	DQA1*2502	
	DQA1*2503	
Mhc-DQA1 IV	DQA1*2401	No human orthologues
	DQA1*2402	
	DQA1*2403	

noting that *Mhc*-DQA1 lineage II contains macaque alleles DQA1*0101, *0102, *0103, *0104, *0105 and *0106, all of which are present in the LABS population. The homologous human alleles, DQA1*0101, *0102, *0103 and *0301, have been associated with either susceptibility to or protection from various infective agents or autoimmune conditions (9, 13-15, 48-52). Since macaques have already been developed as biomedical models for some of these diseases (i.e., HIVS/SIV and *Helicobacter pylori*) the development of prospective studies of associations between alleles of DQ loci and susceptibility or clinical response to infection is now possible and desirable (20, 53).

Primate models for testing the relationship between MHC genotype and disease need not be restricted to Indian rhesus macaques. For example, experimental studies of SIV infection have involved use of Chinese rhesus macaques (54, 55). Macaque alleles DQA1*0501 and *0502 in allelic lineage I were not present in the LABS Indian rhesus macaque sample, but have been observed in Chinese, Vietnamese, and Burmese rhesus macaques (21, 27). Their human homologues, DQA1*0501, *0401 and *0601, have been identified as risk factors in type-I diabetes mellitus, juvenile chronic arthritis, and celiac disease (9, 12, 48, 56). Other macaque species, such as *M. fascicularis*, *M. nemestrina*, and *M. fuscata* have been used in studies of autoimmune and infectious disease, including IDDM, SIV infection, and *H. pylori* (56-64). However, the contribution of genetic susceptibility and disease progression in macaques of various species or subspecies has not yet been systematically investigated, and few examples exist in literature (65).

The genetic similarity between macaques and humans underscores the advantage of developing macaque models of human disease, compared for example, with models based on the more distantly related mouse. The pioneering research conducted by Christ and co-workers (21), Sauermann and co-workers (25), and Sauermann (26), combined with the data presented here should facilitate the application of still-emerging molecular technology toward the characterization of genetic risk factors in nonhuman primate models. Prospective investigation of disease susceptibility and/or progression in macaques with identical and different MHC genotypes could reveal the influence of such risk factors and provide a setting in which heterogeneity of exposure and pathogen can be experimentally controlled. The molecular genetic characterization of monkeys so widely used in biomedical research should foster further development of hypotheses concerning genetic markers of susceptibility and resistance to disease, and a clearer understanding of their statistical effects that can only be experimentally tested in animal models.

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