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Identification of Equine Major Histocompatibility Complex Haplotypes using Polymorphic Microsatellites

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Summary

A system for identifying equine Major Histocompatibility Complex (MHC) haplotypes was developed based on five polymorphic microsatellites located within the MHC region on ECA 20. Molecular signatures for 50 microsatellite haplotypes were recognized from typing 353 horses. Of these, 23 microsatellite haplotypes were associated with 12 established Equine Leukocyte Antigen (ELA) haplotypes in Thoroughbreds and Standardbreds. Five ELA serotypes were associated with multiple microsatellite subhaplotypes, expanding the estimates of diversity in the equine MHC. The strong correlations between serological and microsatellite typing demonstrated a linkage to known MHC class I protein polymorphisms and validated this assay as a useful supplement to ELA serotyping, and in some applications, a feasible alternative method for MHC genotyping in horse families and in population studies.

Keywords

MHC; Microsatellite; Haplotype; Equine; Polymorphism

Diversification of Major Histocompatibility Complex (MHC) class I and class II genes in the vertebrate genome is a key feature of their role in antigen presentation in the adaptive immune system (Hughes and Nei 1992). However, assigning MHC haplotypes to individuals remains challenging even in the age of whole genome sequencing. International workshops convened in the 1980s identified 19 serological specificities as products of the Equine Leukocyte Antigen (ELA) system (Lazary *et al.* 1988). The serological assay is limited by the amount and variety of antibody reagents available, and the complex alloantisera are directed primarily against MHC class I antigens. To address these limitations, alleles at five polymorphic microsatellite loci within the equine MHC (Fig. S1) were used to identify distinct haplotypes based upon the well-known linkage disequilibrium within the MHC (see Appendix S1 for Materials and Methods). In addition, we evaluated these molecular haplotypes as proxies for serologically defined markers by testing their level of correspondence to the serological haplotypes of the ELA system (Lazary *et al.* 1988) within and between horse breeds (Table 1).

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Microsatellite haplotypes were defined in three ways: 1) horses homozygous for microsatellite allele constellations that allowed unambiguous identification of MHC microsatellite haplotypes with supporting serological data; 2) horses belonging to families that allowed definition of microsatellite haplotypes by familial transmission; 3) association of haplotypes with known serotypes that allowed definition of haplotypes in cis configuration. Haplotypes were defined when they could be identified in at least two individuals that were not related within two generations, or when observed segregating in families. When possible, microsatellite haplotype nomenclature was derived from nomenclature of the ELA complex. When a serotype was associated with multiple microsatellite haplotypes, indicating a genetic complexity not reflected by serotyping, the ELA type was followed by a lower-case letter to denote the subtype. Finally, haplotypes not associated with serotypes were given the prefix “COR” (for Cornell) followed by a number as a working title.

A total of 50 microsatellite haplotypes were identified from typing 353 horses using the five intra-MHC microsatellite loci (Table 1; Fig. S1). Variable numbers of alleles were detected for each microsatellite locus (range 8–13, Table S1), but most were not uniquely associated with any single haplotype (Table 1). MHC haplotype definitions were most accurate when based on alleles at all five loci (Fig. S2). Of the 50 haplotypes, 23 were linked to 12 known ELA serotypes based on previous serotyping results (Table 1; Table S2). The remaining 27 haplotypes, designated as “COR” haplotypes, were discovered in horses that carried unidentified haplotypes (negative for known ELA serotypes) or that had not been serotyped.

The equine MHC haplotypes described here represent a large increase in the number of recognized haplotypes segregating within families and detected among unrelated individuals. In addition to defining new levels of diversity in the equine MHC, this study also confirms the distribution of associated broad ELA serotypes in Thoroughbreds and Standardbreds, where a small number of ELA types are carried by the majority of horses in these breeds (Antczak et al., 1986). Overall, the named microsatellite haplotypes defined 90% and 97% of the haplotypes in Thoroughbreds and Standardbreds, respectively, and 63% in Arabian horses (Table S3).

Five ELA serotypes were associated with multiple microsatellite haplotypes. The origin and extent of the diversity within subtypes remain unclear. A strong association was observed between ELA serotypes and alleles at microsatellite locus UMN-JH34-2, located in a cluster of expressed MHC class I genes (Tallmadge *et al.* 2005) (Fig. S2, SOM). Microsatellite variation within serologically defined ELA haplotypes was restricted largely to the MHC class II region. For some subtypes, there was little or no sharing of MHC class II microsatellite alleles (Table 1). In the ELA-A3 haplotype carried by the donor horses of the equine Bacterial Artificial Chromosome library (Gustafson *et al.* 2003) and the genome sequence (Wade *et al.* 2009), there is little evidence of sequence variation in MHC class I or II genes among the subhaplotypes (Tallmadge *et al.* 2005, 2010; Miller and Antczak, personal communication). The subhaplotypes could reflect microsatellite evolution in ancient haplotypes that is independent of alterations in MHC class II structural genes or the recombination between the MHC class I and class II regions that is manifest in new haplotypes. Haplotypes that were serology positive and microsatellite negative could be as yet unrecognized subhaplotypes that were not captured by a single microsatellite haplotype. In contrast, MHC haplotypes that were serology negative and microsatellite positive may have been defined as a result of inaccurate serotyping (Table S2).

The reported high linkage disequilibrium within the equine genome between breeds presumably reflects the recent breed divergence and sharing of founders (Wade *et al.* 2009). While some ELA haplotypes are shared among breeds, Antczak *et al.* (1986) previously

described differences between breeds in the frequency of detection of various ELA haplotypes, including the apparent absence of some haplotypes in certain breeds. Similar restriction was seen in the microsatellite haplotypes defined here (Table 1; Table S3). The high correlation of serotypes with microsatellite haplotypes within certain breeds indicates that intra-MHC microsatellite typing with the described five member panel can be used for MHC genotyping within breeds for population and family studies, particularly in Thoroughbreds and Standardbreds.

The microsatellite typing reported here allows rapid and accurate identification of equine MHC haplotypes in most instances, requires no specialized alloantibody reagents, and as such offers advantages over ELA serotyping. This study used ELA serotyping, known MHC homozygous horses, and MHC gene sequencing (Tallmadge *et al.* 2010) to link key microsatellite constellations to common MHC haplotypes and to expand the estimates of diversity in the equine MHC. Microsatellite typing is thus a powerful complement to classical ELA serotyping and sequencing for identifying MHC haplotypes of the horse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Assigned equine MHC microsatellite constellations with corresponding serological ELA haplotypes[a]

Microsatellit e Haplotype	Serologica 1 ELA Haplotype	r^b	Intra MHC Microsatellite Alleles					Breed(s)[c]	Counts[d]
			Class I	Class II					
			UMN-JH34-2	COR112	COR113	UM011	COR114		
A1	A1	0.93	211	236	266	179	241	SB, TB	57
A2	A2	0.86	211	262	268	174	235	TB, Old, SB	65
A3a	A3		207	254	260	172	243	TB, WB, P, U	21
A3b	A3	0.93	207	262	268	176	247	TB, Han, QH, WB, P, U	53
A3c	A3		207	262	272	168	255	TB	23
A3d	A3		207	262	268	174	235	TB	5
A3e	A3		211	252	282	172	247	SB	8
A4a	A4	0.92	205	236	266	179	241	SB	4
A4b	A4		205	252	276	168	243	SB	8
A4c	A4		217	262	276	168	247	SB	6
A5a	A5	0.88	221	254	260	172	243	TB, SB, Han, QH, U	49
A5b	A5		221	254	268	174	235	OLD	2
A6	A6	0.76	197	243	266	170	245	SB	15
A7	A7	0.83	205	256	270	172	249	SB, TB	11
A8	A8	0.95	219	268	274	180	245	SB, MFT	18
A9a	A9	0.93	217	264	272	168	255	TB, WB, U	26
A9b	A9		217	236	266	180	241	TB	3
A10a	A10	0.86	221	236	264	180	243	SB, QH	53
A10b	A10		221	236	266	179	241	SB, TB, U	17
A10c	A10		221	236	266	168	249	SB, U	9
A10d	A10		221	252	274	168	243	SB	10
A15	A15	0.75	219	254	260	172	243	TB, SB	7
A19	A19	0.75	211	262	270	184	245	TB, U	16
COR1			221	262	268	176	247	TB, U	7
COR2			217	262	270	184	245	TB	3

Microsatellit e Haplotype	Serologica 1 ELA Haplotype	r[b]	Intra MHC Microsatellite Alleles					Breed(s)[c]	Counts[d]
			Class I	Class II					
			UMN-JH34-2	COR112	COR113	UM011	COR114		
COR3			211	254	260	172	243	TB, QH, WB, U	5
COR4			221	264	272	168	255	TB	3
COR5			197	236	266	179	241	TB	5
COR6			221	254	270	172	249	AR, SB	9
COR7			219	262	270	184	245	AR, APP	10
COR8			211	254	268	174	235	AR	9
COR9			211	260	266	170	245	AR, U	6
COR10			207	262	270	184	245	AR	4
COR11			219	264	274	168	247	AR	2
COR12			215	254	260	172	243	APP, U	5
COR13			215	252	274	171	243	U	2
COR14			209	260	260	177	241	P	10
COR15			211	252	270	172	249	P, QH	4
COR16			209	258	274	168	247	P	3
COR17			207	248	270	184	245	P	3
COR18			209	252	270	172	249	APP, Gyp	3
COR19			209	268	266	168	249	P	2
COR20			207	244	268	166	247	P	2
COR21			217	252	280	172	247	P, SB	3
COR22			221	256	270	178	245	TB	2
COR23			197	254	270	172	249	SB	2
COR24			211	243	266	170	245	SB	2
COR25			221	262	270	172	249	SB	3
COR26			203	252	266	184	245	AR	3
COR27			209	262	264	170	238	AR	3

[a] Data collected from a cohort of 353 horses (2n=706 chromosomes) with 600 named haplotypes and 106 blank haplotypes. Microsatellite subtypes of common ELA serotypes are indicated by lowercase letters. Local Cornell (COR) haplotypes did not have an associated ELA serotype.

[b] Correlation coefficient (*r*) was determined for correspondence between ELA serotype and microsatellite-defined MHC haplotype. For sub-haplotypes, *r* values were calculated individually and the combined statistics are reported here. For detailed data and calculations, see Table S2 and Figure S3.

[c] Breeds: APP= Appaloosa; AR= Arabian; Gyp= Gypsy Vanner; Han= Hanoverian; MFT= Missouri Fox Trotter; Old= Oldenburg; P= pony of unknown breeding, presumably mixed breed; QH= Quarter horse; SB= Standardbred; TB= Thoroughbred; WB= warmblood; U= breed of horse unknown, presumed mixed breed.

[d] Number of times each named microsatellite haplotype was identified and counted.