

MHC HAPLOTYPE DIVERSITY IN THE MAIN EQUINE BREEDS OF IBERIAN PENINSULA

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INTRODUCTION

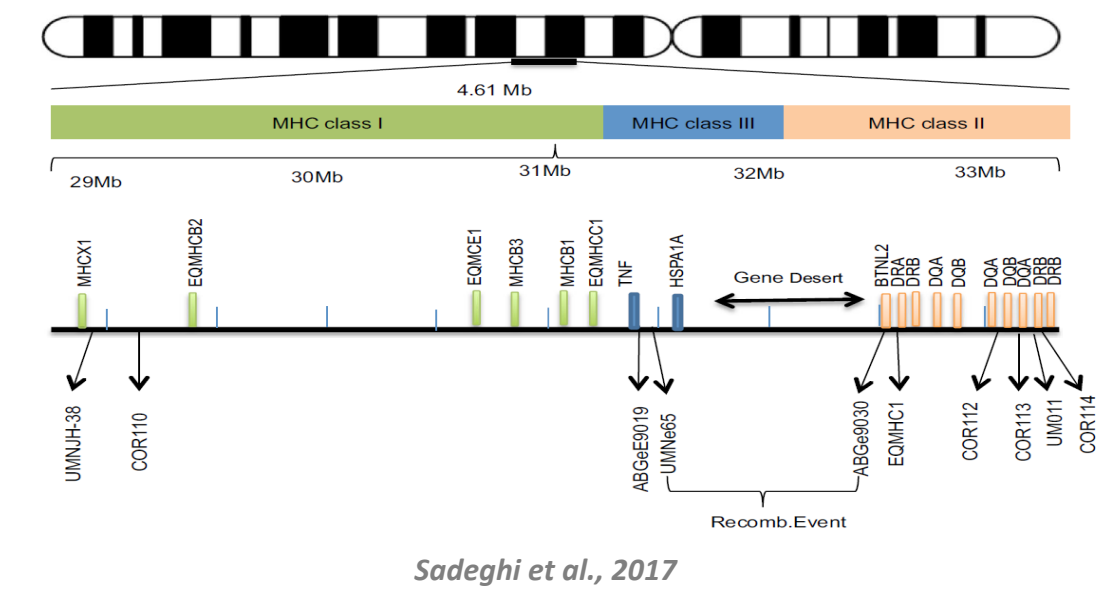
Equine major histocompatibility complex (MHC) genes = Equine Leukocyte Antigen (ELA)

- MHC loci variation → reflects adaptive genetic diversity and immunological fitness of a population.
- Previous studies → high level of MHC heterozygosity and haplotype diversity in Persian Arabian and Icelandic horses.

19 serotypes identified as products of ELA system

⚠ Limitations of serological assay

Assigning MHC-haplotypes to individuals remains challenging



Development of **intra-MHC microsatellite markers** based on polymorphic microsatellite loci and linkage disequilibrium within the **equine MHC (ELA)**

~ 500 MHC-haplotypes described in bibliography in different equine populations

Genetic interest of:



Purebred horses

- Native and widespread breeds in Spain
- Breeding program and Stud Book

Allogeneic cell therapies

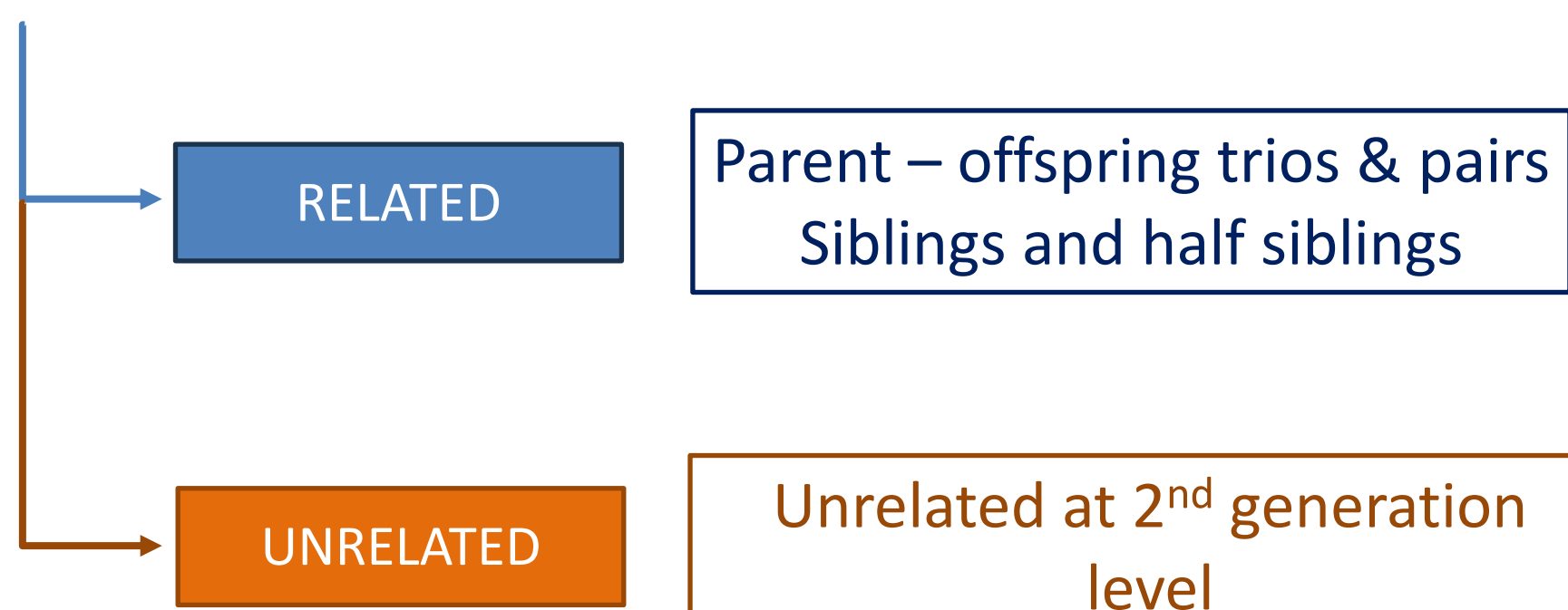
MHC-matching influences immunogenicity of mesenchymal stem cells (MSCs)

Establishing MHC-haplotypes for cell therapy applications: MSCs donor and patient matching can improve safety and effectiveness

This study aimed to enlarge our current knowledge on **MHC-haplotype diversity** focusing on the most common horse breeds in the Iberian Peninsula.

METHODOLOGY

Horse pedigrees



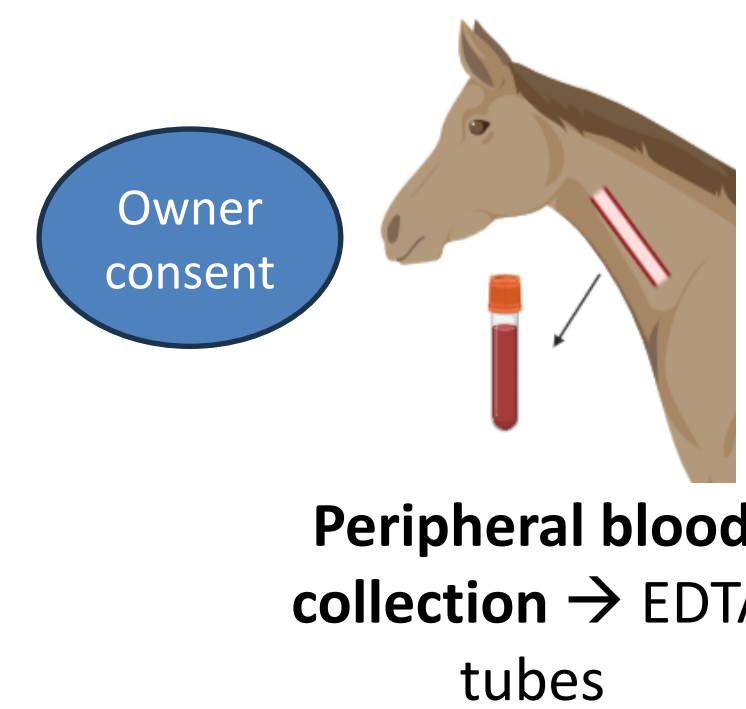
To enlarge **unrelated group** for calculating **MHC-haplotype frequencies**, animals from **related group** that were not related among them were included

Crosscheck with reported MHC-haplotypes in bibliography

Defined MHC-haplotypes by familial transmission

Assignment of **defined** haplotypes found in related group and in bibliography

Remaining microsatellite combinations :
- **Defined** haplotypes if found in at least 2 animals
- **Provisional** haplotypes if found only once



DNA isolation



Multiplex PCR
→ 2 reactions



SeqStudio Genetic Analyzer
GeneMapper® v3.7 software
(Applied Biosystems)

	Microsatellite	Fluorochrome	Primer sequence	Allele range (bp)	Reference	MIX 1	MIX 2
MHC Class I	UMNHJH-38	F'	TGTGTGTGCACCTGTCTCTTT	140-165	Sadeghi et al., 2017	X	
		R'	GATGGGAGGAATGAGGAAT				
	COR110	F'	TTTGTCTTTGCAAGTATGG				
MHC Class II	ABG69019	F'	CTGAGAGAGACAGCATTTGTGG	297-320	Sadeghi et al., 2017	X	
		R'	GAAAGGTGTCTCATTTCTGCT				
	UNMe6S	F'	TGCAAAACCCACAGACTAC				
MHC Class III		R'	TTCTCTTTCTCTCCACTCC	247-269	Sadeghi et al., 2017	X	
		F'	AT550 (NED)				
		R'	AT565 (PET)				
MHC Class II		F'	CCAGCAGACTGCAAGAGTA	205-221	Sadeghi et al., 2017	X	
		R'	AGCATAGCGGAAGAGAGT				
		F'	AT532 (VIC)				
MHC Class II		R'	AGAGACTTCAGTCTCTGTGGTG	180-199	Sadeghi et al., 2017	X	
		F'	TTACTCTGTTATTGGTTATTGG				
		R'	NED				
MHC Class II		F'	TACCCACTAAATCTCAATCC	237-270	Tseng et al., 2010	X	
		R'	TACAGACTCTGGCTCCCTTA				
		F'	GTCTTTCTGCAGCTGTCTCT				
MHC Class II		R'	TGAAAGTAGAAGGGATGTGG	260-280	Tseng et al., 2010	X	
		F'	TCTCAGAGCAGAAAGTCCCTG				
		R'	TCAAATCCAGACTCCCTTC				
MHC Class II		F'	TCATAAAGAGTGGGAGCTG	165-184	Tseng et al., 2010	X	
		R'					
		F'					

RESULTS



Purebred Spanish Horse (PRE)



Arabian Pure Breed (Prá)



Hispano-arabian Horse (Há)



Lusitanian Pure Blood (PSL)

SAMPLES

287

86

37

33

DEFINED HAPLOTYPES

NEW
Previously described

63

9

18

6

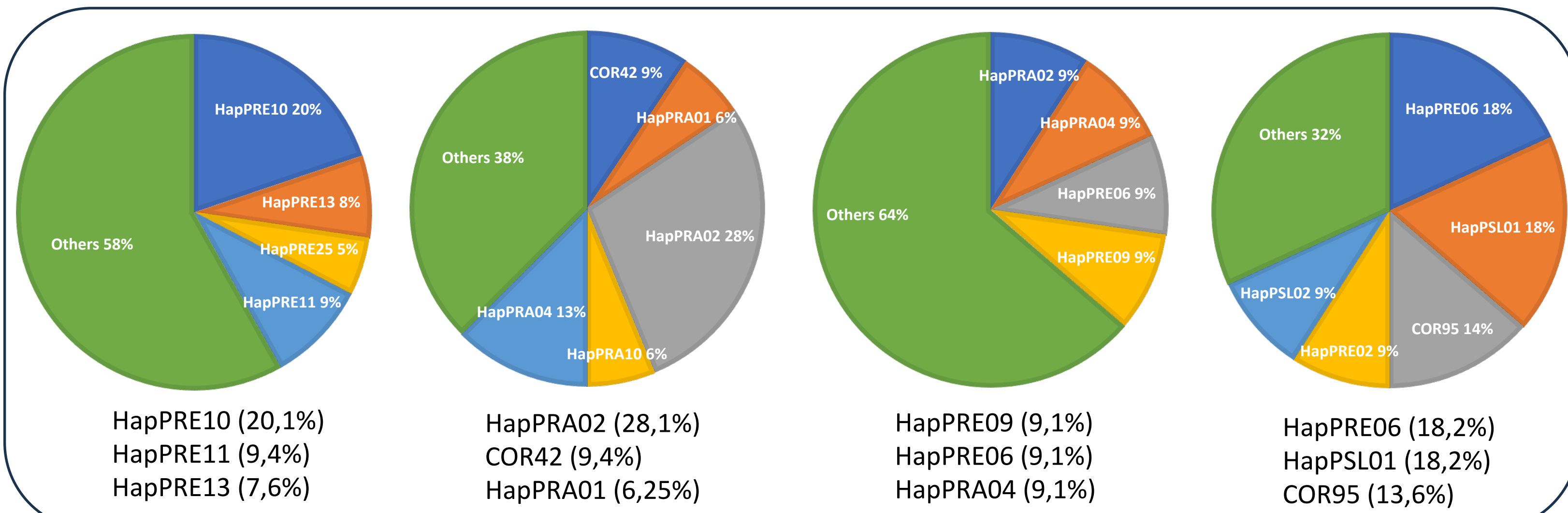
17

10

12

3

HAPLOTYPE FREQUENCIES



HOMOZYGOUS HORSES

11

4

-

1

POTENTIAL equine Mesenchymal Stem Cells donors



ALLELE FREQUENCIES

	PRE	Prá	Há	PSL
UMNHJH-38	52,27%	32,22%	52,5%	47,22%
156				
COR113	39,45%	12,22%	31,25%	30,56%
270				

CONCLUSIONS

- MHC-haplotype diversity in horses** → similar to that reported in other breeds: 2-3 predominant haplotypes + lower frequency of the remaining
- High diversity of MHC-haplotypes:**
 - Among different equine populations: all the defined haplotypes except ten were new in this study (compared to almost 500 previously reported haplotypes)
 - Within Iberian breeds studied: few haplotypes are shared between PRE and Prá → GENE CONSERVATION
- Polymorphism of studied MHC regions** → Allele 156 and 270 highly conserved in the equine species.
- MHC genetic typing** → highly valuable tool to conduct research in allogenic cell therapies (donor-recipient matching).

Larger sample needs to be examined, but this study provides relevant information to gain insight into the extent of MHC-haplotype variability in horses, which is valuable for genetic diversity and future transplant-based therapies requiring donor-receptor MHC matching in horses.