

ORIGINAL ARTICLE

Diversity of major histocompatibility complex (MHC) and natural killer cell receptor (NKR) genes and their interactions in domestic horses

Jana Bubenikova¹  | Martin Plasil¹  | Jan Futas¹  | Karla Stejskalova²  |
Marie Klumplerova¹  | Jan Oppelt¹  | Franz Suchentrunk³  |
Pamela A. Burger³  | Petr Horin^{1,2} 

¹Research Group Animal Immunogenomics, CEITEC VETUNI, University of Veterinary Sciences Brno, Brno, Czechia

²Department of Animal Genetics, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Brno, Czechia

³Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna, Vienna, Austria

Correspondence

Petr Horin, Research Group Animal Immunogenomics, CEITEC VETUNI, University of Veterinary Sciences Brno, Palackeho trida 1946/1, Brno 612 42, Czechia.
Email: horin@ics.muni.cz; horin@med.muni.cz

Funding information

VETUNI Brno, Grant/Award Numbers: DSP_2022_4, Ceitec/Horin/ITA/2020

The immunogenome is the part of the genome that underlies immune mechanisms and evolves under various selective pressures. Two complex regions of the immunogenome, major histocompatibility complex (MHC) and natural killer cell receptor (NKR) genes, play an important role in the response to selective pressures of pathogens. Their importance is expressed by their genetic polymorphism at the molecular level, and their diversity associated with different types of diseases at the population level. Findings of associations between specific combinations of MHC/NKR haplotypes with different diseases in model species suggest that these gene complexes did not evolve independently. No such associations have been described in horses so far. The aim of the study was to detect associations between MHC and NKR gene/microsatellite haplotypes in three horse breed groups (Camargue, African, and Romanian) by statistical methods; chi-square test, Fisher's exact test, Pearson's goodness-of-fit test and logistic regression. Associations were detected for both MHC/NKR genes and microsatellites; the most significant associations were found between the most variable *KLRA3* gene and the *EQCA-1* or *EQCA-2* genes. This finding supports the assumption that the *KLRA3* is an important receptor for MHC I and that interactions of these molecules play important roles in the horse immunity and reproduction. Despite some limitations of the study such as low numbers of horses or lack of knowledge of the selected genes functions, the results were consistent across different statistical methods and remained significant even after overconservative Bonferroni corrections. We therefore consider them biologically plausible.

KEYWORDS

horse, immunogenome, MHC genes, NKR genes

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Authors. HLA: Immune Response Genetics published by John Wiley & Sons Ltd.

1 | INTRODUCTION

The immunogenome is the part of the genome that underlies immune mechanisms and evolves under various selective pressures, particularly under the constant pressure of pathogens. This force is considered one of the most important drivers of evolution.¹ The immunogenome comprises complex genomic regions and single genes belonging to various gene families. To cope with the variability of pathogens, some of immunity-related (IR) genes involved in antigen presentation and recognition have developed a high degree of polymorphism. The complex organization of specific IR regions allowed an accumulation of single nucleotide polymorphisms (SNPs), which, combined with the expansion of some genes and their copy number variation, increases the potential of effective recognition of non-self molecules.² In this context, the major histocompatibility complex (MHC) encoding antigen presenting molecules is the most complex and the most polymorphic region of the immunogenome.³

The genetic diversity of the MHC is associated with different physiological as well as pathological phenotypes in humans and in other mammalian species.^{4–8} At the population level, MHC genes are considered as prominent examples of adaptive genetic diversity maintained by balancing selection.^{9,10} The MHC gene diversity is usually studied and interpreted in the context of the role of MHC class I and class II molecules in antigen presentation.¹¹ However, from an evolutionary perspective, MHC molecules still play another important role related to the process of self versus non-self recognition. During this process, MHC class I molecules can be recognized as markers of self by receptors expressed on natural killer (NK) cells. NK cells are a functionally heterogeneous population involved in several immune processes. Their functional heterogeneity is based on the heterogeneity of their receptors, NKR. The MHC class I molecules thus function as NKR ligands.¹² In these molecular interactions, the high variability of MHC ligands is reflected by the variability of NKR molecules and encoded by highly variable genes. An important group of NKR coding genes is organized in clusters within complex genomic regions, NKR complexes. The genomic organization of the NKR clusters is evolutionarily very flexible. In mammals, two regions known as LRC (leukocyte receptor complex) and NKC (natural killer complex) have been identified. They encode two types of structurally different NKRs capable of interacting with MHC ligands, immunoglobulin-like receptors and lectin-like receptors. The killer cell immunoglobulin-like receptors (KIRs), and the leukocyte immunoglobulin-like receptors (LILRs), encoded in the LRC, and the Ly49 molecules, more recently named

killer cell lectin-like receptor A, (KLRA), are the most studied representatives of the two groups of NK cell receptors. The two complexes are encoded on two different mammalian chromosomes, separately from the chromosome containing the MHC region. The rapid evolution of killer cell receptor (KR) complexes can result in important interspecific differences, when different groups of mammals may use different types of NKR molecules for the same purpose.¹³ Due to this evolutionarily dynamic genomic structure and large interspecific differences, extrapolation from a species to another is often difficult, if not impossible.^{14,15}

The role of MHC and NKR genes in response to selective pressures of pathogens, expressed by their genetic polymorphism at the molecular level, is reflected by their genetic diversity^{10,16} and associations with different types of diseases¹⁷ at the population level. Furthermore, combinations of MHC/NKR variants present at the maternal-fetal interface determine the success of gravidity; specific combinations may be related to complications occurring during fetal development.^{18,19} Although located on different chromosomes and segregating independently, MHC and NKR genes did not evolve independently, at least in humans and other primates.^{20–22} The idea of MHC/NKR co-evolution is supported by findings of associations between specific combinations—compound genotypes—of MHC/NKR haplotypes and different diseases in model species.^{23,24}

Virtually no information on MHC class I/NKR interactions is available for domestic animals, including horses, due mainly to a limited knowledge of both MHC class I and/or NKR genes in these non-model species. While the genetic structure of the equine MHC on the horse chromosome ECA 20q14-q22 has been well annotated in the latest version of the horse genome EquCab3.0,²⁵ and the extent of polymorphism and population diversity of MHC genes have been studied in a number of specific populations, mostly only MHC class II genes were studied. Recently, we have described the variability in physical exon 2/exon 3 haplotypes of MHC I genes *Eqca-1*, *Eqca-2*, *Eqca-7*, and *Eqca-Ψ*.²⁶ Most of MHC class I sequences reported previously were inferred haplotypes. In our study, we reported a so far unrecognized variability of four class I genes in three horse breeds of different geographical and genetic origins, consisting of 112 novel *Eqca* class I sequences. As for the NKR genes, the LRC and NKC clusters are located on the horse chromosomes ECA10 and ECA6, respectively.²⁵ An expansion of the Ly49/KLRA genes was observed in horses²⁷ as well as in other equids.²⁸ We have shown that horses have one probably functional KIR gene²⁸ and have analyzed the SNP polymorphisms of KLRA genes in selected equine populations.²⁹ We also developed a panel

of molecular markers of the MHC and NKR genomic regions as a tool for studying the adaptive population diversity of MHC and NKR genes.³⁰ Currently, there is no information about the functional significance of the diversity of NKR genes and/or about their associations with disease. In this situation, a potential evolutionary relationship between the diversity of expressed MHC class I and NKR genes remains unknown as well.

2 | OBJECTIVES

The objective of this study was to analyze associations between the MHC and NKR gene diversity in three breed groups previously characterized for their MHC class I diversity. Since no associations between MHC and NKR compound genotypes have been observed in horses so far, we tested the null hypothesis that the observed frequencies of MHC and NKR allelic haplotypes are independently combined in compound genotypes.

3 | MATERIALS AND METHODS

3.1 | Samples

A panel consisting of a total of 94 peripheral blood samples from horses of three breeds, Camargue horses ($n = 35$), Romanian horses ($n = 35$) and African (Côte d'Ivoire) horses ($n = 24$) was analyzed. More detailed information about the samples and the genetic diversity of the panel is available in our previous study.²⁶ The three breed groups, originating from different geographical regions with different pathogens should have been exposed to different selection pressures over a long period of time.

3.2 | Genotyping

Genotypes of selected expressed NKR and MHC genes were determined for each horse. A non-MHC gene, *MR1*, encoding the antigen-presenting molecule MR1 was added to the panel for comparison. The diversity of the entire NKR and MHC genomic regions was assessed using a panel of microsatellite markers.

3.2.1 | Next generation sequencing

Standard Illumina MiSeq and MinION sequencing was carried out as described in detail by Plasil et al.²⁶

3.2.2 | Genotyping of NKR genes

Three best characterized NKR genes in terms of their annotation and polymorphism were selected and genotyped by next generation sequencing (NGS) of long-range (up to 5 kb) amplicons. Full-length nucleotide sequence of *KIR3DL* along with the sequence of the lectin-like domain of *KLRA3* and *KLRA7* genes were analyzed. Amplicons were obtained using the KAPA2G Robust PCR Kit (Roche, Basel, Switzerland) with Buffer A and Enhacer 1 according to the manufacturer's protocol. The list of primer sequences and annealing temperatures used for PCR is in Table S1. All amplicons were checked by 0.5% agarose gel electrophoresis and quantified using TECAN fluorimeter and Qubit DNA BR Kit (Invitrogen, Waltham, Massachusetts, USA).

NGS reads were mapped against reference sequences retrieved from the domestic horse (*KIR3DL*, *KLRA3*) and Przewalski's horse (*KLRA7*) genomes (EquCab3.0, GCA_002863925.1 and Burgud, GCA_000696695.1 respectively). Variable positions in the CDS were manually inspected in IGV and recorded using mRNA sequences (*KIR3DL* – NM_001082518.2, *KLRA3* – NM_001081923.1, *KLRA7* – AB120379) as a guide. Haplotypes for *KIR3DL* and *KLRA3* loci were determined for individual exons directly from reads and inferred from individual genotypes of exons by Arlequin 3.5³¹ using the ELB algorithm. For the *KLRA3* gene, the exonic haplotypes were merged into one “complete haplotype,” used in subsequent calculations.

3.2.3 | Genotyping of MHC I and MHC II genes

Genotyping of MHC class I and class II genes *Eqca-1*, *Eqca-2*, *Eqca-7*, *Eqca-Ψ*, and *Eqca-DRA*, *Eqca-DQA1*, respectively, was performed as described in detail previously.²⁶

Briefly, all PCRs were performed using the Kapa 2G Robust HotStart PCR kit (Roche, Basel, Switzerland) in a 12.5 μL total volume according to the manufacturer's instructions. For MHC I genes, locus non-specific primers were designed first, and PCR amplicons were sequenced using MinION (Oxford Nanopore, Oxford, United Kingdom). A bioinformatic sequence analysis then allowed distinguishing individual loci and determining their exon 2-exon 3 physical haplotype sequences as described in Plasil et al.²⁶ For MHC II genes, *Eqca-DRA*, *Eqca-DQA1* locus-specific primers were designed, and PCR amplicons were sequenced using an Illumina MiSeq (Illumina, San Diego, CA). Exon 2 haplotypes were determined based on reads fully matching the length of the

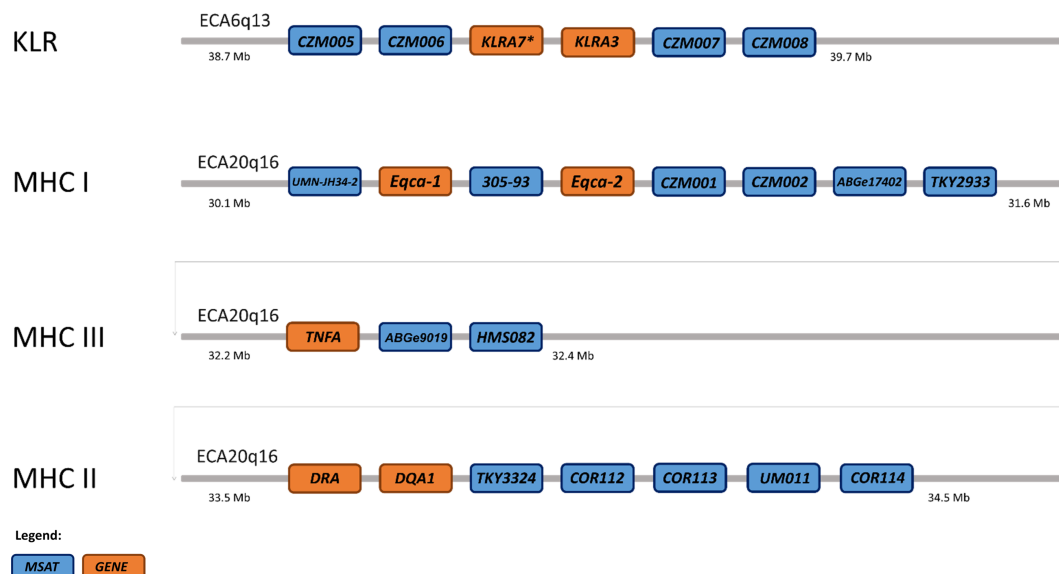


FIGURE 1 Analyzed microsatellite loci linked to the KLR (top), MHC I (upper middle), MHC III (lower middle) and MHC II (bottom) expressed genes; *gene not annotated in the EquCab3.0 genome, missing in some individuals.

reference exon 2 sequence. For MHC class I primers, primers were located in exons 1 and 4; for MHC class II primers, primers were located in introns 1 and 2. The list of the primers used is in Table S1.

3.2.4 | Genotyping of MHC III (TNFA) and MR genes

Primers used for *TNFA* and *MR1* amplification are in Table S1. The sequences covered the whole CDS in the *TNFA* gene and exons 2 and 3 of the *MR1* gene. Amplicons were obtained using PCR amplification mixes Elzyme RobHS Premix RED (Elisabeth Pharmacon, Brno, Czech Republic) for *TNFA* and Ultramix PCRBio (PCR Biosystems, London, UK) for *MR1*. All PCR protocols were done according to manufacturer's instructions. PCR volume was 12.5 μ L. Sequencing was performed on Illumina MiSeq. NGS reads were mapped against reference genome EquCab3.0. Variable positions (SNPs and indels) in the CDS were manually inspected in IGV software. Haplotypes were determined manually where possible.

3.2.5 | Microsatellite genotyping

Microsatellite loci distributed across the MHC and KLR regions were studied. Four KLR microsatellite loci were genotyped as described previously³⁰ while data on genotypes in 13 MHC loci were taken over from Plasil et al.²⁶ Their location within the MHC and KLR regions and their linkage relationships to expressed MHC and KLR are shown in Figure 1. Haplotypes were inferred using

the Arlequin 3.5 software (ELB algorithm). Due to possible differences in haplotype phasing between breed groups, haplotypes were inferred for each group separately. As for the NKR microsatellite loci, only microsatellite markers located within the KLR region were available for analysis.

3.3 | Parameters of genetic diversity and population characteristics

Numbers of synonymous/nonsynonymous SNPs, allele frequencies, and observed heterozygosities were calculated for all studied loci using standard Excel functions. The Hardy-Weinberg equilibrium was assessed using a web calculator (<http://www.dr-petrek.eu/documents/HWE.xls>). The characteristics were determined for each breed group separately and for all horses together. Linkage disequilibrium analysis was conducted in Arlequin 3.5.

3.4 | Frequencies of MHC/NKR haplotype combinations

The occurrence of specific combinations of MHC class I and NKR haplotypes was analyzed using the chi-square test of independence, Fisher's exact test and Pearson's goodness-of-fit test.^{32,33}

Calculations were performed for each breed separately. All possible combinations of MHC and NKR expressed and microsatellite loci, that is, MHC versus NKR expressed genes, MHC versus NKR microsatellites,

MHC expressed genes versus NKR microsatellite loci and vice versa, were analyzed. MHC class II (*DRA*, *DQA1*) and class III (*TNFA*) genes and microsatellites as well as *MR1*, a gene encoding an antigen presenting molecule located outside the MHC region, were included in the comparisons. The *KLRA3* gene was used for calculations involving MHC II and MHC III genes, as only this gene showed significant associations with MHC I.

Deviation from the null hypothesis that the observed MHC and NKR allelic haplotypes are independently combined in compound genotypes was tested for both the non-additive and the additive models. For the former, numbers of carriers of particular haplotypes were considered, for the latter, total numbers of particular haplotypes in the population were counted. Only haplotypes with frequencies higher than 0.1 were analyzed. If no such haplotype was found, the most frequent one was analyzed.

The deviation of observed frequencies of MHC/NKR compound genotype carriers from those expected for random combinations of individual NKR and MHC haplotypes was tested based on absolute frequencies of all four theoretically possible MHC/NKR combinations. The chi-square test of independence and/or the Fisher's exact test were then used to assess differences between expected and observed frequencies of carriers of compound genotypes. For both additive and non-additive models, observed relative haplotype frequencies were used in the Pearson's goodness-of-fit test to calculate expected relative frequencies. Expected absolute frequencies were then calculated from the values of the expected relative frequencies. All calculations were performed using the Excel functions CHITEST and CHIINV, in the case of the Fisher's exact test, the VassarStats web calculator was used (<http://vassarstats.net/tab2x2.html>). Bonferroni corrections for multiple comparisons were made. Compound genotypes significant at $p < 0.05$ for both additive and non-additive models were further analyzed using the SPSS software (www.ibm.com/spss); binomial logistic regression analyzes with MHC haplotypes as dependent variables and NKR haplotypes as independent variables were conducted. SPSS chi-square test was used to confirm results obtained with the Excel calculations. Phi coefficient of association was determined using the VassarStats web calculator as an indicator of the strength of significant associations.

4 | RESULTS

4.1 | Genetic diversity of expressed MHC class I and II genes

The data on genetic diversity detected in the MHC I (exon 2/3 allelic haplotypes) and MHC II (exon 2 allelic haplotypes) were taken over from Plasil et al.²⁶ as

summarized in Table 1. In 28 horses, we were unable, even with different sets of primers, to identify any of the known class I alleles. Since for those horses it was impossible to conduct the MHC class I/NKR association analysis, they were eliminated from the dataset.

The *EQCA-7* gene was excluded from further analyses due to the low number of successfully amplified and sequenced samples (13 samples in total; 9 of African, one of Camargue and 3 of Romanian horses). Complete information on MHC genotyping including data related to non-significant results are available in Plasil et al.²⁶

4.2 | Genetic diversity of expressed NKR genes

A total of 21 polymorphisms were found within the *KIR3DL* sequence (Table 2). Three of them showed frequencies higher than 0.1. Two of them were found in all three breeds, the third was present only in Romanian horses. A very low variability of the *KLRA7* gene was observed, with only one non-synonymous SNP found in exon 5. This gene was missing in some of horses. Associations with MHC genes were calculated for the presence/absence of the gene. The *KLRA3* gene with 17 SNPs was the most polymorphic NKR gene in terms of the numbers of polymorphisms per the sequence analyzed. Thirteen SNPs had frequencies higher than 0.1 in all three breeds.

All characteristics of the diversity of the breed groups analyzed are available in Supplementary File SF1. Haplotypes found in *KIR3DL* and *KLRA3* genes are in Table S2 and Supplementary File SF2.

4.3 | Genetic diversity of expressed MHC III and non-MHC antigen presenting genes

Eight SNPs were found within the *TNFA* gene (Table 3). Four SNPs were shared by all three breed groups, and three of them showed frequencies higher than 0.1 in the merged group. A total of 19 SNPs were detected within the *MR1* gene. Two SNPs were common to all breed groups with only one with frequency higher than 0.1.

Further characteristics of the diversity of the overall group analyzed are available in Supplementary File SF1. Haplotypes detected in *TNFA* and *MR1* genes are in Supplementary File SF2.

4.4 | MHC and NKR diversity defined by microsatellites

The numbers of alleles detected for microsatellite markers of the MHC and KLR regions are in Table 4.

TABLE 1 Selected parameters of genetic diversity within MHC I and MHC II genes (from Plasil et al.²⁶).

Gene	Number of polymorphisms	Non-synonymous	Number of alleles	Heterozygosity
<i>Eqca-1</i>	103	57	47	0.53
<i>Eqca-2</i>	49	38	42	0.79
<i>Eqca-7</i>	40	25	10	0.31
<i>Eqca-Ψ</i>	29	18	17	0.29
<i>DRA</i>	8	5	5	0.36
<i>DQA1</i>	59	42	20	0.53

TABLE 2 Diversity within NKR genes.

Gene	Number of polymorphisms	Non-synonymous	Number of haplotypes	Heterozygosity	Sequence analyzed
<i>KIR3DL</i>	21	15	3	0.04	CDS
<i>KLRA7</i>	1	1	0	0.3 ^a	Exon 4–6
<i>KLRA3</i>	17	10	17	0.24	Exon 4–6

^aGenotypes could not be determined in all individuals.**TABLE 3** Diversity within *TNFA* and *MR1* genes.

Gene	Number of polymorphisms	Non-synonymous	Number of haplotypes	Heterozygosity	Sequence analyzed
<i>TNFA</i>	8	0	4	0.18	Whole gene
<i>MR1</i>	19	1	4	0.04	Exon 2 to 3

TABLE 4 Numbers of alleles of MHC and KLR microsatellites.

Region	Microsatellite	Number of alleles
KLR	<i>CZM005</i>	4
KLR	<i>CZM006</i>	6
KLR	<i>CZM007</i>	2
KLR	<i>CZM008</i>	4
MHC I	<i>UMN-JH34-2</i>	15
MHC I	<i>305-93</i>	13
MHC I	<i>CZM001</i>	7
MHC I	<i>CZM002</i>	18
MHC I	<i>ABGe17402</i>	9
MHC I	<i>TKY2933</i>	11
MHC II	<i>TKY3324</i>	13
MHC II	<i>COR112</i>	14
MHC II	<i>COR113</i>	11
MHC II	<i>UM011</i>	12
MHC II	<i>COR114</i>	16
MHC III	<i>ABGe9019</i>	16
MHC III	<i>HMS082</i>	14

Detailed information on allele frequencies of these alleles in the breed populations is available in Table S3.

4.5 | MHC and NKR microsatellite haplotypes

Tested MHC and NKR microsatellite haplotypes are in Figure 2. A list of all microsatellite haplotypes detected is in Supplementary File SF2.

Three types of haplotypes covering areas of different extent could be constructed for MHC I microsatellite markers. MHC I haplotypes “HapA” comprising *CZM001* and *CZM002* microsatellites; “HapB” haplotype comprising *CZM001*, *CZM002*, *UMN-JH34-2*, and *305-93* microsatellites and “HapC” haplotype comprising *CZM001*, *CZM002*, *ABGe17402*, and *TKY2933* microsatellites. Two microsatellite MHC II haplotypes were identified. The “HapD” haplotype comprised the *COR113*, *UM011*, *COR114* loci, the “HapE” haplotype comprised the *TKY3324*, *COR112*, *UM011*, and *COR114* loci. The MHC III “HapF” haplotype contained the *ABGe9019* and *HMS082* loci. The “HapG” haplotype comprising all four analyzed markers (*CZM005*, *CZM006*, *CZM007*, *CZM008*) spans the entire KLR region.

The variability in KLR microsatellite loci was similar to the variability of the *KLRA3* gene. Four, six, two and four alleles of the *CZM005*, *CZM006*, *CZM007*, and *CZM008* loci, respectively, were identified in the individual microsatellites within the entire cohort of horses

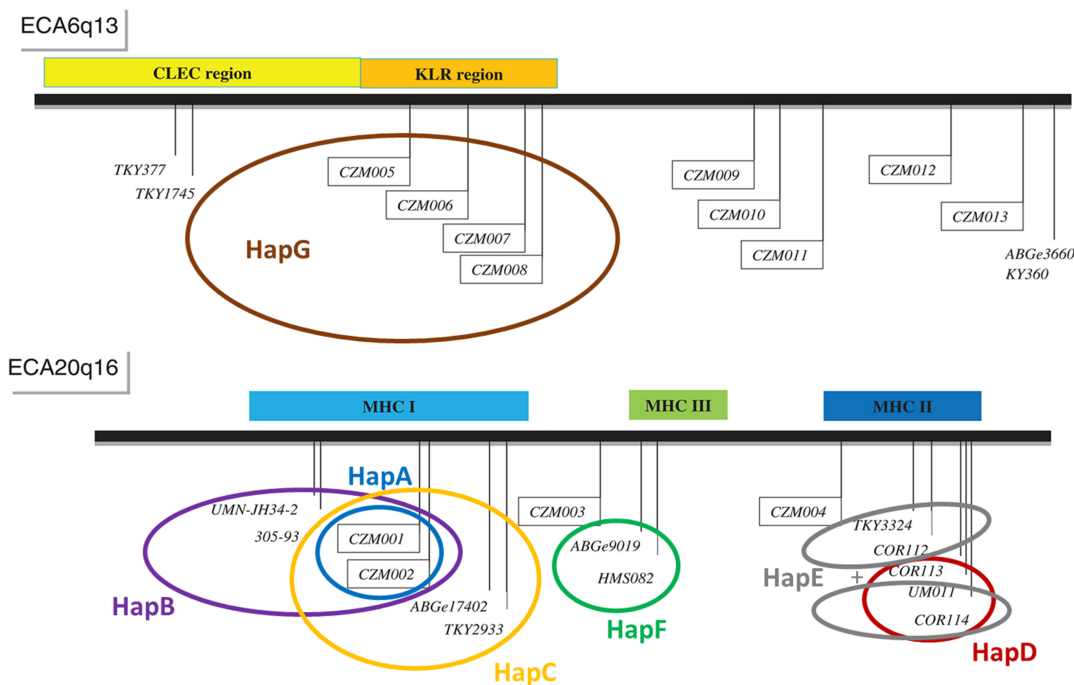


FIGURE 2 Microsatellite haplotypes identified in the MHC and KLR regions (adapted from Horecky et al. ³⁰).

TABLE 5 MHC versus NKR haplotypes associated at $P_{\text{corrected}} \leq 0.05$.

MHC gene	NKR gene	Breed	Combination MHC_NKR	Method	$P_{\text{corrected}}$	Phi coefficient
EQCA-1	KLRA3	Camargue	<i>Eqca-1</i> *022:01_3	Fisher exact test, A	0.039	−0.31
	KLRA3	African	<i>Eqca-1</i> *020:01_1	Fisher exact test, A	0.026	−0.44
EQCA-2	KLRA3	African	<i>Eqca-2</i> *003:05_3	Fisher exact test, NA	0.047	−0.76

Note: The haplotype identification refers always to the same sequence across the three breed groups.

Abbreviations: A, additive method; NA, non-additive method.

analyzed, while 6, 3 and 4 haplotypes were identified for the *KLRA3* exons 4, 5, and 6, respectively.

4.6 | Non-random MHC/NKR combinations

4.6.1 | Associations between haplotypes of expressed genes

MHC/NKR haplotypes associated at $p \leq 0.05$ after Bonferroni correction are shown in Table 5. Complete results of all calculations are available in Table S4. An overview of horse genotypes with highlighted associated combinations is available in SF3.

The results of the regression analysis based on data obtained at $p \leq 0.05$ along with calculations for

an insignificant MHC/NKR combination are in Table 6.

4.6.2 | Associations between microsatellite marker haplotypes

Combinations of MHC/NKR microsatellite marker haplotypes associated at $p \leq 0.05$ after Bonferroni correction are presented in Table 7. Since haplotypes were created for each breed population separately, an identical numerical identification does not refer to the same genotype in different breeding groups. Subscripts indicating the breed group were therefore added to the numerical identification (e.g., 1_C/1_R/1_A). SF2 contains information about particular haplotypes shared across the breed groups. As associations between MHC class II and III with KLR

MHC gene	NKR gene	Breed	Combination MHC_NKR	<i>p</i>
EQCA-1	KLRA3	Romanian	<i>Eqca-1*007:02_1</i>	0.012
	KLRA3	African	<i>Eqca-1*007:02_3</i>	0.001
	KLRA3	African	<i>Eqca-1*017:01_4</i>	0.011
EQCA-2	KLRA3	African	<i>Eqca-2*003:05_3</i>	<0.001
	KLRA3	African	<i>Eqca-2*001:05_11</i>	0.005
Negative control				
EQCA-1	KLRA3	Camargue	<i>Eqca-1*022:01_8</i>	0.468

TABLE 6 Linear regression analysis of selected MHC class I versus KLRA combinations.

Note: The haplotype identification refers always to the same sequence across the three breed groups.

TABLE 7 Associations between MHC and KLR microsatellite haplotypes at $P_{\text{corrected}} \leq 0.05$.

MHC haplotype	NKR haplotype	Breed	Combination MHC_NKR	Method	$P_{\text{corrected}}$	Phi coefficient
Combinations MHC class I_NKR						
HapA (<i>CZM001</i> + <i>CZM002</i>)	HapG	Romanian	3 _R _9 _R	Fisher exact test, NA	0.016	−0.68
HapB (<i>CZM001</i> + <i>CZM002</i> + <i>UMN-JH34-2</i> + <i>305-93</i>)	HapG	Camargue	26 _C _15 _C	Fisher exact test, NA	0.028	−0.55
HapC (<i>CZM001</i> + <i>CZM002</i> + <i>ABGe17402</i> + <i>TKY2933</i>)	HapG	Romanian ^F	16 _R _9 _R	Fisher exact test, A	0.002	−0.47
		Romanian ^F	16 _R _9 _R	Fisher exact test, NA	<0.001	−0.88
Combinations MHC class II_NKR						
HapD (<i>COR113</i> + <i>UM011</i> + <i>COR114</i>)	HapG	Camargue	12 _C _15 _C	Fisher exact test, A	0.029	−0.29
		Camargue	12 _C _15 _C	Fisher exact test, NA	0.027	−0.56
HapE (<i>TKY3324</i> + <i>COR112</i> + <i>UM011</i> + <i>COR114</i>)	HapG	Camargue	13 _C _15 _C	Fisher exact test, A	0.019	−0.29
		Camargue	13 _C _15 _C	Fisher exact test, NA	0.018	−0.56
Combinations MHC class III_NKR						
HapF (<i>ABGe9019</i> + <i>HMS082</i>)	HapG	Camargue	12 _C _15 _C	Fisher exact test, A	0.041	−0.27

Note: Subscripts C, R denotes the Camargue, Romanian. Superscript F denotes the frequency of the most common alleles in the population below 0.1 (0.06–0.08).

Abbreviations: A, additive method; NA, non-additive method.

markers were observed, an additional analysis of MHC class I and MHC class II/III expressed gene haplotypes was performed (see below for the results). Complete results are available in Table S5. A list of horses with highlighted associated combinations is available in SF3.

4.6.3 | Associations between haplotypes of expressed genes and microsatellites

Associations between expressed genes and microsatellite haplotypes at $p \leq 0.05$ after Bonferroni correction are in Table 8. Complete results are in Table S6.

Table 9 and Figure 3 provide a synopsis of all results obtained.

4.6.4 | Associations between MHC class I and MHC class II/III microsatellite haplotypes

Based on the finding of associations between NKR and MHC class II/III microsatellite haplotypes in the Camargue breed (15_C_12_C and 15_C_13_C in MHC II_NKR and 15_C_12_C in MHC III_NKR combinations; see Table 7), we analyzed linkage disequilibrium between MHC class I and MHC class II/III loci.

TABLE 8 Gene versus microsatellite combinations of significantly different frequency at $P_{\text{corrected}} \leq 0.05$.

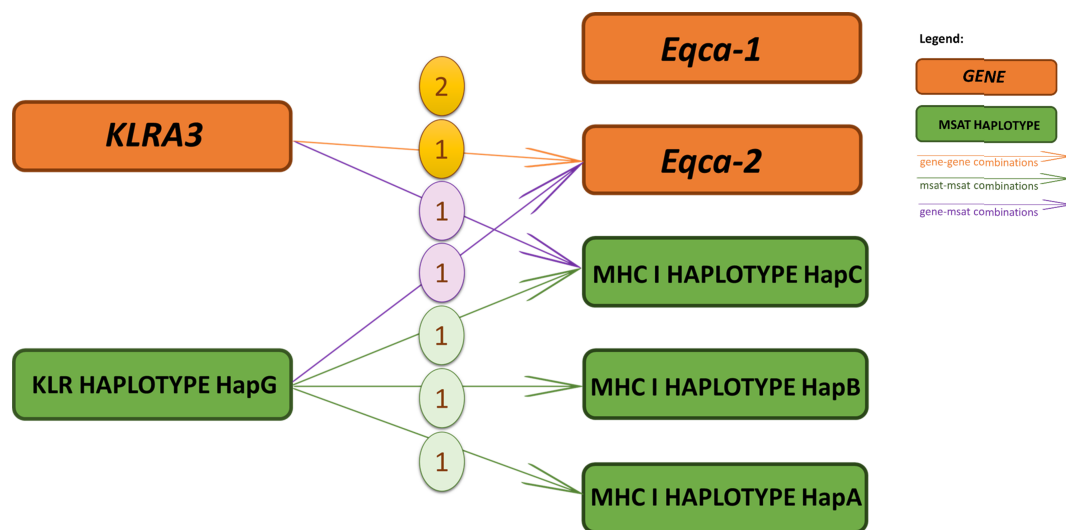
Gene	Microsatellites	Breed	Combination MHC_NKR	Method	$P_{\text{corrected}}$	Phi coefficient
Combinations MHC class I_NKR						
<i>EQCA-2</i>	HapG	Romanian	<i>Eqca-2*001:04_9_R</i>	Fisher exact test, NA	0.030	−0.6
<i>KLRA3</i>	HapC	Romanian ^F	16 _{R_3}	Fisher exact test, NA	0.022	−0.6
Combinations MHC class II_NKR						
<i>Eqca-DQA1</i>	HapG	Camargue	<i>Eqca-DQA*007:02_15_C</i>	Fisher exact test, A	0.030	−0.3

Note: Subscripts C, R denotes the Camargue, Romanian. Superscript F denotes the frequency of the most common alleles in the population below 0.1 (0.07). Abbreviations: A, additive method; NA, non-additive method.

TABLE 9 Significant MHC_NKR associations in all three horse breed groups.

Combination type	Numbers of results significant at $p \leq 0.05$ after BC			
	MHC I_NKR	MHC II_NKR	MHC III_NKR	MR1_NKR
Gene-gene	3	0	0	1
Msat-msat	3	2	1	NA
Gene-msat	2	1	0	NA

Abbreviations: BC, Bonferroni correction; NA, not available.

**FIGURE 3** Numbers of significant MHC I_NKR associations in all three horse breeds.

The chi-square test performed for all possible combinations of MHC I_MHC II microsatellite haplotypes confirmed a significant association for the combination 13_{C_12C} ($P_{\text{corrected}} = 0.01$). The same results were obtained in the case of MHC I_MHC III comparison, with the 13_{C_12C} combination showing a very significant result ($P_{\text{corrected}} = 0.0006$). The results for both the additive and non-additive method are shown in Table 10. The analysis of linkage disequilibrium (LD) between the MHC I-MHC III-MHC II loci by Arlequin 3.5 confirmed a strong LD between these loci (Table 11).

5 | DISCUSSION

Associations of individual haplotypes with different types of infections, autoimmune disorders or cancer have been reported separately for MHC I and NKR genes in humans.^{5,6,34,35} Due to the NKR/MHC receptor/ligand functional relationships, they evolved under the same selection constraints. Although MHC and NKR loci are located on different chromosomes, their advantageous combinations can be maintained in a population and inherited together under long-lasting selection pressures.^{33,36} The role of MHC-NKR interactions is also

TABLE 10 *P* values of control MHC I_MHC II and MHC I_MHC III calculations.

Combination		Additive method		Non-additive method	
		<i>P</i> _{corrected}	Phi coefficient	<i>P</i> _{corrected}	Phi coefficient
MHC I_MHC II	13 _C _12 _C	0.02	−0.3	0.01	−0.62
MHC I_MHC III	13 _C _12 _C	0.004	−0.35	<0.0001	−0.74

TABLE 11 Linkage disequilibrium test results by Arlequin.

Loci in comparison	<i>p</i>	χ^2 test value
MHC I versus MHC III	<0.000001	247.8
MHC I versus MHC II	<0.000001	205.8
MHC III versus MHC II	<0.000001	200.1

essential in the process of NK cell education²² Interactions of NK cell receptor genes and of gene encoding their MHC class I ligands both at the molecular and population level have been well established in humans,³⁷ and were interpreted in terms of a coevolution between KIR and MHC genes.^{24,32,33} Compound MHC/KIR genotypes revealed differences in their adaptive values documented by association studies of specific MHC-NKR combinations.^{38,39} Specific combinations of maternal KIR and paternal HLA-C are also important for a successful pregnancy.⁴⁰

Taking into consideration the high interspecific variability in the genomic organization and expression of NKR genes, and the resulting specificity of their biological roles in primates,⁴¹ no extrapolation of these findings to other mammalian species is possible. While some information is known about associations between infections and individual MHC or NKR genes in other well-studied mammalian species,^{7,8} their co-evolution has not been studied so far in species other than primates.^{21,22} In this situation, population studies looking for associations among specific MHC and NKR genes and/or alleles represent an alternative approach.^{32,33}

Therefore, our aim was to identify compound MHC/NKR genotypes occurring in frequencies different from theoretical expectations based upon the assumption of independent assortment of individual haplotypes. Multiple MHC/NKR genotypes composed of various combinations of expressed gene and microsatellite haplotypes were found to be non-randomly associated across the three horse breed groups studied. Since we were primarily interested about the MHC sub-regions, with class I as the candidate and class II and III as controls, intra-MHC sub-haplotypes were inferred for this purpose. The most significant associations were found between the *KLRA3* gene in combination with the *EQCA-1* or *EQCA-2* genes when accounting for multiple tests; all significant

combinations contained the haplotype of the *KLRA3* gene. *KLRA3* belongs to the group of expanded and expressed horse Ly49 genes originally identified by Takahashi et al.²⁷ Their expansion suggests their potential functional importance for the horse immune and/or reproductive system. Transcriptome analysis performed by Todd et al.¹⁹ found expression of the *KLRA3* gene in equine trophoblast tissue from day 23 of development implying that this gene is important for successful embryo implantation. The non-random combination of *KLRA3* with putative MHC class I ligands supports this assumption. On the other hand, no significant combinations were found for *KIR3DL*, despite its relatively high level of variability comparable to *KLRA3*. It is not clear whether this gene could have a different function in horse immune responses. This also might be an explanation for the low polymorphism observed in the *KLRA7* gene with a single SNP.

As expected, no deviations from theoretical assumptions were detected for various NKR combinations with MHC class II, MHC class III expressed genes, although these calculations always involved the *KLRA3* gene associated with MHC class I genotypes. On the other hand, associations between KLR microsatellite loci with microsatellites located in the MHC class II and MHC class III regions were observed and subsequently confirmed by associations between MHC class I and MHC class II/III microsatellite haplotypes. As MHC class II/III molecules are not ligands for NK cell receptors, these associations are probably secondary, due to a linkage disequilibrium detected between the MHC class I and MHC class II/III genomic regions and to the existence of extended haplotypes, observed in multiple species.^{42,43} The association of *KLRA3* with *MR1*, a non-MHC gene, where observed frequencies of a particular compound genotype were lower than was the theoretical expectation might be a false positive result due to a low absolute number of horses with this specific genotype. On the other hand, it also could be due to functional interactions between NK cells and MR1 restricted lymphocytes observed in mice and humans.^{44,45}

Most of the significant MHC/NKR associations concerned compound genotypes different between the three breeds. However, certain individual haplotypes have been

detected in significant, although different, combinations across breeds (e.g., the *KLRA3* gene haplotype 1 was found associated with MHC in the Romanian and African breed groups; for more examples see Table S4). These findings could reflect overall different selection pressures in the areas of their origin. However, other explanations, such as a sampling bias or founder effect, are possible. Similarly, our detailed analysis of the MHC variability of the same breed groups showed differences in the allelic contents and frequencies among them. Although some haplotypes were specific for one breed, no breed-specific clustering was observed.²⁶

There are some limitations for the interpretation of the data obtained such as low numbers of horses, selection of expressed genes for this analysis and/or the lack of knowledge of their functions. Low numbers of horses available for the study are due to ethical, logistic, and legal limitations of sampling horse populations from specific areas, including free-ranging horses. These demands are permanently increasing over time; we were thus limited to archived samples collected for other purposes.

For the same reason, no pedigree data were available for these particular groups. Therefore, homo/heterozygosity could be determined only from sequencing data. The existence of horses that could not be genotyped indicated the presence of non-identified alleles. This could explain an apparent increase of homozygosity in the two MHC class I genes. Due to a non-recognition of some alleles, some individuals might be identified as homozygous although in fact, they are heterozygous for the “null” allele. Still, the overall frequency of *Eqca-1* heterozygotes (0.53) was similar to heterozygosities of MHC class I microsatellite loci (0.45–0.73; based on data by Plasil et al.²⁶). Some horses also might miss the *Eqca-1* locus, but the current knowledge of equine MHC class I genes did not allow to address this issue.

In terms of the association study, two MHC class I alleles showing the most significant associations were identified as homozygous in three horses for *Eqca-1*022:01*, and one homozygous and two heterozygous horses were identified for *Eqca-1*020:01*. For comparison, two non-associated alleles *Eqca-1*007:02* and *Eqca-1*011:01* were recognized in 5 homozygous and 6 heterozygous horses, and in 3 homozygous and 6 heterozygous horses, respectively. The same calculations including the null allele, performed for both the additive and non-additive models with the associated combinations *Eqca-1*022:01_KLRA3_3* and *Eqca-1*020:01_KLRA3_1*, produced similar results, showing $p_{\text{uncorr}} = 0.0048$ and 0.0025, respectively. It thus does not seem that the presence of a null allele influenced the results of the association analyses.

Despite the low numbers of horses analyzed, different statistical approaches used for testing the same hypotheses gave similar results significant even after Bonferroni corrections; some of them showed strong correlations as assessed by the phi coefficient. In this context, the data obtained look consistent and are biologically plausible since MHC class I genes and microsatellites were primarily associated with NKR genes and/or microsatellites. Expressed loci selected for this analysis do not necessarily represent the entire variability of the NKR and MHC genomic regions. The addition of microsatellite markers documenting their overall variability³⁰ contributed to a more informative context for the data interpretation. However, the almost absolute lack of knowledge of the functions of NK cell receptors and no information on their putative MHC ligands in horses remains a challenge for future studies.

6 | CONCLUSIONS

- A so far unrecognized variability in NKR genes of three domestic horse breeds was reported;
- Non-random occurrence of several MHC/NKR compound genotypes was observed in the three breeds suggesting their adaptive value;
- The most variable *KLRA3* gene was involved in all MHC/NKR associations, which suggests its role in interactions with MHC class I ligand;
- Little or no information on the functions of expressed NKR genes and no information on MHC class I molecules that could be used as NKR ligands represent a major limitation for the interpretation of the data obtained and a major challenge for future studies in this field.

AUTHOR CONTRIBUTIONS

JB: Data curation; population statistics and association analyses; writing—original draft; editing. **MP:** Laboratory—genotyping of MHC class I genes; NGS sequencing; writing—review and editing. **JF:** Laboratory—genotyping of NKR genes; writing—review and editing. **KS:** Laboratory—genotyping of *MR1* and *TNFA* genes; writing—review and editing. **JO:** NGS data curation; writing—review and editing. **MK:** Laboratory—genotyping of MHC class II genes; writing—review and editing. **PB:** Conceptualization; supervision; project administration; writing—review and editing. **FS:** Consulting statistical procedures; writing—review and editing. **PH:** Conceptualization; resources; supervision; project administration; funding acquisition; writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

The authors thank Michaela Cvanova for consulting the statistical calculation procedure and Drs. A. Mihalca, A. Leblond, G. D'Amico for sharing blood samples. Open access publishing facilitated by Veterinarni univerzita Brno, as part of the Wiley - CzechELib agreement.

FUNDING INFORMATION

The project was supported by the institutional projects of VETUNI Brno; Ceitec/Horin/ITA/2020 and DSP_2022_4.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All peripheral blood samples used for DNA extraction were originally collected during standard diagnostic and/or therapeutic procedures unrelated to this study. The work was conducted in compliance with all national and international ethical and legal standards for animal welfare by licensed veterinarians, based on owners' consent. Therefore, in agreement with its rules, no special approval of the Committee for Animal Welfare and Ethics of the University of Veterinary Sciences Brno was needed for working on shared, archived, and re-used samples.

ORCID

Jana Bubenikova  <https://orcid.org/0000-0002-8470-8326>

Martin Plasil  <https://orcid.org/0000-0003-4721-5685>

Jan Futas  <https://orcid.org/0000-0002-2847-879X>

Karla Stejskalova  <https://orcid.org/0000-0002-4372-1508>

Marie Klumplerova  <https://orcid.org/0000-0001-7113-1962>

Jan Oppelt  <https://orcid.org/0000-0002-3076-4840>

Franz Suchentrunk  <https://orcid.org/0000-0001-9862-5591>

Pamela A. Burger  <https://orcid.org/0000-0002-6941-0257>

Petr Horin  <https://orcid.org/0000-0002-5548-4212>

REFERENCES

- Akkaya M, Barclay AN. How do pathogens drive the evolution of paired receptors? *Eur J Immunol*. 2013;43(2):303-313. doi:10.1002/eji.201242896
- Tizard IR. Chapter 7 – The mammalian major histocompatibility complex. In: Tizard IR, ed. *Comparative Mammalian Immunology. Developments in Immunology*. Academic Press; 2023:89-99. doi:10.1016/B978-0-323-95219-4.00011-3
- Murphy K, Weaver C. Janeway's Immunobiology. 9th ed. *Garland Science*. Taylor & Francis Group; 2017.
- Trowsdale J, Knight JC. Major histocompatibility complex genomics and human disease. *Annu Rev Genomics Hum Genet*. 2013;14(1):301-323. doi:10.1146/annurev-genom-091212-153455
- Debebe BJ, Boelen L, Lee JC, et al. Identifying the immune interactions underlying HLA class I disease associations. *eLife*. 2020;9:e54558. doi:10.7554/eLife.54558
- Gao X, Nelson GW, Karacki P, et al. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. *N Engl J Med*. 2001;344(22):1668-1675. doi:10.1056/NEJM200105313442203
- Takeshima S-n, Sasaki S, Meripet P, Sugimoto Y, Aida Y. Single nucleotide polymorphisms in the bovine MHC region of Japanese Black cattle are associated with bovine leukemia virus proviral load. *Retrovirology*. 2017;14:24. doi:10.1186/s12977-017-0348-3
- Rastislav M, Mangesh B. BoLA-DRB3 exon 2 mutations associated with paratuberculosis in cattle. *Vet J*. 2012;192(3):517-519. doi:10.1016/j.tvjl.2011.07.005
- Minias P, Vinkler M. Selection balancing at innate immune genes: adaptive polymorphism maintenance in toll-like receptors. *Mol Biol Evol*. 2022;39(5):msac102. doi:10.1093/molbev/msac102
- Niskanen AK, Kennedy LJ, Ruokonen M, et al. Balancing selection and heterozygote advantage in major histocompatibility complex loci of the bottlenecked Finnish wolf population. *Mol Ecol*. 2014;23(4):875-889. doi:10.1111/mec.12647
- Rock KL, Reits E, Neefjes J. Present yourself! By MHC class I and MHC class II molecules. *Trends Immunol*. 2016;37(11):724-737. doi:10.1016/j.it.2016.08.010
- Trowsdale J. Genetic and functional relationships between MHC and NK receptor genes. *Immunity*. 2001;15(3):363-374. doi:10.1016/S1074-7613(01)00197-2
- Sambrook JG, Beck S. Evolutionary vignettes of natural killer cell receptors. *Curr Opin Immunol*. 2007;19(5):553-560. doi:10.1016/j.coi.2007.08.002
- Brown MG, Scalzo AA. NK gene complex dynamics and selection for NK cell receptors. *Semin Immunol*. 2008;20(6):361-368. doi:10.1016/j.smim.2008.06.004
- Kelley J, Walter L, Trowsdale J. Comparative genomics of natural killer cell receptor gene clusters. *PLoS Genet*. 2005;1(2):129-139. doi:10.1371/journal.pgen.0010027
- Prugnolle F, Manica A, Charpentier M, Guégan JF, Guernier V, Balloux F. Pathogen-driven selection and worldwide HLA class I diversity. *Curr Biol*. 2005;15(11):1022-1027. doi:10.1016/j.cub.2005.04.050
- Khor CC, Hibberd ML. Host-pathogen interactions revealed by human genome-wide surveys. *Trends Genet*. 2012;28(5):233-243. doi:10.1016/j.tig.2012.02.001
- Orgul G, Dalva K, Dalva-Aydemir S, et al. Significance of inhibitory maternal killer-cell immunoglobulin-like receptor (KIR) and fetal KIR ligand genotype combinations in placenta related obstetric complications. *J Reprod Immunol*. 2021;148:103425. doi:10.1016/j.jri.2021.103425
- Todd ET, Thomson PC, Hamilton NA, et al. A genome-wide scan for candidate lethal variants in Thoroughbred horses. *Sci Rep*. 2020;10:13153. doi:10.1038/s41598-020-68946-8

20. Guethlein LA, Norman PJ, Hilton HG, Parham P. Co-evolution of MHC class I and variable NK cell receptors in placental mammals. *Immunol Rev*. 2015;267(1):259-282. doi:[10.1111/imr.12326](https://doi.org/10.1111/imr.12326)
21. de Groot NG, Blokhuis JH, Otting N, Doxiadis GGM, Bontrop RE. Co-evolution of the MHC class I and KIR gene families in rhesus macaques: ancestry and plasticity. *Immunol Rev*. 2015;267(1):228-245. doi:[10.1111/imr.12313](https://doi.org/10.1111/imr.12313)
22. Wroblewski EE, Parham P, Guethlein LA. Two to tango: co-evolution of hominid natural killer cell receptors and MHC. *Front Immunol*. 2019;10:177. doi:[10.3389/fimmu.2019.00177](https://doi.org/10.3389/fimmu.2019.00177)
23. Kelley J, Trowsdale J. Features of MHC and NK gene clusters. *Transpl Immunol*. 2005;14(3-4):129-134. doi:[10.1016/j.trim.2005.03.001](https://doi.org/10.1016/j.trim.2005.03.001)
24. Pollock NR, Harrison GF, Norman PJ. Immunogenomics of killer cell immunoglobulin-like receptor (KIR) and HLA class I: coevolution and consequences for human health. *J Allergy Clin Immunol Pract*. 2022;10(7):1763-1775. doi:[10.1016/j.jaip.2022.04.036](https://doi.org/10.1016/j.jaip.2022.04.036)
25. Kalbfleisch TS, Rice ES, DePriest MS, et al. EquCab3, an Updated Reference Genome for the Domestic Horse. 306928. Published online April 25 2018. doi:[10.1101/306928](https://doi.org/10.1101/306928)
26. Plasil M, Oppelt J, Klumplerova M, et al. Newly identified variability of the antigen binding site coding sequences of the equine major histocompatibility complex class I and class II genes. *HLA*. 2023;102(4):489-500. doi:[10.1111/tan.15078](https://doi.org/10.1111/tan.15078)
27. Takahashi T, Yawata M, Raudsepp T, et al. Natural killer cell receptors in the horse: evidence for the existence of multiple transcribed LY49 genes. *Eur J Immunol*. 2004;34(3):773-784. doi:[10.1002/eji.200324695](https://doi.org/10.1002/eji.200324695)
28. Futas J, Horin P. Natural killer cell receptor genes in the family *Equidae*: not only Ly49. *PloS One*. 2013;8(5):e64736. doi:[10.1371/journal.pone.0064736](https://doi.org/10.1371/journal.pone.0064736)
29. Futas J, Oppelt J, Janova E, Musilova P, Horin P. Complex variation in the KLRA (LY49) immunity-related genomic region in horses. *HLA*. 2020;96(3):257-267. doi:[10.1111/tan.13939](https://doi.org/10.1111/tan.13939)
30. Horecky C, Horecka E, Futas J, Janova E, Horin P, Knoll A. Microsatellite markers for evaluating the diversity of the natural killer complex and major histocompatibility complex genomic regions in domestic horses. *HLA*. 2018;91(4):271-279. doi:[10.1111/tan.13211](https://doi.org/10.1111/tan.13211)
31. Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour*. 2010;10(3):564-567. doi:[10.1111/j.1755-0998.2010.02847.x](https://doi.org/10.1111/j.1755-0998.2010.02847.x)
32. Fasano ME, Rendine S, Pasi A, et al. The distribution of KIR-HLA functional blocks is different from North to South of Italy. *Tissue Antigens*. 2014;83(3):168-173. doi:[10.1111/tan.12299](https://doi.org/10.1111/tan.12299)
33. Guinan KJ, Cunningham RT, Meenagh A, et al. Signatures of natural selection and coevolution between killer cell immunoglobulin-like receptors (KIR) and HLA class I genes. *Genes Immun*. 2010;11(6):467-478. doi:[10.1038/gene.2010.9](https://doi.org/10.1038/gene.2010.9)
34. Pelak K, Need AC, Fellay J, et al. Copy number variation of KIR genes influences HIV-1 control. *PLoS Biol*. 2011;9(11):e1001208. doi:[10.1371/journal.pbio.1001208](https://doi.org/10.1371/journal.pbio.1001208)
35. Chaisri S, Jayaraman J, Mongkolsapaya J, et al. KIR copy number variations in dengue-infected patients from northeastern Thailand. *Hum Immunol*. 2022;83(4):328-334. doi:[10.1016/j.humimm.2022.01.005](https://doi.org/10.1016/j.humimm.2022.01.005)
36. Kulminski AM. Complex phenotypes and phenomenon of genome-wide inter-chromosomal linkage disequilibrium in the human genome. *Exp Gerontol*. 2011;46(12):979-986. doi:[10.1016/j.exger.2011.08.010](https://doi.org/10.1016/j.exger.2011.08.010)
37. Mora-Bitria L, Asquith B. Innate receptors modulating adaptive T cell responses: KIR-HLA interactions and T cell-mediated control of chronic viral infections. *Immunogenetics*. 2023;75(3):269-282. doi:[10.1007/s00251-023-01293-w](https://doi.org/10.1007/s00251-023-01293-w)
38. Hollenbach JA, Pando MJ, Caillier SJ, Gourraud PA, Oksenberg JR. The killer immunoglobulin-like receptor KIR3DL1 in combination with HLA-Bw4 is protective against multiple sclerosis in African Americans. *Genes Immun*. 2016;17(3):199-202. doi:[10.1038/gene.2016.5](https://doi.org/10.1038/gene.2016.5)
39. Khakoo SI, Thio CL, Martin MP, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science*. 2004;305(5685):872-874. doi:[10.1126/science.1097670](https://doi.org/10.1126/science.1097670)
40. Hiby SE, Apps R, Chazara O, et al. Maternal KIR in combination with paternal HLA-C2 regulate human birth weight. *J Immunol*. 2014;192(11):5069-5073. doi:[10.4049/jimmunol.1400577](https://doi.org/10.4049/jimmunol.1400577)
41. Parham P, Abi-Rached L, Matevosyan L, et al. Primate-specific regulation of natural killer cells. *J Med Primatol*. 2010;39(4):194-212. doi:[10.1111/j.1600-0684.2010.00432.x](https://doi.org/10.1111/j.1600-0684.2010.00432.x)
42. Stenzel A, Lu T, Koch WA, et al. Patterns of linkage disequilibrium in the MHC region on human chromosome 6p. *Hum Genet*. 2004;114(4):377-385. doi:[10.1007/s00439-003-1075-5](https://doi.org/10.1007/s00439-003-1075-5)
43. Almawi WY, Nemr R, Finan RR, Saldhana FL, Hajjej A. HLA-A, -B, -C, -DRB1 and -DQB1 allele and haplotype frequencies in Lebanese and their relatedness to neighboring and distant populations. *BMC Genomics*. 2022;23(1):456. doi:[10.1186/s12864-022-08682-7](https://doi.org/10.1186/s12864-022-08682-7)
44. Jiang X, Peng Y, Liu L, et al. MAIT cells ameliorate liver fibrosis by enhancing the cytotoxicity of NK cells in cholestatic murine models. *Liver Int Off J Int Assoc Study Liver*. 2022;42(12):2743-2758. doi:[10.1111/liv.15445](https://doi.org/10.1111/liv.15445)
45. Vacchini A, Chancellor A, Spagnuolo J, Mori L, De Libero G. MR1-restricted T cells are unprecedented cancer fighters. *Front Immunol*. 2020;11: 751. Accessed July 28, 2023. <https://www.frontiersin.org/articles/10.3389/fimmu.2020.00751>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bubenikova J, Plasil M, Futas J, et al. Diversity of major histocompatibility complex (MHC) and natural killer cell receptor (NKR) genes and their interactions in domestic horses. *HLA*. 2024;103(2):e15387. doi:[10.1111/tan.15387](https://doi.org/10.1111/tan.15387)