EXTENSION OF THE MICROSATELLITE PANEL FOR DIVERSITY STUDIES IN THE EQUINE *Ly49* GENES REGION

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Abstract: The genetic variability and different expression of genes for receptors underlies functional variability of individual natural killer cells (NK). Like in the mouse model, the Ly49 receptors on the horse NK cells are believed to bind MHC class I molecules of target cells. Six *Ly49* genes constitute a gene family located on the horse chromosome 6, between 38 200 Kbp – 38 520 Kbp. Immune-response genes represent a functionally important region of the vertebrate genome subject to selection pressure. NK cells are involved in the antigen recognition process through their highly variable receptors. Our work may contribute to better estimating the genetic diversity of this functionally important region. In this work, we identified and genotyped three new polymorphic microsatellite markers that expand the original panel of microsatellites and are located in the *Ly49* region. This methodology will be used for assessment genetic diversity and association analyses with selected diseases of horses.

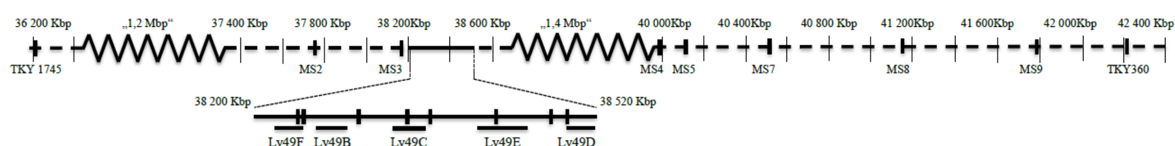
Key Words: NKR, Ly49, microsatellite, genetic diversity, horse

INTRODUCTION

Immune cells have evolved to possess a vast repertoire of cell surface receptors recognizing a diverse array of ligands expressed on the surface of normal as well as abnormal and infected cells (Hirano et al. 2011).Natural killer cells express cell surface receptors that recognize class I major histocompatibility complex (MHC-I) molecules to distinguish between healthy and unhealthy cells. The multigenic and polymorphic nature of the MHC-I genes have influenced convergent evolution of similarly polymorphic and diversified NK cell receptor families: leucine-rich repeat modules (Ly49) in mice, assemblage of immunoglobulin domains (KIR) in human or Ly49 with KIR receptors in horses (Futas, Horin 2013; Parham 2015, Rahim, Makrigiannis 2015). Interactions of different combinations of NK receptors and MHC class I molecules may contribute significantly to selection and disease resistance (Kelley et al. 2005).

Radiation hybrid mapping and fluorescence *in situ* hybridization localized horse *Ly49* genes to chromosomes 6q13 (Figure 1) (Takahashi et al. 2004).

Figure 1 Schema of the NKR region on equine chromosome 6 including Ly49 genes



Domestic mammals represent suitable models for evolutionary biology in general. Among them, the family Equidae consisting of a single genus, *Equus* with different free-living and domesticated species exposed to a variety of pathogens in different habitats is a suitable model for analyzing diversity and evolution of immunity-related genes. It is a rapidly evolving mammalian family, both

at the karyotype and molecular level. Therefore, the Equidae might also be interesting models for studying evolution of NKR and *Ly49* genes (Futas, Horin 2013).

The aim of this work is the extension of the panel of genetic markers (microsatellites) (Horecky et al. 2014) to study the genetic diversity of *Ly49* genes family and natural killer cell receptor (NKR) region. Selected microsatellites will also be used to characterize the genetic variability *Ly49* genes and NKR region in selected populations and for association analysis of selected diseases in horses.

MATERIAL AND METHODS

Animals and their DNA

48 individuals from six populations of different horse breeds (Hucul, Czech Warmblood, Danish Warmblood, Quarterhorse, American Miniature Horse and Andalusian horse) were genotyped. Samples of isolated DNA were provided from DNA bank of Genomic laboratory, Department of Animal Morphology, Physiology and Genetics, Mendel University in Brno.

Microsatellite markers and primers

The whole genome sequences of six horses (Orlando et al. 2013) in the areas of *Ly49* gene family and adjacent parts (35–43 Mbp) and database NCBI Map Viewer were used to select markers *in silico*. Suitable panel of microsatellites for the study of Ly49 region was selected *in silico* by the number of repeats in available horse whole genome sequences. Only microsatellites with the highest number of alleles were selected.

Primers were designed using the OLIGO software v4.0 (Molecular Biology Insights, Inc., Colorado Springs, CO, USA).

Fragmentation analysis for selection of microsatellites

Eight markers were designed and tested using a fragment analysis with fluorescently labelled nucleotides (fdCTP) on the panel of horse breeds (Hucul, Czech Warmblood, Danish Warmblood, Quarterhorse, American Miniature Horse, Andalusian horse and Camargue).

Three markers that showed the highest variability in the test panel of animals were selected. This set of markers was subsequently tested using fluorescent fragment analysis on genetic analyser ABI PRISM 3500 (Life Technologies, Corp., Carlsbad, USA). The obtained data were analysed in GeneMapper software v4.1 (Life Technologies, Corp., Carlsbad, USA).

RESULTS AND DISCUSSION

The total of 8 microsatellite markers was selected *in silico* into gene family Ly49 region (Table 1). The pilot testing was performed using 6 breeds DNA specimens from the DNA bank of the Department of Animal Morphology, Physiology and Genetics of Mendel University in Brno. For subsequent research there were only 3 markers selected, due to the lack of polymorphism in the next 5 markers. Allele frequencies of polymorphic microsatellite markers are summarized in Table 2.

Table 1 New microsatellites in Ly49 region

Marker	Repetition	Range of amplicon (bp)	Number of identified alleles
Ly49F_MS1	$(TAAA)_n$	**monomorphic**	
Ly49F_MS2	$(CAAA)_n$	**monomorphic**	
Ly49B_MS3	$(TAAA)_n$	**monomorphic**	
Ly49C_MS4	$(TA)_n$	**monomorphic**	
Ly49C_MS5	$(TA)_n$	234-236	2
Ly49E_MS6	$(TA)_n$	240-242	2
Ly49E_MS7	$(TAAA)_n$	**monomorphic**	
Ly49E_MS8	$(CA)_n$	261-271	6

Table 2 Allele frequencies of polymorphic markers

Marker	Allele	Frequencies of alleles
Ly49C_MS5	234	0.09
	236	0.91
Ly49E_MS6	240	0.09
	242	0.91
Ly49E_MS8	261	0.23
	263	0.04
	265	0.39
	267	0.24
	269	0.02
	271	0.08

The three polymorphic markers will extend the original eight microsatellite panel, which will be applied to test genetic diversity of populations (Horecky et al., In preparation) and association analyzes of particular Horses Diseases (Futas et al., In preparation). Although two of the markers seem to be biallelic, they will be used to describe the genetic diversity and association analyzes. The relevance is the same as in single nucleotide polymorphisms (SNPs), which are also biallelic (Fernandez et al. 2013).

CONCLUSION

This work extends the number of genetic markers for analysis of Ly49 NK cell receptors genetic variability. We enriched the set of previously described alleles, characterized by single nucleotide polymorphisms (SNPs) (Takahashi et al. 2004) and microsatellites (MSATs) (Horecky et al. 2014) of *Ly49* genes by three utilizable microsatellites. Combined genotyping of SNPs and MSATs may help to define haplotypes of *Ly49* genes. Haplotypes may be more informative for describing the genetic variability in this functionally significant and important part of the immune system.

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