COMMUNICATION

Diversity of equine major histocompatiblity complex class II DRA locus in Posavina and Croatian Coldblood horse: a new polymorphism detected

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ABSTRACT: Domestic equidae display polymorphism within ELA-DRA locus which is not characteristic for other species. We characterised sequence polymorphism present at ELA-DRA locus exon 2 and estimated allele frequencies in two autochthonous breeds, Posavina and Croatian Coldblood. In 88 horses, four different alleles were found, one of them not reported before in horses. The new allele shows non-synonymous mutation at position 65 (T→A) causing amino acid change (Phe→Tyr) in antigen binding site and synonymous mutation at position 105 (C→T). Our findings emphasize the importance of DRA polymorphism among equids and some specific DRA frequency pattern potentially specific in draught horses.

Key words: DRA, SNP, Draught horse.

Introduction - The vertebrate major histocompatiblility complex (MHC) contains genes important for the immune response. They encode cell-surface glycoproteins that bind and present antigens to T helper cells. The MHC family includes two major subfamilies, class I molecules present on all nucleated cells where they bind endogenously derived peptides and class II molecules present on antigen-presenting cells where they bind foreign peptides derived from extracellular parasites. High levels of diversity at MHC loci increase ability of MHC receptor to bind different antigen peptides.

Variability at MHC reflects relevant evolutionary and adaptive processes within and among populations which are of interest for evolutionary ecology and conservation. The equine major histocompatiblity complex class II DRA locus (ELA-DRA) encodes the alpha unit of a heterodimer DR receptor. While there is little or no variation at DRA locus in other species, equids are characteristic for the presence of DRA polymorphism (Albright-Fraser *et al.*, 1996; Brown *et al.,* 2004) and the most variable region of the ELA-DRA receptor is its peptide binding region (PBR), which is encoded by exon 2. Four ELA-DRA alleles have been characterized in horses, namely; DRA*0101, DRA*0201, DRA*0301 and DRA*JBH11.

Our objective was to characterise sequence polymorphism present at ELA-DRA locus

exon 2 and to estimate allele frequencies in PH (Posavina) and CCH (Croatian Coldblood) horses.

Material and methods - Blood samples were collected from PH (n=55) and CCH (n=33). The breed membership was based on microsatellite data and analysis performed in Druml *et al.* (2007) with q>50% (STRUCTURE). DNA was isolated from whole blood using a standard chloroform extraction. The exon 2 was amplified using primers Be3 (5'-GCTTCTCATC-CTAGTTCCCTT-3') and Be4 (5'-GCCTAGGAGTGCAGCAGA-3') according to Albright-Fraser *et al.* (1996). PCRs were performed in a 25µL volume containing 0.4µM of each primer and using Promega PCR master Mix according to manufacturer's protocol. Thermal cycling conditions comprised of an initial denaturation at 94º for 2min, 30 cycles composed of denaturation at 94º for 30sec., annealing at 57º for 1min., extension at 72º for 1min., and final extension at 72º for 10min. PCR products were visualized in 1% agarose gels stained with SYBR Safe (Invitrogen). PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega) and directly sequenced using ABI dye-terminator method with Be4 primer. To confirm the new allele, in one individual, sequencing was performed in both directions (with primers Be4 and Be3), from two independent PCRs. We estimated paternal haplotypes (alleles) for each individual using a Bayesian approach. From estimated haplotypes, we calculated frequencies of alleles and tested deviation from HWE (Hardy-Weinberg equilibrium). All calculations were done by PROC Haplotype and PROC Allele implemented in SAS/Genetics 9.1.3. (SAS Institute, Cary, NC).

Results and conclusions - Distribution of allele frequencies, observed heterozygosity, allelic diversity and HWE test across two populations are shown in Table 1. While frequencies of alleles DRA*0101 and DRA*0201 were comparable with other breeds, we did not found horse specific allele DRA*301 which, except Sorraia, is present in all other breeds (Albright-Fraser *et al*., 1996; Curik *et al*., 2003; Brown *et al*., 2004; Luis *et al*., 2005). The frequencies of DRA*JBH11 observed in PH (14.1%) and CCH (12.1%) were similar to Arab and Cob horses but much higher than those observed in other breeds (Brown *et al*., 2004).

A new allele was found as a heterozygote in four horses and it was named DRA*Hld105

Table 1. DRA allele frequencies (±SE), observed heterozygosity (Ho) and allelic diversity (He).

Breed (n)	DRA*0101	DRA*0201	DRA*JBH11	DRA*Hld105	Ho	He	HWE#
Croatian coldblood (33)	0.80±0.04	0.03±0.02	0.12±0.04	0.05±0.03	0.39	0.34	P=1.00
Posavina horse (55)	0.83±0.04	0.02±0.01	0.14±0.03	0.01±0.01	0.28	0.29	P=0.054

#Exact probability that locus is in Hardy Weinberg equilibrium.

(Accession number FJ716134). The DRA*Hld105 allele is characterized by the non-synonymous nucleotide substitution at position 65 which results in amino acid change (F→Y) at PBR and synonymous nucleotide substitution at position 105 (see table 2). This mutation has been reported only in donkeys for allele DRA*JBD3 (Data collected from GenBank;

Table 2. Horse specific DRA alleles (numbering according to Brown et al., 2004).

Allele	Nucleotide sequence polymorphic site												
	18	45	50	65	75	99	105	132	149	154	165	198	204
DRA*0101	A	G	G	T	C	C	T	G	G	G	G	C	C
DRA*0201	A	G	G	T	C	C	T	G	G	A	G	C	C
DRA*JBH11	G	C	G	T	C	C	T	G	G	G	G	C	T
DRA*HId105	A	G	G	**A**	C	C	**C**	G	G	G	G	C	C

Underlined nucleotides take part in peptide binding region constitution (Brown et al., 1998).
Specific nucleotide mutations are bolded.

Family Equidae). Slight deviation from HWE, due to an excess of heterozygotes, was noticed for Posavina horse at DRA locus while individual SNPs did not deviate from HWE. Increased heterozygosity was close to significance (P=0.054) and might be the consequence of selection. The specific polymorphism found at DRA locus is of particular interest as PH and CCH are kept in a free-range system where they are exposed to a large number of parasites and other diseases.

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