COMMUNICATION

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

Haidi Arbanasic¹, Ana Galov¹, Kresimir Salajpal², Ino Curik²

¹Department of Animal Physiology. University of Zagreb, Croatia
²Department of Animal Science. University of Zagreb, Croatia

Corresponding author: Dr. Haidi Arbanasic. Department of Animal Physiology, University of Zagreb, Roosevelt trg 6, 10.000 Zagreb, Croatia - Tel +385 1 4877700 - Email: haidi.arbanasic@zg.htnet.hr

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 histocompatibility 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 histocompatibility 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'-GCTTCTCATCCTAGTCCCCTT-3') and Be4 (5'-GCCTAGGAGTGCAGA-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 find 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 (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;
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.

REFERENCES – Albright-Fraser, D.G., Reid R., Gerber V., Bailey, E., 1996. Polymorphism of DRA among equids. Immunogenetics 43: 315–317. Brown, J.H., Jardetzky T., Saper M.A., Samraoui, B., Bjorkman, P.J., Wiley, D.C., 1988. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. Nature 332: 845-850. Brown, J.J., Thomson, W., Clegg, P., Eyre, S., Kennedy, L.J., Matthews, J., Carter, S., Ollier, W.E.R., 2004. Polymorphism of the equine major histocompatibility complex class II DRA locus. Tissue antigens 64: 173-179. Curik, I., Fraser, D.G., Eder, C., Achmann, R., Swinburne,. J.E., Binns, M., Brem, G., Sölkner, J., Marti, E., 2003. Association between the MHC gene region and variation of serum IgE levels against specific mould allergens in the horse. Gen. Sel. Evol. 35: 17-190. Druml, T., Curik, I., Baumung, R., Aberle, K., Distl, O., Sölkner, J., 2007. Individual-based assessment of population structure and admixture in Austrian, Croatian and German draught horses. Heredity 98: 114-122. Luis, C., Cothran, E.G., Oom, M.M., Bailey, E., 2005. Major histocompatibility complex locus DRA polymorphism in the endangered Sorraia horse and related breeds. J. Anim. Breed. Gen. 122: 69–72.

| Allele         | Nucleotide sequence polymorphic site |
|---------------|-------------------------------------|
| 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*Hld105    | 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.

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