Analysis of upstream promoter region and corresponding 5’ UTR of glucokinase (GCK) gene in horse breeds

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ABSTRACT: A region of glucokinase (GCK) gene was sequenced in 14 horses of 14 different breeds. The resulting GCK nucleotide sequence (GenBank number EF136885) showed 77% homology with human GCK gene portion containing the upstream promoter region and the corresponding 5’ UTR of the exon 1. Conserved regulatory sequences near the putative transcriptional start site were identified. The obtained sequences were aligned to detect polymorphism. A new C>T transition within the 5’ UTR of exon 1 was found. Allele frequencies of this polymorphism were studied by PCR-RFLP in 193 horses of 14 breeds (Bardigiano, 21; Esperia Pony, 5; Haflinger, 10; Italian Heavy Draught Horse, 28; Italian Saddle, 25; Italian Trotter, 16; Maremmano, 15; Murgese, 14; Norico, 10; Salernitano, 12; Thoroughbred, 10; Tolletano, 7 and Ventasso Horse, 8). The polymorphism was found in all breeds and differences in allelic frequencies among the breeds were observed. The new SNP identified within a regulative region of GCK gene, which plays an important role in insulin secretion and feeding behaviour, could be used for association studies with performance traits of the horses.

Key words: GCK gene, Horses, Breeds, SNP.

INTRODUCTION – The glucokinase (GK) is an enzyme that catalyses the first step in glycolysis. The enzyme is expressed only in hepatocytes and in certain neuro-endocrine cells of the pancreas, pituitary, gut and brain. In hepatocytes, the enzyme plays a key regulatory role in glucose uptake and release, while in pancreatic beta-cells and other cells the enzyme acts as glucose sensor and is involved in the regulation of insulin secretion (Matschinsky, 2002). Besides, the enzyme has a role in feeding behaviour (Roncero et al., 2004). The glucokinase gene (GCK) exists as a single copy with two widely separated cell-specific promoters. The upstream promoter is active in the neuro-endocrine cells; the downstream promoter is transcribed only in hepatocytes (Magnuson and Shelton, 1989; Postic et al., 1995). Multiple tissue-specific isoforms of glucokinase were identified (Matschinsky, 2002). The GCK gene has been studied mainly in humans and it is a candidate for at least three syndromes, including diabetes mellitus (Glyn, 2003). The GCK gene is not yet been studied in farm animals; the characterization of this gene is relevant because it is postulated to affects glucose homeostasis and feeding behaviour. Recently, partial horse GCK gene sequence, was determined and some SNPs were found (Dall'Olio and Minieri, 2006). This study aimed to analyse the regulative upstream region of horse GCK gene and to look for new equine markers useful in association studies with performance traits.

MATERIAL AND METHODS – DNA samples were isolated from hair roots (Healy et al., 1995). Fourteen DNA samples belonging to the 14 horse breeds listed in table 1 were used for sequence analysis of PCR products. PCR primer pairs (F: 5’-cagtcccagttttatgcatgg-3’; R: 5’-cctccatcctggctctgtc-3’) were designed on conserved sequenced of human (GeneBank n° M90297) and mouse (GeneBank n° L41629) GCK gene. The PCR conditions were standard with annealing at 62°C. Amplicons were purified with QIAquick PCR purification kit (QIAGEN, Italy) and then sequenced on both strands by commercial service. The obtained sequences were analysed with BLASTn versus the GenBank databases (Altschul et al., 1997). The sequences were aligned using ClustalW program (Thompson et al., 1994). Additional 193 samples (Table 1) were analysed by PCR-RFLP using FdD II and the resulting products were resolved on 2% agarose gels stained with ethidium bromide.
RESULTS AND CONCLUSIONS – Amplicons of 670 nt were obtained from horse samples. The horse sequence, deposited in GeneBank with accession number EF136885, shows 77% of homology with human (GeneBank n° M90297) and 68% with mouse (GeneBank n° L41629) GCK gene. Based on human GCK gene structure (Tanizawa et al., 1992), our sequences should correspond to 147 nt of upstream promoter region and 523 nt of corresponding exon 1. This exon contains the 5' UTR and the initial translated codons for the isoform expressed in neuroendocrine cells GK containing (Figure 1). In the horses upstream promoter region of GCK gene no TATA box and CCAAT consensus sequences were found confirming results obtained in humans and rodents (Tanizawa, 1992). The horses sequences contain two A-box and four E-box motifs (Figure 1) showing TAAT and CANNTG core sequences, respectively.

Figure 1. Partial nucleotide sequence of the 5' flanking region of the horse GCK gene. The putative transcriptional start site is shown in boldface, the translation start codon is shown in boldface italic type. The A-boxes and the E-boxes DNA motifs are underlined.

The A-box, also known as upstream promoter element (UPE), is recognized by pancreatic and duodenal homeobox 1 (PDX-1) transcription factor essential for pancreas development and beta cell function. E-box motif binds BETA2/NeuroD1 transcription factor expressed in pancreas and gut (Moates et al., 2003). Since the 1st A-box and the 1st E-box motifs of the figure 1 resulted conserved among humans (GeneBank n° M90297), mouse (GeneBank n° L41629) and horses GCK gene sequences, they may be functionally important. Additional analyses are needed to determine the functional interaction of these transcriptional factors with the equine GCK promoter. The obtained horse sequences were aligned and analyzed to detect putative SNPs. A new C>T transition in 5'UTR of exon 1, located 460 nt upstream of the ATG start codon, was detected and then confirmed by PCR-RFLP. The presence of the mutation creates one Fnd II restriction site only with the C allele giving two fragments of 486 bp and 184 bp. The frequencies of the polymorphism per breed are shown in table 1. A predominance was found for the C allele in all breeds, except for the Lipizzan where the T allele frequency was 0.875. This results is possibly linked to the characteristics of the Herd Book of this breed. The T allele occurred in all breeds with differences in its frequency. These results add a new horse marker and it is interesting to point out that this polymorphism, considering its location within the regulative region, could have a role on the expression level of the GCK gene. The identification of a SNP within GCK gene, which has an important role in insulin secretion and feeding behaviour, might be used for association analysis between the SNP and performance traits of the horses. Indeed, because other SNPs were reported within horse GCK gene (Dall'Olio and Minieri, 2006), further studies could be carried out to analyse the horse GCK intragenic haplotypes and their influence on productive traits.
The research was supported by Ex 60% 2004 founds.

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Table 1. Allele frequencies of the polymorphism located within the 5’UTR of exon 1.

| Horse breeds                  | Samples | T allele | C allele | Horse breeds                  | Samples | T allele | C allele |
|-------------------------------|---------|----------|----------|-------------------------------|---------|----------|----------|
| Bardigiano                    | 21      | 0.214    | 0.786    | Maremmano                     | 15      | 0.367    | 0.633    |
| Esperia Pony                  | 5       | 0.400    | 0.600    | Murgese                       | 14      | 0.107    | 0.893    |
| Haflinger                     | 10      | 0.050    | 0.950    | Norico                        | 10      | 0.200    | 0.800    |
| Italian Heavy Draught Horse   | 28      | 0.071    | 0.929    | Salernitano                   | 12      | 0.083    | 0.917    |
| Italian Saddle                | 25      | 0.360    | 0.640    | Thoroughbred                  | 10      | 0.100    | 0.900    |
| Italian Trotter               | 16      | 0.250    | 0.750    | Tolfetano                     | 7       | 0.357    | 0.643    |
| Lipizzan                       | 12      | 0.875    | 0.125    | Ventasso Horse                | 8       | 0.250    | 0.750    |

The Authors want to thank A.N.A.C.A.I.T.P.R., A.I.A, A.P.As and ex Istituto Sperimentale per la Zootecnia of Rome for providing samples.