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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Primer sequences used to sequence *SPINK5* genomic DNA and cDNA, pyrosequencing primers, as well as a list of detected SNPs.

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A point mutation in the splice donor site of intron 7 in the αs2-casein encoding gene of the Mediterranean River buffalo results in an allele-specific exon skipping

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Source/description: The *CSN1S2* cDNA of 10 unrelated Mediterranean River Buffaloes reared in Southern Italy was amplified by RT-PCR, while the region from the 6th to the 8th exon of the *CSN1S2* gene was amplified from genomic template. All amplicons were sequenced twice and in both directions. Fifty-three individuals randomly chosen from four breeding herds were genotyped for an AluI-restriction fragment length polymorphism (RFLP). Primer sequences and PCR conditions are given in Tables S1 & S2. Three individual milk samples from buffaloes with different genotypes at *CSN1S2* were analysed by reverse-phase-high pressure liquid chromatography (RP-HPLC).

Polymorphism detection: cDNA sequence comparisons showed that five individuals had a normal transcript only (lodged on EMBL, accession FM865618, named *CSN1S2A*), one had a deleted transcript only (lodged on EMBL, accession FM865619, named *CSN1S2B*), because of the splicing out of the 27-bp of exon 7, and the remaining four had a heterozygous pattern. Analysis of the genomic sequences revealed a FM865620: g.773G>C transversion that caused inactivation of the intron 7 splice donor site and, consequently, the allele-specific exon-skipping characteristic of the *CSN1S2*B allele. The g.773G>C mutation creates a new AluI restriction site enabling a PCR–RFLP rapid genotyping assay (Fig. S1). PCR–RFLP genotypes for the AluI site were consistent with the cDNA sequence data for all 10 animals. The cDNA sequences showed three additional exonic mutations forming an extended haplotype with the g.773G>C polymorphism: FM865618: c.459C>T, c.484A>T and c.568A>G homozygous and heterozygous respectively in the *CSN1S2*BB and *CSN1S2*AB buffaloes. The first is silent, while the remaining two are non-conservative (p.Ile162Phe and p.Thp200Ala respectively). Chromatographic analysis of three individual samples with *CSN1S2* AA, AB and BB genotypes showed the same retention time for the αs2 casein fraction (Fig. S2), but the hydrophobic characteristics of each allele do not allow their chromatographic separation.

Allelic frequencies: The genotype frequencies (37 *CSN1S2*A/A, 15 *CSN1S2*A/B and one *CSN1S2*B/B) are in agreement with Hardy–Weinberg equilibrium ($\chi^2 = 0.13$, d.f. = 1), with the frequency of the deleted B allele being 0.16.

Comments: The results indicate that buffalo, similar to goats[1,2] and cattle,[3,4] have a *CSN1S2* allele resulting from a non-constitutive splicing event. The predicted bubaline αs2B protein is 198 aa long instead of 207 aa and would also be characterized by the presence of Phe at position 147 and Ala at 185.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Oligonucleotide primer sequences and positions.

Table S2 Thermal amplification programmes for (a) RT-PCR, (b) PCR and (c) AluI PCR-RFLP.

Figure S1 Observed genotypes after Alu*I* digestion of fragments obtained by PCR of the DNA region spanning the 7th exon and flanking regions of the Mediterranean river buffalo *CSN1S2* gene.

Figure S2 RP-HPLC chromatogram of individual buffalo milk samples.

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