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Technical note: A simplified PCR-based assay for the characterization of two prolactin variants that affect milk traits in sheep breeds

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ABSTRACT

In the present study, a rapid and cost-effective PCR-based assay was developed for the genetic identification of 2 different variants within intron 2 of the prolactin gene. This polymorphism has previously been associated with milk traits in some ovine breeds and was recently proposed as a potential marker for future breeding schemes in dairy sheep. Until now, 2 alleles (A and B) have been identified by PCR-RFLP that included *Hae*III digestion of a 2.5-kb PCR fragment. By partial sequencing of the prolactin gene intron 2, it was found that the B variant results from a 23-bp deletion of the A variant of the prolactin gene and not from an extra *Hae*III digestion site, as had been reported. This finding assisted the design of new primers for analysis of prolactin intron 2 variants based on the size of an easily amplified short PCR product, thereby avoiding the need and cost for additional digestions. The method was validated by genotyping 80 animals from 2 breeds and showed 100% sensitivity and specificity compared with the PCR-RFLP assay. The established simplified PCR assay was then successfully used to genotype 356 Chios sheep.

Key words: genotyping, marker assisted selection, polymerase chain reaction–restriction fragment length polymorphism, prolactin

The dairy sheep industry plays a vital commercial role in many Mediterranean countries. Milk is used mainly for the production of high-quality cheese from local dairy breeds; therefore, both milk yield and content are important selection objectives (Carta et al., 2009). However, because of financial and practical restrictions, genome-wide selection is usually unfeasible for most dairy sheep breeds, making the application of selection schemes assisted by molecular information on causal mutations of genes affecting milk traits an attractive alternative in dairy sheep (Carta et al., 2009).

Prolactin (***PRL***) is a lactogenic hormone that plays a significant role in milk production; its depletion in sheep provokes a severe reduction of milk secretion (Knight, 2001), suggesting that *PRL* is a functional candidate gene that could contribute to variations in milk yield. In addition, the *PRL* gene is located in a region of the ovine chromosome 20 where putative QTL for fat percentage (Gutiérrez-Gil et al., 2009) and milk, fat, and protein yield (Barillet et al., 2005) have been proposed. Therefore, *PRL* also could be used as a positional marker gene associated with milk production and composition traits.

Among the limited polymorphisms that have been identified in the ovine *PRL* gene, 2 variants (A and B) within intron 2 have been associated with milk-related traits (Ramos et al., 2009; Staiger et al., 2010). The *PRL* genotypes significantly affected milk yield and fat and protein content in Serra da Estrela sheep (Ramos et al., 2009). More recently, Staiger et al. (2010) suggested that the *PRL* intron 2 polymorphism significantly affects milk yield in East Friesian sheep and could therefore be used as a potential marker in selection breeding programs.

To date, the 2 variants in the ovine *PRL* gene have been distinguished based on *Hae*III digestions of PCR products, as first described by Vincent and Rothschild (1997), although the precise genetic nature of these forms have until now remained uncharacterized. Because little has been published on the ovine intronic sequence, the original assay relied on the generation and subsequent restriction digestion of a 2.5-kb PCR product from genomic DNA, using primers that anneal in known flanking exonic sequences. However, the generation of such long PCR fragments from genomic DNA is often difficult and requires extra handling (Schwarz et al., 1990; Wilton and Lim, 1996). In addition, the use of restriction enzymes increases the genotyping cost and time to complete the analysis.

In the present study, the development of a simplified, rapid, and cost-effective method of genotyping prolactin intron 2 variants is described. By direct DNA sequencing of PCR products generated from AA and BB homozygous animals, we determined that the B allele is the result of a 23-bp deletion in the region flanked

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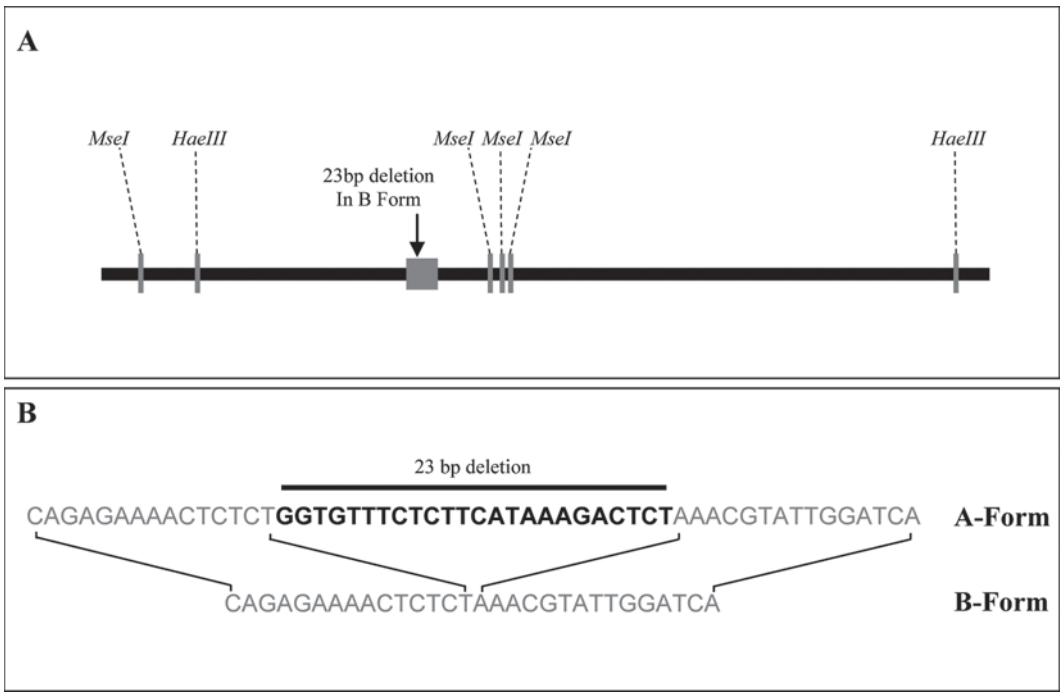

Figure 1. Molecular characterization of ovine prolactin (*PRL*) variants A and B. (A) Schematic representation of the intron 2 region of the ovine *PRL* gene harboring the deletion; (B) precise DNA sequence of the deletion within intron 2 of the ovine *PRL* gene.

by 2 *Hae*III sites (Figure 1). This finding facilitated the design of new primers flanking the deletion; therefore, genotyping of the 2 alleles is possible by using a simplified PCR assay, based solely on the size of a short PCR product without the need for digestions (Figure 2).

Genomic DNA was extracted from whole blood samples with a genomic DNA blood kit (Macherey-Nagel GmbH, Düren, Germany) according to the manufacturer's instructions. Primers PRL-F or PRL-R used for the amplification of the ovine prolactin intron 2 sequence were designed against the bovine prolactin genomic sequence (GenBank accession no. NC 007324), using the online program Primer3 (http://frodo.wi.mit.edu/primer3/input.htm). Amplification of DNA with primers PRL-F or PRL-R from AA and BB homozygous animals, as characterized by a modification of the method described by Vincent and Rothschild (1997), yielded 1,209- and 1,186-bp PCR products, respectively. The DNA sequencing of the agarose gel–purified PCR products was performed from both ends, using either primer PRL-F or PRL-R with the BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Carlsbad, CA), and analyzed on an ABI 3130 genetic analyzer. Each obtained sequence was confirmed to be a *PRL* intron 2 sequence because each exhibited 89% identity to the bovine counterpart and included *Hae*III and *Mse*I restriction enzymes sites in locations consistent with the original method of Vincent and Rothschild (1997; Figure 1). The novel sequences were submitted to GenBank with accession numbers HM234397 and HM234398 for the A and B alleles, respectively.

Based on the obtained intronic sequences, primers PRLDel-F (5′-TCTGCTAAGGGCTCTGCCTA-3′) and PRLDel-R (5′-ACAAGGGAAGCCCAGAAGAT-3′) were designed to flank the deletion. Amplifications were performed in a final volume of 25 μL using 10 ng of genomic DNA, 2.5 m*M* $MgCl_2$, 0.4 μ*M* PRLDel-F, 0.4 μ*M* PRLDel-R, and 1 U of Taq DNA polymerase (Qiagen, Valencia, CA). Following an initial 5-min denaturation step at 94°C, the PCR reactions were subject to 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and a final elongation step for 5 min at 72°C. Clear, high-intensity bands of the expected size (213 bp for the A allele and 190 bp for the B allele) could be resolved on 2% agarose gels run for 45 min. Allele sizes were compared to a 100-bp DNA ladder (Figure 2).

The sensitivity and specificity of the method was evaluated in a double-blind experiment that included 80 animals from 2 breeds, 40 of which were Chios sheep randomly selected from an experimental farm based at the Agricultural Research Institute (Nicosia, Cyprus) and 40 Cyprus Fat-Tailed sheep provided by a commercial farm in Cyprus raising purebred animals of this rare breed. All animals were genotyped by both the proposed simplified PCR assay and a modification of the method described by Vincent and Rothschild