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## **PCR-SSCP and *Hin6I* PCR-RFLP detecting novel anonymous SNPs within ovine *PROPI* gene (Brief report)**

(Anonyme SNPs im ovinen *PROPI*-Gen detektiert mittels PCR-SSCP und *Hin6I* PCR-RFLP)

**Background:** *PROPI* plays a direct or indirect key role in the ontogenesis of pituitary gonadotropes, as well as somatotropes, lactotropes and caudomedial thyrotropes (WU et al., 1998). Mutations of *PROPI* are responsible for deficiencies of POU1F1, GH, prolactin (PRL) and thyroid stimulating hormone (TSH) in Snell and Jackson dwarf mice and in man, as well as deficiencies of LH and FSH, suggesting that the *PROPI* gene is a potential candidate gene associating with production traits in animal breeding and genetics. To date, no polymorphism of *PROPI* gene has been reported in ruminant animals except bovine (PAN et al., 2007). So, the objective of this study is to investigate the polymorphism of all coding regions within ovine *PROPI* gene by PCR-SSCP, DNA sequencing and PCR-RFLP methods, which will possibly contribute to conducting association analysis and evaluating them as genetic markers in animal breeding and genetics.

### **Procedures:**

#### *PCR primers and conditions*

Based on ovine *PROPI* gene sequence (GenBank accession no. AY533708) and bovine *PROPI* gene sequence (GenBank accession no. AF453512), 3 pairs of primers were designed to amplify entire coding regions and its flanking regions:

P1F: 5'-atcaagataccagcatagagg-3'; P1R: 5'-cccaaagattcactcacc-3' (exon 1, 203 bp);

P2F: 5'-atgtggtctggatggatg-3'; P2R: 5'-ctggtgaaggttgggttag-3' (exon 2, 418 bp);

P3F: 5'-ctgatgctgctcttcttc-3'; P3R: 5'-actttagtccaggacttgg-3' (exon 3, 371 bp).

The 20 µL PCR amplification contained 50 ng of genomic DNA, 10 pM of each primer, dNTPs (0.2 mM), MgCl<sub>2</sub> (1.5 mM), and 0.50 U *Taq* DNA polymerase (MBI manufactory). The cycling protocol was 5 min at 95 °C, 35 cycles of 94 °C for 30 s, annealing for 30 s, 72 °C for 1 min, with a final extension at 72 °C for 10 min.

#### *PCR-SSCP and DNA sequencing and *Hin6I* PCR-RFLP*

Polymorphism of *PROPI* was detected by SSCP in 10% PAGE (80×73×0.75 mm) in constant voltage (180 V) for 1.5-2.5 h after its PCR product was denatured 10 min at 98 °C. The gel was stained with silver nitrate and visualized with 2.0% NaOH solution (supplied with 0.1% formaldehyde) (LAN et al., 2007). DNA samples showing different patterns on SSCP gels were selected for DNA sequencing. For each pattern of SSCP at least three random DNA samples were sequenced by both directions in ABI 377.

Sequence analysis revealed that a *Hin6I* PCR-RFLP could be used to genotype the mutation at P2 locus. Aliquots of 20 µL PCR products obtained with primers P2F/P2R were digested with 10 U *Hin6I* (MBI, Vilnius, Lithuania) following the supplier's

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directions for buffer condition. The digested products were detected by electrophoresis in 3.0% agarose gel stained with ethidium bromide.

**Results:** SSCP banding patterns of unrelated healthy female individuals in German Merino (DM, n=22) and Karakul (KA, n=21) populations revealed a polymorphism in the fragment amplified with primers P2F/P2R (EU340144 and EU340145). Comparisons between nucleotide sequences of ovine *PROPI* revealed one novel mutation AY533708:g.1402G>A identifying an anonymous mutation (A79A). The novel mutation could be detected by a *Hin6I* endonuclease restriction site (gcgc). The genotypic and allelic frequencies of the novel anonymous SNP (A79A) within ovine *PROPI* by PCR-SSCP and *Hin6I* PCR-RFLP methods were shown in Table 1.

Table 1

Genotypic and allelic frequencies of the novel anonymous SNP (A79A) within ovine *PROPI* gene by PCR-SSCP and *Hin6I* PCR-RFLP methods

| Breed    | Frequencies of genotypes and alleles |       |                             |       |       | X <sup>2</sup> value (HWE) |
|----------|--------------------------------------|-------|-----------------------------|-------|-------|----------------------------|
|          | GG                                   | GA    | AA                          | G     | A     |                            |
| DM       | 0.864                                | 0.136 | 0                           | 0.932 | 0.068 | P>0.05                     |
| KA       | 0.667                                | 0.333 | 0                           | 0.833 | 0.167 | P>0.05                     |
| PCR-SSCP | 4 SSCP bands                         |       | 5 SSCP bands                |       |       |                            |
| PCR-RFLP | 167bp+126bp+97bp+28bp                |       | 167bp+154bp+126bp+97bp+28bp |       |       |                            |

HWE = Hardy-Weinberg equilibrium

There was a low genetic diversity within ovine *PROPI* gene in analyzed populations. The novel mutation AY533708:g.1402G>A extended the spectrum of genetic variation of ovine *PROPI* gene. Previous reports showed that silence mutations in coding region of candidate genes associated with production traits in goat *POUIF1* gene (LAN et al., 2007). Hence, the above described SNP of *PROPI* possibly contributed to conducting association analysis and evaluating it as genetic markers in production traits for ovine industry.

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