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A novel *EcoR*II PCR-RFLP detecting genetic variation of goat *NF1-C2* gene and its association with milk yield (Brief Report)

Eine neuer *EcoR*II PCR-RFLP Nachweis genetischer Variation des Ziegengens *NF1-C2* und dessen Assoziation mit der Milchleistung (Brief Report)

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Background

The transcription factor nuclear factor1-C2 (*NF1-C2*) mediates the action of prolactin in the mammary gland. Research on the molecular genetic mechanism of model system have indicated that the *NF1-C2* gene plays an important role for the activation of several mammary gland specific genes (Nilsson *et al.* 2006). Moreover, the effects of prolactin on milk production traits have been reported in ruminant (Seriwatanachai *et al.* 2008). *NF1-C2* may have a similar function in goat. To our knowledge polymorphisms of *NF1-C2* gene have not been described in animals. Here a SNP of the caprine *NF1-C2* was detected and a *EcoR*II PCR-RFLP was derived, that allowed to clearly detect *NF1-C2* genotypes. Further investigation was conduct to evaluate the association between polymorphisms and milk yield at different lactation stages.

Procedures

Primer sequences and PCR conditions

One pair of primers was designed to amplify a 485bp fragment based on the bovine *NF1-C2* gene (GenBank accession No. g. NC_007305).

P1F:5'-CCC TAC CTT AAC CCT AAC CA-3' (nt44604-nt44623);

P1R:5'-CAG GGA AAC GAA TGA ACC-3' (nt45072-nt45089).

The 25 µL polymerase chain reaction (PCR) contained 50 ng of genomic caprine DNA 0.2 µM of each primer, 1×Reaction Mix (including dNTP, KCl and $MgCl^2$) and 0.5 U Taq DNA polymerase (Tiangen Biotech, Beijing, China). The cycling protocol was 4 min at 94 °C, 34 cycles of 94 °C for 30 s, annealing at 51.6 °C for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 10 min. To detect single nucleotide polymorphism (SNP) within the caprine *NF1-C2* gene DNA pool (Khatib *et al.* 2008) was constructed from 50 different goat blood samples and the PCR products of the pooled DNA samples were sequenced in ABI 377.

EcoRII PCR-RFLP conditions

A G>A transition detected at the position corresponding to nt44654 of GenBank acc. no. NC_007305 was genotyped using a *EcoR*II PCR-RFLP. Therefore, an alternative reverse primer was designed in which the original 'A' at nt44823 of the bovine sequence was substituted by a 'C' (bold) to avoid a *EcoR*II restriction site (P2R: GTC C**C**G GAC TGT GAT TTG C (nt44819-nt44837). PCR was carried out to amplify the 233 bp fragment of caprine *NF1-C2* gene based on primers P1F/P2R. Genotyping of *EcoR*II PCR-RFLP within *NF1-C2* allele was according to the following protocol: aliquots of 20 µL PCR products of *NF1-C2* gene were digested with 10 U *EcoR*II (TaKaRa, Dalian, China) for 8 h at 37 °C. The digested products were detected by 3.0 % agarose gels stained with ethidium bromide.

Results

One SNP 44654G>A (published in GenBank g. NC_007305; corresponding to position 51 in GenBank GQ169554) was detected in 776 unrelated healthy goats from three breeds in China (Xinong Saanen dairy 249, Guanzhong dairy 408, Xinjiang white cashmere 119) based on PCR and DNA-pooling sequencing approach (Khatib *et al.* 2008). As shown in Table 1, three different patterns were observed. The frequency of mutant allele was similar to that of wild type in dairy goats, which implied that *NF1-C2* gene was less conservative at *EcoR*II locus.

Table 1
The genotypic and allelic frequencies of the novel SNP within goat *NF1-C2* by *EcoR*II PCR-RFLP

	AA	AG	GG	A	G
Xinong Saanen dairy	0.422	0.056	0.522	0.450	0.550
Guanzhong dairy	0.402	0.206	0.392	0.505	0.495
Xinjiang white cashmere	0.176	0	0.824	0.176	0.824
*EcoR*II PCR-RFLP	233	233+186+47	186+47		

As demonstrated in a novel mechanism of prolactin signaling (Nilsson *et al.* 2006), prolactin modulates several mammary gland specific genes through Jak2/*NF1-C2*. If that is tenable, the *NF1-C2* gene may have significantly association with milk production traits. To address the relationship, the association of genotype with milk yield at different lactation was analyzed (Table 2).

Table 2
Least square mean (means±standard error of means) of milk yield at different lactation for the alternative *NF1-C2-EcoR*II genotype in Xinong Saanen dairy goat

Milk yield	AA genotype	GG genotype	*P*-value
The first lactation	612.967±8.760	609.852±7.280	0.785
The second lactation	834.006±13.071	853.164±11.265	0.269
The third lactation	925.225±25.116	985.146±20.756	0.070

A total of 200 goats of Xinong Saanen dairy originated from our institute was used. Effects of year, season of birth (spring versus fall), age of dam and sire were not included in the linear model, as the preliminary statistical analyses indicated that these effects did not have

significant influences on variability of traits in the population. The fixed effect of genotype was included as independent variable in the linear model. There were only 7 GA animals in the tested population that were excluded from the analysis. The result demonstrated that the polymorphism of the *Eco*RII PCR-RFLP in the goat *NF1-C2* gene was not associated with milk yield; only in the third lactation genotype GG tended to be associated with higher milk yield than genotype AA. Although *NF1-C2* has an important role in milk gene activation, the *NF1-C2* protein levels are reduced at lactation, when milk genes are most highly expressed (Johansson *et al.* 2005). Our results are in accordance with the hypothesis that the *NF1-C2* gene participates in the establishment of mammary gland but not the maintenance of milk gene expression which influences milk yield.

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References

Johansson EM, Kannius-Janson M, Gritli-Linde A, Bjursell G, Nilsson J (2005) Nuclear factor 1-C2 is regulated by prolactin and shows a distinct expression pattern in the mouse mammary epithelial cells during development, Mol Endocrinol 19, 992-1003

Khatib H, Monson RL, Schutzkus V, Kohl DM, Rosa GJM, Rutledge JJ (2008) Mutations in the *STAT5A* gene are associated with embryonic survival and milk composition in cattle, J Dairy Sci 91, 784-793

Nilsson J, Bjursell G, Kannius-Janson M (2006) Nuclear jak2 and transcription factor *NF1-C2*: A novel mechanism of prolactin signaling in mammary epithelial cells, Mol Cell Biol 26, 5663-5674

Seriwatanachai D, Charoenphandhu N, Suthiphongchai T, Krishnamra N (2008) Prolactin decreases the expression ratio of receptor activator of nuclear factor kappa b ligand/osteoprotegerin in human fetal osteoblast cells, Cell Biology International 32, 1126-1135

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