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# The novel SNPs of the *IGFBP3* gene and their associations with litter size and weight traits in goat (Brief report)

(Neue SNPs im IGFBP3 und ihre Assoziation mit Wurfgröße und Körpergewicht bei der Ziege)

**Background:** The insulin-like growth factor binding protein-3 (*IGFBP-3*) gene is a structural gene responsible for the multiple effects of insulin-like growth factors (IGFs) playing a key role in mammalian growth, development and reproduction (BALE et al., 1992; HASTIE et al., 2004). Single nucleotide polymorphisms (SNPs) have been described in the bovine *IGFBP3* gene which was associated with production traits, as well as in the buffalo (MACIULLA et al., 1997; PADMA et al., 2004). No polymorphism was detected in the sheep *IGFBP3* gene (KUMAR et al., 2006). In present experiment most of the coding region and part of introns of *IGFBP3* were scanned for SNPs in predominant goat breeds of China. Associations of SNPs of *IGFBP3* with litter size and weight traits were analyzed.

**Procedures:**

*Primer sequences:*

As complete goat *IGFBP3* gene sequences was not available, the primer pairs P1-P3 were design based on bovine IGFBP-3 gene (Acc. No. AF305712).

P1: 5'-AGATGCGAGCGCAGCAGCTATTCC-3',
5'-CCTGACGCGGACGGTAGCAGGTAA-3';
P2:5'-GAAATGGCAGTGAGTCGG-3',
5'-TGGGCTCTTGAGTAATGGTG-3'; P3:5'-CCAAGCGTGAGACAGAATAC-3',
5'-AGGAGGGATAGGAGCAAGTT-3'.

*PCR conditions:*

The 15 μL PCR amplification contained 50 ng of genomic goat DNA, 10 p M of each primer, dNTPs (0.2 m M), $MgCl_2$ (1.5 m M), and 0.50 U *Taq* DNA polymerase (MBI manufactory). The cycling protocol was 4 min at 95℃, 35 cycles of 94℃ for 45 s, annealing at 65℃, 63℃ or 60℃ corresponding to 3 different primer pairs for 45 s, 72℃ for 1 min, with a final extension at 72℃ for 10 min. Polymorphism of IGFBP3 was detected by SSCP in 10% PAGE (80×73×0.75 mm) in constant voltage (200V) for 1.5-2.5 h after its PCR product was denatured 10 min at 98°C. The PCR fragments from different SSCP patterns were subcloned and sequenced (Acc. No.AY526114 and AY785559).

**Results**: According to Acc. No.AF305712 and 526114, four SNPs, namely, C>T(nt58 of exon II ), C>G (nt67 of exon II), A>G (nt78 of intron II) and G>A (nt217 of intron II) were detected in 767 unrelated goats from the wool breed (452 Inner Mongolia White Cashmere(IMWC)), three dairy breeds (74 Xinong Sannen (XS), 80 Laoshan (LS), 62 Guanzhong (GZ)) and three meat breeds (31 Guizhou White, 34 Shaanan White, 34 Leizhou). The C>T (nt58 of exon II) mutation resulted in a Pro to Ser; C>G (nt67 of exon II) resulted in an Arg to Glu. Interestingly, two SNPs of exon II were in linkage disequilibrium. Moreover, the frequencies of mutation “C” of exon II among

different types breeds (0.181 for Cashmere (n=452), 0.099-0.015 for dairy (n=216) and zero for meat breeds (n=99)) showed significant differences in wool, dairy and meat types ($X^2$=49.077; $P$<0.001). These implied that the two SNPs of *IGFBP3* possibly associated with production traits. As *IGFBP3* binds IGFs which has effects on growth and reproduction, the association of genotypes (the C>G or C>T of exon II) with litter size and weight traits were analyzed in goat (Table). A total of 668 goats of known pedigree originated from the breeding farm of dairy goats (XS, LS and GZ) and IMWC goats were used. Fixed effects of breed, sire, genotype, year, season of birth (spring vs fall), sex were included as independent variables in the linear model (WANG et al., 2006). The 668 goats were daughters of 60 sires. As GG (n=15) animals in the tested population was rare, thus these animals were not in analysis. The result indicated that the *IGFBP3* genotype was significantly associated with litter size ($P$<0.05).

However, significant association of SNP with weight traits were not detected ($P$>0.05). So, two linked mutations in *IGFBP3* showed significant association with reproduction traits. This result provoked the *IGFBP3* gene as important candidate gene for reproduction trait.

Table 1
Least square mean (means ± standard error of means) of litter size and weight traits for *IGFBP3* genotypes (the C>G or C>T in exon II)

| Traits | CC (or CC) (n= 466) | CG(or CT) (n=187) | *P*-value |
|---|---|---|---|
| Average litter size (lamb) | 1.88±0.038 | 1.35±0.03 | 0.027 |
| Birth weight (kg) | 3.03±0.02 | 3.02±0.03 | 0.954 |
| Weight of 6 months(kg) | 36.20±0.97 | 37.75±1.03 | 0.311 |
| Weight of 12 months (kg) | 47.50±1.96 | 48.92±1.83 | 0.609 |

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