Seven Novel SNPs Of *PRDM16* Gene And Their Association With Growth Traits In Chinese Cattle

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Introduction

Prdm (PRDI-BF1 and RIZ homology domain containing) proto-oncogene transcription factor family may significantly participate in the development of the mammalian nervous system, they were also known to control cell proliferation both in cancer (Reid and Nacheva (2004), Pasqualucci, L. Compagno, M. and Houldsworth, J. (2006), Tam, W. Gomez, M. and Chadburn, A. (2006), Nishikawa, N. Toyota, M. and Suzuki, H. (2007)) and normal development (Davis, C. Haberland, M. and Arnold, M. (2006)). Recently, in mice the abilities of PR domain containing 16 (*PRDM16*) genes to control the switch between skeletal muscle and brown fat had attracted a great deal of interests. On the one hand, *PRDM16* gene stimulated brown fat-selective genes ((PGC-1a, UCP1) by binding to PPAR-γ; on the other hand, it suppressed the expression of genes selective for white fat cells. Previous studies showed that the regulated docking of the CtBP proteins on PRDM16 protein controls the brown and white fat-selective gene programs (Seale, P. Kajimura, S. and Yang, W. (2007), Kajimura, S. Seale, P. and Tomaru, T. (2008)). PRDM16 protein stimulates brown adiposeness by binding to PPAR-γ and activating its transcriptional function. Imbalance of a complete *PRDM16* message with a PR domain might be involved in the pathogenesis of MDS patients (Xiao, Z. Zhang, M. and Liu, X. (2006)).

To date, no polymorphisms within the bovine *PRDM16* gene had been reported. Therefore, analyzing the genetic variations of the *PRDM16* gene in 1031 cattle was a preliminary and interesting study.

Materials and Methods

DNA samples. Four Chinese indigenous cattle breeds (Jiaxian (JX, n=446), Nanyang (NY, n=269), Qinchuan (QC, n=236), Chinese Holstein (CH, n=80)) were used in this study. DNA

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samples were extracted from leucocytes according to Chen and Leibenguth (1995).

PCR. Seventeen pairs (involving the 5'UTR, 3'UTR and entire exons) of PCR primers were designed to amplify 17 exons (exon 1-17) of bovine *PRDM16* gene. PCR-SSCP and DNA sequencing were used to detect the genetic variations of *PRDM16* gene. After the polymorphism detection, at least three different PCR products represented each pattern were selected to purify and sequence with the forward and reverse directions.

Statistical analysis. According to Botstein's methods, the population genetic indices (i.e. gene homozygosity, gene heterozygosity, Ne and PIC) were calculated (data not show). The associations between single SNP marker genotypes of the *PRDM16* gene and growth traits in cattle were analysed by the least-squares method as applied in the general liner models (GLM) procedure of SAS.

Results and Discussions

***PRDM16* gene sequencing scanning.** According to the DNA sequencing, no polymorphism was detected in the region of exon 1, 3, 4, 5, 7, 8, and 10-17, seven novel mutations (NC_007314.3: g.577 G>T(exon2), 614 T>C(intron2), 204811 A>G(exon6), 204877 G>A(exon6), 212237 T>C (exon9), 211562T>C(exon9), 211802G>A(exon9)) was found. Among them the mutations in exon 2 and 9 (NC_007314.3: g.577 G>T(synonymous mutation), 212237 T>C (missence mutation), 211562T>C (synonymous mutation), 211802G>A(synonymous mutation)) were detected in four Chinese bovine breeds by forced PCR-RFLP. The electrophoretic patterns on agrose or PAGE after digestion with different endonuclease were showed in Fig.1.

The difference among the bovine breeds. In these loci, three farming and meat utility breeds (JX, NY and QC) possessed moderate genetic diversity. This demonstrated that the genetic diversity within the *PRDM16* gene in the analyzed populations was not very high. In contrast, the milk utility breed (CH) had poor genetic diversity, this maybe caused by the breeding program of Chinese Holstein.When compared with the farming and meat utility breeds (JX, NY, and QC), no TT, AA, CC and CC genotype was detected in these loci in the milk breed (CH). It implies that there were significantly differences in various traits, such as meat and milk production.

The associations of the observed polymorphisms with growth traits in Nanyang cattle. To investigate the effects of these mutations (NC_007314.3: g.577 G>T, 211562T>C, 211802G>A, 212237 T>C), we analyzed the relationship between *PRDM16* genotypes for the effects on variation in body weight and growth rate in 269 Nanyang cows from a

breeding farm (Table 1).

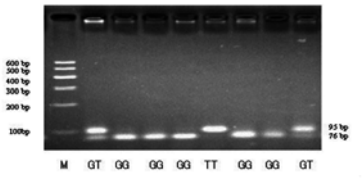

Figure 1a

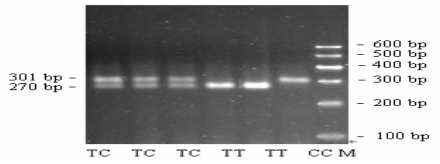

Figure 1b

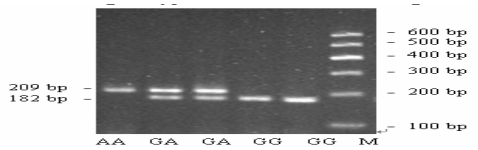

Figure 1c

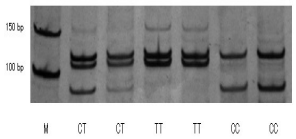

Figure 1d

Figure 1a. Electrophoretic patterns on 3.0% agrose after digestion with *Msp*I endonuclease of the PCR fragment containing g.577 G>T mutation of *PRDM16* gene.

Figure 1b. Electrophoresis patterns of *Pvu*II forced PCR-RFLP analysis of *PRDM16* gene.

Figure. 1c. Electrophoresis patterns of *Hae*III forced PCR-RFLP analysis of *PRDM16* gene.

Figure.1d. Electrophoretic patterns on 10% PAGE after digestion with *Msp*I endonuclease of the PCR fragment containing g.212237 T>C of *PRDM16* gene.

Table 1: Associations of genotypes with growth traits in Nanyang cattle

Loci	Ages	Growth traits	Genotypes		
g.577 G>T	6 Months		GG	GT	TT
		BW (kg)	163.000±1.923^{Aa}	159.727±2.560^{a}	147.333±5.661^{Bb}
		ADG (kg)	0.739±0.010^{a}	0.720±0.014^{ab}	0.658±0.030^{b}
g.211562T>C	12 Months		TT	TC	CC
		BW (kg)	222.048±3.380^{b}	224.302±2.760^{ab}	233.750±4.472^{a}
		ADG (kg)	0.349±0.014^{B}	0.318±0.017^{B}	0.449±0.023^{A}
g.211802G> A	12 Months		GG	GA	AA
		BW (kg)	223.643±3.704^{ab}	220.443±2.510^{b}	233.500±5.658^{a}
		ADG (kg)	0.340±0.021^{B}	0.337±0.014^{B}	0.447±0.032^{A}
g.212237 T>C	6 Months		TT	TC	CC
		BW (kg)	161.667±2.110^{a}	161.094±2.608^{a}	150.571±5.075^{b}
		ADG (kg)	0.729±0.011^{a}	0.729±0.014^{a}	0.668±0.027^{b}

Note: Values with different superscripts within the same line differ significantly $P<0.01$ (A, B) and $P<0.05$ (a, b).

We can find that the birth weight of the bovine has no significant difference, thus we can speculate that these mutations didn't have any effects on the growth traits before birth.

At the 6 months, we find that the individuals with the GG genotype in the g.577 G>T

mutation and TT genotype in the g. 212237 T>C mutation showed better performance in body weight and average daily gain, respectively. These results were in agreement with the development of the brown fat. Brown fat dissipate energy as heat and plays an important role in the regulation of body temperature and fat metabolism in small and newborn mammals.
In 212237 T>C mutation, the animals homozygous for genotype CC had significantly better body weight and average daily gain ($P<0.05$ and $P<0.01$, respectively; Table 1) than those of the homozygous TT genotype animals aged 12 months. Interesting, for the 211562T>C mutation, the animals with AA genotype had significantly better body weight and average daily gain ($P<0.05$ and $P<0.01$, respectively); the rest of the records of growth traits had no significant association with these loci ($P>0.05$; data not show).

Conclusion

In conclusion, seven novel mutations (NC_007314.3: g.577 G>T, 614 T>C, 204811 A>G, 204877 G>A, 212237 T>C, 211562T>C, 211802G>A) not only extend the spectrum of genetic variation of bovine *PRDM16* gene, but also contribute to conducting possible association analysis and evaluating them as genetic marker in bovine breeding, genetics and MDS detection.

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