ONLINE RESOURCES

# Two novel SNPs in the coding region of bovine *VDR* gene and their associations with growth traits

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## Introduction

The vitamin D receptor (*VDR*) gene encodes nuclear hormone receptor for vitamin D3 which plays a crucial role in calcium related metabolism and calcium homeostasis. In humans, *VDR* polymorphisms are associated with several metabolic defects such as type 1 diabetes mellitus (T1DM) and type II vitamin D-resistant rickets. In this study by DNA pool sequencing and PCR-RFLP methods we identified two sequence variants (SV1: g.45515T>C, exon 7; SV2: g.53558C>A, exon 8) of the *VDR* gene in a total of 1166 individuals from five Chinese cattle breeds. Meanwhile, we explored the associations between the two SVs and several growth traits in Nanyang population. At SV1 locus, the individuals with genotype CC showed significantly higher hucklebone width in comparison with TT and TC ($P < 0.05$ or $P < 0.01$) at all the observed stages. At SV2 locus, individuals with genotype CA displayed significantly higher hucklebone width compared to CC ($P = 0.009$) at 6 months old. Our results suggested that *VDR* gene could be used as a candidate gene for marker-assisted selection and management in beef breeding practice.

VDR is nuclear hormone receptor which belongs to the family of trans-acting transcriptional regulatory factors and shows sequence similarity to the steroid and thyroid hormone receptors. Downstream targets of this nuclear hormone receptors are principally involved in mineral metabolism, though the receptor regulates a variety of other metabolic pathways, such as those involved in the immune response and cancer (Haussler *et al*. 1998). Moreover, as a transcription factor, *VDR* regulates transcription of hormone sensitive genes via its association with the WINAC complex, a chromatin-remodelling complex (Kato *et al*. 2004). In addition, VDR as a receptor for the secondary bile acid, lithotomic acid, protects the gut against toxic and carcinogenic effects of these endobiotics (Adachi *et al*. 2005). Besides, *VDR* also plays a central role in calcium homeostasis (Goltzman *et al*. 2004). In humans, it was reported that the *VDR* polymorphisms were associated with several metabolic diseases. In 2002, Györffy's research indicated the significance contribution of *VDR* polymorphisms in T1DM (Gyorffy *et al*. 2002). Meanwhile, Driver found that sequence variants in *VDR* were associated with type II diabetes (Driver *et al*. 2011). Also, mutations in *VDR* are associated with type II vitamin D-resistant rickets (Malloy *et al*. 2011). A single nucleotide polymorphism in the initiation codon resulted in an alternate translation start site three codons downstream which caused low bone mineral density in postmenopausal Mexican–American women (Gross *et al*. 1996). These findings indicate that *VDR* gene is an excellent candidate gene for growth-related traits in humans or livestock.

However, no polymorphisms within the bovine *VDR* gene have been reported so far. Therefore, we first identified two novel genetic variations in the coding region of the bovine *VDR* gene by DNA sequencing, PCR-RFLP and forced PCR-RFLP methods. Then, we assessed the association of polymorphisms in *VDR* gene with growth traits in Nanyang cattle, which is crucial to conducting association analysis and evaluating them as genetic markers for meat production and other function in animal breeding and genetics.

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## Materials and methods

### *Sample animals*

A total of 1166 cattle including four Chinese indigenous breeds: Jiaxian cattle (JX, $n = 410$), Nanyang cattle (NY, $n = 225$), Qinchuan cattle (QC, $n = 224$), Luxi cattle (LX, $n = 168$) and one fostered breed: Chinese Red Steppe cattle (CRS, $n = 139$). All these cattle represent the mian breeds of China. They were from Jiaxian cattle breeding centre in Henan province, Nanyang cattle breeding base in Henan province, Qinchuan cattle breeding centre in Shaanxi province, the fineness breeding centre of Luxi cattle in Shangdong province and Chinese Red Steppe cattle breeding field in Jilin province, China, respectively. Genomic DNA of 1166 animals were isolated from 2% heparin-treated blood samples and stored at −80°C, following the standard procedures (Sambrook *et al*. 2002). The content of DNA was estimated spectrophotometrically, and then the genomic DNA was diluted to 50 ng/$\mu$L. We quantified the growth traits of 225 Nanyang samples. The animals were weaned at an average age of six months and raised from weaning to slaughter on a corn–corn silage diet. The traits under study were the hucklebone width and heart girth at six months, 12 months, 18 months and 24 months of birth. Statistical analysis was performed as described in Gilbert *et al*. (1993).

### *Variants discovery (primer design, commercially sequencing and PCR amplification)*

Polymorphisms of cattle *VDR* gene were detected by DNA pool seqencencing. Ten pairs of PCR primers (table 1) were designed based on the DNA sequence of bovine *VDR* gene (GenBank accession no. NC_007303.5). The results of allelic variation at SVs were detected basd on the electrophoretic pattern of the PCR products. PCR was performed in a 25 $\mu$L of reaction volume containing 50–100 ng genomic DNA, 1 $\mu$mol/L of each primer, 1× buffer (including 1.5 mmol/L $MgCl_2$), 200 $\mu$mol/L dNTPs, and 1.5 U of Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania). The cycling protocol was 5 min at 95°C, 34 cycles of 94°C for 30 s, annealing for 30 s, 72°C for 30 s, with a final extension at 72°C for 10 min.

### *Genotyping*

The SV1 (exon 7: g.45515 T>C) was genotyped by forced PCR-RFLP method with one of the primers containing two nucleotide mismatch, which enables the use of restriction enzyme *Pvu*II for discriminating sequence variation. The SV2 (exon 8: g.53558C>A) was genotyped by PCR-RFLP method. In this study, the 203-bp fragment of the *VDR* gene was amplified by the SV2 primers (size: 203 bp; primers: F2/R2). The PCR products hold a natural *Msp*I endonuclease restriction site (C^CGG). Detailed information of these sequence variants and their respective PCR-RFLP genotyping approaches are listed in table 2. Aliquots of 10 $\mu$L PCR products were digested with 10 U *Pvu*II/*Msp*I for 8 h at 37°C. The digested products were detected by electrophoresis in 3.5% agarose gel stained with ethidium bromide.

### *Data analysis*

Gene frequencies were determined for each breed by direct counting. The Hardy–Weinberg equilibrium (HWE) was

**Table 1.** Primers information for PCR amplification of bovine *VDR* gene.

| Primer | Primer sequence | $T_m$ (°C) | Position (ref. NC_007303.5) | Annotation |
|---|---|---|---|---|
| P1 | E1F: 5′-GTGAAGACTGGACCTGTG-3′<br>E1R: 5′-AACCCTACGCCGCCTAAT-3′ | 57.8 | 35575394–35575617 | 224 bp/exon 1, partial 5′UTR, partial intron 1 |
| P2 | E2F: 5′-TCCAGTGGTGAGATCAAC-3′<br>E2R: 5′-CTCCCATTCAACATCCCT-3′ | 51.2 | 35597259–35597482 | 224 bp/exon 2, partial intron 1, partial intron 2 |
| P3 | E3F: 5′-ATGCCCGCCTGCTGTTCTT-3′<br>E3R: 5′-CCCTCTACTCCTCGCTCTT-3′ | 55.7 | 35600783–35600985 | 203 bp/exon 3, partial intron 2, partial intron 3 |
| P4 | E4F: 5′-TCTCACGGTCACAGGCTCT-3′<br>E4R: 5′-GCCGCGGAAACTCACATT-3′ | 56.8 | 35610201–35610435 | 235 bp/exon 4, partial intron 3, partial intron 4 |
| P5 | E5F: 5′-CCTGGTGAGCTCTGAGTGT-3′<br>E5R: 5′-GGTGTCGTCGTAGGTCTTGT-3′ | 56.1 | 35617537–35617820 | 284 bp/partial intron 4, partial exon 5 |
| P6 | E6F: 5′-GTGGGCTTCTGACATTCC-3′<br>E6R: 5′-GCAGCGCACAGTAGTAAGT-3′ | 53.8 | 35617983–35618231 | 249 bp/exon 6, partial intron 5, partial intron 6 |
| P7 | E7F: 5′-CCTTTTCTTCCCTATTCC-3′<br>E7R: 5′-TCTTTGGGCTCAGGATA-3′ | 52.2 | 35620872–35621128 | 257 bp/exon 7, partial intron 6, partial intron 7 |
| P8 | E8F: 5′-AGTAACTGACCTCCCCCT-3′<br>E8R: 5′-GTGGAGCTTAGGCAGGT-3′ | 53.9 | 35628911–35629137 | 227 bp/exon 8, partial intron 7, partial intron 8 |
| P9 | E9F: 5′-CTTCAGAGGGAGGGAGTTAG-3′<br>E9R: 5′-TCCTCCCGAACTTCTCTG-3′ | 55.7 | 35629168–35629478 | 311 bp/exon 9, partial intron 8, partial intron 9 |
| P10 | E10F: 5′-TGACCGAGGAAGGTGCTGA-3′<br>E10R: 5′-CTCGTTGCCAAACACCTCG-3′ | 59.4 | 35630788–35631151 | 364 bp/exon 10, partial intron 9, partial exon 10 |

$T_m$, annealing temperature.

**Table 2.** Primers used for forced PCR-RFLP analysis of bovine *VDR*.

| Sequence variants | Location | Amino acid changes | Primer sequences | $T_m$ (°C) | Restriction enzyme | F-PCR-RFLP pattern (bp) |
|---|---|---|---|---|---|---|
| g.45515T>C | Exon 7 | 234Tyr>Tyr | F1: 5′-CTTTTCTTCCCTATTCCTTCCCC-3′<br>R1: 5′-GCCGATGACCTTCTGG**CA**GCT-3′ | 58.4 | *Pvu*II | TT:177<br>TC:177, 152, 25<br>CC:152, 25 |
| g.53558C>A | Exon 8 | 290Pro>Gln | F2: 5′-CGAGTGCCATTGAAGTCATCATGC-3′[a]<br>R2: 5′-AACTCCCTCCCTCTGAAGTGCCTG-3′ | 62.5 | *Msp*I | CC:122, 81<br>CA:203, 122, 81 |

Underlined bases show mismatches changed for creating restriction sites; [a]this site contains a natural restriction site; $T_m$, annealing temperature.

tested based on the likelihood ratio for different locus–population combinations and the number of observed and effective alleles by the POPGENE (ver. 1.3.1) software (University of Alberta, Edmonton, Canada). Population genetic indexes such as $H_e$ (gene heterozygosity), $H_o$ (gene homozygosity), $N_e$ (effective allele numbers) were computed by POPGENE software. $H_e$ and $H_o$ are the measure of genic variation of a population and were calculated according to Nei's methods (Nei and Roychoudhurg 1974; eq. 2); polymorphism information content (PIC) was calculated according to Botstein's methods (Botstein *et al*. 1980; eq. 1). The relationships between the variations of the *VDR* gene and growth traits were analysed by analysis of variance (ANOVA) (SPSS software GLM procedure Chicago, USA) using the following model:

$$Y_{ij} = \mu + Age_i + Marker_j + e_{ij},$$

where $Y_{ij}$, observation of the trait; $\mu$, least square mean; $Age_i$, effect of age; $Marker_j$, effect of marker genotype; $e_{ij}$, residual effect.

## Results and discussion

### *Sequence variants identified in bovine VDR gene*

Bovine *VDR* gene maps to chromosome 5. The coding region consists of 10 exons, which are shorter in size than human *VDR* gene (accession number: NC_000012.11). In this study, the coding region of *VDR* in all five cattle breeds was successfully amplified using primer pairs for the *VDR* gene (table 1). We amplified and sequenced all introns and exons of the *VDR* gene from 100 animals; 20 DNA samples were selected randomly from each cattle breed. By comparing with previously reported sequence (GenBank accession number: NC_007303.5), only two novel SVs were identified in these animals: SV1 (g.45515 T>C, exon 7) is a silent mutation (p. Tyr234Tyr); while SV2 (g.53558 C>A, exon 8) resulted in a missense mutation (p. Pro290Gln) (table 2). The genotyping of the two single nucleotide polymorphisms (SNPs) (SV1 and SV2) were successfully implemented employing PCR-RFLP and forced PCR-RFLP methods in these animals (table 2). These sequence variants in the coding region of bovine *VDR* gene were first detected with restriction endonuclease, *Pvu*II/*Msp*I. It is very efficient and effective strategy to scan large sample size sequence variants with the combination of DNA sequencing and forced PCR-RFLP methods, which will overcome the disadvantages of PCR-SSCP: inaccuracy, the complicated technical demands, slow speed and unstable reproducibility. Here, the forced PCR-RFLP method was successfully carried out to accurately detect the polymorphism of the bovine *VDR* gene.

At the SV1-*Pvu*II locus (exon 7), digestion of the 177 bp PCR product with *Pvu*II resulted in fragment lengths of 177 bp band for genotype TT individuals (homozygous); 177 bp, 152 bp and 25 bp bands for genotype TC (heterozygous); 152 bp and 25 bp for genotype CC. At the SV2-*Msp*I locus (exon 8), digestion of 203 bp PCR product with *Msp*I resulted in fragment lengths of 122 bp and 81 bp for genotype CC; 203 bp, 122 bp and 81 bp for genotype CA (figures 1 and 2). Moreover, homozygote AA (SV2) was not detected in the study which revealed that the frequencies of allele A in all five animal populations demonstrated a decreasing trend through artificial selection, migration, and genetic drift function. The observed segregation of the allele A can be explained rather by a negative or sublethal effect

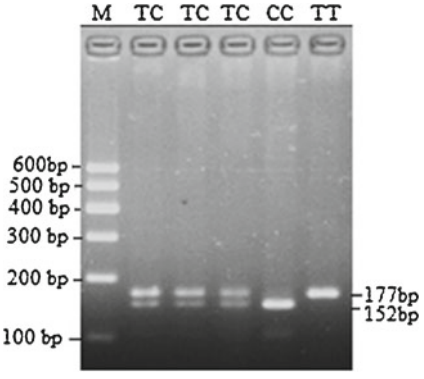


**Figure 1.** Electrophoretic patterns on 3.5% agrose after digestion with *Pvu*II endonuclease of the PCR fragment containing g.45515 T>C mutation of bovine *VDR* gene. TC (177 + 152 bp), CC (152 + 25 bp), TT (177 bp); M, marker I (600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp); 25 bp fragment was too short to be visible.

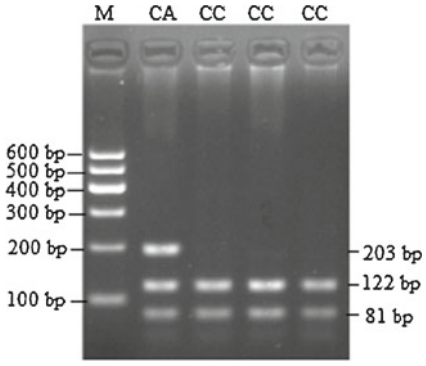


**Figure 2.** Electrophoretic patterns on 3.5% agrose after digestion with *Msp*I endonuclease of the PCR fragment containing g.53558 C>A mutation of bovine *VDR* gene. CA (203 + 122 + 81 bp), CC (122 + 81 bp); M, marker I (600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp).

of the missing genotype. In this situation, the genotype AA may negatively influence the growth of fetuses during prenatal development. Therefore, homozygote AA (SV2) was eliminated during the process of natural selection. Recently, some research results indicated that certain homozygote are missing in livestock. In the POU1F1-*Dde*I locus of goat, a comparable population demonstrated lack of the D2D2 genotype (Lan *et al*. 2007a), in the *PRL* locus almost no AA genotype was detected (Lan *et al*. 2009). In cattle, the *NPM1* locus almost no WW genotype was detected in 1035 individuals of four cattle breeds (Huang *et al*. 2010). Besides, the reason why genotype AA was so rare in these breeding farms still needs a further investigation. Then, the frequencies of genotype and allele of the five Chinese bovine populations were calculated directly (table 3). At the SV1-*Pvu*II locus, the frequency of allele T has shown a high prevalence in the five breeds and TC genotype displayed more frequently than other genotypes. The $\chi^2$ test showed that the genotype distributions in NY and JX cattle were significantly deviated from HWE ($P < 0.01$) which means there was no dynamic equilibrium after experiencing artificial selection, migration, and genetic drift function; while the other three breeds were in HWE ($P > 0.05$), demonstrating there was a dynamic equilibrium despite artificial selection, migration, and genetic drift function.

At the SV2-*Msp*I locus, the frequency of allele C has demonstrated a high prevalence in all populations (NY, 97.8%; QC, 94.2%; JX, 87.3%; LX, 97.3%; CRS, 98.2%) and CC genotype appeared more frequent in most of the populations (NY, 95.6%; QC, 88.4%; JX, 74.6%; LX, 94.6%; CRS, 96.4%). The $\chi^2$-test showed that the genotype distributions within all cattle populations were in the HWE ($P > 0.05$) except JX cattle populations (table 3).

### *Diversity analysis of the bovine VDR in the five Chinese cattle populations*

Genetic diversity is essential for species preservation and improvement of production of potentially selected breeds. In this study, genetic indices ($H_o$, $H_e$, $N_e$ and PIC) in these five Chinese cattle populations are presented in table 3. At the SV1 locus, the values of $H_e$ and $N_e$ were approaching to 0.5 and 2, respectively. While at the SV2 locus, the values of $H_e$ and $N_e$ are rather low. This indicates that SV1 locus displayed a higher sequence variants and the allele distribution is more evenly. According to the classification of PIC (PIC < 0.25, low polymorphism; 0.25 < PIC < 0.5, intermediate polymorphism; and PIC > 0.5, high polymorphism), the maximum and minimum PIC values were 0.425 and 0.035. At the SV1 locus, all the five cattle populations belonged to intermediate genetic diversity; At the SV2 locus, only the NY cattle (intermediate genetic diversity PIC = 0.425) was not defined as low polymorphism. Hence, there was not a very high

**Table 3.** Genotype frequencies and genetic diversity parameters of bovine *VDR*.

| | | Genotypic and allelic frequencies | | | | | | | Diversity parameter | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Loci | Breeds | TT | TC | CC | T | C | $\chi^2$ (HWE) | | $H_o$ | $H_e$ | $N_e$ | PIC |
| g.45515 T>C | NY (225) | 0.182 | 0.707 | 0.111 | 0.536 | 0.464 | 39.788 | $P < 0.01$ | 0.503 | 0.497 | 1.990 | 0.374 |
| | JX (410) | 0.478 | 0.502 | 0.020 | 0.729 | 0.271 | 30.425 | $P < 0.01$ | 0.605 | 0.395 | 1.653 | 0.317 |
| | QC (224) | 0.585 | 0.393 | 0.022 | 0.781 | 0.219 | 4.999 | $P > 0.05$ | 0.658 | 0.342 | 1.519 | 0.283 |
| | LX (168) | 0.292 | 0.476 | 0.232 | 0.530 | 0.470 | 0.329 | $P > 0.05$ | 0.502 | 0.498 | 1.992 | 0.374 |
| | CRS (139) | 0.511 | 0.431 | 0.058 | 0.727 | 0.273 | 1.040 | $P > 0.05$ | 0.603 | 0.397 | 1.659 | 0.318 |
| | | CC | CA | AA | C | A | | | | | | |
| g.53558 C>A | NY (225) | 0.956 | 0.044 | 0.000 | 0.978 | 0.022 | 0.116 | $P > 0.05$ | 0.957 | 0.043 | 1.045 | 0.425 |
| | JX (410) | 0.746 | 0.254 | 0.000 | 0.873 | 0.127 | 8.650 | $P < 0.05$ | 0.779 | 0.221 | 1.285 | 0.197 |
| | QC (224) | 0.884 | 0.116 | 0.000 | 0.942 | 0.058 | 0.850 | $P > 0.05$ | 0.891 | 0.109 | 1.123 | 0.103 |
| | LX (168) | 0.946 | 0.054 | 0.000 | 0.973 | 0.027 | 0.127 | $P > 0.05$ | 0.948 | 0.052 | 1.055 | 0.051 |
| | CRS (139) | 0.964 | 0.036 | 0.000 | 0.982 | 0.018 | 0.047 | $P > 0.05$ | 0.965 | 0.035 | 1.037 | 0.035 |

$\chi^2$ (HWE), Hardy–Weinberg equilibrium $\chi^2$ value. $H_o$, gene homozygosity; $H_e$, gene heterozygosity; $N_e$, effective allele numbers; PIC, polymorphism information content.

genetic diversity within *VDR* gene of Chinese cattle in all the five analysed populations.

***Association analysis of single markers***

We investigated the associations of the two SVs in coding region of *VDR* gene with growth traits in NY (table 4). Growth traits (body height, body length, heart girth, hucklebone width, body weight, and average daily gain) were analysed in NY cattle at 6, 12, 18 and 24 months old.

At SV1 locus, the individuals with genotype CC showed significantly higher hucklebone width when compared to TT and TC ($P < 0.05$ or $P < 0.01$) at all the observed stages. At the age of 18 months, cattle with CC genotype appeared superior in heart girth and body length compared to those with TT and CC ($P < 0.05$). Besides, there was a tendency that individuals with CC genotype had higher heart girth than TT, TC genotypes, although no significant differences appeared ($P > 0.05$) in this study. The allele SV1-C might be associated with an increase in body length, heart girth, especially the hucklebone width. At SV2 locus, individuals with genotype CA displayed significantly higher hucklebone width in comparison with CC ($P = 0.009$) at six months old. However, individuals with genotype CC had higher heart girth and body length than those with genotypes CA at 18 months and 24 months, respectively ($P = 0.023$, $P = 0.026$). Also, there was a trend that individuals with CC genotype had higher body length (data not shown) and heart girth than CA genotypes ($P > 0.05$). The allele SV2-A might be associated with an increase in hucklebone width. Hence, the above results suggested that the cattle with SV1-CC, SV2-CA could be selected to obtain greater body measurement in bovine. Noticeably, the SV1-*Pvu*II mutation (g.45515T>C, exon 7) was a silent mutation (Tyr–Tyr). Recently, there were some reports about the silent mutations impacted on the gene function and phenotype (Komar 2007). A silent polymorphism in the *MDR1* gene resulted in substrate specificity change (Kimchi-Sarfaty *et al*. 2007). In goat, a silent mutation of goat *POU1F1* gene had been found to be associated with milk yield and birth weight (Lan *et al*. 2007b). In humans, a novel nonsense mutation in *VDR* causing hereditary 1, 25-dihydroxyvitamin D3-resistant rickets has been observed (Mechica *et al*. 1997). Additionally, the SV2-*Msp*I mutation (g.53558C>A, exon 8) was a missense mutation (Pro290Gln). Further, this may also affect the translation efficiency of *VDR* itself; thereby, altering their functions, which appear to be a tendency to control body measurement in bovine's each developmental phase. In humans, a single nucleotide mutation in *VDR* gene caused amino acid change from Gly to Asp which is associated with hypokalemic rickets (Hughes *et al*. 1988). Thus, this left us an interesting and significant work, revealing the mechanism of the association of both silent mutations and missense mutations with bovine VDR gene and their growth traits.

The *VDR* gene is a nuclear hormone receptor for vitamin D3. Also as a transcription actor, *VDR* mediates the action of vitamin D3 by controlling the expression of hormone sensitive genes (Akutsu *et al*. 2001). Still, it is involved in cell differentiation and proliferation, bone and cartilage development, and regulation of osteoporosis. *VDR* is expressed in the

**Table 4.** Association of different genotypes with growth traits in Nanyang cattle.

| | | | Mean ± S.E. | | | |
|---|---|---|---|---|---|---|
| Locus | Age | Growth trait (cm) | TT | TC | CC | *P* |
| SV1 | 6 months | HG | 128.000 ± 2.150 | 129.760 ± 0.909 | 132.500 ± 1.093 | 0.161 |
| | | HW | 17.542 ± 0.463 | 18.541 ± 0.146 | 18.313 ± 0.299 | 0.060 |
| | 12 months | HG | 141.830 ± 2.374 | 142.190 ± 0.875 | 144.250 ± 1.178 | 0.450 |
| | | HW | 20.833 ± 0.328$^{AB}$ | 20.824 ± 0.167$^{B}$ | 22.500 ± 0.202$^{A}$ | 0.001 |
| | 18 months | HG | 154.000 ± 1.619$^{B}$ | 155.220 ± 0.964$^{AB}$ | 161.380 ± 1.037$^{A}$ | 0.002 |
| | | HW | 22.708 ± 0.199$^{B}$ | 23.015 ± 0.211$^{B}$ | 24.625 ± 0.416$^{A}$ | 0.003 |
| | 24 months | HG | 167.750 ± 2.230 | 171.360 ± 1.223 | 172.000 ± 2.436 | 0.494 |
| | | HW | 24.667 ± 0.655 | 25.690 ± 0.286 | 26.750 ± 0.587 | 0.057 |
| | | | CC | CA | AA | |
| SV2 | 6 months | HG | 129.330 ± 1.040 | 129.600 ± 1.343 | – | 0.917 |
| | | HW | 18.175 ± 0.194$^{B}$ | 19.500 ± 0.365$^{A}$ | – | 0.009 |
| | 12 months | HG | 141.980 ± 1.070 | 141.400 ± 2.296 | – | 0.832 |
| | | HW | 20.954 ± 0.214 | 21.000 ± 0.422 | – | 0.933 |
| | 18 months | HG | 156.420 ± 1.114$^{a}$ | 150.000 ± 1.700$^{b}$ | – | 0.023 |
| | | HW | 23.193 ± 0.250 | 22.800 ± 0.327 | – | 0.524 |
| | 24 months | HG | 169.610 ± 1.312 | 167.700 ± 4.594 | – | 0.602 |
| | | HW | 25.342 ± 0.321 | 25.600 ± 0.778 | – | 0.758 |

HG, heart girth; HW, hucklebone width; S.E., standard error of means; values with different superscripts within the same line differ significantly at $P < 0.01$ (A, B, C) and $P < 0.05$ (a, b, c).

intestine, thyroid and kidney, and has a vital role in calcium homeostasis. In humans, it is reported that polymorphisms in *VDR* are associated with several metabolic diseases such as type II vitamin D-resistant rickets, low bone mineral density and so on. To date, the mechanism of the association between *VDR* polymorphisms and its function is not clarified. There are mainly two interpretations: *VDR* polymorphisms influenced the expression and stability of *VDR* mRNA which caused a tiny difference either in the numbers of receptor proteins or its activity (Ogunkolade *et al*. 2002); another explanation is that there exist an unknown gene closed to *VDR* gene in linkage disequilibrium with *VDR* alleles through which aspects of calcium related metabolism are affected (Gross *et al*. 1998).

In this study, we reported two novel SVs in the coding region of bovine *VDR* gene and explored their association with body size in NY cattle population for the first time. Additionally, the coding region is an extremely crucial region, which directly decides and affects gene function. Polymorphisms in this region can alter gene expression, thereby the gene function. Hence, further study is needed to clarify the role of the genetic variants of the *VDR* gene and to investigate the mechanism of mRNA expression levels of the *VDR* gene. Admittedly, the eventual goal in the genomic study of candidate genes in animals is to identify these biologically relevant genotype–phenotype associations and to apply them to marker-assisted selection in breeding practice for superior beef cattle in China.

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