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Molecular characterization of the vitamin D receptor (VDR) gene in Holstein cows

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ABSTRACT

Vitamin D plays a vital role in calcium homeostasis, growth, and immunoregulation. Because little is known about the vitamin D receptor (VDR) gene in cattle, the aim of the present investigation was to present the molecular characterization of exons 5 and 6 of the VDR gene in Holstein cows. DNA extraction, genomic sequencing, phylogenetic analysis, synteny mapping and single nucleotide gene polymorphism analysis of the VDR gene were performed to assess blood samples collected from 50 clinically healthy Holstein cows. The results revealed the presence of a 450-base pair (bp) nucleotide sequence that resembled exons 5 and 6 with intron 5 enclosed between these exons. Sequence alignment and phylogenetic analysis revealed a close relationship between the sequenced VDR region and that found in Hereford cattle. A close association between this region and the corresponding region in small ruminants was also documented. Moreover, a single nucleotide polymorphism (SNP) that caused the replacement of a glutamate with an arginine in the deduced amino acid sequence was detected at position 7 of exon 5. In conclusion, Holstein and Hereford cattle differ with respect to exon 5 of the VDR gene. Phylogenetic analysis of the VDR gene based on nucleotide sequence produced different results from prior analyses based on amino acid sequence.

Vitamin D plays a crucial role in calcium homeostasis, growth, and the differentiation of multiple cell types (Adams and Hewison, 2008). Vitamin D stimulates cells responsible for both adaptive and innate immunity, with subsequent expression of the vitamin D receptor (VDR) gene in response to 1,25-dihydroxycholecalciferol (Adorini and Penna, 2008).

The function of vitamin D is controlled by the activity of the VDR gene (Collingwood et al., 1994). The interaction between the VDR gene product, a phospho-protein, and vitamin D leads to control of the intracellular expression of certain genes via zinc finger motifs known as vitamin D-responsive elements (Haussler et al., 1997).

Although mutations in living organisms are attributed to genetic events with multi-factorial causes, certain genes are capable of retaining their ancestral organization under different circumstances; collections of such genes are called synteny blocks (Murphy et al., 2005), and this phenomenon has driven scientists to identify mutational changes in gene sequences (Lucas et al., 2014). In humans, VDR gene polymorphism is associated with a wide range of diseases (Gatto et al., 2016; Pabalan et al., 2017; Zhang et al., 2016). Multiple sequence alignment is essential for identifying functional sites from genomic sequences and subsequently performing phylogenetic analyses to determine evolutionary relationships among genomic sequences from different species (Notredame, 2002). There is a dearth of literature on the VDR gene in Holstein cows. Thus, the purpose of this investigation was to distinguish single nucleotide polymorphisms (SNPs) and genomic sequence polymorphisms in the VDR gene in Holstein cows and compare these features with those of VDR genes of Hereford cattle and other species.

Blood samples were collected from 50 clinically healthy Holstein cows (age, 4–6 years; parity, 3–6) in EDTA-containing tubes and preserved at −80 °C until DNA extraction.

DNA extraction was performed using a standard method (Bardakci and Skibinski, 1994). SNP analysis was restricted to parts of exons 5 and 6 as well as intron 5. Primer sequence design was based on the VDR gene sequence in GenBank under accession number NW_014644792. Alignment of the VDR gene from the complete genome and the VDR

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mRNA sequence recorded under accession number NM_001167932 was conducted to detect the appropriate exons.

Polymerase chain reaction was performed using an equivalent of 1 ng of DNA template mixed with 12.5 µl of master mix (TaKaRa, Japan) and primers with the following sequences: ACTGATGAAGAAG TGCAGCG for VDR1F and AGGAGGTACAGTGATCCGAA for VDR1R. The PCR cycling conditions involved an initial denaturation step at 94 °C for 9 min followed by 40 cycles of denaturation (94 °C for one min), annealing (58 °C for one min) and elongation (72 °C for one min). A final extension step was also performed at 72 °C for 9 min. A PCR product of the expected size (420 base pairs (bp)) was detected. The obtained band was purified using a DNA purification kit (GeneJET Gel Extraction Kit, Thermo Fisher Scientific) and sequenced using both forward and reverse primers. The obtained genomic sequences were assessed using sequences in GenBank to determine percentage identities with the bovine VDR gene.

The sequenced genomic DNA regions of the VDR gene were aligned to specify a conserved region of this gene. Similarities between sequences were determined, and sequences were aligned with a sequence of the VDR gene in cattle in GenBank using MEGA4 software (Tamura et al., 2007). Neighbour-joining and maximum likelihood methods were used for estimation in a phylogenetic analysis of the VDR genes of different animals. With respect to bootstrap statistics, 1000 replicates were used for both the neighbour-joining and maximum likelihood methods (Felsenstein, 1985). The phylogenetic analysis was based on sequences from the following species: Holstein cattle (KX018313), Hereford cattle (AC_000162), sheep (NW_004080166), goats (NC_022297), horses (NC_009149), cats (NC_018729), dogs (NC_006609), rats (NC_005106), chimpanzees (NC_006468), humans (NC_00012), and pigs (NC_010447).

The identification of synteny relationships between the VDR gene sequences of cattle and those of other species, including humans, mice, pigs, cats, sheep and goats, was performed using the Ensembl genome browser (Yates et al., 2015). For confirmation, collagen type II and histone deacetylase were selected as reference genes.

The genomic sequences obtained for the VDR gene and reported for other mammalian species were aligned using Clustal Omega online software (http://www.ebi.ac.uk/Tools/msa/clustalo/help/faq.html) (Goujon et al., 2010). The conserved sequence identified via this alignment process was subjected to SNP analysis. Changes detected in nucleotide sequence analysis were extended to the deduced amino acid sequences.

The obtained sequence of the selected portion of the VDR gene consisted of 2 exons (5 and 6) that had a total length of 249 bp and were separated by an intron (5) of 171 bp. The Ensembl genome browser allowed for alignment between the obtained sequence for the VDR gene of Holstein cattle and the corresponding sequence for Hereford cattle in UMD 3.1 (with accession number NW_014644792), which is located on chromosome 5.

Syntenic relationship analysis revealed that the isolated fragment of the VDR gene was located on chromosomes 12, B4, 15, 5 and 20 in humans, cats, mice, pigs, sheep and goats, respectively (Fig. 1). Two reference genes, histone deacetylase and collagen type 2, were used to confirm findings for the VDR gene (Fig. 2a).

Analysis of the VDR gene revealed the presence of a 141-bp conserved region with a few SNPs between ruminants and other mammals. For Holstein cattle, the frequency of the identified SNP in the VDR gene was 1/11 (0.09). At position 29, cytosine was detected for all ruminant species (Holstein cattle, Hereford cattle, sheep and goats), whereas the remaining species had a thymine. At position 38, all ruminants had a guanine, whereas other mammals had a cytosine. At position 73, thymine was replaced by cytosine in ruminants. At positions 104 and 105, guanine and adenine in ruminants were replaced by two cytosine bases in other mammals. A conserved nucleotide base was detected at position 138; at this location, guanine (in all animals) had been replaced by adenine in both Hereford and Holstein cattle.

Phylogenetic analysis revealed close relationships between Hereford and Holstein cattle; sheep and goats; dogs and cats; and humans and chimpanzees (Fig. 2b). For Holstein cattle, compared with Hereford cattle, genomic alignment revealed a SNP at position 7 in exon 5 associated with an amino acid sequence change involving the replacement of glutamate with arginine.

There are few studies on the VDR gene and the association between VDR gene polymorphisms and diseases in cattle. The expected amplicon size of the VDR gene in Holstein cows, which is located on chromosome 5, is 414 bp. Research has indicated that the coding region of the VDR gene consists of 10 exons (Deiner et al., 2012; Gao et al., 2013). In the current study, exons 5 and 6 were chosen for isolation and sequencing procedures due to the proximity of these exons to each other. This preliminary study was performed to detect differences in nucleotide sequences for Holstein and Hereford cattle, suggesting that the study results could feasibly be used in the diagnosis of clinical manifestations related to VDR gene dysfunction.

Analysis of the syntenic relationships between the bovine VDR gene and the corresponding gene in other mammalian species was performed to determine the shared conserved genes flanking the VDR gene across species and to define the position of the VDR gene on different chromosomes of these species (Bhutkar et al., 2006). Additionally, syntenic relationship and protein coding information could suggest potential orthology among similar genes in different species. In our study, in accordance with findings from previous studies in mammals, the identified syntenic block revealed that the VDR gene lies between the collagen type II and histone deacetylase genes. Five conserved SNPs between ruminants and other mammals were detected. In comparisons with VDR genes in other mammals, one conserved SNP was identified in both bovine VDR genes.

Multiple sequence alignment is important for the creation of a computational tool for predicting the VDR gene structure that might act as a guide to extrapolate from recent results to the interpretation of larger data sets (Reumers et al., 2009). The presence of conserved SNPs in ruminant species may reflect the high identity percentage among all studied ruminants and specifically indicate structural properties of the VDR gene (Yue et al., 2005). In addition, the conserved nucleotide sequence in both bovine VDR genes may reinforce the hypothesis of high identity between Holstein and Hereford cattle, suggesting the conservation of structural properties between these breeds (Fu et al., 2007).

Phylogenetic analysis showed two clades. The first clade had four clusters. The F-A1 and F-A2 clusters involved cattle and small ruminants, respectively, whereas the other two clusters involved horses and carnivores (dog and cats). The second clade was specific to humans, chimpanzees and rats. In our study, the bootstrap percentage for humans and chimpanzees revealed 87% identity. On the other hand, based on amino acid sequence, the bovine VDR gene protein exhibits a close relationship with the corresponding gene in primates, dogs and rats due to the absence of not only other ruminants but also introns that might affect VDR protein identity (Ekins et al., 2008). Therefore, genomic VDR gene nucleotide sequence results differ from VDR protein results.

The amino acid sequence showed the replacement of lysine in Hereford cattle with glutamate in Holstein cattle. Fortunately, this change in amino acid sequence between Hereford and Holstein cattle does not alter the function of the polypeptide chain due to the polarities of both amino acids, which share the same biological role (Ashburner et al., 2000).

A limitation of the present study is that only exons 5 and 6 were selected for analysis; therefore, complete characterization of the VDR gene could not be performed. Because the VDR gene is extremely large (55,000 bp), extensive investigations are warranted for the complete molecular characterization of this gene.

In conclusion, the VDR gene of Holstein cow includes a SNP in exon 5 that is associated with a change in the deduced amino acid sequence. The results of phylogenetic analysis of the VDR gene based on nucleotide sequence differ from prior findings from analyses based on