A genomewide association study identified CYP2J2 as a gene controlling serum vitamin D status in beef cattle¹,²

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ABSTRACT: Vitamin D is an important modulator of calcium homeostasis and has several effects on the immune system. The objective of the study was to estimate its heritability and to identify genomic regions associated with concentration of circulating 25-hydroxyvitamin D (25OHD) in beef cattle. Status of vitamin D was measured in crossbred animals from Cycle VII of the United States Meat Animal Research Center (USMARC) Germplasm Evaluation Project. Progeny were born from March through May in 2008 and in 2010. Heritability was estimated and a genomewide association study was conducted on the concentration of 25OHD measured in 1,432 animals at preconditioning and 1,333 animals at weaning. Genotyping of the population was done by imputing from the parental generation genotyped with a high density array (777,000 SNP) to a target population genotyped with a medium density SNP array (50,000 SNP). After imputation, 675,018 SNP were used in the genomewide association study. Heritability of concentration of circulating 25OHD in cattle at preconditioning and at weaning was 0.41 ± 0.08 and 0.32 ± 0.11, respectively. A region on chromosome 3 was associated with circulating 25OHD. The region on BTA3 had 7 SNP significantly (\(P < 7.4 \times 10^{-8}\)) associated at the genomewide level with serum concentrations of serum 25OHD. Genomewide significant SNP spanned the region between 84.93 and 86.65 megabases (Mb); however, 6 SNP reside between 86.64 and 86.65 Mb. The gene CYP2J2 was identified as a candidate gene associated with concentrations of serum 25OHD in cattle. This is 1 of 6 enzymes involved in metabolizing vitamin D to 25OHD. Results from the present study suggest that CYP2J2 is a gene controlling serum 25OHD concentrations in cattle. CYP2J2 should be considered a prime candidate for understanding both genetic and physiological factors affecting serum 25OHD concentrations in cattle and, therefore, vitamin D status.

Key words: cattle, CYP2J2, genomewide association study, heritability, vitamin D 25-hydroxylase, vitamin D status

INTRODUCTION

Vitamin D is involved in calcium homeostasis and in modulating the immune system (Horst et al., 1994; Van Etten et al., 2008; Nelson et al., 2010).

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Dietary vitamin D can be obtained as ergocalciferol from plants or cholecalciferol from animal sources. Vitamin D can also be obtained by the conversion of 7-dehydrocholesterol in the skin by a reaction catalyzed by the UV rays from the sun. Bioactivation of vitamin D is done in 2 steps: the first step is the conversion of vitamin D (ergocalciferol or cholecalciferol or 7-dehydrocholesterol) to 25-hydroxyvitamin D (25OHD) in the liver. The second step is conversion of 25OHD to the active form, 1,25-dihydroxyvitamin D, in the kidney or production by different organs from external sources. Circulating 25OHD reflects adequacy of intake of vitamin D and indicates vitamin D status (Nelson et al., 2012; Zhu and DeLuca, 2012).
Studies in humans have estimated heritability of circulating 25OHD concentrations using twins (monozygotic and dizygotic) as the target populations. Heritability in these studies ranges from 0.2 to 0.8, depending on the population used (Arguelles et al., 2009; Bu et al., 2010). To our knowledge, heritability of 25OHD has not been estimated in cattle.

Genomewide association studies (GWAS) are now possible due to the availability of technology that permits high throughput genotyping of SNP. This technology allows deciphering the genetics behind the expression of economically important traits. Genetic regions associated with productive traits have been identified in dairy cattle (Cole et al., 2011) and beef cattle (Snelling et al., 2010, 2011) as well as for diseases in cattle (Minozzi et al., 2012) and additional traits of interest (Pausch et al., 2012). The objective of the study was to estimate the heritability and to identify genomic regions associated with concentration of circulating 25OHD in beef cattle.

MATERIALS AND METHODS

Animal experimental procedures were approved and performed in accordance with United States Meat Animal Research Center (USMARC) Animal Care Guidelines and the Guide for Care and Use of Agricultural Animals Research and Teachings (FASS, 1999).

Animals

Measurements of 25OHD were done on sera obtained from animals in advanced generations of the USMARC Germplasm Evaluation Project (GPE), in Clay Center, NE. This particular GPE subset was a product of multiple-sire matings of crossbred cows to F1 bulls of varying breed composition. The resulting animals used within this study consisted of variable fractions of Angus, Hereford, Red Angus, Brahman, Charolais, Gelbvieh, Limousin, Simmental, and MARC III (one-fourth Hereford, one-fourth Angus, one-fourth Red Poll, and one-fourth Pinzgauer).

Progeny were born from March through May in 2008 (n = 683) and in 2010 (n = 780). Male calves were castrated within 24 h after birth. At preconditioning, calves were vaccinated against bovine viral diarrhea virus types 1 and 2, parainfluenza 3, infectious bovine rhinotracheitis virus, and bovine respiratory syncytial virus. Preconditioning spanned from September 1 through 4 in 2008 and from September 2 through 13 in 2010. Approximately 3 wk later (September 22 to 25 in 2008 and from September 23 to October 4 in 2010), calves were revaccinated, sorted, and placed in pens by sex at weaning.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>n</th>
<th>Mean, ng/mL</th>
<th>SD</th>
<th>Heritability ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconditioning</td>
<td>1,432</td>
<td>59.49</td>
<td>17.74</td>
<td>0.41 ± 0.08</td>
</tr>
<tr>
<td>Weaning</td>
<td>1,333</td>
<td>58.34</td>
<td>18.46</td>
<td>0.37 ± 0.11</td>
</tr>
</tbody>
</table>

1 Preconditioning management was done from September 3 to 8, 2008, and from September 2 to 13, 2010.
2 Weaning was done from September 22 to 25, 2008, and from September 23 to October 4, 2010.

Marker Information

High density genotypes in the population under study were imputed from a high density array [BovineHD; all sires (n = 106) were genotyped with 777,000 SNP] in the parental generation, using a medium density SNP array (BovineSNP50; 50,000 SNP) in the target population, using the Illumina platform (Illumina, Inc., San Diego, CA). Imputation procedures have been described elsewhere (Weigel et al., 2010). The imputation algorithm used both pedigree and haplotype information, according to the procedure indicated by VanRaden et al. (2013). Single nucleotide polymorphisms that were monomorphic, that were not in Hardy-Weinberg equilibrium, or that had a minor allele frequency (MAF) less than 5%, were eliminated from the analysis. A total of 675,018 SNP were successfully genotyped and imputed from all autosomal chromosomes.

Statistical Analysis

Open source software, R (R Core Team, 2012), was used to conduct statistical analysis. Genomewide association analysis was performed using the GenABEL package (Aulchenko et al., 2007). Markers were fitted as a linear covariate in the statistical model. Genotypes of the SNP were represented as the number of copies of the Illumina B allele (Illumina, Inc.). An additive polygenic model was initially run (using the “polygenic” command in GenABEL), using a genomic relationship matrix (“ibs” command in GenABEL) to estimate the heritability. The polygenic model estimated the heritability of each trait.
To run the GWAS, “mmscore” (Chen and Abecasis, 2007) was used (within the GenABEL package). The mmscore command accounts for population stratification and familial relationships using the output from the polygenic command by running this mixed model for all traits: $Y_{ijk} = \mu + (CG)_i + S_j + \beta_1 h + a_k + e_{ijk}$, which included birth location and mating group by year of birth (CG) and sex (S) as fixed effects; $\beta_1$, the linear regression on the covariate heterosis (pedigree derived heterosis); $h$, heterosis (minimum $= 0.39$, maximum $= 1$, and average $= 0.77$); $a$, the random effect of animal $a \sim N(0; G)$; in which G is the genomic relationship matrix); and $e_{ijk}$, the residual error $e \sim N(0, \sigma^2_e)$.

A Bonferroni adjustment was applied to determine significance ($P < 0.05/675,018$), establishing the genomewide threshold for significant association to a nominal $P < 7.4 \times 10^{-8}$. Chromosome-wide significance was claimed when it reached a nominal $P < 0.05$/number of markers on the chromosome. Chromosome-wide significance for BTA3 was $P < 1.4 \times 10^{-6}$ ($= 0.05/35,575$). Chromosomal location of SNP is based on the University of Maryland version 3 assembly.

RESULTS

Heritability of 25OHD status is shown in Table 1. Heritability estimates for concentrations of 25OHD at preconditioning and at weaning are considered moderate to high. The direct genetic correlation between 25OHD measured at preconditioning and at weaning was $r_g = 0.61 \pm 0.14$.

The GWAS for 25OHD status at preconditioning is shown in Fig. 1. A region on chromosome 3, where the gene CYP2J2 resides, was associated with concentrations of 25OHD. The region on chromosome 3 had 7 significantly ($P < 7.4 \times 10^{-8}$) associated SNP at the genomewide level with concentration of 25OHD.

Table 2 shows the position, minor allele, effect of the MAF allele, and the significance at the genomewide and chromosomal-wide level for the significant markers on chromosome 3. At the chromosome-wide level, markers located between 84.93 and 86.65 megabases (Mb) were associated with concentration of 25OHD. Genomewide significant SNP spanned the region between 86.64 and 86.65 Mb. However, 6 of the 7 significant SNP reside between 86.64 and 86.65 Mb (Fig. 1; Table 2).

The GWAS for concentration of 25OHD at weaning is shown in Fig. 2. Markers on chromosome 3 were significantly ($P < 7.4 \times 10^{-8}$) associated with 25OHD concentration. Table 2 shows the position, MAF, effect of the minor allele, and the significance at the genomewide and chromosome-wide level for the significant markers. At the chromosome-wide level, markers significantly associated with 25OHD concentrations spanned the region between 57.76 and 86.65 Mb. At the genomewide level, significant markers spanned from 86.49 to 86.65 Mb. Six of the 7 genomewide significant SNP spanned between 86.64 and 86.65 Mb.

The same SNP were significant for concentration of 25OHD at preconditioning and at weaning, between 86.64 and 86.65 Mb. The substitution effect of these SNP was similar at preconditioning and at weaning (Table 2). The average value of the effect was 4.8 ng/mL (Table 2). The effects of the minor allele were all negative in this region except for rs110220315 and rs11039677.

DISCUSSION

The GWAS identified a region on chromosome 3 associated with vitamin D status as measured by serum 25OHD concentration in cattle. Seven of the genomewide significant SNP resided within a 10,000 bases region. This region of chromosome 3 harbors a candidate gene (CYP2J2) involved in the bioactivation of vitamin D sources to 25OHD (Zhu and DeLuca, 2012).

The gene CYP2J2 produces the enzyme cytochrome P450, family 2, subfamily J, polypeptide 2. The cytochrome P450 superfamily (CYP) is a group of enzymes that catalyze the oxidation of small organic compounds. The substrates of CYP enzymes include metabolic intermediates such as lipids and steroidal hormones precursors such as vitamin D as well as drugs and other chemicals (Sugimoto and Shiro, 2012; Zhu and DeLuca, 2012; Jones et al., 2013). The CYP enzymes are the major enzymes involved in drug metabolism and intermediate and final activation of steroid hormones such as vitamin D to 25OHD and finally 1,25 dihydroxy vitamin D \([1,25(\text{OH})_2\text{D}] \). The most common reaction catalyzed by CYP enzymes is a mono-oxygenase reaction, which results in the insertion of oxygen. The CYP enzymes belong to a superfamily of proteins containing a heme cofactor. Most CYP enzymes require a protein partner to deliver 1 or more electrons to reduce the iron molecular oxygen. Based on the nature of the electron transfer proteins, CYP enzymes can be classified into several groups. The 2 groups important in vitamin D metabolism are microsomal and mitochondrial P450 systems. In microsomal P450 systems, electrons are transferred from Nicotinamide Adenine Dinucleotide Phosphate (NADPH) via cytochrome P450 reductase. In mitochondrial P450 systems an adrenodoxin reductase and adrenoxin are used to transfer electrons from NADPH to P450. Gene CYP2J2 is in the microsomal group of the CYP P450 enzymes (Zhu and DeLuca, 2012; Jones et al., 2013).

The gene CYP2J2 resides in the region of interest of BTA3. This gene spans from base 86,609,661 to base pair 86,641,519 on this chromosome. The gene is fewer
Figure 1. Manhattan plot of association of single nucleotide polymorphisms with production of 25-hydroxyvitamin D at preconditioning in beef cattle. Genomewide significant threshold was established at $P < 7.4 \times 10^{-8}$ \text{[log10}(P\text{-value}) = 7.1]. See online version for figure in color.

Table 2. Single nucleotide polymorphism identification on bovine chromosome 3, minor allele frequency (MAF), effect, and SE of the marker, genomewide significance, and chromosome-wide significance of markers associated with 25-hydroxyvitamin D production in beef cattle at preconditioning and at weaning

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position (base pairs)</th>
<th>MAF</th>
<th>Preconditioning</th>
<th>Weaning</th>
<th>Weaning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Effect, ng/ml</td>
<td>SE</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$P^1$ genome</td>
<td>$P^2$ chromosome</td>
<td>$P^1$ genome</td>
</tr>
<tr>
<td>Rs132867616</td>
<td>57,769,692</td>
<td>0.05</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rs43348347</td>
<td>84,933,588</td>
<td>0.26</td>
<td>4.82</td>
<td>0.9</td>
<td>$2.98 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rs133179082</td>
<td>86,429,838</td>
<td>0.41</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rs135930078</td>
<td>86,498,691</td>
<td>0.39</td>
<td>4.04</td>
<td>0.9</td>
<td>$4.13 \times 10^{-7}$</td>
</tr>
<tr>
<td>Rs109751435</td>
<td>86,644,301</td>
<td>0.31</td>
<td>–7.5</td>
<td>0.9</td>
<td>$4.39 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rs108951412</td>
<td>86,645,370</td>
<td>0.31</td>
<td>–7.63</td>
<td>0.9</td>
<td>$4.34 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rs110220315</td>
<td>86,646,396</td>
<td>0.31</td>
<td>4.83</td>
<td>0.9</td>
<td>$2.71 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rs110396677</td>
<td>86,647,218</td>
<td>0.31</td>
<td>4.83</td>
<td>0.9</td>
<td>$2.71 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rs135013274</td>
<td>86,648,039</td>
<td>0.31</td>
<td>–8.22</td>
<td>0.9</td>
<td>$2.74 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rs110910033</td>
<td>86,649,494</td>
<td>0.31</td>
<td>–8.22</td>
<td>0.9</td>
<td>$2.71 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rs110759538</td>
<td>86,650,543</td>
<td>0.38</td>
<td>–4.18</td>
<td>0.8</td>
<td>$3.02 \times 10^{-7}$</td>
</tr>
</tbody>
</table>

1Genomewide significant threshold $P < 7.4 \times 10^{-8}$
2Chromosome-wide significant threshold, $P < 1.4 \times 10^{-6}$
Genomic scan for vitamin D in beef cattle

than 2,800 base pairs away from the most telomeric significant SNP in the region. Zhu and DeLuca (2012) indicate this gene produces 1 of 6 enzymes involved in synthesizing vitamin D to 25OHD in humans.

There are 6 enzymes from the cytochrome P450 superfamily associated with the 25-hydroxylation of vitamin D although the physiological relevance of each in 25-hydroxylation of vitamin D in different species is unknown (Aiba et al., 2006; Schuster, 2010; Zhu and DeLuca, 2012). Zhu and DeLuca (2012) indicate that further studies are needed to establish the relevance of each enzyme in the physiological production of 25OHD. Results from the present studies indicate that CYP2J2 is the physiologically relevant 25-hydroxylase in cattle for the conversion of vitamin D to 25OHD. The gene CYP2J2 in cattle is an ortholog to 1 of the 6 hydroxylases known to be involved in vitamin D synthesis in humans. Results from the present study suggest that CYP2J2 should be a prime candidate for further physiological studies in vitamin D.

We determined the heritability of 25OHD concentrations in cattle. Heritability of 25OHD concentrations has been estimated in human using monozygotic and dizygotic twins. Comparison of estimates of heritability between cattle and monozygotic twins in humans could be biased given the population structure used in estimating heritability. The intraclass correlation of 25OHD concentration in monozygotic twins was 0.71 (Orton et al., 2008) and 0.69 (Karohi et al., 2010). These values are greater than those estimated in the present study. This difference can be attributed to the population structure when estimating heritabilities. The intraclass correlation

Figure 2. Manhattan plot of association of single nucleotide polymorphisms with production of 25-hydroxyvitamin D at weaning in beef cattle. Genomewide significant threshold was established at \( P < 7.4 \times 10^{-8} \). [-log10(P-value) = 7.1]. See online version for figure in color.
would be a broad-sense heritability, which would also include dominance and common environmental variance. Estimates of heritability in dizygotic twins ranged from 0.29 (Karohl et al., 2010) to 0.32 (Orton et al., 2008) in humans. These values are similar to those estimated in cattle. To our knowledge, this is the first heritability report of concentration of 25OHD in livestock.

The direct genetic correlation between measures of 25OHD at 2 different times can be considered moderate to high. Munim et al. (2012) estimated direct genetic correlations among weights at different ages in Japanese Black cattle, ranging from 0 to 4 mo, to be moderate to high. Munim et al. (2012) reported direct genetic correlations ranging from 0.53 to 0.96. Direct genetic correlations observed in the present study are within the range expected given that it is the same phenotype measured within a relatively short interval.

Cattle breeding focuses on production traits. Little effort has been expended to breed for improved health characteristics in cattle. Breeding for greater concentrations of 25OHD could potentially be used to increase animal health. Vitamin D has been shown to play a role in regulating gene expression in immune cells and their ability to kill pathogens. Serum concentrations of 25OHD have been correlated with the efficacy of human macrophages to kill Mycobacterium tuberculosis in culture (Liu et al., 2006). Humans with low 25OHD had immune cells that did not effectively kill bacteria whereas those patients with greater serum concentrations of 25OHD were able to kill bacteria. It was shown that the addition of 25OHD to serum deficient in 25OHD was sufficient to restore the ability of macrophages to kill the bacteria in culture. Screening of human and mouse genomes revealed over 3,000 genes with vitamin D response element to which the fully active 1,25 synthesized. This work demonstrated that the mechanism to convert vitamin D into 1,25(OH)2D is activated during infection. In calves fed milk replacer diets with different levels of vitamin D for 10 wk and then experimentally infected with bovine respiratory syncytial virus (RSV), calves with high serum 25OHD concentrations differed from the low 25OHD group in the expression of cytokines important for the immune response to RSV (Sacco et al., 2012). Similarly, an important association between vitamin D status and lung infection has been reported in humans. This work suggests that intervention of maternal vitamin D status during pregnancy or lactation or supplementation of the infant can reduce the risk of RSV infections in infants (Maxwell et al., 2012).

Infusion of 25OHD into the mammary gland of a cow with mastitis has shown changes in the immune response. Immune cells, specifically CD14+ monocytes, from the mammary gland were isolated and the enzyme required to convert 25OHD into the biologically active 1,25(OH)2D showed increased gene expression in infected versus uninfected animals (Nelson et al., 2010). This work demonstrated that the mechanism to convert 25OHD into 1,25(OH)2D is activated during infection and the active 1,25(OH)2D did cause expression changes of genes that are affected by vitamin D. Infusion of 25OHD into the mammary gland of a cow with mastitis showed that this treatment significantly lowered the severity of the infection (Lippolis et al., 2011). These studies show the potential of vitamin D as a marker of immune system health and as a therapeutic.

Lactating dairy cows are at considerable risk of developing milk fever (parturient paresis). This condition is characterized by changes in endocrine function and metabolism associated with calving and the start of lactation. Calcium homeostasis is disrupted by increased demands for the mineral. After the discovery of vitamin D and its regulation of calcium, treatments for milk fever were developed using vitamin D before calving (Thilsing-Hansen et al., 2002; Horst et al., 2003; DeGaris and Lean, 2008). Prevention of milk fever has been attempted by treating cows with 25OHD 8 to 10 d before calving. However, the difficulty of this treatment is in predicting the date of calving. Genetic selection for SNP associated with concentration of 25OHD in the present study could be used to increase the production of CYP2J2, thus potentially minimizing milk fever. These results can be used as a starting point in identifying genomic variation in 25OHD status in dairy cattle.

The use of the bovine immune system as a model for understanding vitamin D requirements in humans has been previously proposed (Nelson et al., 2012). Nelson et al. (2012) indicate that cattle and humans endocrine physiology of vitamin D is similar. Therefore, the bovine working model should be useful to understand
the physiology of vitamin D in humans. Studies have focused on associating levels of vitamin D with immunity to pulmonary tuberculosis in humans (Battersby et al., 2012; Selvaraj et al., 2012). These studies conclude there is an association and that vitamin D seems to act as an anti-inflammatory agent. Orton et al. (2008) studied the association of SNP in the VDR and CYP27B1, which are involved in the use and conversion of 25OHD to the active form (1,25-dihydroxyvitamin D), in twins with multiple sclerosis. Genes VDR and CYP27B1 reside in human chromosome 12, which is a different chromosome where CYP2J2 resides in human (chromosome 1) and cattle (chromosome 3). Orton et al. (2008) found 1 SNP in the VDR gene and 2 SNP in the CYP27B1 gene associated with 25OHD concentrations but no significant association with multiple sclerosis. It would be essential to evaluate genetic markers on the human CYP2J2 gene to establish if there is an association with onset of multiple sclerosis.

Cattle, like humans, vary widely in their 25OHD serum status although cattle in the present study were housed and fed under similar conditions. Cattle with lower concentrations of 25OHD serum may have a health disadvantage compared with animals with high concentrations. This study demonstrates that the trait of 25OHD serum concentrations is heritable. We predict that breeding of greater 25OHD serum concentrations would have a positive health benefit. Results from the present study suggest that CYP2J2 is a gene controlling serum 25OHD concentrations in cattle. The gene CYP2J2 should be considered a prime candidate for understanding both genetic and physiological factors affecting serum 25OHD concentrations and therefore vitamin D status in cattle.

Results from the present study indicate that a region on BTA3 plays an important role in concentration of 25OHD in cattle. Gene CYP2J2, on chromosome 3, is known to be involved in the pathway to produce active 1,25-dihydroxyvitamin D in cattle and humans and was identified as a putative candidate gene. Gene CYP2J2 should be considered a prime candidate for understanding genetic and physiological factors affecting serum 25OHD concentration in cattle and thus vitamin D status. Serum 25OHD concentrations influence the development of a robust immune response and therefore vitamin D status plays an important role and contributes to cattle health.

LITERATURE CITED


