Genome-wide association study identifies QTLs for displacement of abomasum in Chinese Holstein cattle¹

Hetian Huang,^{*} Jie Cao,[†] Gang Guo,[‡] Xizhi Li,[‡] Yachun Wang,^{*} Ying Yu,^{*} Shengli Zhang,^{*} Qin Zhang,^{*} and Yi Zhang^{*,2,●}

^{*}Key Laboratory of Animal Genetics, Breeding and Reproduction, MARA, National Engineering Laboratory of Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China;

[†]Department of Clinical Veterinary Medicine, College of Veterinary Medicine, China Agricultural University, Beijing, China; and [‡]Beijing Sunlon Livestock Development Co. Ltd., Beijing, China

ABSTRACT: Displacement of abomasum (DA) is one of the most common and important disorders in dairy cattle. The objective of the present study was to detect the quantitative trait loci (QTL) for DA in Chinese Holstein using single-step genomic BLUP methodology. A total of 60,556 producer-recorded DA event records from 32,190 cows, together with 2,336 genotyped animals with 40,054 SNP markers, were used for the analysis. Genomic data were incorporated into a threshold model for variance component estimation, and the estimated heritability of DA was 0.108 (SE = 0.086). Results of genome-wide association

studies were reported as the proportion of genetic variance explained 20-SNP windows. Eight QTLs covering 129 genes on *Bos taurus* autosomes 2, 4, 7, 10, 14, 17, 20 showed associations with DA. Ten genes, namely *BMP4*, *SOCS4*, *GCH1*, *DDHD1*, *ATG14*, *ACBP/DBI*, *SMO*, *AHCYL2*, *CYP7A1*, and *CACNA1A*, involved in insulin metabolism and lipid metabolism pathways may be considered as candidate genes of DA in dairy. The identified QTLs, biological pathways, and associated genes underlying DA identified from the present study will contribute to the understanding of the genetic architecture of this complex disease.

Key words: dairy cattle, displacement of abomasum, metabolism pathway, producer-recorded data, quantitative trait loci (QTL), single-step genomic BLUP approach

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INTRODUCTION

Displacement of abomasum (DA) is one of the energy metabolic disorders described in

high-performing dairy cattle in their postpartum and early lactation period (Sexton et al., 2007; Pryce et al., 2016). In affected animals, the abomasum is displaced from its normal position on the ventral part of the abdomen to the left (LDA) or right (RDA) abdominal wall. The incidence rate of DA ranged from 1.6% to 5% in the Holstein cows of several countries (Kelton et al., 1998; Neuenschwander et al., 2012; Koeck et al., 2013). The average loss caused by DA per case was estimated at \$432 for primiparous cows and \$639 for multiparous cows (Liang et al., 2017). In China, the concurrent incidence of DA has become one of the most important factors for high culling rate (Wu et al., 2012).

Complex pathophysiological mechanisms contribute to the pathogenesis of DA. Although

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²Corresponding author: yizhang@cau.edu.cn

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abomasal hypomotility and increased gas accumulation may predispose the disease (Geishauser et al., 2000), epidemiological studies have identified various risk factors including twin pregnancy, body condition score, and concurrent disease such as ketosis (Rohrbach et al., 1999; Toni et al., 2011). Metabolic and biochemical factors such as insulin concentration, high milk fat to protein ratio, calcium metabolism, potassium homeostasis, metabolic alkalosis, and the parasympathetic nervous system are reported to play significant roles (Doll et al., 2009; Turck and Leonhard-Marek, 2010; Zurr and Leonhard-Marek, 2012). However, the primary cause of DA still remains unknown.

In beef cattle and dual-purpose breeds such as German Fleckvieh DA is rarely observed, but is prevalent in the common dairy breeds, for instance, Holstein, Jersey, Guernsey, and Ayrshire, which suggests a genetic predisposition (Constable et al., 1992; Zerbin et al., 2015). In a quantitative trait loci (QTL) mapping study using a paternal half-sib design, QTLs for DA were identified across 13 chromosomes in German Holsteins (Mömke et al., 2008). Recently, genome-wide association studies (GWAS) have detected several genes associated with DA (Mömke et al., 2013; Lehner et al., 2018). Refining the position of QTL regions in a separate population and identifying genes underlying this complex disease may further contribute to elucidation of the genetic background of DA (Zerbin et al., 2015).

The classical GWAS is based on covariance model fitting a single marker, which has been performed on German and Italian Holstein (Mömke et al., 2013; Biffani et al., 2014). However, given the limited sample size and low heritability nature of the complex disease, the power and precision of GWAS is compromised (Korte and Farlow, 2013). In the present study, the GWAS approach called GWAS by single-step genomic BLUP (ssGBLUP) also known as single-step GWAS (ssGWAS) was employed (Wang et al., 2012). In this approach, all the pedigree information, phenotypic and genotypic data can be interpreted simultaneously when compared with the classical GWAS. In addition, it allows unequal variances for SNP, which results in improved precision of the estimation of SNP effects (Wang et al., 2012). It has been implemented in cattle (Tiezzi et al., 2015; Lemos et al., 2016), chicken (Wang et al., 2014), and pigs (Wu et al., 2018).

The objective of the present study was to perform a GWAS in Chinese Holstein cattle from available producer-recorded data to identify potential

candidate genomic regions and genes associated with DA.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of China Agricultural University approved the procedures used with animals in this experiment.

Phenotype and Genotype Data

Producer-recorded health event data of DA in the period from 2009 to 2016 were collected from 21 farms in Beijing, China. Diagnosis of DA was based on physical examination of suspected animals by veterinarians. The cases were those cows that have been diagnosed with DA surgically and vice versa for the controls. Data were trimmed to ensure data plausibility following the criterion in the literature (Parker Gaddis et al., 2012). Cows were considered healthy if they did not have a veterinarian clinical operative records of DA. To avoid data bias, only the records from parities 1 to 4 were retained for analysis. Minimum and maximum restrictions were imposed on the data by herd-year basis to avoid herds that either over- or under-reported DA. The minimum restriction was applied to select records from herd-years with at least one reported incidence of DA and herd-years with more than 5 cows. For the maximum reporting incidence restriction, only herd-years with a reporting incidence greater than 2 SD beyond the overall average incidence were considered. Affected animals did not have subsequent records after being declared a case given they were treated surgically and removed. Phenotypes were defined as binary traits.

A total of 2,510 Holstein cows were genotyped using the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA). The genotype data were further filtered by the PLINK software (v1.07; Purcell et al., 2007). The resulting SNP data set was filtered by excluding individuals with more than 10% missing genotypes. We also removed SNPs with a minimum allele frequency lower than 0.05 or a call rate lower than 90%. Markers that departed significantly from the Hardy–Weinberg equilibrium expectations ($P < 1 \times 10^6$) were all discarded. After editing, 40,054 SNP markers for 2,336 cows (case: 363, control: 1,973) were kept for analysis.

The final data set consisted of 60,556 records from 32,190 cows including 3,265 DA cases, of which 2,336 cows (case: 363, control: 1,973) had both phenotype and genotype data. Pedigree information

was obtained from the Dairy Association of China (Beijing, China) by tracing the pedigrees of cows more than 3 generations, including 62,773 animals. The summary of the phenotype data and pedigree information is shown in Table 1.

Model and Analysis

The ssGBLUP approach (Aguilar et al., 2010; Christensen and Lund, 2010; Wang et al., 2012) was applied for the GWAS analyses. Given the binary nature of the trait, a threshold animal model was used with the model specification,

$$\lambda = \mathbf{X}\beta + \mathbf{Z}_h\mathbf{h} + \mathbf{Z}_a\mathbf{a} + \mathbf{Z}_p\mathbf{p} + \mathbf{e}$$

where λ is the vector of unobserved liabilities to phenotype (0/1), β is a vector of fixed effects including overall mean, parity, and year-season with 4 seasons defined within each calving year: spring (March–May), summer (June–August), autumn (September, October), winter (December–February); $\mathbf{h}$ is the random herd-year effect, where $\mathbf{h} \sim N(0, \mathbf{I}\sigma_h^2)$, with $\mathbf{I}$ as an identity matrix and σ_h^2 as the herd-year variance; $\mathbf{a}$ is the random animal effect, where $\mathbf{a} \sim N(0, \mathbf{H}\sigma_a^2)$, with $\mathbf{H}$ representing the additive relationship matrix based on pedigree file and genomic relationship matrix and σ_a^2 as the additive genetic variance; $\mathbf{p}$ is the random effect of permanent environment with $\mathbf{p} \sim N(0, \mathbf{I}\sigma_{pe}^2)$ and $\mathbf{e}$ is the random residual, where $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, with residual variance restricted to be 1. $\mathbf{X}$, $\mathbf{Z}_h$, $\mathbf{Z}_a$, and $\mathbf{Z}_p$ are incidence matrix corresponding to the fixed effects in β and the random effects of herd-year, animal, and permanent environment, respectively.

The model implemented BLUP with combined pedigree–genomic relationship matrix, which facilitated the combination of genotyped and ungenotyped animals (Aguilar et al., 2010; Christensen

and Lund, 2010). Bayesian inference (Gianola and Fernando, 1986) implemented in THRGIBBS1F90 and POSTGIBBSF90 program (Misztal et al., 2002; Tsuruta and Misztal, 2006) was used to estimate genomic breeding value (GEBV) and variance components. A total of 500,000 iterations were run with the first 50,000 discarded as burn-in, thinning every 50 samples. The chain convergence was verified with the graphical evaluation of sampled values by using the ‘coda’ package in R (Plummer et al., 2006). Reliabilities of GEBV were estimated following Misztal et al. (2013).

Conversion from GEBV to SNP effects was carried out as described in (Wang et al., 2012) by solving

$$\hat{\mathbf{u}} = \mathbf{D}\mathbf{Z}'[\mathbf{ZDZ}']^{-1}\hat{\mathbf{a}}$$

where $\hat{\mathbf{u}}$ is the vector with estimated SNP effects; $\mathbf{D}$ is a diagonal matrix of weights for variances of SNP effects; $\mathbf{Z}$ is a matrix relating genotypes of each locus to each individual; $\hat{\mathbf{a}}$ is the vector of GEBV for genotyped animals. The $\hat{\mathbf{u}}$ vector and the matrix $\mathbf{D}$ were iteratively recomputed using an iterative procedure proposed by Wang et al. (2012). The percentage of genetic variance explained by the i th window has been calculated as follows:

$$\frac{\text{Var}(a_i)}{\sigma_a^2} \times 100\% = \frac{\text{Var}(\sum_{j=1}^n Z_j \hat{u}_j)}{\sigma_a^2} \times 100\%$$

where a_i is the genetic value of the i th window that consists of contiguous n SNPs; σ_a^2 is the total genetic variance; Z_j is a vector of the gene content of the j th SNP for all individuals; $\hat{u}_i$ is marker effect of the j th SNP within the i th window. SNP effects and genetic variance explained by 20-SNP windows (approximately equivalent to 1 Mbp), as in Abdalla et al. (2016), was calculated in the postGSF90 program (Aguilar et al., 2014).

Searching for Gene and Functional Annotation

Following the studies that have been reported using the ssGWAS approach (Utsunomiya et al., 2014; Tiezzi et al., 2015; Guarini et al., 2019), we selected the windows explaining the largest genetic variances as potentially important QTL regions. The candidate genes were identified on the basis of the *Bos taurus* UMD3.1 genome assembly (Zimin et al., 2009) and the Ensembl Genome Browser (Zerbino et al., 2018). For functional annotation, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were used for getting GO terms and pathways by using the

Table 1. Summary statistics of the final data set used for genetic analyses

Statistic category	Statistic
No. of records	60,556
No. of animals	32,190
Animals in pedigree	62,773
Average incidence ¹	5.30%
No. of herd-year classes	167
No. of year-season classes	32
No. of parity	1 to 4
Animals with genotype	2,336
No. of markers after QC	40,054

¹The incidence of displacement of abomasum was calculated following Parker Gaddis et al. (2012).

“cluster Profiler” package for R program (Yu et al., 2012), with the cutoff of $P < 0.05$.

RESULTS AND DISCUSSION

The estimate of heritability was 0.108 (SE = 0.086) for DA in Chinese Holstein. This result was similar to several previous studies reporting heritability ranging from 0.081 to 0.360 for DA (Neuenschwander et al., 2012; Parker Gaddis et al., 2014; Vukasinovic et al., 2017). The reliabilities of GEBV for DA were generally low. Average reliability was 0.29 and 0.22 for genotyped and ungenotyped animals, respectively. The reason for the low reliability might be due to a combination of low heritability and limited availability of phenotypes.

To highlight the genomic regions of significance, a sliding window approach was used to calculate the genetic variance of 20 adjacent SNP (Fig. 1). It was seen that many windows across the genome were found to be associated with DA. However, the genetic effect captured by a window was small, with the genetic variance ranging between 0 and 0.58%, which is consistent with the nature of low heritability ($h^2 = 0.108$). Significantly, a total of 58 windows were detected to explain more than 0.4% of genetic variance. These top windows were reasonably considered as important QTL regions, as in Guarini et al. (2019). Windows that had an adjacent boundary were combined as one genomic region. Consequently, these windows were grouped into 8 QTL regions on 7 chromosomes [*Bos taurus* autosome (BTA) 2, 4, 7, 10, 14, 17, 20; Table 2 and Supplementary Table S1]. The 8 QTL regions were used to identify putative candidate genes. A total of 129 genes were annotated in these regions (Supplementary Table S1).

The region with the largest genetic variance was located between 66.6 and 68.0 Mbp with peak signal at the SNP (rs109320355) on BTA10. This region is entirely located within the QTL region reported in German Holsteins (from 63.6 to 72.4 Mbp on BTA10; Mömke et al., 2008) and also close to the significant SNPs identified by a GWAS study of LDA in German Holsteins (Mömke et al., 2013).

Interestingly, a recent GWAS study also identified a significant genomic region associated with ketosis at 66.3 Mbp on BTA10 (Parker Gaddis et al., 2018). Both ketosis and DA are common metabolic disorders related to energy metabolism. The relationship between DA and ketosis has been identified as bidirectional (Gröhn et al., 1989). Ketosis can be a risk factor as well as a consequence of DA (Rohrbach et al., 1999; Stengärde et al., 2012). Moderate genetic correlations have been reported between DA and ketosis in previous genetic analysis (Koeck et al., 2012; Neuenschwander et al., 2012; Parker Gaddis et al., 2014), which suggest that this region has an effect on an underlying biological process affecting both disorders.

Insulin plays a key role in regulating energy metabolism during the transition period in lactating dairy cows (Weber et al., 2016). In late pregnancy, decreased insulin sensitivity in peripheral tissue is part of this physiological response. In cattle with high concentrations of glucose reduced insulin sensitivity that has been considered as a prerequisite for DA (Turck and Leonhard-Marek, 2010).

In the present study, 3 candidate genes, that is, *BMP4*, *SOCS4*, *GCHI*, related to insulin regulation were located on BTA10 from 65.9 to 68.3 Mbp. The most promising candidate gene was *BMP4*, which is known to be related to the development of

Figure 1. Manhattan plot for the proportion of the genetic variance explained by 20-SNP sliding windows for displacement of abomasum (DA) in a GWAS study using single-step genomic BLUP methodology. The dotted line represents the windows that explained higher than 0.4% of genetic variance.

Table 2. Genomic regions associated with DA in lactating Holstein dairy cows¹

BTA ²	Regions, Mb ³	N ⁴	Variance explained, % ⁵	Genes ⁶
2	70.2 to 71.5	1	0.41	<i>ACBP/DBI</i>
4	69.8 to 71.4	9	0.49	<i>SNX10</i>
4	93.3 to 94.4	5	0.42	<i>SMO</i>
7	13.4 to 14.3	2	0.41	<i>CACNA1A</i>
10	65.9 to 68.3	17	0.47	<i>BMP4, SOCS4, GCH1, DDHD, ATG14</i>
14	26.1 to 27.4	13	0.43	<i>CYP7A1</i>
17	5.5 to 6.6	7	0.45	<i>GATB</i>
20	40.6 to 41.5	3	0.42	<i>ZFR</i>

¹DA = displacement of abomasum.

²BTA = *Bos taurus* autosome.

³Genomic regions of the continuous 20-SNP windows.

⁴Number of 20-SNP windows contained in a genomic region.

⁵Averaged genetic variance over all 20-SNP windows in the genomic region.

⁶Functionally relevant candidate genes. The full list of annotated genes is given in [Supplementary Table S1](#).

metabolic pathologies such as type 2 diabetes mellitus and obesity ([Grgurevic et al., 2016](#)). Signaling of BMP4, along within the BMP type 1a receptor, play its role in signaling pancreatic β cells in impaired glucose-stimulated insulin secretion ([Goulley et al., 2007](#)). BMP4-deficient mice have been shown to have impaired insulin sensitivity, indicating the genetic- and metabolic-based role of BMP4 in altering insulin sensitivity ([Qian et al., 2013](#)).

A further functional candidate gene is *SOCS4*, belonging to the suppressor of cytokine signaling family, which affects insulin resistance and appears to play an important role in the pathological processes leading to both type 1 and type 2 diabetes mellitus ([Ueki et al., 2004](#); [Feng et al., 2014](#)). The *GCH1* gene encodes a member of the GTP cyclohydrolase family. Genetic variation in the *GCH1* gene was associated with endothelial dysfunction and oxidative stress in type 2 diabetes mellitus ([Wolkow et al., 2014](#)).

Similarly, the QTL regions located on other chromosomes also harbor interesting candidate genes related to insulin metabolism. The *ACBP* gene also known as *DBI* gene, located on BTA2 at 70.2 to 71.5 Mbp, is a soluble 10-kDa lipid-binding protein ubiquitously expressed in all tissues of eukaryotic species examined, also implicated in the control of steroidogenesis in mitochondria and glucose-stimulated insulin release ([Gossett et al., 1996](#)). ACBP-overexpressing rats fed with a medium-chain fatty acid-rich diet improved glucose tolerance and lower serum insulin levels ([Oikari et al., 2008](#)). Moreover, an SNP in the human *ACBP* gene promoter was associated with reduced risk of type 2 diabetes in 2 German human populations, probably due to increased transcriptional activity of *ACBP* ([Fisher et al., 2007](#)).

The *SMO* and *AHCYL2* genes, located on BTA4, were found to be associated with DA in this study. The *SMO* has previously been associated with pancreas morphogenesis and β -cell expansion in mice ([Lau and Hebrok, 2010](#)). Smo-deficient mice displayed glucose intolerance, increased insulin sensitivity, and reduced total insulin production ([Lau and Hebrok, 2010](#)). The *AHCYL2* was found to be associated with insulin signaling and inflammation, which mediate lipoprotein responses to fenofibrate in human ([Frazier-Wood et al., 2012](#)). The SNP in *AHCYL2* were associated with a decrease in the concentration of very low-density lipoprotein ([Frazier-Wood et al., 2012](#)).

In addition, the region identified on BTA7 at 13.4 Mbp associated with the *CACNA1A* gene has been implicated in Reactome pathways including regulation of insulin secretion (R-BTA-422356; <https://reactome.org/content/detail/R-BTA-422356>) and integration of energy metabolism (R-BTA-163685; <https://reactome.org/content/detail/R-BTA-163685>).

The gene *CYP7A1*, a member of the cytochrome P450 system gene family, located on BTA14 at 26.3 Mbp, is responsible for bile acid synthesis ([Li et al., 2012](#)). Glucose induction of bile acid synthesis was an important mechanism by which the liver regulates postprandial glucose and lipid homeostasis ([Li et al., 2012](#)). Glucose and insulin act as key physiological regulators of *CYP7A1* gene expression during the postprandial state ([Li et al., 2012](#)). Interestingly, the 2 genes *CYP26B1* and *CYP11B1* belonging to the same gene family as *CYP7A1* and located on BTA14 were associated with ketosis in US Jersey dairy cattle ([Parker Gaddis et al., 2018](#)) and the fat to protein ratio in primiparous lactating Holstein cows ([Tetens et al., 2013](#)).

Insulin resistance has been proposed as part of the etiology of DA (Pravettoni et al., 2004). Cows that have undergone surgical correction for DA by omentopexy have a prolonged period of abnormal abomasal motility when insulin concentrations persisted on a higher level (Pravettoni et al., 2004). Previous GWAS for DA also detected several candidate genes related to insulin metabolic and insulin-dependent diabetes mellitus (Mömke et al., 2013). Therefore, the metabolism disturbance caused by insulin deficiency should be an important pathogenic factor for DA.

Notably, we also detected 2 candidate genes, that is, *DDHD1*, *ATG14* on BTA10 at 65.9 and 68.3 Mbp, respectively, which are related to lipid metabolism. The *DDHD1* gene encodes fatty acid enzyme and involved in pathways including metabolism of lipids, lipoproteins, and glycerophospholipid biosynthesis (Tesson et al., 2012). The *ATG14* gene is regulated by fork head box O transcription factors and plays an important role in hepatic lipid metabolism (Xiong et al., 2012). Health disorders in dairy cattle are attributed to the process of uncontrolled lipid mobilization in response to excessive negative energy balance in early lactation. Cows with DA are preceded by significant changes in adipose mobilization and energy metabolism up

to 3 wk before the disease event (LeBlanc et al., 2005). Elevated NEFA concentration in plasma is a prerequisite for the development of hepatic lipodosis that occurs in DA cows (Rehage et al., 1996). Therefore, the genes related to lipid metabolism have been considered as a plausible candidate gene associated with DA.

There are several genes found in our study that have not been reported before to be associated with DA. The *GATB* gene located on BTA17 at 5.9 Mbp is a protein-coding gene. Gene ontology annotations related to this gene include ligase activity, translation factor activity, and RNA binding. The *SNX10* gene on BTA4 encodes a member of the sorting nexin family and plays a role in regulating endosome homeostasis. The *ZFR* gene on BTA20 played an essential role in cell growth and associated with the induction of pancreatic cancer in human (Zhao et al., 2016). These genes could influence the affection risk for DA by currently unknown mechanisms.

All of the 129 candidate genes were then used to perform GO enrichment and KEGG pathway analyses. Six GO terms related to cell development and immune response (GO:0033687, GO:0003309, GO:0003323, GO:0070232, GO:0033087, GO:0033085) and 2 GO terms related to metabolic process

Table 3. The 10 most significantly enriched gene ontology (GO) terms under the biological processes category associated with DA in lactating Holstein dairy cows¹

GO ID	Term	P
GO:0033687	Osteoblast proliferation	0.0002
GO:0035196	Production of miRNAs involved in gene silencing by miRNA	0.0017
GO:0003309	Type B pancreatic cell differentiation	0.0036
GO:0070232	Regulation of T-cell apoptotic process	0.0041
GO:0009953	Dorsal/ventral neural pattern formation	0.0044
GO:0032436	Positive regulation of proteasomal ubiquitin-dependent protein catabolic process	0.0046
GO:0044106	Cellular amine metabolic process	0.0057
GO:0003323	Type B pancreatic cell development	0.0058
GO:0033087	Negative regulation of immature T-cell proliferation	0.0058
GO:0033085	Negative regulation of T-cell differentiation in thymus	0.0058

¹DA = displacement of abomasum.

Table 4. KEGG terms significantly over-represented with genes statistically associated with DA in lactating Holstein dairy cows¹

KEGG ID	Term	Genes count	Genes	P
Map4930	Type II diabetes mellitus	2	<i>CACNA1A</i> , <i>SOCS4</i>	0.016
Map4340	Hedgehog signaling pathway	2	<i>BMP4</i> , <i>SMO</i>	0.022
Map5217	Basal cell carcinoma	2	<i>BMP4</i> , <i>SMO</i>	0.023
Map4115	p53 signaling pathway	2	<i>STEAP3</i> , <i>CYCS</i>	0.034
Map3320	PPAR signaling pathway	2	<i>CYP7A1</i> , <i>ACBP/DBI</i>	0.037
Map790	Folate biosynthesis	1	<i>GCHI</i>	0.045

¹KEGG = Kyoto Encyclopedia of Genes and Genomes; DA = displacement of abomasum.

(GO:0032436, GO:0044106) were identified (Table 3). Among the 6 KEGG pathways, the type 2 diabetes mellitus pathway ($P = 0.016$) was the most significant (Table 4).

Gene-set enrichment and pathway analyses may provide deeper biological knowledge of complex traits (Ramanan et al., 2012). In this study, 5 of the enrichment of GO terms are related to the type B pancreatic cell and T-cell development (Table 3). Previous studies reported the strong interactions between metabolic disorders and immune response in transition cows (Esposito et al., 2014). Interestingly, the KEGG pathway 'type 2 diabetes mellitus' was enriched for DA. In addition, the PPAR signaling pathway (Table 4), which plays a pivotal role in the physiology and metabolism of dairy cattle (Bionaz et al., 2013), appeared to have a key role in the development of DA. There are 3 members of the PPAR family encoded by the separate gene, that is, PPAR α , PPAR γ , and PPAR δ , binding fatty acids as well as eicosanoids (Abbott, 2009; Bionaz et al., 2013). PPAR γ controls the expression of leptin, which is an adipocyte hormone that maintains homeostatic control of adipose tissue mass and functions as an afferent signal in a negative feedback loop (Ahmadian et al., 2013). In addition, the gene *leptin* has already been reported to be associated with DA (Chebel et al., 2008).

Identification of candidate genes involved in DA may provide insights into disease mechanisms. The present study is interesting in that notable association signals, and several putative genes in energy metabolism pathway were detected. However, given the low heritability of DA, the power of this study is compromised because of relatively smaller sample size of DA cases used to interpret the findings. Therefore, further studies with higher detective power are needed to confirm the findings and refine the positions of QTL regions. Meanwhile, most recently, a new bovine reference genome named ARS-UCD1.2, which was assembled by long-read sequencing technology, has been published (https://www.ncbi.nlm.nih.gov/genome/82?genome_assembly_id=371813). This new reference genome may provide additional gene annotations in the future studies.

CONCLUSIONS

By incorporating genomic information, low heritable nature of the DA in dairy is confirmed in the present study, and DA is highly polygenic influenced by numerous regions throughout the genome based on results from ssGWAS. Although only a small proportion of genetic variance contributed by

the identified genomic regions, the plausible candidate genes, enriched in insulin metabolism and lipid metabolism pathways, may help to understand the genetics and pathogenesis of DA in dairy.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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