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High-density genome-wide association study for residual feed intake in Holstein dairy cattle

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ABSTRACT

Improving feed efficiency (FE) of dairy cattle may boost farm profitability and reduce the environmental footprint of the dairy industry. Residual feed intake (RFI), a candidate FE trait in dairy cattle, can be defined to be genetically uncorrelated with major energy sink traits (e.g., milk production, body weight) by including genomic predicted transmitting ability of such traits in genetic analyses for RFI. We examined the genetic basis of RFI through genome-wide association (GWA) analyses and post-GWA enrichment analyses and identified candidate genes and biological pathways associated with RFI in dairy cattle. Data were collected from 4,823 lactations of 3,947 Holstein cows in 9 research herds in the United States. Of these cows, 3,555 were genotyped and were imputed to a high-density list of 312,614 SNP. We used a single-step GWA method to combine information from genotyped and nongenotyped animals with phenotypes as well as their ancestors' information. The estimated genomic breeding values from a single-step genomic BLUP were back-solved to obtain the individual SNP effects for RFI. The proportion of genetic variance explained by each 5-SNP sliding window was also calculated for RFI. Our GWA analyses suggested that RFI is a highly polygenic trait regulated by many genes with small effects. The closest genes to the top SNP and sliding windows were associated with dry matter intake (DMI), RFI, energy homeostasis and energy balance regulation, digestion and metabolism of carbohydrates and proteins, immune regulation, leptin signaling, mitochondrial ATP activities, rumen development, skeletal muscle development, and spermatogenesis. The region of 40.7 to 41.5 Mb on BTA25 (UMD3.1 reference genome) was the top associated region for RFI. The closest genes to this region, *CARD11* and *EIF3B*, were previously shown to be related to RFI of dairy cattle and FE of broilers, respectively. Another candidate region, 57.7 to 58.2 Mb on BTA18, which is associated with DMI and leptin signaling, was also associated with RFI in this study. Post-GWA enrichment analyses used a sum-based marker-set test based on 4 public annotation databases: Gene Ontology, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, Reactome pathways, and medical subject heading (MeSH) terms. Results of these analyses were consistent with those from the top GWA signals. Across the 4 databases, GWA signals for RFI were highly enriched in the biosynthesis and metabolism of amino acids and proteins, digestion and metabolism of carbohydrates, skeletal development, mitochondrial electron transport, immunity, rumen bacteria activities, and sperm motility. Our findings offer novel insight into the genetic basis of RFI and identify candidate regions and biological pathways associated with RFI in dairy cattle.

Key words: feed efficiency, dairy cow, genome-wide association study, enrichment analysis

INTRODUCTION

Feed accounts for the largest part of operating costs in dairy production (European Commission, 2018; USDA, 2018). Improving feed efficiency (**FE**) of dairy cattle has the potential to increase farm profitability and reduce the environmental footprint of dairy production (VandeHaar et al., 2016). The widely recognized genetic variation in FE has created possibilities for improving FE of dairy cattle using genetics and breeding. Genetic

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studies of FE have been carried out in several dairy populations worldwide, covering some important topics including genetic parameter estimation (Berry et al., 2014; Li et al., 2018), genomic evaluation (de Haas et al., 2015; Pryce et al., 2015), and genome-wide association (**GWA**; Hardie et al., 2017; Lu et al., 2018). In addition, alternative FE definitions were investigated to appropriately define FE of dairy cattle in different populations (Lu et al., 2015; Pryce et al., 2015; Hurley et al., 2017; Li et al., 2017).

Residual feed intake (**RFI**), as one proposed FE definition trait, has been widely studied in pigs (Patience et al., 2015), chickens (Wolc et al., 2013), beef cattle (Crews, 2005), and dairy cattle (Berry and Crowley, 2013; Tempelman et al., 2015). Generally, RFI is defined as the difference between an animal's actual feed intake and its expected feed intake based on energy requirements for production and maintenance (Koch et al., 1963). In dairy cattle, RFI is calculated as the deviation of actual intake of a cow from the average intake of other cows that are fed and managed in the same way (cohort), after adjusting for major energy sinks [milk production, metabolic body weight (**MBW**), and change in BW (**ΔBW**)] (VandeHaar et al., 2016). The calculated RFI is phenotypically independent of energy sink traits (e.g., milk production, MBW) but may still be genetically correlated with these traits. Lu et al. (2015) applied a multiple-trait modeling method to define RFI in dairy cattle in order to derive RFI that was genetically uncorrelated with energy sink traits. VanRaden et al. (2018) developed an alternative method to derive RFI that was genetically uncorrelated with milk energy and body weight composite (**BWC**) by including genomic predicted transmitting ability (**GPTA**) of milk energy and GPTA of BWC in the genetic analyses for RFI. An RFI value that is genetically uncorrelated with major energy sink traits (e.g., milk yield, BW) is of interest because milk production traits and BW-related traits are often part of the selection objectives in dairy cattle selection indices (Cole and VanRaden, 2018). After the removal of the genetic correlations of RFI with milk production and BW, RFI becomes a more independent trait representing FE in the selection index.

Defining RFI that is genetically independent from yield traits and BW could also improve understanding of the genetic basis of RFI in dairy cattle. Genome-wide association analysis is a useful tool for understanding the underlying biology of a trait by identifying genomic regions associated with genetic variation in traits, as well as identifying genes that may be associated with those traits (Cole et al., 2011). When RFI is defined as being genetically independent from energy sink traits, the GWA signals for RFI could be more associated with RFI itself and free from the influence of milk yield and BW. In addition, previous GWA studies (**GWAS**) for RFI in lactating dairy cows were mostly carried out using medium-density (50–60k) SNP genotypes (Hardie et al., 2017; Lu et al., 2018). Applying high-density (**HD**) SNP chips for a GWAS may help to refine the candidate genomic regions for RFI and offer new insight into the genetic basis of RFI.

In this study, RFI was analyzed using the model of VanRaden et al. (2018), in which RFI was genetically uncorrelated with milk energy and BWC. The objectives were to understand the genetic basis for RFI using high-density genotypes and to identify candidate genes, biological processes, and pathways associated with RFI through GWA and post-GWA enrichment analyses.

MATERIALS AND METHODS

Phenotypes

The current study included 4,823 lactations of 3,947 Holstein cows. The cows were from 9 research herds in the central and eastern United States, including Iowa State University (Ames), University of Wisconsin-Madison, the USDA Animal Genomics and Improvement Laboratory (Beltsville, MD), University of Florida (Gainesville), the US Dairy Forage Research Center (Madison, WI), Michigan State University (East Lansing), the Purina Animal Nutrition Center (Gray Summit, MO), Virginia Polytechnic Institute and State University (Blacksburg), and the Dairy Research Facility at the Miner Institute (Chazy, NY). Data were collected between 2007 and 2016. The cows were in lactation 1 to lactation 8, and the number of cows in each parity was 3,889, 3,482, 2,407, 1,308, 543, 37, 13, and 2, respectively. The calving age of the cows ranged from 19 to 94 mo, with an average of 40 mo. Pedigree information included 42,057 animals going back as many generations as possible for all animals with records.

The cows were involved in 44 experiments in the 9 research herds. The experimental designs and ingredients of diets in these experiments were described in detail previously (Ferraretto et al., 2012; He et al., 2012; Spurlock et al., 2012; Connor et al., 2013; Tempelman et al., 2015; Manzanilla-Pech et al., 2016). In general, the studied cows were fed TMR, and feed intakes were measured using electronic feeding systems. The experimental designs varied from single-ration studies dedicated only to FE genetic studies, to randomized designs, simple crossover designs, and multiple-treatment Latin square designs (Tempelman et al., 2015).

Daily DMI and milk yield, weekly or biweekly BW, and milk composition were recorded for each cow. Only

measurements collected between 50 and 200 DIM were used and edited to form one 28-d average phenotype for DMI, milk energy, MBW, and ΔBW (Tempelman et al., 2015). Energy sinks of milk energy, MBW, ΔBW, and several environmental effects were removed from DMI to obtain RFI records from previous studies (Tempelman et al., 2015). Most RFI records were from 6-wk trials, but 202 records were from 4-wk trials. The records from 4-wk trials were given less weight (weight $= 0.96^2 = 0.92$) in the genetic analyses because the standard deviation was higher in 4-wk trials than 6-wk trials (1.75 vs. 1.68 kg/d) and the correlation of 4- and 6-wk trials was 0.96.

Genotypes

High-density genotypes were used in this study, including 312,614 SNP spanning the entire bovine genome. The 312,614-SNP panel was derived from 777k Illumina BovineHD Genotyping BeadChip (Illumina Inc., San Diego, CA) genotypes after editing for linkage disequilibrium, minor allele frequency, Hardy-Weinberg equilibrium, and Mendelian errors, using the same method as Wiggans et al. (2016). Of the 3,947 cows with phenotypes, 3,555 cows were genotyped, including 502 on the HD panel; the remaining 3,053 were imputed to HD as part of a larger study that included 2,394 HD genotypes and 592,757 genotyped Holsteins (VanRaden et al., 2017). Of the 42,057 animals in the pedigree, 6,151 were genotyped, including genotyped cows with phenotype information as well as genotyped sires and other ancestors in the pedigree. In the quality control process, animals and SNP with call rates <0.90, SNP with minor allele frequency <0.05, monomorphic SNP, SNP deviating from Hardy-Weinberg equilibrium expectation, and animals with parent-progeny Mendelian conflicts were omitted from the data set, using the preGSf90 program (version 1.10; Misztal, 2013). After data filtering, genotypes of 278,524 SNP from 5,610 genotyped individuals in the pedigree remained in the data set.

GWA Analyses

A single-step GWAS method (**ssGWAS**) proposed by Wang et al. (2012) was used to combine information from genotyped and nongenotyped animals with phenotypes as well as their ancestors' information into GWA analyses. In ssGWAS, a single-step genomic BLUP was first done to estimate genomic breeding values (**GEBV**) for all animals in the pedigree by combining their pedigree and genomic information. The model for single-step genomic BLUP analyses for RFI was as follows:

$$\mathrm{y} = \text{Age-parity-grp} + \mathrm{b}_1 \times (\mathrm{GPTA}_{\text{milk net energy}}) + \mathrm{b}_2 \times (\mathrm{GPTA}_{\mathrm{BWC}}) + a + pe + e,$$

where y is the RFI phenotype adjusted for energy sinks of milk yield, MBW, ΔBW by phenotypic regressions, and several environmental effects, according to Tempelman et al. (2015); Age-parity-grp is the fixed effect of the age and parity group; $\mathrm{GPTA}_{\text{milk net energy}}$ is the cow's GPTA for milk net energy and $\mathrm{GPTA}_{\mathrm{BWC}}$ is the cow's GPTA for BWC, where the GPTA values for the studied cows were obtained from the US national genomic evaluation database for milk net energy and BWC; $\mathrm{GPTA}_{\text{milk net energy}}$ and $\mathrm{GPTA}_{\mathrm{BWC}}$ were included to remove the remaining genetic correlations of RFI with milk production and BW that were not completely removed by the phenotypic regressions; b_1 is the regression coefficient of cow's RFI on GPTA of milk net energy, which was different from 0 ($P < 0.05$); b_2 is the regression coefficient of cow's RFI on GPTA of BWC, which was not significantly different from 0; a is the random additive genetic effect with $\mathrm{var}(a) \sim N\left(0, \mathbf{H}\sigma_a^2\right)$, where σ_a^2 is the additive genetic variance and $\mathbf{H}$ is the relationship matrix incorporating pedigree and genomic information as defined in Legarra et al. (2009); pe is the random permanent environmental effect to account for repeated measurements from an animal, with $\mathrm{var}(pe) \sim N\left(0, \mathbf{I}\sigma_{pe}^2\right)$, where σ_{pe}^2 is the permanent environmental variance and $\mathbf{I}$ is the identity matrix; and e is the random residual with $\mathrm{var}(e) \sim N\left(0, \mathbf{R}\sigma_e^2\right)$, where σ_e^2 is the residual error variance and $\mathbf{R}$ is a diagonal matrix to adjust for the residual variance of each record based on their weights. Almost all RFI records were from 6-wk trials, whereas 202 records were from 4-wk trials. The records from 4-wk trials were given less weight (weight = 0.92), as described earlier in this article. Therefore, the diagonals of the $\mathbf{R}$ matrix are mostly 1 for records from 6-wk trials and 1/0.92 for records from 4-wk trials.

The variance components for RFI were estimated by an average information-restricted maximum likelihood algorithm using pedigree information, implemented in the airemlf90 program (version 1.134; Misztal, 2013). The estimated variance components were then applied to single-step genomic prediction implemented by the program blupf90 (version 1.58; Misztal, 2013). The GEBV were estimated for all animals in the pedigree by combining their pedigree and genomic information, and the inverse of the relationship matrix was as follows:

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix},$$

where $\mathbf{G}^{-1}$ is the inverse of the genomic relationship matrix and $\mathbf{A}_{22}^{-1}$ is the inverse of the pedigree-based relationship matrix for genotyped animals. $\mathbf{G}$ was calculated as $\mathbf{G} = \mathrm{w}\mathbf{Gr} + (1 - \mathrm{w})\mathbf{A_{22}}$ (Forni et al., 2011), where w = 0.95 and $\mathbf{Gr}$ is a genomic matrix before weighting calculated as VanRaden (2008).

In the next step, GEBV were back-solved to obtain the SNP effects for RFI implemented by the program postGSf90 (version 1.46) (Wang et al., 2012). The program assumes that the markers explain 100% of the genetic variance in the back-solving process (Wang et al., 2012). The absolute values of estimated SNP effects were divided by the empirical standard deviation of estimated SNP effects to obtain standardized SNP effects. In addition to the single-marker estimates, a 5-SNP sliding window (average window size 38.4 kb) was also constructed to calculate the proportion of genetic variance explained by 5-SNP sliding windows. In the postGSf90 program, the proportion of genetic variance explained by each sliding window was calculated by the variance explained by the window divided by the total genetic variance (Wang et al., 2014), as follows:

$$\frac{\mathrm{var}(a_i)}{\sigma_a^2} \times 100\% = \frac{\mathrm{var}\left(\sum_{j=1}^{5} \mathbf{Z}_{ij}\hat{u}_{ij}\right)}{\sigma_a^2} \times 100\%,$$

where a_i is genetic value of the ith sliding window that consists of contiguous 5 SNP; $\mathrm{var}(a_i)$ is the genetic variance explained by the ith sliding window; σ_a^2 is the total genetic variance obtained from the variance component estimate for RFI; $\mathbf{Z}_{ij}$ is the vector of gene content of the jth SNP for all individuals in the ith window; and $\hat{u}_{ij}$ is the estimate of marker effect of the jth SNP within the ith window.

GWA Signal Enrichment Analyses

For the public annotation databases, R packages of org.Bt.eg.db (version 3.6.0), reactome.db (version 1.64.0), and MeSH (version 1.10.0) as distributed in Bioconductor (version 3.7) (https://www.bioconductor.org/) were used to obtain Gene Ontology (**GO**) terms, Kyoto Encyclopedia of Genes and Genomes (**KEGG**) pathways, Reactome pathways, and medical subject heading (**MeSH**) terms (Morota et al., 2015), respectively. The biological terms or pathways with fewer than 10 genes were excluded, so that each term or pathway contained at least 10 genes for analyses. In total, 898 GO terms, 225 KEGG pathways, 820 Reactome pathways, 436 MeSH terms, and 248 trait-related terms were available for enrichment analyses.

A SNP was considered associated with one biological term or pathway if the SNP is located within ±10 kb of genes in the term or pathway. Then, the enrichment of GWA signals in each term or pathway was tested using the effects of the SNP associated with the term or pathway. A marker-set test method was applied to the enrichment analyses (Rohde et al., 2016; Fang et al., 2017), implemented by the R package for Quantitative Genetic and Genomic analyses (Rohde et al., 2018). The summary statistics (T_{sum}) for each term or pathway was calculated as follows using the SNP effects associated with each term or pathway:

$$T_{sum} = \sum_{i=1}^{m_f} t^2,$$

where T_{sum} is the summary statistics for each biological term or pathway; t is the standardized SNP effect (i.e., absolute value of estimated SNP effect divided by the standard deviation of estimated SNP effects) of the SNP associated with each term or pathway; and m_f is the number of SNP that are associated with each term or pathway.

The degree [i.e., $-\log_{10}(P\text{-value})$] of enrichment of GWA signals in each term or pathway was then determined by a 10,000-times permutation test for T_{sum} of each term or pathway. The empirical P-value for each term or pathway was calculated as the proportion of random T_{sum} from permutation greater than the observed T_{sum} (Rohde et al., 2016; Fang et al., 2017).

RESULTS AND DISCUSSION

GWA Signals for RFI

Residual feed intake was genetically regulated by many small-sized effects, indicating that RFI is a highly polygenic trait in dairy cattle (Figure 1a). Our findings in this study were consistent with previous GWAS for RFI in which no large peaks were observed for RFI in single-marker GWA analyses (Hardie et al., 2017; Lu et al., 2018). The 20 SNP with the highest standardized genetic effects for RFI are rich in BTA25 and BTA18 and were also observed in BTA1, 5, 9, 11, 14, 16, and 22 (Table 1). The top SNP for RFI are all common variants based on their minor allele frequencies (Table 1). Of the top 20 SNP, 13 are only available in the HD genotype and not within the standard genomic evaluation set of 60,671 SNP markers (Wiggans et al., 2016), implying some benefit of using denser markers for GWAS on RFI. The genes located closest to the top SNP are related to DMI and RFI, digestion and

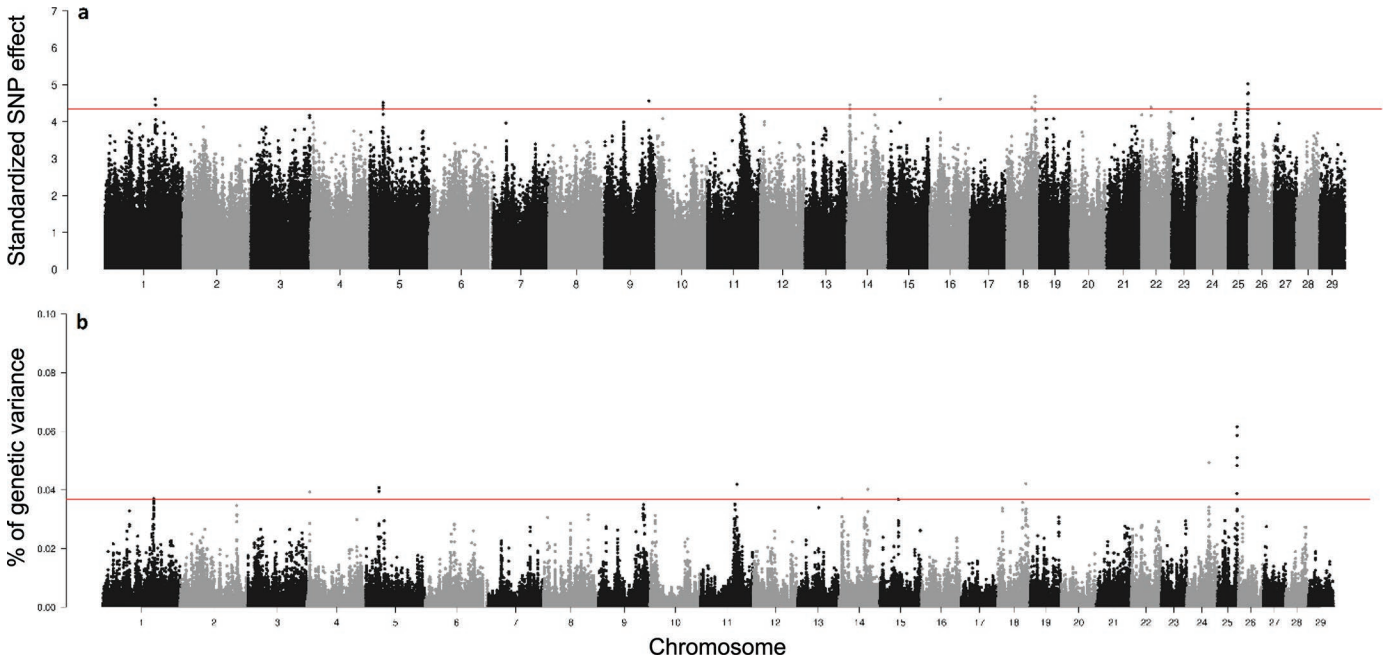

Figure 1. Manhattan plot of (a) the standardized SNP effect for residual feed intake (RFI), (b) the genetic variance (%) explained by each 5-SNP sliding window (average window size 38.4 kb) for RFI in Holstein dairy cattle. The horizontal line in (a) shows the threshold of the top 20 SNP with the largest standardized effects for RFI, and the horizontal line in (b) is the threshold of the top 20 SNP explaining the largest genetic variance for RFI.

metabolism of carbohydrates and proteins, immune regulation, and mitochondrial ATP activities (Table 1).

The strongest SNP effects for RFI were clustered in the region of 40.7 to 41.5 Mb on BTA25 (UMD3.1 reference genome), close to the *CARD11* and *EIF3B* genes. The *CARD11* (caspase recruitment domain family member 11) gene is a protein coding gene in *Bos taurus*, and its homolog in humans is widely reported to be associated with peripheral B-cell differentiation and a variety of critical T-cell effector functions (Stepensky et al., 2013; Ma et al., 2017). For FE in dairy cattle, Salleh et al. (2017) reported several immune genes and pathways associated with RFI in Danish Holstein cattle, among which *CARD11* gene was found to be downregulated in animals with high RFI compared with those with low RFI. The *EIF3B* gene is a protein-coding gene related to the initiation of protein synthesis (Lee et al., 2015). The protein expression of *EIF3B* gene was upregulated in broilers with high FE (FE = BW gain/feed intake) (Kong et al., 2016).

Apart from the signals on BTA25, top effects for RFI were also rich in the region of 57.7 to 58.2 Mb on BTA18. This candidate QTL region for RFI overlaps with a QTL region for DMI on BTA18 (Lu et al., 2018) and is also close to a QTL region affecting several calving and type traits in dairy cattle (Cole et al., 2009). Because RFI is mathematically defined from DMI, it seems likely that QTL associated with DMI also influence RFI. The potential link among cows' RFI, DMI, sizes, and calving traits on BTA18 could be further studied by calculating regional genetic correlations between these traits for this particular region on BTA18. In addition, the top SNP in this candidate QTL region on BTA18 for RFI falls into the *VSIG10L* gene (V-set and immunoglobulin domain containing 10 like), a protein-coding gene highly expressed in the normal esophagus with a putative role in maintaining healthy esophageal homeostasis in humans (Fecteau et al., 2016).

Variance in RFI Explained by Sliding Windows

The proportion of genetic variance explained by 5-SNP sliding windows is shown in Figure 1b. Each sliding window explained a small proportion of genetic variance for RFI, with the highest variance explained by 5-SNP sliding windows being <1%. The 20 sliding windows that explained the largest variance for RFI were observed in BTA25, 24, 18, 11, 5, 4, 14, 1, and 15 (Table 2). Most of the top SNP detected in the earlier single-marker GWA analysis were found within or close to these top sliding windows. The genes overlapping with, or closest to, these top windows were associated with DMI and RFI, energy homeostasis and energy

Table 1. The 20 SNP with the highest standardized genetic effects for residual feed intake (RFI) and their chromosome (Chr), position (SNP_Pos), standardized SNP effect (Effect), minor allele frequency of the SNP (MAF), the closest gene to the SNP, gene description, and the related references to the gene or the SNP

Chr	SNP_Pos (bp)	Effect	MAF	Gene	Gene description	Related references[1]
25	40,739,131	5.03	0.45	*CARD11*	Caspase recruitment domain family member 11	Expression of the *CARD11* gene is related to RFI in Holstein (Salleh et al., 2017).
25	41,455,328	4.78	0.43	*EIF3B*	Eukaryotic translation initiation factor 3 subunit B	*EIF3B* encoded protein is upregulated in broilers with high FE (Kong et al., 2016).
25	40,716,490	4.75	0.43	*CARD11*	Caspase recruitment domain family member 11	Expression of the *CARD11* gene is related to RFI in Holstein (Salleh et al., 2017).
18	57,856,652	4.69	0.32	ENSBTAG00000008851		The SNP is close to the QTL region for DMI, MBW, stature, and strength in Holstein (Cole et al., 2009; Lu et al., 2018).
18	57,782,107	4.68	0.33	*VSIG10L*	V-set and immunoglobulin domain containing 10 like	The SNP is close to the QTL region for DMI, MBW, stature, and strength in Holstein (Cole et al., 2009; Lu et al., 2018).
1	102,438,912	4.61	0.41	*BCHE*	Butyrylcholinesterase	The *BCHE* gene affects food intake and glucose homeostasis in mice and is related to obesity and metabolic syndrome in humans (Benyamin et al., 2011; Chen et al., 2017).
16	21,469,268	4.61	0.39	*GPATCH2*	G-patch domain containing 2	*GPATCH2* is associated with milk fat percentage in dairy buffaloes (de Camargo et al., 2015).
9	91,092,009	4.56	0.30	*MTRF1L*	Mitochondrial translational release factor 1 like	
18	58,198,582	4.53	0.42	ENSBTAG00000037906		The SNP is close to the QTL region for DMI, MBW, stature, and strength in Holstein (Cole et al., 2009; Lu et al., 2018)
5	26,683,762	4.52	0.49	*TARBP2*	TARBP2, RISC loading complex RNA binding subunit	The SNP is close to the QTL region for DMI in beef cattle (Nkrumah et al., 2007)
25	40,730,047	4.48	0.49	*CARD11*	Caspase recruitment domain family member 11	Expression of the *CARD11* gene is related to RFI in Holstein (Salleh et al., 2017)
1	103,185,339	4.46	0.43	*SI*	Sucrase-isomaltase	Sucrose-isomaltase complex is essential for digestion of dietary carbohydrates including starch, sucrose, and isomaltose (Lin et al., 2012).
14	7,342,696	4.46	0.40	*RF00402*		
5	26,526,934	4.44	0.47	*ATP5MC2*	ATP synthase membrane subunit c locus 2	*ATP5MC2* encodes a subunit of mitochondrial ATP synthase. It is also known as *ATP5G2*. ATP5G2 was found to be associated with body height in humans (Okada et al., 2010; He et al., 2015).
22	20,036,123	4.39	0.40	*GRM7*	Glutamate metabotropic receptor 7	
18	50,629,799	4.38	0.41	ENSBTAG00000047196		
25	40,746,377	4.36	0.47	*CARD11*	Caspase recruitment domain family member 11	Expression of *CARD11* is related to RFI in Holstein (Salleh et al., 2017).
5	26,776,071	4.35	0.48	*SP1*	Sp1 transcription factor	*SP1* is related to RFI in Holstein cows (Hou et al., 2012).
18	57,855,715	4.35	0.31	ENSBTAG00000008851		The SNP is close to the QTL region for DMI, MBW, stature, and strength in Holstein cows (Cole et al., 2009; Lu et al., 2018).
14	7,313,228	4.35	0.40	*RF00402*		

[1]FE = feed efficiency; MBW = metabolic body weight.

balance regulation, immune regulation, rumen development, skeletal muscle development, and spermatogenesis (Table 2).

In general, among all genes residing in or near the top sliding windows for RFI, several (*C5H12orf10*, *PFDN5*, *CALCA*, *SDK1*) were found to be related to RFI itself or to energy intake, and several genes (e.g., *DNMT3A*, *MFSD5*) are related to energy balance regulation (Table 2). These findings imply a general association of energy utilization and balance with RFI in dairy cows. A strong and positive genetic correlation was reported between RFI and energy balance in dairy cattle (Liinamo et al., 2015; Hurley et al., 2017), in agreement with our findings of a genetic association of energy balance with RFI.

Similar to earlier findings in the single-marker GWA analysis, the window that explains the highest genetic variance for RFI spans 40.71 to 40.77 Mb on BTA25 (Figure 1; Table 2). The closest gene to this window, *CARD11*, is related to immune regulation and was related to RFI in dairy cattle (Salleh et al., 2017), as discussed earlier in the single-marker GWA analysis. The region of 57.81 to 57.86 Mb on BTA18 was also seen among the top windows, in agreement with single-marker GWA results. In addition, the top sliding windows of 57.81 to 57.86 Mb on BTA18 and 45.93 to 46.05 Mb on BTA24 are found to overlap with *SIGLEC10* (sialic acid binding Ig-like lectin-10) gene and *SIGLEC15* gene, respectively. The *SIGLEC10* and *SIGLEC15* genes are members of the SIGLEC family of immune regulatory receptors (von Gunten and Bochner, 2008; Bornhöfft et al., 2018), and several members of the *SIGLEC* gene family (e.g., *SIGLEC6*, *SIGLEC5*) are known to bind to leptin (Patel et al., 1999). Leptin is widely recognized in the regulation of food intake, energy expenditure, fat distribution, whole-body energy balance, glucose homeostasis, and reproduction in rodents and humans (Houseknecht et al., 1998; Carter et al., 2013). In dairy cattle, Ehrhardt et al. (2016) reported that increased plasma leptin attenuates adaptive metabolism (e.g., mobilization of endogenous reserves) in early-lactating dairy cows. Based on our current findings, leptin and SIGLEC-family genes may play a role in regulating RFI and FE in dairy cattle.

GWA Signal Enrichment

The top biological terms or pathways highly enriching GWA signals for RFI are shown in Figure 2. Across the 4 annotation databases, GWA signals for RFI were highly enriched in the biosynthesis and metabolism of amino acids and proteins, digestion and metabolism of carbohydrates, skeletal development, mitochondrial electron transport, immunity, rumen bacteria activities, and sperm motility. These results are highly consistent with earlier findings in the top SNP or sliding windows. Overall, RFI was found to be related to diverse biological processes and pathways, suggesting RFI is a highly polygenic trait regulated by many genes.

More specifically, GWA signals for RFI were highly enriched in genes associated with digestion and metabolism of carbohydrates and proteins in all annotation databases, including carbohydrate digestion and absorption, gluconeogenesis, biosynthesis of various amino acids, and clathrin-coated vesicle activity. This finding indicates a systematic influence of energy metabolism on regulating FE estimated using RFI. In addition, the GWA signals for RFI were also highly enriched in skeletal development, including skeletal muscle cell differentiation (from GO), biosynthesis and metabolism of keratan sulfate (from both KEGG and Reactome), transforming growth factor-β (**TGF-β**) signaling, and SMAD activities (from Reactome). The biosynthesis of keratan sulfate was the top pathway related to RFI in both KEGG and Reactome pathways. Keratan sulfate, as a complex glycosaminoglycan, is observed in diverse tissues and is an important component of cartilage and bone matrix (Wendel et al., 1998; Funderburgh, 2000). The TGF-β signaling and SMAD proteins play key roles in body development and regulate the composition of bone matrix and bone architecture (Derynck and Zhang, 2003; Balooch et al., 2005).

Mitochondrial electron activity is another top enrichment term related to RFI, including mitochondrial electron transport, NADH to ubiquinone (from GO), and the pathway of ubiquinone and other terpenoid-quinone biosynthesis (from KEGG). The association of RFI with mitochondrial ATP activity was also observed previously from the top GWA signals. Recent studies in poultry (Bottje et al., 2006) and in beef cattle (Kolath et al., 2006) provided evidence of a link between inefficient mitochondrial respiration and decreased FE. In Angus steers, steers with low RFI exhibited a greater rate of mitochondrial respiration than those with high RFI (Kolath et al., 2006). In broilers, mitochondria obtained from inefficient broilers exhibited greater uncoupling of the electron transport chain and greater oxidative stress compared with efficient broilers (Bottje et al., 2006; Bottje and Kong, 2013).

In addition, terms or pathways associated with immunity (Toll-Like Receptors Cascades from Reactome), rumen bacterial activities (Ruminants and bacteria from MeSH, Porphyrin and chlorophyll metabolism from KEGG), and sperm motility (from KEGG) were also enriching GWA signals for RFI. The associations between RFI with immunity and with rumen bacterial activities were observed earlier in our top sliding windows and also in the recent literature (Jewell et al.,

Table 2. The 20 sliding windows explaining the highest proportion of genetic variance for residual feed intake (RFI), and their chromosome (Chr), start and end positions of the window, the proportion of genetic variance explained by the window (Var%), the closest gene to the window, the gene description, and the related references to the gene or to the window

Chr	Start (bp)	End (bp)	Var%	Gene	Description	Related references[1]
25	40,716,490	40,766,665	0.062	*CARD11*	Caspase recruitment domain family member 11	Expression of *CARD11* is related to RFI in Holstein (Salleh et al., 2017).
25	40,697,080	40,753,128	0.059	*CARD11*	Caspase recruitment domain family member 11	
25	40,730,047	40,768,748	0.051	*CARD11*	Caspase recruitment domain family member 11	
24	45,933,554	46,045,120	0.049	*SIGLEC15*	Sialic acid binding Ig like lectin 15	*SIGLEC15* is in the Siglec family as immune regulatory receptors (von Gunten and Bochner, 2008; Bornhöfft et al., 2018).
25	40,655,616	40,746,377	0.048	*CARD11*	Caspase recruitment domain family member 11	Expression of *CARD11* is related to RFI in Holstein (Salleh et al., 2017).
18	57,818,432	57,860,111	0.042	*SIGLEC10*, *LIM2*	Sialic acid binding Ig like lectin 10, lens intrinsic membrane protein 2	*SIGLEC10* is in the Siglec family as immune regulatory receptors (von Gunten and Bochner, 2008; Bornhöfft et al., 2018). The SNP is close to the QTL regions for DMI, MBW, stature, and strength in Holstein (Cole et al., 2009; Lu et al., 2018).
11	74,035,522	74,074,496	0.042	*DNMT3A*	DNA methyltransferase 3 α	DNMT3A in Sim1 neurons is necessary for normal energy homeostasis and energy balance regulation (Kohno et al., 2014).
5	26,879,626	26,938,546	0.041	*C5H12orf10*, *PFDN5*, *ESPL1*, *MFSD5*, *RARG*	Chromosome 5 C12orf10 homolog, Prefoldin Subunit 5, extra spindle pole bodies like 1, major facilitator superfamily domain containing 5, retinoic acid receptor gamma	*C12orf10* (homolog of C5H12orf10) is associated with energy intake in Holstein (Loor et al., 2006); *PFDN5* is differentially expressed in beef cattle when feed intake increases in compensatory growth (Connor et al., 2010); *ESPL1* is related to calf rumen epithelial tissue development and function during weaning (Connor et al., 2014); *MFSD5* is related to energy homeostasis and regulation (Perland et al., 2016); and *RARG* is related to immune response, development of bovine embryo (Mohan et al., 2001; Channabasappa et al., 2014).
14	59,679,712	59,742,596	0.040	*ABRA*	Actin binding Rho activating protein	*ABRA* signaling pathway is responsive to changes in skeletal muscle loading (Lamon et al., 2009).
5	26,894,204	26,948,778	0.040	*ESPL1*, *MFSD5*, *RARG*	Extra spindle pole bodies like 1, major facilitator superfamily domain containing 5, retinoic acid receptor gamma	*ESPL1* is related to calf rumen epithelial tissue development and function during weaning (Connor et al., 2014); *MFSD5* is related to energy homeostasis and regulation (Perland et al., 2016); and *RARG* is related to immune response, development of bovine embryo (Mohan et al., 2001; Channabasappa et al., 2014).
4	5,604,707	5,683,973	0.039	*SPATA48*	Spermatogenesis associated 48	*SPATA48* is essential for spermatogenesis in humans and mice (Zhang et al., 2018).
25	40,457,032	40,478,151	0.039	*SDK1*	Sidekick cell adhesion molecule 1	*SDK1* is associated with RFI in beef cattle (Barendse et al., 2007).
14	7,104,148	7,127,478	0.037	*RF00402*		
1	104,055,079	104,073,286	0.037	ENSBTAG00000018312		
1	104,049,927	104,056,337	0.037	ENSBTAG00000018312		
1	104,051,734	104,057,596	0.037	ENSBTAG00000018312		
1	104,053,062	104,059,287	0.037	ENSBTAG00000018312		
1	104,054,034	104,066,487	0.037	ENSBTAG00000018312		
15	38,178,809	38,219,116	0.037	*CALCA*	Calcitonin-related polypeptide α	Calcitonin gene-related peptide is involved in modulating food intake and appetite in mice (Krahn et al., 1984; Essner et al., 2017).
1	104,027,056	104,048,241	0.037	ENSBTAG00000018312		

[1]MBW = metabolic body weight.

2015; Salleh et al., 2017; Elolimy et al., 2018). Studies also reported that beef bulls with lower RFI have decreased sperm motility and sperm viability compared with the inefficient beef bulls, indicating an undesirable effect of selection for improved FE on reproduction in beef cattle (Awda et al., 2013; Fontoura et al., 2016). However, the genetic correlation between RFI and fertility of dairy cattle remains unclear. In dairy cattle, studies indicate genetic correlation between residual energy intake and energy balance (Liinamo et al., 2015), and negative energy balance in cows has been reported to have unfavorable effect on fertility (e.g., Banos and Coffey, 2010). However, the direct estimation of genetic correlation between RFI and cow fertility has very rarely been studied. Assessing accurate genetic correlation between RFI and fertility in dairy cattle is highly recommended in future genetic studies for FE. It is also worth noting that the estimates of genetic correlation between RFI and other traits can be affected by the methods used to model RFI (e.g., different energy sink traits included in the RFI model).

GWA Using the Single-Step Method

Genome-wide association analyses can be conducted using various methodologies (Wang et al., 2012; Hayes,

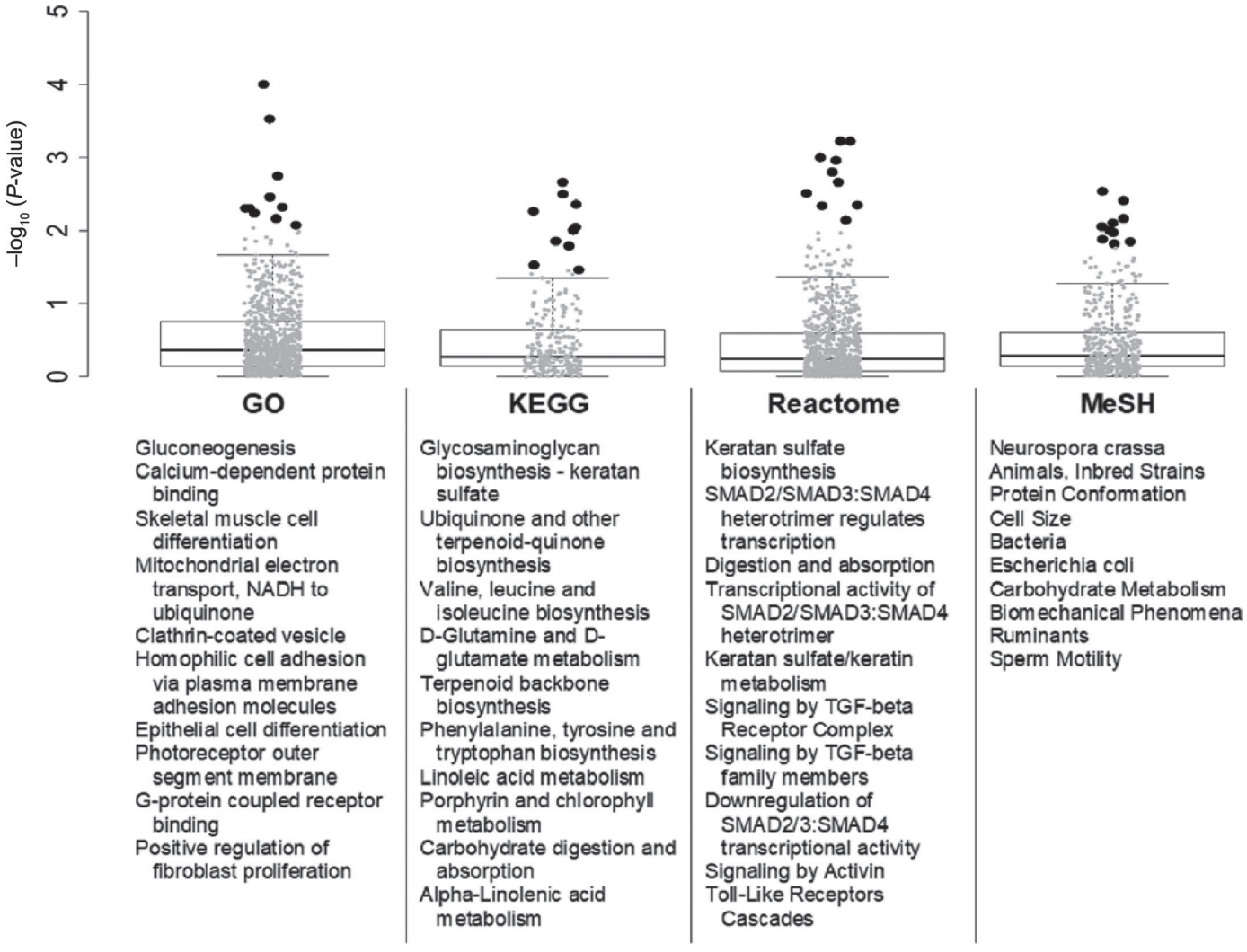

Figure 2. The top biological terms and pathways highly enriching genome-wide association (GWA) signals for residual feed intake (RFI). Each dot in the boxplot represents one tested term or pathway in 4 public gene annotation databases of 898 Gene Ontology (GO) terms, 225 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, 820 Reactome pathways, and 436 medical subject heading (MeSH) terms. The degree [i.e., $-\log_{10}(P\text{-value})$] of GWA signal enrichment in each term or pathway was determined by a 10,000-times permutation test for the summary statistics (T_{sum}) of each term or pathway. The top 10 biological terms or pathways enriching GWA signals of RFI are shown as black dots in a boxplot and are tabulated under the boxplot for each annotation database. The midline, box edges, the upper bound and lower bound lines of each boxplot represent median, interquartile range (IQR), third quartile + 1.5 × IQR, and first quartile − 1.5 × IQR, respectively.

2013; Schmid and Bennewitz, 2017), including classical single-marker regressions, Bayesian methods, and ssGWAS, as applied in this study. In single-marker regressions, the level of multiple testing can be enormous with dense SNP data, and stringent thresholds are needed to prevent an inflation of type-I errors (Schmid and Bennewitz, 2017). The GWA analyses using Bayesian methods or ssGWAS evaluate all SNP simultaneously, considering that the effect of a gene is only partly captured by a single marker due to imperfect linkage disequilibrium but might be better explained jointly by multiple SNP surrounding the gene (Hayes, 2013; Schmid and Bennewitz, 2017). In the ssGWAS used in this study, all phenotypic information from genotyped and ungenotyped animals, as well as their ancestors' information, was used simultaneously through joint genomic and pedigree information. The main advantage of ssGWAS is the ability to incorporate phenotypes of ungenotyped subjects directly in the association analyses without the need to construct pseudo-observations (Wang et al., 2012). Therefore, ssGWAS could be more useful when a large number of phenotyped subjects are not genotyped or when obtaining accurate pseudo-data (e.g., de-regressed proofs) is difficult (Wang et al., 2014).

In Bayesian analyses or single-marker regressions, information from nongenotyped animals can be indirectly included in the association in multistep procedures using de-regressed proofs. In our previous study (unpublished data), an approximate BayesA method was applied to estimate GEBV and SNP effects for RFI using the same data set as this study, but with 60,671 SNP markers. The SNP that explained the largest genetic variances for RFI from the BayesA analysis agree with those found in this study using ssGWAS. The top SNP for RFI found from BayesA and from ssGWAS were identical, on BTA25 in the region of 40.7 to 41.5 Mb. A more comprehensive comparison between ssGWAS and Bayesian method for GWA analyses was described by Wang et al. (2012, 2014), who compared ssGWAS and BayesB for GWAS through a simulation study and a real-data study in chicken. Their results showed that ssGWAS and BayesB delivered similar predictions of QTL, but the magnitude of SNP effect estimates can be very different due to the different assumptions in the distribution of SNP effect and variance (Wang et al., 2012, 2014).

A limitation of the current study using ssGWAS is that it assumes equal variance for all SNP effects, which may not be accurate in all cases. One way to offset this limitation is to relax this assumption through combining single-step method with Bayesian framework. However, the cost of computing time in such a method would also need to be considered. In addition, a proper significance test for SNP effects needs to be implemented in ssGWAS. In the current version of postGSf90 program used in this study, a standardized SNP effect was calculated to account for the empirical standard deviation of estimated SNP effects. A significance test in ssGWAS is under development in a new version of postGSf90 program, so that *P*-value test statistics could be applied to future genomic association analyses using ssGWAS.

Genomic Prediction for RFI

Residual feed intake is regulated by many small-sized effects and is related to diverse biological processes and pathways. Despite the complexity of the genetic basis of RFI, RFI is still a promising FE candidate trait in dairy cattle breeding, considering its independence of other index traits. In this study, GPTA of energy sink traits was included in the genetic model of RFI, so that RFI was genetically uncorrelated with major energy sink traits (milk production, BWC) in the selection indexes. The heritability of RFI was estimated to be 0.14 in our data set (VanRaden et al., 2018). The SNP of importance detected from this study could be used to provide weight information to the SNP used for future genomic prediction for RFI. Minor allele frequencies of the top SNP in this study indicated that the top SNP for RFI were common variants, indicating the potential of applying genomic selection to RFI given the variation.

CONCLUSIONS

Residual feed intake can be defined to be genetically uncorrelated with major energy sink traits (e.g., milk production, BW), by including animals' GPTA of these energy sink traits in the genetic analyses for RFI. Based on our GWA analysis and post-GWA enrichment analyses, RFI was a highly polygenic trait regulated by many small-sized effects. Residual feed intake was related to diverse biological processes and pathways, including DMI, energy balance, carbohydrate and protein metabolism, skeletal development, mitochondrial energy generation, leptin signaling, immunity, rumen bacteria activities, and sperm motility. The region of 40.7 to 41.5 Mb on BTA25 was the top associated region for RFI, with the closest gene to this region (*CARD11*) being related to immune regulation and having been reported to be related to RFI in dairy cattle. The region of 57.7 to 58.2 Mb on BTA18, which is associated with leptin signaling and DMI, was also associated with RFI in this study. Despite the complexity of the genetic basis of RFI, RFI is still a promising FE candidate

trait in dairy cattle breeding, with genomic selection as an important tool in selecting for FE in dairy cattle. The SNP of importance detected from this study could be used to provide weight information to the SNP for future genomic prediction for RFI.

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