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The role of obesity genes for milk fat yield in Holstein dairy cattle

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ABSTRACT: Under the assumption that human obesity genes not only regulate fat deposition but also energy homeostasis, we expected effects of those genes on milk fat yield. Therefore, we performed an association study with EBVs for milk fat yield of 2,402 German Holstein bulls. We used SNPs in 2 Mb regions around 317 bovine homologues of human obesity candidate genes. 78 (24.6%) regions harbored 141 SNPs significantly (P<0.05) associated with milk fat yield. Three SNPs were highly significant (P<0.001). 36 SNPs were significantly associated with milk fat and protein yield and 12 SNPs were significant for fat, protein and milk yield. The results confirm that many genes affecting obesity also influence milk fat yield. The information on potential pleiotropic effects can be used to influence the milk fat to protein ratio and can be considered for selecting robust dairy cows.
Keywords: dairy cattle; lactation; milk fat yield; health; genetics

Introduction

In dairy cows, energy partitioning is a crucial metabolic challenge, in particular in the transition time pre and postpartum and the first third of lactation. In this period, a negative energy balance occurs because energy intake by fodder is lower than energy required for milk production in addition to all other biological functions. Cows in this part of lactation are metabolically stressed, and, therefore, are more vulnerable to diseases, such as ketosis or mammary infections, or impaired fertility (Jamrozik and Schaeffer (2012)). The amount of fat secreted in milk is a crucial part of the energy needed of a lactating cow. Approximately a half of the energy in the milk is present in form of milk fat (Schlimme and Buchheim (1999)). Therefore, the regulation of fat secreted in milk is extremely important for the energy homeostasis of a cow. To obtain the energy needed for the synthesis of milk compounds, bodily energy resources are mobilized as fat from fat tissue, but also as protein from muscles. Since fat storage, mobilization, utilization, and secretion are central for the energy homeostasis of a lactating cow, we expect that the regulation of these processes is crucial besides fat synthesis and fat oxidation itself. The genetic factors determining energy homeostasis are also likely to be responsible for the maintenance of other energy requiring biological function like fertility and defense against diseases.

Previous genome-wide association studies (GWAS) in humans and dairy cattle were performed to uncover genetic variation contributing to obesity and milk production, respectively. Although joined pathways can be assumed for the control of body weight and milk fat synthesis neither human nor cattle studies have covered both aspects of energy balance and partitioning. The objective of this study was to test, if bovine homologues of obesity candidate genes identified in human association studies affect also the milk fat yield in dairy cattle.

Materials and Methods

Animals and phenotypic data. The study was performed with 2,402 breeding bulls of the German Holstein Friesian population. We used estimated breeding values (EBVs) of the bulls for milk fat yield, milk protein yield and milk yield. EBV's averaged for the first three lactations were chosen to detect genes which have an impact on milk fat yield for the entire productive live span. EBVs were obtained from the national center for breeding value estimation (Vereinigte Informationssysteme Tierhaltung w.V. (VIT), Verden, Germany).

Genotypes and quality check. The bulls were genotyped with the Illumina BovineSNP50 v1 BeadChip (54,001 SNPs). The SNP data were subjected to rigorous validation to exclude un-positioned SNPs (Schmitt et al. (2010)), SNPs with a minor allele frequency (MAF) <0.01 and SNPs with a missing genotype rate >10%. In addition, individuals with a SNP call rate <90% were removed. The remaining data set included 43,062 SNPs and 2,360 bulls with a genotyping rate of 99.6%.

Human obesity candidate genes. We conducted a database search with the phrases: "genome-wide association", "GWA", in combination with either "obesity", "fat", "body mass index", "BMI" or "adiposity". Furthermore, the GWAS catalogue (www.genome.gov/ gwastudies) was inspected for additional candidate genes reported for: "adiposity", "BMI", "BMI and fat mass", "lipid traits", "obesity", "obesity (early onset extreme)", "obesity (extreme)", "obesity-related traits", "visceral adipose tissue adjusted for BMI", "waist circumference", "waist circumference triglycerides (WC-TG)", "waist circumference and related phenotypes" and "weight". After removing gene name bias and overlapping genes, this strategy resulted in 338 non-redundant human obesity candidate genes. 317 (94%) genes, which had a bovine homolog (Sayers et al. (2011)) and an unambiguous chromosomal annotation in the bovine genome (Ensembl database version 69/UMD3.1) were included in the association analysis.

Association analysis. We used the software PLINK 1.06 (Purcell et al. (2007)). A linear regression model was used to estimate the additive effect of the SNP alleles. To account for population stratification, we performed a pairwise population concordance test. The test identified 124 significant clusters of related animals ($P < 10^{-4}$), which were fitted in our model using a multidimensional scaling approach. To account for the known *DGAT1* effect on milk fat yield, we implemented the SNP *ARS-BFGL-NGS-4939* having the lowest P value in the *DGAT1* region as a fixed effect in our model. We performed an association analysis with all 317 candidate genes simultaneously. For every candidate gene, a center-SNP was chosen from the Illumina BovineSNP50K BeadChip, which was ideally located in or adjacent to the candidate. Since linkage disequilibrium remains at distances up to 1 Mb in cattle (Gibbs et al. (2009), Hayes et al. (2010)), we extended the candidate region to 1 Mb- up- and downstream of the center-SNP. A similar partitioning of the bovine genome in chromosomal segments for association analysis was previously reported. All candidate gene regions contained 8,643 SNPs. The association results were adjusted for multiple testing using the false discovery rate (FDR) by Benjamini und Hochberg (1995).

Results and Discussion

Association of obesity candidate genes with milk fat yield. Sixty five different 2 Mb regions surrounding bovine homologues of human obesity candidate genes showed significant association ($P < 0.05$) with the averaged milk fat yield over the first three lactations. In these regions, 78 (24.6%) out of 317 tested genes of the candidate gene set were located, harboring 141 significant SNPs ($P<0.05$). Thirteen associated SNPs were located directly within six candidate genes; the other significant SNPs were in close neighborhood to the suggested human candidate genes. Among the obesity candidate genes that were associated with milk fat yield were the *fat mass and obesity associated* (*FTO*) gene and the *brain-derived neurotrophic factor* (*BDNF*) gene, for which the effects on milk fat yield were verified in a cow population (Zielke et al. (2011); Zielke et al. 2013)).

The most significant SNPs associated with milk fat yield were located outside the suggested human candidate genes. These SNPs were *Hapmap53294-rs29016908* on BTA5 (P=9.62E-11), *BTB-00318021* on BTA7 (P=4.23E-04) and *Hapmap40333-BTA-10479* on BTA18 (P=4.85E-05) (Table 1).

The most significant SNP *Hapmap53294-rs29016908* (BTA5) had a minor allele frequency of 0.25. The effect of the minor allele was 6.08 kg milk fat per lactation. The gene in closest neighborhood (28,057 bp downstream) of this SNP is the *epidermal growth factor receptor substrate 8* (*EPS8*) gene. Recently, *EPS8* has been associated with milk fat content in the same German Holstein population we used (Wang et al. (2012)). EPS8 is a substrate for the epidermal growth factor receptor (EGFR) kinase (Fazioli et al. (1993)). EGFR itself is suggested to be essential for the morphogenesis of the mammary ducts (Sebastian et al. (1998)) and for increasing the concentration of intracellular fatty acids by initiating the transcription of fatty acid synthesis (Guo et al. (2009)). However, the *microsomal glutathione S-transferase* (*MGST1*) gene, which is prioritized as the most likely obesity candidate gene in the associated region in humans (Fox et al. (2012)), is in high linkage disequilibrium (LD) with *EPS8*. The biological function as an enzyme that protects mitochondria from oxidative stress by suppressing lipid peroxidation (Johansson et al. (2010)) qualifies *MGST1* as a functional candidate gene. As such, it could contribute to the balance of fatty acids availability for milk fat or fat depots. Therefore, additional association and functional studies are necessary to distinguish between the account of each of the two genes *EPS8* and *MGST1* to the amount of fat secreted during lactation.

The second highly significant SNP *BTB-00318021* (BTA7) associated with milk fat yield had a frequency of 0.44. The minor allele had a positive effect of 3.70 kg fat per lactation. This SNP and seven additional significant SNPs reside within the *glutamate receptor, ionotropic, AMPA 1* (*GRIA1*) gene. GRIA1 is one of the major mediators of excitatory synaptic transmission. The highest density of GRIA1 can be found in the hippocampus and prefrontal cortex. It is thought to influence cognitive functions, such as reward learning and working memory (Schmitt et al. (2005); Chiesa et al. (2012)). As such, the protein could affect milk fat yield via ensuring signaling in specific nuclei of the nervous system required for stimulating feed intake. The two suggested human obesity genes in the associated region encode *the microfibrillar-associated protein 3 isoform 1* (*MFAP3*) and *UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10* (*GALNT10*) (Ng et al. (2012)). However, SNPs in close distance to these two candidate genes showed no significant association with milk fat yield in our study.

The third highly significant SNP *Hapmap40333-BTA-10479* (BTA18) had a minor allele frequency of 0.23. The minor allele effect was negative, reducing milk fat yield by -4.27 kg fat per lactation. This SNP is located in the *cysteine-rich secretory protein LCCL domain containing 2* (*CRISPLD2*) gene. CRISPLD2 is suggested to be a critical serum protein for the endotoxin function (Wang et al. (2009)), involved in cell motility and to play a role in the folate pathway (Chiquet et al. (2011)). The SNP was in high LD ($D' > 0.95$) with two other SNPs in the *ubiquitin specific peptidase 10* (*USP10*) gene and with one SNP in the *kelch-like 36* (*KLHL36*) gene; but none of the linked genes was significantly associated with milk fat yield.

Potential pleiotropic effects on other milk yield traits. Under the assumption that the human obesity candidate genes affect energy partitioning, we would also expect pleiotropic effects on other milk yield traits. Since milk pro-

tein and total milk yield data were available, we tested these two traits with the same candidate gene regions as for milk fat yield. For protein yield, 23 candidate gene regions had the same lead SNP (SNP with the lowest P value in a region) for fat and protein yield and 36 SNPs were significant for both traits (Figure 1). While most SNPs had minor allele effects acting into the same direction of effect, two SNPs showed opposite direction of effect, where the effect for fat yield was decreasing and for protein increasing. Twelve SNPs showed a significant association with fat, protein and milk yield (Figure 1).

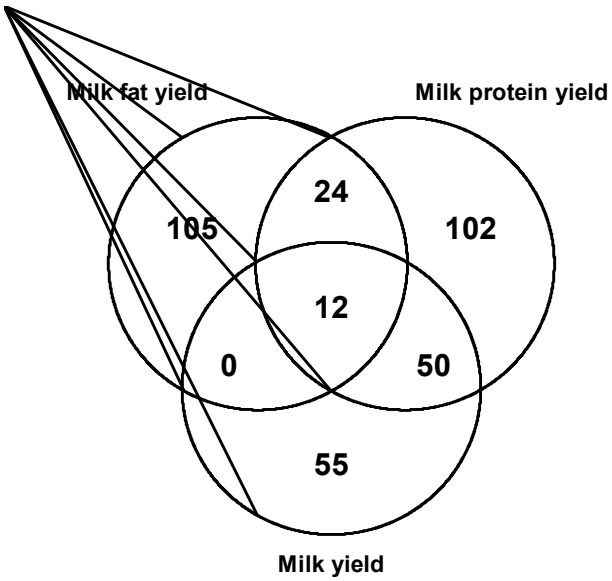

Figure 1. Venn diagram showing the number (n) and the overlap of significant SNPs identified in the obesity candidate gene analysis affecting milk fat yield, milk protein yield, and milk yield. Data used for the association study are estimated breeding values averaged for the first three lactations.

Conclusion

The results of testing more than 300 candidate genes for body composition traits, in particular for fat deposition, suggest that about a quarter of those genes affect also the amount of fat secreted during lactation. This provides evidence that genes controlling milk energy content also control body composition. This hypothesis is further supported by the finding that some SNPs are associated with both milk fat and protein yield and most of those SNPs affect the amount of fat and protein into the same direction. Nevertheless, the few SNPs affecting these traits into opposite directions could be considered to change the fat to protein ratio, which is used to characterize the metabolic state of a cow. In addition to the estimated whole-genome breeding value, that SNP information could be used for specific breeding decisions. For the selection of robust cows it will be necessary to account for pleiotropic effects of genes affecting milk fat content on the whole-body energy regulation.

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