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Abstract

Lameness in dairy cattle is primarily caused by foot lesions including sole haemorrhage (SH) and sole ulcers (SU). This study investigated the genetic architecture of SH and SU in different lactation stages based on accurate phenotyping, genetic parameter estimation, genome-wide association (GWA) and functional enrichment analyses. Foot lesion records were collected from 2,353 Holstein cows on four herds at four time-points: prior to calving, immediately after calving, early lactation, and late lactation stages. Heritability estimates were 0.11-0.20 for SH and 0.05-0.13 for SU across stages, and genetic correlations between stages were high for both traits. Candidate genes associated with SH and SU link to immune functions such as complement activation and inflammation, nervous system and muscle functions. To conclude, SH and SU are under genetic control and foot health can be improved with selective breeding.

Introduction

Lameness in dairy cattle is primarily caused by foot lesions including sole haemorrhage (SH) and sole ulcers (SU) as two common claw horn lesions (CHL). Recent studies have shown genetic variation in resistance to foot lesions (Heringstad *et al.*, 2018). A few genome-wide association (GWA) analyses have shown a complex genetic background for foot health (e.g. Swalve *et al.*, 2014; Sánchez-Molano *et al.*, 2019; Butty *et al.*, 2021; Lai *et al.*, 2021), but results generally differ between studies. Most previous studies utilized foot-trimming data that may be subject to variability between recorders and populations and may miss some mild lesion cases.

In the present study we closely monitored cohorts of cows across lactation and collected detailed foot lesion records. The objectives were to: (1) estimate genetic parameters of CHL in different lactation stages; and (2) characterise the genomic architecture of CHL.

Materials & methods

Animals and data recording. A total of 2,353 Holstein cows from four herds in the United Kingdom were included. The studied cows calved between April and September 2019. Individual animals were assessed for SH and SU at four time points: prior to calving (pre-calving stage, mean=55.3 days before calving), immediately after calving (calving stage, mean=5.4 days in milk), at peak yield time (early lactation stage, mean=83.9 days in milk), and in mid-late lactation (late lactation stage, mean=199.6 days in milk).

Foot lesions were recorded by qualified veterinary surgeons using case definitions in the ICAR claw health atlas (Egger-Danner *et al.*, 2020). Over 90% of foot lesion identification and recording was performed by a single researcher. All four feet were examined for each lesion, which were scored as 0 (no lesion), 1 (mild lesion), 2 (moderate lesion) and 3 (severe lesion). The pedigree spanning seven generations was available for all phenotyped animals.

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Phenotypes. The phenotype of each lesion severity was calculated as the average score across all assessed feet. The phenotype was calculated for each studied time-point, so each animal had repeated records across lactation stages.

Genotypes. Genotypes were available for 2,250 of the studied animals. The genotypes had been imputed to 80K SNPs within the national dairy cattle genomic evaluation. Chromosome locations of the 80K SNP panel were based on ARS-UCD 1.2. Genotype quality control kept SNP and animal call rates higher than 90%, SNPs with minor allele frequency higher than 0.05, and animals with no parent-progeny Mendelian conflicts.

Genetic parameter estimation. Variance components and heritability for SH and SU were estimated for each lactation stage using the airemlf90 software (Misztal, 2013). Multivariate analyses were conducted to estimate correlations between stages. The model used was:

$$y = Xb + Za + e \quad (1)$$

where y is the phenotype; b is the fixed effect for parity and herd-year-season of recording; age effect was not included because variance explained by age effect has been explained by its correlated effect of parity; a is additive genetic effect with var(a) ~ $N(0, H\sigma_a^2)$, where σ_a^2 is the additive genetic variance and H is the relationship matrix incorporating pedigree and genomic information (Legarra *et al.*, 2009); e is random residual with var(e) ~ $N(0, I\sigma_e^2)$; and X and Z are incidence matrices for b and a, respectively.

Subsequently, repeated records of individuals across lactation were combined and analysed with an animal repeatability model. The model was as model (1) with the addition of a fixed effect for lactation stage and a random permanent environmental effect. Single-step GBLUP animal GEBVs were estimated by the animal repeatability model and blupf90 software (Misztal, 2013).

Genome-wide association analyses. Animal GEBVs were back-solved to obtain SNP effects for each trait, using the postGSf90 software (Wang *et al.*, 2012). In addition, sliding genomic windows with a size of 0.65 Mb were constructed to calculate the proportion of genetic variance explained by each genomic window. The window size of 0.65 Mb was determined based on the average distance in the genome where linkage disequilibrium between markers halved. The proportion of genetic variance explained by sliding windows was calculated with the method described by Wang *et al.* (2014) with postGSf90 software.

Candidate genes located close to the large-effect variants within 0.2 Mb upstream and downstream or within the candidate regions were examined with functional enrichment analyses using the DAVID bioinformatic resource.

Results & discussion

Incidence rates of SH and SU. The incidence rate of SH in the study population (83.0%) was higher than that of SU (13.9%) over the whole lactation. The higher prevalence of SH than SU can be partly explained by the fact that SU cases may be developed from severe SH cases. The incidence rates of SH and SU were at the highest at early lactation stage (57.5% for SH, and 6.1% for SU). Our findings infer a higher risk of SH and SU in early lactation with high yield. Unfavourable genetic correlations between foot lesions and milk yield were reported previously (Koenig *et al.*, 2005).

Genetic parameters for CHL. The heritability estimates ranged from 0.11 to 0.20 for SH, and 0.05 to 0.13 for SU across stages, with no significant differences between stages (Table 1). Although the phenotypic correlations between stages were generally lower than 0.50 for both traits, their genetic correlations were

much higher, especially for SU (Table 1). The high genetic correlations suggest that CHL may have similar genetic background across lactation.

Single-marker GWA results. Significant SNPs were identified on BTA1 for SH, and on BTA8 and BTA5 for SU (Table 2). Genes located closest to these SNPs were *PGM5, TAC3, and BACE2*. The *PGM5* gene was associated with human lower-limb muscle injury (Aguilar *et al.*, 2015). The *TAC3* gene links to nervous system function, smooth muscle contraction, and leukocyte gene expression after burn injury or trauma-haemorrhage in mice (Topaloglu *et al.*, 2009). The *BACE2* gene is related to bovine muscle formation (Lee *et al.*, 2012).

Genomic windows associated with CHL. For SH, highest proportions of genetic variance were explained by genomic regions on BTA20, BTA22 and BTA3 (Table 3). The region on BTA3 was associated with digital dermatitis (Sánchez-Molano *et al.*, 2019). Genes within the region of BTA3 (*C8B, C8A*) link to complement activation. Genes within the regions on BTA20 and BTA22 link to immune functions including inflammation regulation (*OTULIN, OTULINL*), leukocyte migration (*TRIO),* and T cells cytokine production (*BHLHE40*).

Respective regions for SU were found on BTA18 and BTA14 (Table 3). The *CHD9* gene within the region on BTA18 is involved in osteogenesis. Genes within the regions on BTA14 link to neutral system function (*KCNQ3*) and inhibition of cell growth and proliferation (*KHDRBS3*).

Gene enrichment. Enrichment of the above-mentioned genes link to Gene Ontology terms of DNA transcription regulation (*OTULIN, BHLHE40*), KEGG pathways of complement and coagulation cascades (*C8A, C8B*), cholinergic synapse (*ITPR1, KCNQ3*), and Reactome pathway of regulation of complement cascade (*C8A, C8B*).

Table 1. Heritability estimates (diagonal, in bold) for sole haemorrhage (SH) and sole ulcer (SU) at pre-calving stage, calving stage, early and late lactation stages, and genetic correlations (upper-diagonal) and phenotypic correlations (below diagonal) between stages; standard errors of estimates are in brackets.

Trait		Pre-calving	Calving	Early	Late
SH	Pre-calving	**0.11** (0.03)	0.98 (0.02)	0.78 (0.05)	0.80 (0.04)
	Calving	0.35 (0.02)	**0.14** (0.03)	0.74 (0.05)	0.72 (0.07)
	Early	0.27 (0.02)	0.21 (0.02)	**0.20** (0.04)	0.98 (0.02)
	Late	0.25 (0.02)	0.16 (0.02)	0.36 (0.02)	**0.13** (0.04)
SU	Pre-calving	**0.13** (0.04)	0.97 (0.01)	0.96 (0.01)	0.96 (0.02)
	Calving	0.38 (0.02)	**0.07** (0.03)	0.92 (0.03)	0.97 (0.01)
	Early	0.29 (0.02)	0.21 (0.02)	**0.05** (0.02)	0.96 (0.01)
	Late	0.37 (0.02)	0.39 (0.02)	0.44 (0.02)	**0.08** (0.04)

Table 2. Significant markers for sole haemorrhage (SH) and sole ulcer (SU) in single-marker GWA analyses with respective chromosome (BTA) and position (Pos), minor allele frequency (MAF), *P*-value, closest gene, and distance to the gene.

Lesion	BTA	Pos	MAF	*P*-value	Gene	Distance (bp)
SU	8	44,652,431	0.08	6.76E-06	*PGM5*	39,594
	8	44,735,178	0.08	8.21E-06	*PGM5*	122,341
	5	56,420,099	0.06	1.37E-05	*TAC3*	2,087
SH	1	141,365,527	0.21	2.22E-05	*BACE2*	16,070

Table 3. Genomic regions that explain the highest proportions of genetic variance (Var%) for sole haemorrhage (SH) and sole ulcer (SU), including chromosome (BTA) and positions (Pos_start, Pos_end) and genes located within.

Trait	BTA	Pos_start	Pos_end	Var%	Genes
SH	20	58,161,594	58,795,440	0.78	*ANKH, OTULIN, OTULINL, TRIO*
	22	21,284,764	21,911,533	0.63	*BHLHE40, ITPR1, SUMF1*
	3	88,750,416	89,386,272	0.50	*C8B, C8A*
SU	14	8,954,477	9,597,429	0.93	*KCNQ3, EFR3A*
	18	21,454,669	22,081,129	0.69	*CHD9, RBL2, AKTIP, FTO*
	14	5,922,777	6,526,644	0.53	*KHDRBS3*

Conclusions

SH and SU are under genetic control and animal resistance to CHL may be improved with selective breeding. The CHL may have highly similar genetic background in different lactation stages with high genetic correlations. As polygenic traits, SH and SU were associated with immune functions such as complement activation and inflammation, nervous system and muscle functions, DNA transcription regulation, and cell growth and proliferation.

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