Genomic analyses of claw disorders in Holstein cows: Genetic parameters, trait associations, and genome-wide associations considering interactions of SNP and heat stress

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ABSTRACT

The aim of the present study was an in-depth genomic analysis to understand the genomic mechanisms of the 3 claw disorders dermatitis digitalis (DD), interdigital hyperplasia (HYP), and sole ulcer (SU). In this regard, we estimated genetic parameters based on genomic relationship matrices, performed genome-wide association studies, annotated potential candidate genes, and inferred genetic associations with breeding goal traits considering the most important chromosomal segments. As a further novelty of this study, we inferred possible SNP × heat stress interactions for claw disorders. The study consisted of 17,264 first-lactation Holstein Friesian cows kept in 50 large-scale contract herds. The disease prevalence was 15.96, 2.36, and 8.20% for DD, HYP, and SU, respectively. The remaining breeding goal traits consisted of type traits of the feet and leg composite, female fertility, health traits, and 305-d production traits. The final genotype data set included 44,474 SNPs from the 17,264 genotyped cows. Heritabilities for DD, HYP, and SU were estimated in linear and threshold models considering the genomic relationship matrix (G matrix). Genetic correlations with breeding goal traits based on G were estimated in a series of bivariate linear models, which were verified via SNP effect correlations for specific chromosome segments (i.e., segments harboring potential candidate segments for DD, HYP, and SU). Genome-wide association studies were performed for all traits in a case-control design by applying a single SNP linear mixed model. Furthermore, for DD, HYP, and SU, we modeled SNP × heat stress interactions in genome-wide association studies. Single nucleotide polymorphism-based heritabilities were 0.04 and 0.08 for DD, 0.03 and 0.10 for SU, and 0.03 and 0.23 for HYP from linear and threshold models, respectively. The genetic correlations between DD, HYP, and SU with conformation traits from the feet and leg composite were positive throughout, indicating the value of indirect selection on conformation traits to improve claw health. Genetic correlations between DD, SU, and HYP with other breeding goal traits indicated impaired female fertility, impairedudder health status, and productivity decline of diseased cows. Genetic correlations among DD, SU, and HYP were moderate to large, indicating that different claw disorders have similar genetic mechanisms. Nevertheless, we identified disease-specific potential candidate genes, and genetic associations based on the surrounding SNPs partly differed from the genetic correlations. Especially for candidate genes contributing to 2 traits simultaneously, correlations based on SNP effects from the respective chromosome segment were close to 1 or to −1. In this regard, we annotated the candidate genes KRT33A and KRT33B for HYP and DD, KIF27 for HYP and calving to first insemination, and MAN1A1 for SU and the production traits. For SNP × heat stress interactions, we identified significant SNPs on BTA 2, 4, 5, 7, 8, 9, 13, 22, 25, and 28, and we annotated the potential candidate genes FSIP2, CLCN1, ADGRV1, DOP1A, THBD, and RHOBTB1. Results indicate gene-specific mechanisms of the claw disorders only in specific environments.

Key words: claw disorders, genomic analyses, genetic parameters, genome-wide associations

INTRODUCTION

An increasing fraction of involuntary cullings in dairy cows is due to claw disorders, implying a detrimental effect on farm economy (Bruijnis et al., 2010; Mülling and Hagen, 2012). The claw disorder displaying the highest incidences is dermatitis digitalis (DD), also referred as Mortellaro’s disease (Klitgaard et al., 2014; Solano et al., 2016). Dermatitis digitalis is a multifactorial claw infection (Blowey and Sharp, 1988; Read and Walker, 1998), including effects of the pathogenic environment through bacteria of the genus *Treponema* spp., of housing conditions, of feeding ration characteristics, and of host genetics (Döpfer et al., 2012; Solano et
al., 2017). Recently, in some countries, official national genetic evaluations for DD have been introduced, considering diagnoses from producers and claw trimmers (Rensing, 2019). The estimated heritabilities for DD were in small to moderate range from 7.3 to 14.2% from pedigree-based approaches (Koenig et al., 2005; van der Linde et al., 2010; Schöpke et al., 2015), and from 5 to 36.7% when considering genomic relationship matrices (Biemans et al., 2019; Shabalina et al., 2021). The development of claw selection indices requires genetic correlations among all diseases. Malchiodi et al. (2017) estimated positive genetic correlations throughout between DD with other claw diseases, and identified the largest correlation between DD and interdigital hyperplasia (HYP) with 0.57. Accordingly, Gernand et al. (2012) estimated quite large genetic correlations between DD and HYP, ranging from 0.50 to 0.61 in the course of lactation; additionally, among all claw disorders, HYP was the trait with the highest heritability (up to 0.35 at the end of lactation). Götze (1952) described a strong genetic predisposition for HYP decades ago. A third claw disorder displaying increasing incidences in German dairy cow herds is sole ulceration (SU; Gernand et al., 2012). van der Spek et al. (2013) and Gernand et al. (2012) estimated a heritability for SU in threshold models of 0.08 and 0.07, respectively. The genetic correlation between SU and HYP was 0.18 (van der Waaij et al., 2005). For the development of selection indices, it is also imperative to study genetic associations between DD with conformation traits, female fertility, and productivity. In this regard, opposite pedigree-based genetic correlations were estimated between DD with milk yield, such as −0.31 by Onyiro et al. (2008), but 0.24 by Koenig et al. (2005). The genetic correlations between DD and the interval from calving to first insemination was 0.01 (Buch et al., 2011).

Regarding genomic analyses, GWAS for DD with ongoing gene annotations suggested potential candidate genes on chromosomes 3, 6, 9, 11, 12, 19, and 26 (Naderi et al., 2018; Sánchez-Molano et al., 2019; Kopke et al., 2020; Shabalina et al., 2020). For HYP, Zhang et al. (2019) identified a missense mutation in the gene ROR2 on BTA 8, which was associated with distal limb ossification and brachydactyly in humans. For SU, van der Spek et al. (2015), Sánchez-Molano et al. (2019), and Butty et al. (2021) reported QTL or potential candidate genes on BTA 8, 10, 11, 12, 18, 22, and 29. In summary, some genomic studies for DD, HYP, and SU have been conducted, but ignored possible environmental effects on genetic mechanisms. Recently in middle Europe, periods of heat stress (HS) have increased gradually. Brügemann et al. (2013) and Gernand et al. (2019) especially identified effects of HS directly after calving on genetic parameter estimates for low heritability functional traits, and hypothesized HS effects on genetic mechanisms. In such context, consideration of high-throughput genomic marker data combined with innovative statistical modeling approaches allows deeper insights, with possibilities to infer SNP marker × HS interactions (Halli et al., 2021).

Consequently, the aim of the present study was to conduct comprehensive genomic analyses for DD, HYP, and SU including (1) the estimation of heritabilities based on genomic relationship matrices, (2) the estimation of SNP-marker effects for DD, HYP, and SU and the annotation of potential candidate genes from models considering or ignoring possible HS interactions, and (3) the estimation of genetic correlations based on genomic relationship matrices between DD, HYP, and SU with breeding goal traits reflecting health, female fertility, and productivity, and of respective correlations based on SNP effects with focus on the identified candidate gene segments.

MATERIALS AND METHODS

Cow Traits

The genotypes and phenotypes were from databases for national official genetic evaluations. Hence, no additional animal care statement was necessary. We considered 17,264 first-lactation Holstein Friesian cows from 50 large-scale co-operator herds located in the German federal states of Brandenburg and Mecklenburg-West Pomerania. The calving years covered the period from 2010 to 2016. The age at first calving ranged from 20 to 40 mo. All cows were diagnosed (1 = diseased; 0 = healthy) for the claw disorders DD, HYP, and SU, for endometritis (EM), and for clinical mastitis (CM) by veterinarians and herd managers based on the “central diagnosis key for health data recording” according to the official ICAR nomenclature (Stock et al., 2013). A cow was classified as diseased if at least one diagnosis was recorded until lactation d 365. All cows without any diagnosis until lactation d 365 were defined as healthy for the corresponding disease. Repeated entries for the same diagnosis were ignored. The average prevalence was 15.96% for DD, 8.20% for SU, 2.36% for HYP, 25.12% for EM, and 25.77% for CM.

Conformation traits included linear scores from official type trait recording for rear leg side view (SV), foot angle (FA), and rear leg rear view (RV) for a subset of 14,648 cows. For the conformation trait SV with an intermediate optimum (score = 5), deviations in both directions were treated equally; that is, scores 1 and 9 were allocated to class 1, scores 2 and 7 to class...
2, scores 3 and 6 to class 4, and the score 5 represented the best value in class 5. For RV and FA on a scale from 1 to 9, 9 was the best value.

Records for the female fertility traits nonreturn at d 90 (NR90) and for the interval from calving to first insemination (CFI) were available for a subset of 15,452 cows. For calving interval (CIN) between the calving dates in lactation 1 and 2, we considered records from 13,732 cows. Production data for 305-d lactation milk yield (MY), fat yield (FY), and protein yield (PY) were available from 16,147 cows. Table 1 shows the descriptive statistics for all traits.

### Genotypes

The cows were genotyped with the Illumina BovineSNP50 v2 BeadChip (Illumina Inc.; 4,589 cows) or with the Illumina Bovine Eurogenomics 10k chip (12,675 cows). Cows with low-density 10k genotypes were imputed to the 50k panel by the project partner VIT (Vereinigte Informationssysteme Tierhaltung w.V., Verden, Germany) using the algorithm by Segelke et al. (2012). The SNP data set included 44,474 SNPs from 17,264 genotyped cows with phenotypic records. Quality control of the genotype data was performed with the software package PLINK (Purcell et al., 2007). Single nucleotide polymorphisms with a minor allele frequency <1% and a deviation from the Hardy-Weinberg equilibrium (P-value < 1 × 10⁻⁶) were discarded. Finally, 44,046 SNPs from 17,264 cows remained for the genomic studies.

### Meteorological Data

For the identification of the nearest weather station for each herd, distances (km) between each herd and all public weather stations were calculated using the R-package GEOSPHERE (Hijmans et al., 2019), considering the respective longitudes and latitudes of the herds and the weather stations. Finally, we allocated 30 weather stations to 50 herds. The maximum distance between a herd and a weather station was 27.88 km, the minimum distance was 0.74 km, and the average distance was 13.96 km. We computed the hourly temperature-humidity index (THI) as follows (NRC, 1971):

\[
\text{THI} = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) 
\times (1.8 \times T - 26),
\]

where T = hourly temperature and RH = relative humidity. Afterward, we calculated the average daily THI for all days within the period from −1 d before to 21 d after calving. We focused on this interval because HS, especially during the early lactation period, contributed to an increase of claw disorder incidences (Gernand et al., 2019). The range of the daily THI within the defined period was from 32.11 to 76.95. In a next step, we defined a HS threshold with THI 68 as identified by Brügemann et al. (2012) for functional traits in different production systems in Germany. A genotyped cow underwent HS after first calving if at least one of the daily THI within the defined period exceeded 68. Otherwise, the cow was allocated to the cow group without HS. The number of cows allocated to THI >68 was 3,866, implying 22.39% of the cows from all 50 herds were exposed to HS.

### Statistical Models

#### Estimation of Heritabilities for Claw Disorders

Single nucleotide polymorphism-based heritabilities for DD, HYP, and SU were estimated via both linear (on the observed scale) and threshold (on the...
underlying liability scale) single-trait animal models using the GREML function as implemented in the GCTA software package (Yang et al., 2011). The single-trait model 1 was

\[ y = X\beta + Zg + e, \]  

where \( y \) = vector of observations for DD, HYP, and SU; \( \beta \) = a vector of fixed effects including herd (50 herds), calving year (2010–2016), calving season (4 quarters: Jan–Mar, Apr–Jun, Jul–Sep, Oct–Dec), and first calving age (linear regression); \( g \) = a vector of additive genetic effects following \( N(0, \sigma_g^2) \); respectively. \( e \) = a vector of random residuals following \( N(0, \sigma_e^2) \), where \( X \) = incidence matrices for fixed and random genetic effects, respectively. In the threshold model, variances from the observed scale were transformed to the underlying liability scale according to Lee et al. (2011).

Estimation of Genetic Correlations Among Claws Disorders, and Between Claw Disorders with Female Fertility, Conformation, Health, and Production Traits. Genetic correlations among DD, HYP, and SU and between DD, HYP, and SU with conformation, female fertility, health, and 305-d lactation traits, were estimated via bivariate linear animal models using the GREML algorithm as implemented in GCTA (Yang et al., 2011). The bivariate model 2 was

\[
\begin{bmatrix}
  y_1 \\
  y_2
\end{bmatrix} = \begin{bmatrix}
  X_1 \beta_1 + Z_1 g_1 + e_1 \\
  X_2 \beta_2 + Z_2 g_2 + e_2
\end{bmatrix},
\]

where the subscripts 1 and 2 represent the first and the second trait, respectively. The effects were the same as described in model 1. The bivariate model considered the following (co)variance structure for random effects:

\[
\begin{bmatrix}
  \sigma_{g_1}^2 & \sigma_{g_2}^2 & 0 & 0 \\
  \sigma_{g_2}^2 & \sigma_{g_1}^2 & 0 & 0 \\
  0 & 0 & \sigma_{e_1}^2 & \sigma_{e_2}^2 \\
  0 & 0 & \sigma_{e_2}^2 & \sigma_{e_1}^2
\end{bmatrix},
\]

where \( G \) = genomic relationship matrix; \( \sigma_{g_1}^2 \) and \( \sigma_{g_2}^2 \) = genomic variances for the first and the second trait, and genomic covariance between both traits, respectively; \( \sigma_{e_1}^2 \) and \( \sigma_{e_2}^2 \) = residual variances for the first and second trait and residual covariance between both traits, respectively.

**Genome-Wide Associations and Gene Annotations.** We performed a GWAS for DD, HYP, and SU in a case-control design by applying a single SNP linear mixed model and using the mlma-loco “leaving one chromosome out” option as implemented in the software GCTA (Yang et al., 2011). Statistical model 3 was defined as follows:

\[ y = X\beta + x_{\text{snp}} u_{\text{snp}} + Zg + e, \]

where \( x_{\text{snp}} \) = SNP genotypes and \( u_{\text{snp}} \) = SNP effect. The remaining effects were the same as described in model 1.

The number of false positives associations was indicated by QQ plots and inflation factors (\( \lambda \)).

The genome-wide significance level according to Bonferroni (\( p_{BF} = 0.05/\text{NSNP} = 44,046 \)) was \( 1.14 \times 10^{-6} \). Furthermore, following Kurz et al. (2018) and Klein et al. (2020), a less conservative normative significance threshold was used to identify candidate SNPs defined as \( p_{CD} = 1 \times 10^{-4} \). Potential candidate genes from the Ensembl database based on the Bos taurus ARS-UCD1.2 genome assembly (Yates et al., 2019) were identified, and assigned to the corresponding significant or candidate SNPs. In this regard, a gene was considered as a candidate gene if at least one significantly associated SNP was located in the gene or within a window size of 200 kb up- and downstream. In the last step, physiological functions of potential candidate genes were inferred based on information from the Ensembl (Yates et al., 2019) and Genecard (Stelzer et al., 2016) databases.

Model 3 was also applied to estimate SNP effects for conformation traits, female fertility traits, EM, CM, and 305-d lactation traits. For conformation and 305-d lactation traits, we applied linear mixed model association analyses (option mlma instead of mlma-loco) as a significantly greater population stratification was observed in the GWAS for these traits (\( \lambda > 2 \)).

**Calculation of SNP Effect Correlations.** We calculated correlations for the estimated SNP effects from model 3 between DD, HYP, and SU with conformation traits (SV, FA, and RV), female fertility traits (NR90, CFI, and CIN), health traits (EM and CM), and 305-d lactation traits (MY, FY, and PY). In this regard, we considered the SNPs located 200 kb up- and downstream of the respective identified potential candidate gene for DD, HYP, and SU. The total number of variants within the defined chromosome segments of all identified potential candidate genes for the 3 claw
disorders DD, HYP, and SU ranged from 5 to 24, with an average number of 10.84 variants per interval.

**Genome-Wide Associations for Claw Disorders with SNP by Heat Stress Interactions.** We estimated main and interaction SNP effects for DD, HYP, and SU via generalized least squares equations according to the algorithm as introduced by Yang et al. (2014). Statistical model 4 to estimate main and interaction SNP effects was

\[
y = X\beta + x_{\text{snpi}}u_{\text{snpi}} + x_{\text{interi}}u_{\text{interi}} + Zg + Wg_{\text{hs}} + e, \tag{4}
\]

where \( y \) = a vector of observations for DD, HYP, and SU; \( x_{\text{snpi}} \) = SNP genotypes; \( u_{\text{snpi}} \) = SNP main effect; \( x_{\text{interi}} \) = a vector of genotypes for cows undergoing HS (i.e., THI >68) after first calving; \( u_{\text{interi}} \) = SNP by HS interaction effect; \( g_{\text{hs}} \) = a vector of genotype by HS interaction effects for cows with HS following \( N(0, G_{\text{hs}}\sigma^2_{\text{ghs}}) \), where \( G_{\text{hs}} \) = the genomic relationship matrix for the cows with HS and \( \sigma^2_{\text{ghs}} \) = the variance of genotype by HS interactions (estimated using the gxe option in GCTA); and \( W \) = a design matrix allocating phenotypic records to \( g_{\text{hs}} \). Hence, the term \( u_{\text{interi}} \) with the respective design matrix \( x_{\text{interi}} \) was used to indicate whether the genotyped cow was exposed to HS. The regression coefficients of genotypes were nested within the 2 THI classes THI \( \leq 68 \) or THI > 68. The remaining effects were the same as described in model 1. The significance values of main and interaction effects for each SNP were estimated using our own R-package “GWAInter.R” (Halli et al., 2021). Hence, each SNP has 2 \( P \)-values, specifically one for the significance of the main effect and another for the significance of the interaction effect. In the next step, potential candidate genes for interaction effects were annotated as described above for the SNPs from model 3.

**RESULTS AND DISCUSSION**

**Heritabilities for Claw Disorders**

The SNP based heritability for DD from the linear model on the observed scale was 0.04 (SE 0.005), reflecting pedigree-based estimates from linear models by Onyiro et al. (2008) and Johansson et al. (2011) (Table 2). As expected from theory (Fahrmeir and Tutz, 2001; Mrode 2005), the heritability on the underlying liability scale from the threshold model for DD was slightly larger with 0.08 (SE 0.01). Dermatitis digitalis heritabilities in Holstein populations from threshold models were 0.10 (van der Waaij et al., 2005), 0.07 (Koenig et al., 2005), and 0.09 (Gernand et al., 2012). The differences in heritability estimates from linear and threshold models for HYP and SU were obvious, but expected due to their low prevalence (Table 2). The incidence of a disease strongly determines the value of the transformed heritability when applying the transformation equation as developed by Dempster and Lerner (1954), explaining the large heritability differences on the observed and on the underlying liability scale for the diseases with low incidences. Accordingly, the linear estimated heritability for HYP was 0.03 (SE 0.006), but 0.23 (SE 0.04) from the threshold model. The threshold model heritabilities for HYP agree with estimates in Holstein populations conducted by Gernand et al. (2012) with 0.22 (SE 0.04) and by Burnester (2005) with 0.28. Similarly, for SU, the heritability was 0.10 (SE 0.02) from the threshold model, and 0.03 (SE 0.005) from the linear model. Similar heritabilities for SU were estimated by van der Spek et al. (2013), such as a heritability of 0.03 from the linear model, and 0.11 from the threshold model.

**Genome-Wide Associations and Potential Candidate Genes for Claw Disorders**

The Manhattan plot for the DD GWAS from model 3 is presented in Figure 1a. The corresponding inflation factor \( \lambda \) was 1.20. We identified 13 significant SNPs associated with 14 potential candidate genes (Table 3). One of the SNPs located on BTA 10 exceeded pBF. The other 12 significant SNP markers according to pCD are located on BTA 9, 10, 16, 19, and 24. The largest number of significantly associated SNPs was detected on BTA 10. Table 3 shows the significant SNPs, as well as the annotated potential candidate genes for DD. The potential candidate gene \( \text{NEO1} \) on BTA 10 encodes a cell surface protein that belongs to the immunoglobulin superfamily (Vielmetter et al., 1997). The involvement in cell growth and differentiation might explain the significant effect on DD. Accordingly, in the context...
of cell growth, Cole et al. (2018) discussed associations between genotypes of the NEO1 gene with fertility and embryonic size in humans and mice. Another identified potential candidate gene for DD is DAPK2 on BTA 10. Britschgi et al. (2008) described functions of DAPK2 on granulocytic differentiations. Granulocytes represent a subgroup of leukocytes, explaining the effects of DAPK2 on DD, because leukocytes defend infections caused by bacteria (e.g., treponemes), fungi, or parasites. The annotated potential candidate gene USP3 on BTA 10 is involved in protein ubiquitination (Das et al., 2020). This process, in turn, is directly or indirectly involved in numerous cellular processes, explaining the entrance of treponemes due to damaged skin cells. Keogh et al. (2021) addressed similar cell repairing mechanisms regulated by USP3 in the context of calving difficulties and recovering after calving in dairy cows and beef heifers. The potential candidate gene CA12 on BTA 10 was related to feed intake in Hereford and Angus cattle (Seabury et al., 2017) and with uterine pH in Holstein Friesian cattle (Kiser et al., 2019), but not directly with disease resistances. The potential candidate genes KRT33A and KRT33B on BTA 19 are involved in keratin formation (McKenzie et al., 2010). Keratin is a major component of the claw horn (Litzke, 2019), implying the penetration of treponemes in case of insufficient keratin production.

The Manhattan plot for the GWAS of HYP is presented in Figure 1b. For HYP, we identified 27 significant SNPs associated with 12 potential candidate genes. The corresponding inflation factor λ was 1.16. One of the SNPs located on BTA 13 exceeded pBF. The largest number of significant SNPs according to pCD is located on BTA 8. Table 4 includes the significant SNPs, as well as the associated candidate genes for HYP. The potential candidate gene UBQLN1 on BTA 8 influences protein degradation in vivo, and proteins interacted with diseases such as the foot and mouth disease virus (Gladue et al., 2014). The potential candidate gene WDPCP on BTA 11 plays a crucial role in collective cell movement and cilia formation, explaining the involvement in disease resistance mechanisms (e.g., de las Heras-Saldana et al., 2019; Afonso et al., 2020). The potential candidate gene ITPR1 on BTA 22 encodes an intracellular receptor for inositol-1, 4, 5-triphosphate, and was active in the presence of external stressors (Cherniyov et al., 2021).

The Manhattan plot for the GWAS of SU is presented in Figure 1c. For SU, we identified 14 significant SNPs associated with 6 potential candidate genes. The corresponding inflation factor λ was 1.16. None of the SNPs exceeded pBF. The significant SNP markers according to pCD are located on BTA 4, 5, 6, 7, 9, 12, 13, and 27. The largest number of significant SNPs was found on BTA 13. Table 5 shows the significant SNPs for SU and the annotated potential candidate genes. However, direct relationships between the identified genes and diseases were only reported for C2orf202 on BTA 13, due to the effect on Na+/K+/2CL cotransporter mechanisms (Shiozaki et al., 2014). The other potential candidate genes for SU were associated with body size (JAZF1; Zhao et al., 2015 and RSP04; Duan et al., 2021), MY (CCDC159; Du et al., 2019), female fertility (MAN1A1; Tarekeng et al., 2019), and male fertility (CTCFL; Han and Peñagaricano, 2016; Chen et al., 2021) in cattle.

Visual inspections of the Manhattan plots for the 3 claw disorders DD, HYP, and SU in Figures 1a–c indicate regions containing potential candidate genes on BTA 9. Especially for DD and HYP, a large number of potential candidate genes are located on BTA 8, 9, 10, and 11. Furthermore, both diseases have several potential candidate genes on BTA 19 between base pair positions 34,000,000 and 55,000,000, again indicating similar genetic mechanisms and supporting the quite large genetic correlation. From a pathological perspective and in the context of shared diseases pathways, HYP often occurs after a DD infection due to external stressors (de Jesús Argáez-Rodríguez et al., 1997). Nevertheless, we also identified a large number of variants, which did not overlap across the different claw disorders, indicating that the genetic correlation is mostly due to polygenetic effects.

Genetic Correlations and SNP Effect Correlations Among Claw Disorders, and Between Claw Disorders and Remaining Traits

Dermatitis Digitalis. Genetic and SNP effect correlations between DD with all other traits are presented in Figure 2. The genetic correlation between DD and the conformation traits SV, FA, and RV was negative throughout, but close to zero and in a narrow range from 0.05 to −0.14. From a breeding perspective, a negative correlation is favorable, implying that bulls with a fewer number of diseased daughters have the desired scores for linear type traits from the feet and leg composite. Onyiro et al. (2008) and van der Linde et al. (2010) estimated stronger genetic correlations between DD with feet and leg type traits of −0.31 and −0.67, respectively, for locomotion, and of −0.27 and −0.63, respectively, for the overall fundament score. Genetically, Kopke et al. (2020) associated favorable DD breeding values with steeper and parallel legs, supporting the results from the present study. The SNP effect correlations between DD and FA considering the SNPs from the potential candidate genes were highly variable, and ranged from −0.87 (SHC4) to 0.75
Figure 1. (a) Manhattan plot for $-\log(10) P$-values of SNP effects for dermatitis digitalis; (b) Manhattan plot for $-\log(10) P$-values of SNP effects for interdigital hyperplasia; (c) Manhattan plot for $-\log(10) P$-values of SNP effects for sole ulcer. Genome-wide significance threshold ($p_{BF} = 1.14 \times 10^{-6}$); suggestive candidate threshold ($p_{CD} = 1 \times 10^{-4}$). $p_{BF}$ = genome-wide significance level according to Bonferroni; $p_{CD}$ = normative significance threshold.
The correlation was positive (0.75) and significantly ($P < 0.05$) different from zero for SNPs from the $NEO1$ gene segment, as well as for SNPs surrounding the $LRRC38$ gene segment (0.85). There was a highly significant positive correlation (0.85) between DD and SV for SNPs from the $HERC1$ segment. With regard to RV, the strongest SNP correlation (0.96) with DD was estimated based on SNPs from the segments surrounding the genes $KRT33A$ and $KRT33B$. However, most of the SNP effect correlations for specific chromosome segments between DD and conformation traits were negative, supporting the genetic correlation estimates.

Genetic correlations between DD and female fertility traits were −0.16 for NR90, 0.12 for CFI, and 0.28 for CIN. The positive genetic correlations between DD and fertility interval traits indicate longer periods from calving to first estrus for diseased or lame cows. Accordingly, Buch et al. (2011) estimated a positive, but very small genetic correlation of 0.01 between DD with CFI. The negative genetic correlation between DD and $Sölzer et al.: GENOMICS OF CLAW DISORDERS$

Table 3. Significantly associated SNP markers and annotated potential candidate genes for dermatitis digitalis

<table>
<thead>
<tr>
<th>BTA</th>
<th>SNP-ID</th>
<th>SNP position (bp)</th>
<th>$P$-value</th>
<th>Gene1</th>
<th>Gene position (bp)1</th>
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<td>$FGFR1OP$</td>
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<td>$6.67 \times 10^{-5}$</td>
<td>$ENSBTAG00000054087$</td>
<td>101,855,408–101,856,823</td>
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<td>10</td>
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<td>$5.11 \times 10^{-5}$</td>
<td>$NEO1$</td>
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1No gene name or no gene position: SNP was not located in the gene or within a window size of 200 kb up- and downstream.

Table 4. Significantly associated SNP markers and annotated potential candidate genes for interdigital hyperplasia

<table>
<thead>
<tr>
<th>BTA</th>
<th>SNP-ID</th>
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<th>$P$-value</th>
<th>Gene1</th>
<th>Gene position (bp)1</th>
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<td>54,822,569</td>
<td>$2.23 \times 10^{-5}$</td>
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</tr>
</tbody>
</table>

1No gene name or no gene position: SNP was not located in the gene or within a window size of 200 kb up- and downstream.
NR90 indicates improved conception for healthy cows. With focus on some specific gene segments, SNP effect correlations differed from the genetic correlations. In this regard, the SNP effect correlation between DD and NR90 was −0.89 for the DAPK2 gene segment, and −0.91 for the LRRC38 gene segment. For the fertility interval traits, the sign of SNP effect correlations mostly differed when compared with the genetic correlation. The genetic correlations between DD and interval traits were positive and favorable from a breeding perspective, but negative for most of the relevant chromosome segments.

The genetic correlation between DD with SU was 0.20, and 0.64 with HYP. Fiedler (2014) explained positive phenotypic correlations among DD, HYP, and SU through causalities in infection pathways. Single nucleotide polymorphism effect correlations between DD and SU considering the gene segments varied widely in the range from −0.63 (ENSBTAG00000054087) to 0.42 (HERC1). We identified significant positive SNP effect correlations between DD and HYP for 11 of the 14 segments, with largest correlations considering the SNPs from the segments KRT33A (0.93), KRT33B (0.93), HERC1 (0.90), and DAPK2 (0.84). The weak to moderate negative genetic correlations between DD with EM (−0.22) and with CM (−0.07) indicate different pathways for bacterial infections of different disease categories and a broad variety of bacteria species that can trigger the respective disease. Accordingly, Gernand et al. (2012) and Buch et al. (2011) found genetic correlations close to zero between DD with diseases from other health categories. For SNP effect correlations for EM, the range varied from −0.94 (HERC1) to 0.79 (ENSBTAG00000054087). Most of the SNP effect correlations between DD and EM, and between DD and CM were negative.

With regard to 305-d lactation traits, genetic correlations between DD with MY (0.13), FK (0.03), and PK (0.10) indicate slight genetic antagonistic relationships between bacterial claw infections and productivity as previously shown by Koenig et al. (2005). However, with focus on the most important genes or chromosome segments for DD, SNP effect correlations with 305-d lactation MY ranged from −0.58 (ENSBTAG00000054087) to 0.79 (LRRC38), with 10 of the 14 correlations being positive. Regarding FY, SNP effect correlations with DD varied in the range from −0.91 (RNF112) to 0.73 (LRRC38), and for PY from −0.75 (RNF112) to 0.82 (LRRC38). With regard to the LRRC38 segment, the SNP effect correlations between DD and the production traits MY, FY, and PY were significant and positive. LRRC38 is actively involved in K transport and is associated with postpartum Ca concentration in blood and in milk of Holstein cows (Cavani et al., 2022). Calcium in turn plays an important role in milk production (Breves et al., 2016), as well as in keratinization and mature horn cell formation of the claw (Langova et al., 2020). Rodriguez et al. (2017) associated increased calcium losses at the beginning of lactation and hypocalcemia of especially high yielding cows with several diseases, which may explain the positive correlations between DD and the production traits based on the SNPs of the LRRC38 segment.

**Interdigital Hyperplasia.** Genetic and SNP effect correlations for HYP with all other traits are presented in Figure 3. The genetic correlations between HYP and the conformation traits SV, FA, and RV were 0.03, −0.18, and −0.21, respectively. Hence, genetic improvements of FA and RV imply indirect favorable selection response in the HYP health status. Similarly, in pedigree-based approaches, van der Waaij et al. (2005) estimated negative genetic correlations between HYP

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**Table 5. Significantly associated SNP markers and annotated potential candidate genes for sole ulcer**

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<th>SNP position (bp)</th>
<th>P-value</th>
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<td>MAN1A1</td>
<td>31,675,672–31,849,229</td>
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<td>MAN1A1</td>
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<td>2.86 × 10⁻⁶</td>
<td>JAZF1</td>
<td>67,992,971–68,321,145</td>
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</tbody>
</table>

¹No gene name or no gene position: SNP was not located in the gene or within a window size of 200 kb up- and downstream.
with FA (−0.15) and with RV (−0.35), but a weak positive genetic correlation with SV (0.04). Again, the SNP effect correlations with SV based on the identified candidate genes for HYP display a heterogeneous pattern from −0.89 (KIF27) to 0.80 (ITPR1). Regarding FA, SNP effect correlations with HYP ranged from −0.46 (KIF27) to 0.68 (ITPR1).

The genetic correlations between HYP with NR90 (−0.06), CFI (0.17), and CIN (0.11) reflect the estimates between female fertility traits with DD. Hence, increased susceptibility to HYP is genetically related to a delayed estrus activity after calving and with impaired conception. Regarding the identified important chromosome segments for HYP, 7 genes contributed to

<table>
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<tr>
<th>Trait</th>
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<th>FA</th>
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<th>CFI</th>
<th>CIN</th>
<th>SU</th>
<th>HYP</th>
<th>EM</th>
<th>CM</th>
<th>MY</th>
<th>FY</th>
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<td>−0.13 (0.08)</td>
<td>−0.16 (0.15)</td>
<td>0.12 (0.09)</td>
<td>0.28 (0.09)</td>
<td>0.2 (0.12)</td>
<td>0.64 (0.10)</td>
<td>−0.22 (0.19)</td>
<td>−0.07 (0.11)</td>
<td>0.13 (0.06)</td>
<td>0.03 (0.08)</td>
<td>0.1 (0.01)</td>
</tr>
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<td>NEO1</td>
<td>DAPK2</td>
<td>USP3</td>
<td>HERC1</td>
<td>CA12</td>
<td>RORA</td>
<td>SHC4</td>
<td>LRRC38</td>
<td>RNFL12</td>
<td>KRT33A</td>
<td>KRT33B</td>
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<td></td>
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<td>−0.25 −0.3 0.75 −0.46 −0.47 −0.63 −0.5 −0.37 −0.87 0.85 0.05 0.75 0.75 −0.41</td>
<td>0.08 0.06 0.56 −0.27 0.21 −0.19 0.12 0.28 −0.01 0.72 −0.65 0.56 0.96 −0.03</td>
<td>0.18 0.18 −0.27 −0.89 −0.68 −0.86 −0.55 −0.47 −0.11 −0.91 0.5 0.67 0.67 0.24</td>
<td>−0.37 −0.39 −0.62 −0.25 −0.85 −0.24 −0.67 −0.21 −0.39 −0.43 0.95 0.22 0.22 −0.03</td>
<td>−0.38 −0.38 −0.12 0.08 −0.85 −0.1 −0.44 −0.11 0.57 0.19 0.8 0.6 0.6 −0.45</td>
<td>−0.57 −0.63 0.07 0.33 0.1 0.42 0.24 −0.01 −0.19 −0.39 0.21 −0.29 −0.29 0.4</td>
<td>−0.24 −0.29 −0.09 0.84 0.81 0.9 0.78 0.32 0.1 0.83 0.36 0.93 0.93 0.2</td>
<td>−0.22 0.73 0.79 0.62 0.81 0.81 0.94 0.33 −0.22 −0.09 −0.03 −0.84 −0.69 −0.69 −0.49</td>
<td>−0.4 −0.43 0.19 −0.41 −0.33 −0.52 −0.06 −0.53 0.09 −0.27 0.11 0.63 0.63 −0.18</td>
<td>0.13 −0.57 −0.58 0.01 0.27 −0.03 0.31 0.05 0.11 0.75 0.79 −0.35 0.31 0.31 0</td>
<td>−0.63 −0.66 −0.05 −0.07 0.35 −0.33 0.32 0.24 0.26 0.73 −0.91 0.29 0.29 0.55</td>
<td>−0.55 −0.55 0 0.13 −0.01 −0.16 0.12 0.12 0.66 0.82 −0.75 0.04 0.04 0.49</td>
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</table>

**Figure 2.** Genetic correlations and SNP effect correlations considering SNPs surrounding potential candidate genes for dermatitis digitalis (DD) between DD and remaining traits. Significance of the Pearson correlation: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; SV = rear leg side view; FA = foot angle; RV = rear leg rear view; NR90 = nonreturn rate at d 90; CFI = days from calving to first insemination; CIN = calving interval; SU = sole ulcer; HYP = interdigital hyperplasia; EM = endometritis; CM = clinical mastitis; MY = milk yield; FY = fat yield; PY = protein yield; rg = genetic correlation between dermatitis digitalis and the listed traits.
negative correlations with NR90, and 5 genes to positive correlations with NR90. Such a “balance” of gene segments contributing to either favorable or unfavorable SNP effect correlations with HYP also was identified for the fertility interval traits CFI and CIN. Highly significant correlations were found between HYP and CFI (0.90), as well as between HYP and CIN (0.74) for SNPs surrounding the KIF27 gene. With regard to the potential candidate gene KIF27 on BTA 8, Parker Gaddis et al. (2016) confirmed the effect on female fertility. The genetic correlation between HYP and SU was 0.14 (0.13), reflecting estimates by van der Waaij.
et al. (2005) based on pedigree data. Accordingly, Koenig et al. (2005) estimated positive genetic correlations among DD, SU, and HYP, and they indicated that claw disorders “genetically appear to occur in clusters.” This means that a cow with a DD infection has an increased genetic risk to show symptoms for HYP or SU, and vice versa. Phenotypically, 580 cows had more than one claw disorder; specifically, 155 cows with a diagnosis for DD and HYP, 336 cows with a diagnosis for DD and SU, 25 cows with a diagnosis for DD, HYP, and SU, and 64 cows with a diagnosis for HYP and SU. Very strong positive correlations between SNP effects for HYP and SU were calculated based on the SNPs surrounding the genes MFSD11 (0.87), MXRA7 (0.84), and ST6GALNAC1 (0.83), supporting the favorable genetic correlation between HYP and SU. With regard to WDPCP, the correlation between DD and HYP was 0.89. WDPCP was associated with inflammatory response mechanisms to infections (de las Heras-Saldana et al., 2019; Afonso et al., 2020), which might play a role for both claw disorders DD and HYP. The genetic correlation between HYP and CM was 0.08, and −0.04 between HYP and EM. Similarly, Gernand et al. (2012) estimated genetic correlations of 0.03 between HYP with EM, and of 0.00 between HYP and CM. For production traits, genetic correlations with HYP were close to zero, but always positive; in particular, correlations were 0.09 with MY, 0.05 with FY, and 0.08 with PY. Accordingly, SNP effects correlations between HYP and MY were positive for 11 gene segments, and only negative based on the SNPs surrounding WDPCP.

Sole Ulceration. Figure 4 displays the genetic and SNP effect correlations between SU and the selected traits. Genetic correlations between SU and the conformation traits SV, FA, and RV were throughout negative with −0.21, −0.07, and −0.19, respectively. SNP effect correlations between SU and SV ranged from −0.90 to −0.06. Pronounced negative correlations were calculated between SU with SV (−0.81), with FA (−0.66), and with RV (−0.58) for the SNPs located in the MAN1A1 segment. With regard to SU and female fertility associations, genetic correlations were 0.05 with NR90, 0.31 with CFI, and 0.23 with CIN. Accordingly, Buch et al. (2011) estimated a genetic correlation of 0.33 between SU and CFI based on pedigree data. The SNP effect correlations with focus on the SU candidate genes and female fertility traits showed a heterogeneous pattern. Again, strong correlations were found for the MAN1A1 gene segment, such as 0.92 between the SNP effects for SU and for CIN. MAN1A1 was suggested as a candidate gene for human health and fertility disorders, such as in the context of mesenchymal tumor development (Alonso-Garcia et al., 2020).

With regard to direct health trait diagnoses, the genetic correlation between SU and EM was 0.65, and 0.22 between SU and CM. The respective SNP effect correlations between SU and EM ranged from −0.90 (CTCFL) to 0.34 (CDC159). Genetic correlations between SU and 305-d lactation traits were throughout antagonistic; specifically, they were 0.25 with MY, 0.26 with FY, and 0.27 with PY, reflecting estimates by Koenig et al. (2005) and Oliveira Junior et al. (2021) when using pedigree data.

**Genome-Wide Associations for SNP by Heat Stress Interactions**

The Manhattan plot for the SNP interaction effects with HS for DD is presented in Figure 5. A significant interaction means that the respective SNP is relevant for DD susceptibility under HS, but not under thermoneutral conditions, or vice versa. The corresponding inflation factor λ was 1.07. Two SNPs exceeded the candidate threshold. The annotated potential candidate genes for interactions effects are listed in Table 6. One significant SNP was located in close distance to the gene THBD on BTA 13. This protein-coding gene is involved in blood clotting and associated with tuberculosis disease in mice (Weijer et al., 2005). With regard to the main effects from model 4, the Manhattan plot (not shown) was similar with the pattern from model 3 as presented in Figure 1a. Again, most of the significant SNPs are located on BTA 10 and on BTA 16 (see Figure 1a).

The Manhattan plot for the SNP interaction effects for HYP is presented in Figure 6. The corresponding inflation factor λ was 1.01. Two of the SNPs exceeded the candidate threshold (see Table 6). For the HYP × HS interaction, a significant SNP was found in the segment of ADGRV1 on BTA 7. ADGRV1 was associated with metabolic BW in Holstein Friesian cows (Hardie et al., 2017). With regard to the main effects, the Manhattan plot from the interaction model 4 (not shown) was similar with the pattern from model 3 as presented in Figure 1b. Again, most of the significant SNPs are located on BTA 19 (see Figure 1b).

The Manhattan plot for the SNP interaction effects for SU with HS is presented in Figure 7. The corresponding inflation factor λ was 1.15. Fifteen of the SNPs exceed the candidate threshold (see Table 6). For the interaction term, a significant SNP was found in the CLCN1 segment on BTA 4. CLCN1 is involved in the electrical excitability of skeletal muscle cells, explaining its role in the occurrence of myotonia hereditaria in Australian cattle dogs (Finnigan et al., 2007). Another significant SNP is located in close distance to the segment of TRH.
Figure 4. Genetic correlations and SNP effect correlations considering SNPs surrounding potential candidate genes for sole ulcer (SU) between SU and remaining traits. Significance of the Pearson correlation: *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; SV = rear leg side view; FA = foot angle; RV = rear leg rear view; NR90 = nonreturn rate at d 90; CFI = days from calving to first insemination; CIN = calving interval; DD = dermatitis digitalis; HYP = interdigital hyperplasia; EM = endometritis; CM = clinical mastitis; MY = milk yield; FY = fat yield; PY = protein yield; $rg =$ genetic correlation between sole ulcer and the listed traits.
on BTA 22, which is related to growth hormone and prolactin secretion (Kaiser et al., 1994). One significant SNP is located within the segment of RHOBTB1 on BTA 28. This protein-coding gene is involved in actin filament assembly and affected meat quality in cattle (Silva et al., 2020).

Studies addressing genotype x environment interactions for claw disorders are quite rare. Applying the classical multiple-trait approach, Shabalina et al. (2021) proved genotype × environment interactions for DD based on genetic correlations smaller than 0.80 between DD recorded in conventional or in organic farms.

Table 6. Significantly associated SNP markers and annotated potential candidate genes for SNP × heat stress interaction effects

<table>
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<tr>
<th>Claw disorder</th>
<th>BTA</th>
<th>SNP-ID</th>
<th>SNP position (bp)</th>
<th>P-value</th>
<th>Gene1</th>
<th>Gene position (bp)1</th>
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<td>7.23 × 10⁻⁵</td>
<td>ADGRV1</td>
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<td>rs110912511</td>
<td>12,724,331</td>
<td>8.00 × 10⁻⁵</td>
<td>ERCC4</td>
<td>12,833,435-12,874,714</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>rs110136757</td>
<td>16,691,439</td>
<td>4.49 × 10⁻⁵</td>
<td>RHOBTB1</td>
<td>16,637,438-16,777,483</td>
</tr>
</tbody>
</table>

1No gene name or no gene position: SNP was not located in the gene or within a window size of 200 kb up- and downstream.
Accordingly, for the claw disorder sole hemorrhage, Swalve et al. (2014) indicated that the etiology of a disease is highly dependent on the particular environment. In their study, they found strong associations of genotypes for *IQGAP1* on sole hemorrhage. However, in a previous study (Urao et al., 2010), effects of *IQGAP1* on blood circulation in mice feet depended on the environmental stress conditions. Brenig et al. (2003) indicated that the *SLC26a2* Gen (bovine diastrophic sulfate transporter) is involved in mechanisms regulating cell sulfate intake in the cattle claw. However, in associated analyses conducted in bulls kept on station, the same authors reported stable surface particularities on *SLC26a2* expressions.

**CONCLUSIONS**

Single nucleotide polymorphism-based heritabilities were 0.04 and 0.03 for DD, 0.08 and 0.10 for SU, and 0.03 and 0.23 for HYP from linear and threshold models, respectively. The heritability differences from linear and threshold models reflect theoretical expectations when considering the respective disease prevalence. The genetic and the SNP based correlations between DD, HYP, and SU with conformation traits from the feet and leg composite were throughout favorable from a breeding point of view. Genetic correlations between DD, SU, and HYP with other breeding goal traits indicated impaired female fertility, an impaired udder health status, and a productivity decline of diseased cows. Genetic correlations among DD, SU, and HYP were moderate to large, indicating that different claw disorders have similar genetic mechanisms. Nevertheless, we identified disease specific potential candidate genes, and genetic associations based on the surrounding SNPs partly differed from the genetic relationships. Especially for candidate genes affecting 2 traits simultaneously, SNP effect correlations were close to 1 or to −1. With regard to SNP × HS interactions, we identified significant SNPs on several chromosomes, and annotated the potential candidate genes *FSIP2*, *CLCN1*, *ADGRV1*, *DOP1A*, *THBD*, and *RHOBTB1*. These results indicate gene specific mechanisms of claw disorders only in specific environments.

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Figure 7. Manhattan plot for −log(10) P-values of SNP × heat stress interaction effects for sole ulcer. Genome-wide significance threshold (pBF = 1.14 × 10⁻⁶); suggestive candidate threshold (pCD = 1 × 10⁻⁴). pBF = genome-wide significance level according to Bonferroni; pCD = normative significance threshold.

ship to Niklas Sölzer. The authors have not stated any conflicts of interest.

REFERENCES


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