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Association analysis for feet and legs disorders with whole-genome sequence variants in 3 dairy cattle breeds

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ABSTRACT

Identification of genetic variants associated with feet and legs disorders (FLD) will aid in the genetic improvement of these traits by providing knowledge on genes that influence trait variations. In Denmark, FLD in cattle has been recorded since the 1990s. In this report, we used deregressed breeding values as response variables for a genome-wide association study. Bulls (5,334 Danish Holstein, 4,237 Nordic Red Dairy Cattle, and 1,180 Danish Jersey) with deregressed estimated breeding values were genotyped with the Illumina Bovine 54k single nucleotide polymorphism (SNP) genotyping array. Genotypes were imputed to whole-genome sequence variants, and then 22,751,039 SNP on 29 autosomes were used for an association analysis. A modified linear mixed-model approach (efficient mixed-model association eXpedited, EMMAX) and a linear mixed model were used for association analysis. We identified 5 (3,854 SNP), 3 (13,642 SNP), and 0 quantitative trait locus (QTL) regions associated with the FLD index in Danish Holstein, Nordic Red Dairy Cattle, and Danish Jersey populations, respectively. We did not identify any QTL that were common among the 3 breeds. In a meta-analysis of the 3 breeds, 4 QTL regions were significant, but no additional QTL region was identified compared with within-breed analyses. Comparison between top SNP locations within these QTL regions and known genes suggested that *RASGRP1*, *LCORL*, *MOS*, and *MITF* may be candidate genes for FLD in dairy cattle.

Key words: feet and legs disorders, genome-wide association, mixed model, meta-analysis

INTRODUCTION

Diseases affect animal welfare and cause economic losses for farmers. Cows with feet and legs problems may incur treatment costs, may suffer loss of BW, reduced milk production (Warnick et al., 2001), and suboptimal reproduction (Morris et al., 2011) and may have an increased risk of culling (Booth et al., 2004). Feet and legs disorders (**FLD**) caused by genetic factors, management conditions, or other reasons are common in dairy cattle (Clarkson et al., 1996; Rutherford et al., 2009); however, the heritability of FLD is low (~1%; Nielson et al., 1999).

Both FLD and digestive problems are strongly affected by the environment. Nielsen et al. (1997) reported a strong genetic correlation between digestive diseases and FLD (0.93 for Danish Holstein and 0.89 for Danish Red cattle in first lactation). This strong correlation is due to the fact that a common foot disease, laminitis, is caused by digestive problems (Nielsen et al., 1997). Previous studies have shown that FLD have an unfavorable genetic correlation with production of milk (0.26–0.32; Lyons et al., 1991; Groen et al., 1994; Uribe et al., 1995) and an unfavorable genetic correlation (0.27–0.36) with early reproductive, late reproductive, and metabolic disorders in the first lactation in Holstein (Nordic Cattle Genetic Evaluation, 2013). This finding indicates that high selection pressure on production and reproduction traits might lead to increased FLD susceptibility. Therefore, inclusion of FLD in breeding goals would be helpful. Identification of genes associated with FLD would be extremely useful for reducing the incidence of FLD, by providing knowledge on genes that influence variations in this trait.

A few studies have detected QTL for some FLD, such as hoof trimming, lameness, and claw disorders (Buitenhuis et al., 2007; Swalve et al., 2014; van der Spek et al., 2015). However, genetic studies on feet and legs traits are limited due to a lack of recording in most countries. In Denmark, recording of FLD in cattle started in 1990s and is part of the routine genetic evaluation of dairy cattle. The aim of the present study was to detect QTL regions for FLD in 3 dairy cattle

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Table 1. Descriptive statistics of DRP[1] and reliability of feet and legs disorders in Danish Holstein, Nordic Red Dairy Cattle, and Danish Jersey populations

	Danish Holstein		Nordic Red		Danish Jersey	
Summary	DRP	Reliability	DRP	Reliability	DRP	Reliability
Number	5,334	5,334	4,237	4,237	1,180	1,180
Mean	94.93	0.57	96.64	0.57	97.47	0.53
SD	18.29	0.13	14.96	0.13	19.18	0.13
Minimum	44.6	0.20	55.80	0.20	44.90	0.20
Maximum	147.20	0.99	137.10	0.98	149.80	0.98

[1]DRP = deregressed estimated breeding value.

breeds. This information will aid in studies of the genetic architecture of FLD and can be exploited for more reliable breeding value estimates for this trait.

MATERIALS AND METHODS

Phenotype and Genotype Data

We used 5,334 Danish Holstein (**HOL**), 4,237 Nordic Red Dairy Cattle (**RDC**), and 1,180 Danish Jersey (**JER**) bulls with deregressed EBV (**DRP**) for FLD in the analyses. Table 1 lists descriptive statistics of the DRP and reliabilities of FLD for the 3 breeds. Histograms of DRP for the 3 breeds are also available (Supplemental Figure S1; http://dx.doi.org/10.3168/jds.2015-10705). The FLD index includes heel erosion, interdigital dermatitis, claw trimming by veterinarian, interdigital necrobacillosis, interdigital skin hyperplasia, laminitis, arthritis, sole ulcer, pressure injuries, tenosynovitis of the hoofs, and other leg diseases (Nielson et al., 2000). Veterinarians or farmers register FLD in the period 15 d before to 305 d after calving for the first, second, and third parity (Nordic Cattle Genetic Evaluation, 2013).

The FLD index was calculated by applying the EBV of FLD from the Nordic Cattle Genetic Evaluation (2013), as follows:

$$\text{FLD} = 0.5 \times \text{FLD}_1 + 0.3 \times \text{FLD}_2 + 0.2 \times \text{FLD}_3,$$

where FLD_1, FLD_2, and FLD_3 are EBV for FLD from 15 d before to 305 d after calving in the first, second, and third lactation, respectively. Cows with and without FLD were recorded as 1 and 0, respectively. Phenotypic data were precorrected for heterogeneous variance due to breed, year of calving, and country. Breeding values for FLD in each lactation were estimated by a linear multitrait, multilactation sire model. Records on early reproductive, late reproductive, and metabolic disorders from the first, second, and third lactations, together with records for clinical mastitis from the first lactation, were included as correlated traits to improve the accuracy of EBV for FLD. However, EBV for these correlated traits were not included in the FLD index. Feet and legs disorders DRP (Goddard, 1985; Schaeffer, 1985) were derived from the EBV of FLD evaluated from Nordic Cattle Genetic Evaluation (2013).

We carried out an association study for FLD, using imputed whole-genome sequence (**WGS**) data. All bulls were genotyped with Illumina 54k chip version 1 or 2 (Illumina Inc., San Diego, CA). For imputation to WGS variants, we followed the procedure described by Iso-Touru et al. (2016). Briefly, 54k genotypes were imputed to WGS variants by using a 2-step approach. First, using a multibreed reference of 3,383 animals (1,222 HOL, 1,326 RDC, and 835 JER) that had been genotyped with the Illumina BovineHD chip (Illumina Inc.), all animals were imputed to the high-density (**HD**) level. Next, the imputed HD genotypes were imputed to the WGS level by using a multibreed reference of 1,228 animals from run4 of the 1,000 Bull Genomes Project (1,148 cattle, including 288 individuals from the global Holstein-Friesian population, 56 RDC, and 61 JER, as well as 743 individuals from different breeds; Daetwyler et al., 2014) and private data from Aarhus University (80 cattle, including 23 HOL, 30 RDC, and 27 JER). Imputation to HD genotypes was undertaken by using IMPUTE2 v2.3.1 (Howie et al., 2011), and imputation to the whole-genome level was done by using Minimac2 (Fuchsberger et al., 2015).

A total of 22,751,039 biallelic variants were present in the imputed sequence data. For each breed, SNP with a minor allele frequency (**MAF**) below 1% or with a large deviation from Hardy–Weinberg equilibrium ($P < 0.000001$) were excluded. After this quality-control filtering step was completed, 15,355,402 SNP for HOL, 15,243,842 SNP for RDC, and 13,403,941 SNP for JER remained. We obtained an imputation accuracy (squared correlation between imputed genotypes and true, unobserved genotypes) of 0.85 for the across-breed imputation of 19,498,365 SNP through the quality control step. Imputation accuracy results are shown