Laminitis-related claw disorders in dairy cattle: A genome-wide association study

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ABSTRACT: 10 significant and 31 suggestive SNP were identified for laminitis-related claw disorders in a dataset with 1,771 genotyped cows. The SNP were spread across the genome indicating that many SNP, each explaining a small proportion of the genetic variance, influence claw disorders. Some significant and suggestive SNP were closely located to SNP found in previous research on feet and leg conformation traits. Major genes influencing claw disorders were not identified, but in order to reduce the incidence of claw disorders by breeding, genomic selection is a promising approach.

Keywords: GWAS; Health; Holstein Friesian

Introduction

Because of impaired welfare (Enting et al., 1997) and economic losses (Brijnits et al., 2012), claw disorders have become important breeding goal traits. Previous studies have identified quantitative trait loci (QTL) for lameness and feet and leg conformation (e.g. Buitenheus et al., 2007). However, to our knowledge, no linkage studies or genome-wide association studies (GWAS) have been published on claw disorders. Identifying chromosomal regions associated with claw disorders might add to a better understanding of the development of claw disorders when underlying genes can be identified. Therefore, the aim of the current study was to perform a GWAS on laminitis-related claw disorders in dairy cows.

Materials and methods

Phenotypic and genotypic data. The data set contained 1,771 genotyped Holstein Friesian cows with a total of 2,723 phenotypic records. Claw disorders were recorded for the hind legs as a binary trait: 0 = no claw disorder, 1 = claw disorder in at least one hind leg. Analysed claw disorders were double sole (DS), sole haemorrhage (SH), sole ulcer (SU), and white line separation (WLS). 41,761 SNP were retained after quality control.

Statistical analysis. The association of an individual SNP with a phenotype was estimated using the following linear animal model:

\[ Y_{ijklmn} = \mu + H_i + YS_j + P_k + L_l + SNP_m + \text{Animal}_n + PE_o + e_{ijklmn} \]

where \( Y_{ijklmn} \) is a claw disorder, \( \mu \) is the overall mean, \( H_i \) is the fixed effect of herd \( i \), \( YS_j \) is the fixed effect of year-season of trimming \( j \) (season is defined as spring = March – May, summer = June – August, autumn = September – November, winter = December – February), \( P_k \) is the fixed effect of the \( k \)th parity (\( k = 1, 2, 3, \) and \( \geq 4 \)), \( L_l \) is the fixed effect of the \( l \)th lactation stage at trimming (\( l = 1 \) to 10, group 1-9 are 50 days each, with the first group from 1-50 days, the second group from 50-100 days, etc. Cows with lactation stage \( \geq 450 \) days were combined in group 10), \( SNP_m \) is the fixed effect of the \( m \)th SNP, \( \text{Animal}_n \) is the random additive genetic effect of the \( n \)th cow \( \sim N(0, \sigma^2_A) \), where \( A \) is the additive genetic relationships matrix among cows. \( PE_o \) is the random permanent environment effect \( \sim N(0, \sigma^2_{pe}) \) and \( e_{ijklmn} \) is the random residual effect \( \sim N(0, \sigma^2_e) \), where \( I \) is the identity matrix. The heritabilities for the claw disorders were fixed at the estimates given by Van der Spek et al. (2013). Analyses were performed using ASReml v3.0 (Gilmour et al., 2009). The significance threshold for the GWAS was adjusted for multiple testing using the false discovery rate (FDR). A SNP with FDR<0.20 will be referred to as suggestive and with FDR<0.05 as significant.

Results

Figure 1 shows the Manhattan plot for the laminitis-related claw disorders. In total, 17 significant and 42 suggestive SNP were identified. A sensitivity test was performed when a genotype class contained less than 5 cows by omitting this genotype class and re-evaluating the association based on the remaining two genotype classes. From the total of 17 significant associations, 7 were retested and from the 42 suggestive associations, 21 were retested. None of the re-evaluated associations had a P-value <0.05 and were consequently omitted. The remaining SNP will be discussed in more detail. 10 significant and 20 suggestive SNP associations were found for SU, and one suggestive SNP association was found for DS (Fig. 1). For WLS and SH no significant or suggestive SNP associations were found (Fig. 1).

The -log\(_{10}\) P-values for the 31 associations ranged from 3.67 to 6.79. The suggestive SNP associated with DS had a -log\(_{10}\) P-value of 5.08. The AA genotype corresponds to an increase of DS with 48% when compared to BB. The most significant SNP (-log\(_{10}\) P-value of 6.79) was associated with SU. The AA genotype corresponds to an increase of SU with 27% as compared to the genotype BB. The 10 significant SNP were located on BTA8, BTA10, BTA11, BTA18 and BTA22. Most significant SNP (n=5) were lo-
cated on BTA8. The 21 suggestive SNP were located on 13 different chromosomes; BTA1, BTA5, BTA7-BTA11, BTA13-BTA15, BTA17, BTA18, and BTA21-BTA23. Most suggestive SNP were also located on BTA8 (n=5).

Figure 1. GWAS for laminitis-related claw disorders. The false discovery rate was set at 0.05 for significant SNP (dashed line) and at 0.20 for suggestive SNP (solid line).

Discussion

Ten significant and 21 suggestive SNP were identified for laminitis-related claw disorders. The SNP were spread across 13 chromosomes. This suggests that each claw disorder is influenced by many genes, each explaining a small part of the genetic variance, dispersed across the entire genome. Genetic correlations between claw disorders are different from 1 (Van der Spek et al., 2013), indicating they are different traits. Therefore, it was expected that different claw disorders are influenced by different genes as they have a different aetiology. Claw disorders have a low heritability (ranging from 0.02 to 0.14, Van der Spek et al., 2013) and consequently the power to detect QTL based on the available 1,771 cows is low. With 1,777 genotyped cows, an $r^2$ between marker and QTL of 0.6, marker and QTL allele frequencies of 0.5, 2% of the total variance explained by the QTL and a Type I error equal to 0.001, the detection power is equal to 85% (calculated using the function “luo.ld.power” (Luo 1988) from the package “IdDesign” for the statistical software R). The detection power shows that we should be able to identify SNP explaining at least 2% of the total variance in our study. Such a SNP would explain 40% of the genetic variance when the heritability of the trait is 0.05. Many SNP are expected to explain less than 4% of the genetic variance (Goddard and Hayes, 2009). The power to detect SNP which explain such a small fraction of the genetic variance is low in the current study.

SNP with a MAF of less than or equal to 2% were omitted from our analyses. However, this could not prevent several SNP with a genotype class of less than 5 cows. These SNP were re-evaluated by omitting the smallest genotype class. Alleles were expected to have additive effects, where both homozygotes perform different from each other but also different from the heterozygote. From the total of 59 significant or suggestive SNP, 28 were re-evaluated and removed as the P-value was higher than 0.05. The difference between the homozygote and heterozygote was not significant. This might be due to dominance effects but seems unlikely and therefore these SNP were removed. A large number of significant SNP was not found. Instead, few SNP with large effects were found associated with laminitis-related claw disorders. Previous studies have shown that alleles with low frequencies can have a large effect on complex traits (e.g. Mackay et al. 2012; Weber et al. 2012). Alleles with low frequency are difficult to detect with GWAS as they might explain only a small part of the variance (e.g. Pritchard, J.K. 2001; Cirulli, E.T. and D.B. Goldstein, 2010). Another reason for poor identification of SNP in LD with low frequency alleles is that SNP used in GWAS are a selected sample resulting in ascertainment bias. These reasons might explain why only few associations, with large effects, were identified.

Literature. No other genome-wide association studies on claw disorders have been published. However, QTL studies and a genome-wide study have been published on other feet and leg traits, e.g. rear leg rear view, rear leg side view, foot angle, general feet and legs score, hock quality, and lameness, which have shown to be genetically correlated to claw disorders (e.g. Van der Waaij et al., 2005, Weber et al., 2013). Cole et al. (2011) found most significant associations with feet and leg traits on BTA11, BTA13, BTA18, BTA20, and BTA26. In the current study, 2 significant and 3 suggestive SNP associations were found on the same chromosomes, but the regions do not overlap. One suggestive SNP identified in our study on BTA5 was closely located to a SNP found by Cole et al. (2011) and Hiendleder et al. (2003). The SNP on BTA5 was suggestively associated with SU in our study, with general feet and leg score in the study by Cole et al. (2011) and with foot angle in the study by Hiendleder et al. (2003). Buitenhuis et al. (2007) identified several QTL associated with lameness and leg conformation traits. They found a QTL marker on BTA8 associated with foot angle which appears to be in the middle of a region with 3 significant and 5 suggestive SNP, associated with SU in our study. A recent study of Swalve et al. (2014) using a preselected set of 384 SNP, found a strong association on BTA21 with SH. In our study, we did not find a significant or suggestive SNP on BTA21 associated with laminitis-related claw disorders. The SNP identified by Swalve et al. (2014) had a P-value of 0.06 for SU in our study. For other traits the P-value of this SNP was higher than 0.20.

Conclusion

The current study is to our knowledge the first GWAS which revealed significant and suggestive SNP associated with laminitis-related claw disorders. Although a major gene could not be identified, 10 significant and 21
suggestive SNP associations were identified. Some significant and suggestive SNP were closely located to SNP found in previous research on feet and leg conformation traits. The SNP associations were located on 13 chromosomes. This suggests that claw disorders are controlled by many loci distributed across the genome, each explaining a small proportion of the genetic variance. Therefore, identifying and characterising individual genes underlying claw disorders might not be feasible. In order to reduce the incidence of claw disorders by breeding, genomic selection, which does not require knowledge about individual genes might be a promising approach.

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Literature Cited