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Association analysis for young stock survival index with imputed whole-genome sequence variants in Nordic Holstein cattle

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ABSTRACT

Identification of the genetic variants associated with calf survival in dairy cattle will aid in the elimination of harmful mutations from the cattle population and the reduction of calf and young stock mortality rates. We used de-regressed estimated breeding values for the young stock survival (YSS) index as response variables in a genome-wide association study with imputed whole-genome sequence variants. A total of 4,610 bulls with estimated breeding values were genotyped with the Illumina BovineSNP50 (Illumina, San Diego, CA) single nucleotide polymorphism (SNP) genotyping array. Genotypes were imputed to whole-genome sequence variants. After quality control, 15,419,550 SNP on 29 *Bos taurus* autosomes (BTA) were used for association analysis. A modified mixed-model association analysis was used for a genome scan, followed by a linear mixed-model analysis for selected genetic variants. We identified 498 SNP on BTA5 and BTA18 that were associated with the YSS index in Nordic Holstein. The SNP rs440345507 (Chr5:94721790) on BTA5 was the putative causal mutation affecting YSS. Two haplotype-based models were used to identify haplotypes with the largest detrimental effects on YSS index. For each association signal, 1 haplotype region with harmful effects and the lead associated SNP were identified. Detected haplotypes on BTA5 and BTA18 explained 1.16 and 1.20%, respectively, of genetic variance for the YSS index. We examined whether YSS quantitative trait loci (QTL) on BTA5 and BTA18 were associated with stillbirth. YSS QTL on BTA18 overlapped a QTL region for stillbirth, but most likely 2 different causal variants were responsible for these 2 QTL. Four component traits of the YSS index, defined by sex and age, were analyzed separately by the modified mixed-model approach. The same genomic regions were associated with both bull and heifer calf mortality. Several genes (*EPS8*, *LOC100138951*, and *KLK* family genes) contained a lead associated SNP or were included in haplotypes with large detrimental effects on YSS in Nordic Holstein cattle.

Key words: young stock survival, calf mortality, genome-wide association, quantitative trait loci

INTRODUCTION

Calves and young stock that die during the rearing period result in lost revenue for dairy farmers, fewer heifers for replacement, veterinary costs, and adverse effects on animal welfare. Juvenile death rates among Danish Holstein calves born between 2008 and 2012 were 7.5% in heifer calves and 10% in bull calves (Pedersen et al., 2014). Mortality during the rearing period has greater economic consequences than early embryo loss, abortion, or stillbirth.

Part of the variation in survival has a genetic basis. Fuerst-Waltl and Sørensen (2010) reported heritability values between 0 and 0.08, depending on the age of calves investigated. Norberg et al. (2013) presented heritability for mortality between 0 and 0.03 in the period from 24 h after birth to 180 d in Danish Jersey heifer calves. Some of these deaths may be due to the action of recessive lethal alleles. Due to the widespread use of a limited number of elite dairy cattle bulls, some harmful recessive alleles have spread in the population. These deleterious alleles frequently go unnoticed because the identification of a carrier bull requires mating between its descendants. Mutations causing embryonic lethality and stillbirth have been reported in Nordic dairy cattle by using genomic data (Sahana et al., 2013, 2016; Kadri et al., 2014). However, recessive lethal mutations have not been reported for young stock mortality. An index for young stock survival (**YSS**) in calves was included in the Nordic total merit index by the Nordic Cattle Genetic Evaluation (**NAV**; www.nordicebv.info). Pedersen et al. (2015) reported that correlations between the YSS index and other Nordic total merit indices were generally close to 0. The strongest positive correlations (between 0.1 and 0.2) were observed with 2

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indices, health index and longevity. To improve YSS, it is necessary to select for YSS directly.

The number of dairy cattle with genomic SNP array data has increased rapidly in recent years. The combination of genomic information and breeding values for YSS creates an opportunity to identify genomic variants with harmful effects on YSS. Once these variants have been identified, it will be possible to select against them and prevent at-risk matings between carriers. This selection will improve calf survival and reduce the cost per live cattle produced. Quantitative trait locus information can be used to improve accuracies for genomic prediction (Brøndum et al., 2015). The aim of this study was to identify QTL affecting YSS in Nordic Holstein (**NH**) cattle.

MATERIALS AND METHODS

Phenotype and Genotype Data

We analyzed data from 4,610 NH bulls born in 1998 or later with EBV for YSS. The YSS index was calculated based on 4 survival traits: survival from 2 to 30 d for bull calves (**BP1**) and heifer calves (**HP1**), from 31 to 184 d for young bull calves (**BP2**), and from 31 to 458 d for young heifer calves (**HP2**). Calf death and survival during this period were recorded as 0 and 1, respectively. Calves slaughtered, exported, or with missing records were recorded as missing. The YSS index was calculated by combining EBV for BP1, BP2, HP1, and HP2 by NAV (Denmark), which were weighted by their relative economic values and standardized (Pedersen, 2015).

The breeding value estimation procedure for YSS by NAV was described by Pedersen et al. (2014). Phenotypic data for YSS traits were precorrected for heterosis (by applying regression on the expected total heterosis of all included populations), country (Danish, Swedish, and Finnish data were combined), calf size, calving ease, effect of transfer to another herd, and herd-by-year effect. De-regressed EBV (**DRP**) were derived for animals based on the effective daughter contributions of sire and maternal grandsire (Goddard, 1985; Schaeffer, 1985) by using MiX99 software (Vuori et al., 2006). Supplemental Table S1 (https://doi.org/10.3168/jds.2017-12688) lists descriptive statistics of DRP and reliabilities of the YSS index, its components traits, and stillbirth. Histograms of DRP distributions for BP1, BP2, HP1, HP2, and the YSS index are presented in Supplemental Figure S1 (https://doi.org/10.3168/jds.2017-12688). Heritability estimates for the YSS index and its component traits were reported as being in the range of 0.007 to 0.027 (Pedersen et al., 2014). The phenotypic correlation (DRP) among the YSS index and its components traits was ~0.78 on average (Supplemental Table S2; https://doi.org/10.3168/jds.2017-12688).

An association study for the YSS index was carried out by using imputed whole-genome sequence (**WGS**) data. All bulls were genotyped with the Illumina BovineSNP50 BeadChip (54k; version 1 or 2; Illumina, San Diego, CA). The 54k genotypes were first imputed to the Illumina BovineHD marker set and then to the full WGS level (Iso-Touru et al., 2016; Wu et al., 2016). A total of 22,751,039 biallelic variants (SNP and indels) were imputed from WGS reference data. The SNP with a minor allele frequency less than 1% or those deviating from Hardy-Weinberg proportions ($P < 10^{-6}$) were removed. Eventually, 15,419,550 SNP remained for association analysis. The position of each SNP was defined according to the *Bos taurus* genome assembly UMD3.1 (Zimin et al., 2009). Genes located within or overlapping with top associated SNP were determined by using information from the Variant Effect Predictor tool version 87 of Ensembl (McLaren et al., 2010).

To examine whether YSS QTL were associated with stillbirth, detected QTL regions for the YSS index were analyzed for 5,484 NH bulls with DRP for stillbirth for first parity (4,575 records overlap with YSS). Stillbirth was defined as a calf that was born dead or died within 24 h after birth. Stillbirth might be affected by some of the same genetic factors as mortality at a very early age in life. The EBV of stillbirth was estimated by a multitrait sire model with direct and maternal effects by NAV (http://www.nordicebv.info/dk/).

Association Analyses

A modified linear mixed-model approach (efficient mixed-model association expedited, **EMMAX**) (Kang et al., 2008, 2010) was used to detect associations between imputed sequence variants and YSS index. For details of model description and analysis, see Iso-Touru et al. (2016) and Wu et al. (2016). Significantly associated SNP from the above analysis were reanalyzed using a linear mixed model (**LMM**; Yu et al., 2006), which included a polygenic effect to adjust for familial relatedness and population structures and a fixed effect of the marker. Details of the statistical models and analytical approach were presented by Wu et al. (2016).

Association analyses for BP1, BP2, HP1, and HP2 were only carried out using EMMAX. For each model, a Bonferroni correction was applied to control for false-positive associations that arose due to multiple testing. A SNP was declared significant if its *P*-value was less than 0.05/M, where M is the number of SNP (= 15,419,550). The resulting threshold was $-\log_{10}(P) > 8.49$. For each chromosome, SNP were considered