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Genome-wide association study identifies functional genomic variants associated with young stock survival in Nordic Red Dairy Cattle

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ABSTRACT

Identifying quantitative trait loci (QTL) associated with calf survival is essential for both reducing economic loss in cattle industry and understanding the genetic basis of the trait. To identify mutations and genes underlying young stock survival (YSS), we performed GWAS using de-regressed estimated breeding values of a YSS index and its component traits defined by sex and age in 3,077 Nordic Red Dairy Cattle (RDC) bulls and 2 stillbirth traits (first lactation and later lactations) in 5,141 RDC bulls. Two associated QTL regions on *Bos taurus* autosome (BTA) 4 and 6 were identified for the YSS index. The results of 4 YSS component traits indicate that same QTL regions were associated with bull and heifer calf mortality, but the effects were different over the growing period and suggested an additional QTL on BTA23. The GWAS on stillbirth identified 3 additional QTL regions on BTA5, 14, and 24 compared with YSS and its component traits. The conditional test of BTA6 showed at least 2 closely located QTL segregating for YSS component traits and stillbirth. We found 2 independent QTL for stillbirth on BTA23. The post-GWAS revealed *LCORL*, *PPM1K*, *SSP1*, *MED28*, and *LAP3* are putative causal genes on BTA6, and a frame shift variant within *LCORL*, BTA6:37401770 (rs384548488) could be the putative causal variant. On BTA4, the *GRB10* gene is the putative causal gene and BTA4:5296018 is the putative causal variant. In addition, *NDUFA9* and *FGF23* on BTA5, *LYN* on BTA14, and *KCNK5* on BTA23 are putative causal genes for QTL for stillbirth. The gene analysis also proposed several candidate genes. Our findings shed new light on the candidate genes affecting calf survival, and the knowledge could be utilized to reduce calf mortality and thereby enhance welfare of dairy cattle.

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

INTRODUCTION

Mortality of cattle during the rearing period causes economic losses for dairy farmers and has adverse impacts on animal welfare. In the Nordic countries, recording of the exact date of death or disposal of young stock has been routine since the 1998, and data shows that the prevalence of juvenile death accounts for 8.3% in heifer calves and 10.8% in bull calves in Nordic Red Dairy Cattle (**RDC**; Pedersen et al., 2014). The young stock survival (**YSS**) index, which describes the genetic ability of bulls' offspring to survive from birth to a certain predefined age, has been included in the breeding goals in the Nordic Cattle Genetic Evaluation (**NAV**; www.nordicebv.info).

Identification and characterization of genes associated with calves and young stock mortality traits is essential for both reducing economic loss in cattle industry and understanding the genetic basis of the trait. Genome-wide association studies (GWAS) have been extensively used to identify genetic variants associated with quantitative traits in cattle. Previously, recessive lethal mutations in RDC have been identified through QTL affecting fertility (early embryonic mortality; Kadri et al., 2014b) or stillbirth (Sahana et al., 2016). Following identification, it is possible to remove the mutations from populations by selecting against heterozygote individuals and avoiding mating between mutation carriers to prevent calves being homozygous for the mutant allele. The removal of harmful mutations or avoidance of mating between mutation carriers from the dairy cattle population will reduce calf mortality and thereby reduce the costs per live cattle produced for the farmer. The first step to identify causal variants through the QTL route is to detect the genomic regions harboring variants affecting the phenotype of interest. Using whole-genome sequence data, a previous study (Wu et al., 2017) has detected QTL on chromosome 5 and 18 for the YSS index in the Nordic Holstein population. The intensive selection within population has largely shaped the QTL allele frequencies for different dairy cattle populations; therefore, the knowledge gained from one population is not directly transferable

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to another population to exploit in breeding programs. The aim of the present study was to detect QTL regions for YSS in RDC. This might lead to identification of causal mutations responsible of mortality in growing calves. This information will reduce calf mortality by avoiding carrier mating and may also improve genomic prediction accuracy for YSS.

MATERIALS AND METHODS

Phenotype and Genotype Data

In this study, no animal handling was involved. All the phenotype and genotype data were from the routine breeding and genomics evaluations. Therefore, this analysis did not require approval by an Institutional Animal Care and Use Committee.

Death and disposal of young stock has been recorded in the Knowledge Center of Agriculture in Denmark since 1998 (https://www.seges.dk/en), and the YSS index has been included in the Nordic Total Merit index from 2016 (Carlen et al., 2016). The YSS index is constructed from its 4 components traits, which are based on sex and period: bull calf survival in the period from 2 to 30 d (**BP1**), bull calf survival in the period from 31 to 184 d (**BP2**), heifer survival in the period from 2 to 30 d (**HP1**), and heifer survival in the period from 31 to 458 d (**HP2**). The details of the YSS index were described by Pedersen et al. (2015).

Breeding values of the YSS index are routinely estimated by the NAV (Pedersen et al., 2015). Heritability estimates for the YSS index and its components traits were in the range of 0.007 to 0.034 (Pedersen et al., 2014). A total of 3,114 RDC progeny-tested bulls with EBV for YSS traits were analyzed in this study. Deregressed EBV (**DRP**) for YSS traits were derived for these bulls based on the effective daughter contributions of sire and maternal grandsire (Goddard, 1985; Schaeffer, 1985) by using Mix99 software (Vuori et al., 2006). The phenotypic correlation (DRP) among the YSS index and its components traits was ~0.90 on average (https://nordicebv.info/).

A calf is considered stillborn if it was born dead or died within 24 h after parturition. It is worth considering whether some of the genetic factors for stillbirth are shared with mortality at early age. Therefore, we analyzed 5,141 RDC bulls with DRP for stillbirth (3,113 records overlap with YSS). We carried out GWAS separately for stillbirth in first lactation (**SBF**) and stillbirth in later lactations (**SBL**). The descriptive statistics of DRP and their reliabilities of stillbirth are available on NAV (https://nordic.mloy.fi/NAVBull/Phenotypes/ENG/RDC).

All bulls were genotyped with BovineSNP50 BeadChip (Illumina, San Diego, CA). All genotypes were imputed to the high-density level and then imputed to the whole-genome sequence (**WGS**) level. The methodology to impute the low-density genotype to WGS level was described in (Cai et al., 2022). Briefly, the probe sequences of low-density and high-density marker sets were extracted from Illumina Chip manual files. The remaining sequences of each SNP were mapped to the *Bos taurus* genome assembly ARS-UCD1.2 (Rosen et al., 2020) by Burrows-Wheeler alignment (https://github.com/lh3/bwa). The unambiguous mapped SNPs were retained for downstream analysis. The low-density and high-density marker sets were phased using BEAGLE 5 (Browning et al., 2021) with default settings. The HD panel included 3,531 animals from various breeds. The WGS panel included 2,685 animals of various breeds from run7 of the 1000 Bull Genomes Project and in-house sequencing data. For phasing the WGS panel, we applied pre-phasing with BEAGLE4 (Browning et al., 2021) and corrected the calling error by SHAPEIT2 (Delaneau et al., 2011). Approximate of 16.11 million bi-allelic variants with imputation accuracy $R^2 > 0.4$, minor allele frequency larger than 1%, and following Hardy-Weinberg proportions ($P > 10^{-6}$) in the imputed WGS data were used for association analyses.

Association Analyses

First, GCTA *mlma* (Yang et al., 2011) was used for GWAS with bulls having both genotype and phenotype (DRP) data, to detect SNP associated with BP1 (3,072) and HP1 (3,072), for BP2 (3,076) and HP2 (3,077), YSS index (3,077), SBF (5,141), and SBL (5,141). Then, to investigate whether one or multiple causal factors were located within a targeted region on a chromosome, the conditional and joint (**COJO**) algorithm implemented in GCTA was used to detect the independent association signal.

Bonferroni correction was applied to control for false positive associations, and we declared a SNP significant if its P-value was less than 0.05/M, where M is the number of SNP; that is, $-\log_{10}(P) > 8.51$. For each QTL region, the SNP with the lowest P-value was designated as the lead SNP.

LocusZoom. We used the standalone version of LocusZoom (Pruim et al., 2010) to plot some QTL regions. To prepare the database of the LocusZoom, the *refflat* annotation file was retrieved from UCSC Genome Browser (Karolchik et al., 2003). The vcf files from *run7* of the 1000 Bull Genomes Project were converted into binary files using PLINK (Purcell et

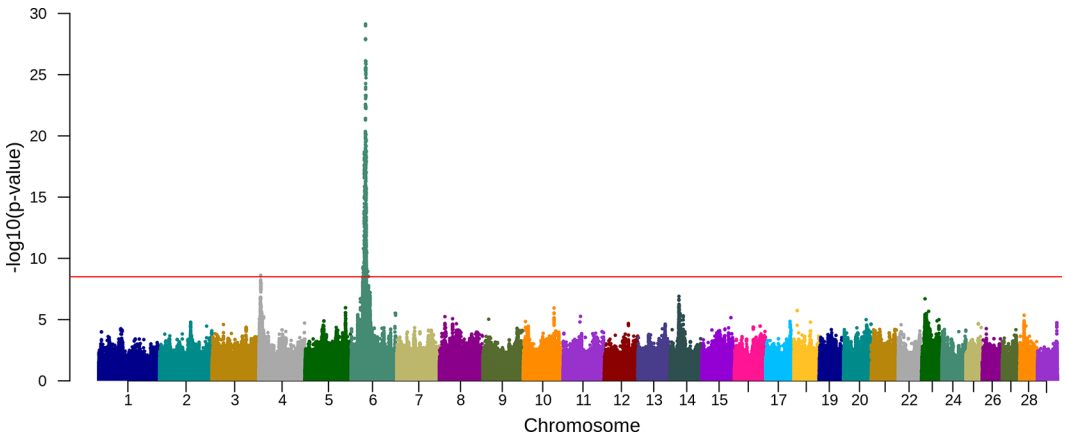

Figure 1. Manhattan plot for the association test for young stock survival in Nordic Red Dairy Cattle population. The Manhattan plot shows association test ($-\log_{10}$ *P*-value) on the y-axis against physical autosomal location on the x-axis. The standard genome-wide significance cutoff of $-\log_{10}$ *P*-value = 8.5 is shown by the horizontal red line.

al., 2007). Both the annotation file and genotype files were converted with the script provided by LocusZoom.

Multi-Trait Meta-Analysis. We also used MTAG (Turley et al., 2018) to perform the multi-trait meta-analysis of YSS index with stillbirth. The default parameter was used to run MTAG with the assumption that the samples are overlapping and trait definition of YSS index and stillbirth are different.

Variant Effect Predictor. The *run8* 1000 Bull Genomes Project reference panel (Daetwyler et al., 2014) was used to annotate the consequence of the variants with Variant Effect Predictor (**VEP**) v. 100 (McLaren et al., 2016) with the merged cache file to annotate the genomic feature for both ENSEMBLE and NCBI. All the significant SNPs are treated as a pool of candidate causal variants to extract the annotation of these variants.

Gene Analysis. We ran gene-based association analysis with MAGMA (de Leeuw et al., 2015). To set up the database for MAGMA, we generated the cattle gene location file using the bed file download from Ensembl v. 104 (Flicek et al., 2013). The RDC animals from r*un7* of the 1000 Bull Genomes Project (Daetwyler et al., 2014) reference panel were converted to plink binary format for marker location information. Before gene analysis, we used MAGMA annotate to prepare the annotation file for each chromosome. To run MAGMA, we used the mean SNP association test model with an adaptive permutation procedure to run possible model with summary statistics. Bonferroni correction was applied to find the genome-wide significant genes with *P*-value < 1.92e-6 (i.e., nominal type I error of 0.05 with a total number of 26,071 gene tests). The significant genes were queried against the Mammalian Phenotype Database (**MPD**; Blake et al., 2011) to find biological support.

Detection of SNP Homozygous Status in the 1000 Bull Genomes Project Sequence Data. To check whether the detected SNP were recessive lethal mutations, we examined the existence of homozygote animals for the alternative (i.e., non-reference) allele for detected SNP among the 2,333 animals (including 128 sequenced RDC) in *run7* of the 1000 Bull Genomes Project. If there is a recessive genetic factor that causes calf mortality, we expect that the variants will not be present in a homozygous state in populations other than RDC and also that it will have a negative effect on phenotype in GWAS.

RESULTS

Association Analysis for YSS Index

We identified 2 loci significantly associated with YSS index in RDC: one on BTA6 and the other on BTA4 (Figure 1 and Table 1). The lead SNP on BTA6, BTA6: 36929541 (rs380367705) is an intergenic variant close to the *IBSP* gene. The LocusZoom plot (Figure 2) showed that the lead SNP is located in a gene-rich region, which indicated the possibility that the nearest gene to the lead SNP might not be the causal gene. In this QTL region, we detected 2,244 significant SNPs. The lead SNP on BTA4, rs447889413 (BTA4:4890044) is an intergenic variant close to the *COBL* gene.

Association Analysis for Component Traits of YSS

To better interpret the association for YSS, we also conducted association test for BP1 (Figure 3a and

Table 1. Associated region and its lead SNPs on BTA for the young stock survival index, its 4 component traits and 2 stillbirth traits[1]

		Associated region		Lead SNPs					
Trait	BTA	QTL (bp)	Position (bp)	RS ID	MAF	Effect	*P*-value	Annotation	Nearest gene
YSS	4	3,914,509–5,140,071	4890044	rs447889413	0.20	−3.89	2.42e-09	Intergenic	*COBL*
	6	36,679,547–37,179,665	36929541	rs380367705	0.35	−6.38	7.32e-30	Intron	*IBSP*
BP1	6	36,679,547–37,179,665	36929541	rs380367705	0.35	−3.42	1.74e-22	Intron	*IBSP*
	23	11,978,619–12,478,646	12228376	rs379326817	0.09	−3.04	9.12e-12	Intron	*BTBD9*
BP2	6	36,679,547–37,179,665	36929541	rs380367705	0.35	−3.73	2.00e-30	Intron	*IBSP*
HP1	6	6,903,437–37,430,251	37180233	NA	0.21	−3.55	1.64e-21	3′ UTR	*FAM184B*
	23	11,807,356–12,307,529	12057334	NA	0.06	−4.13	2.47e-12	Downstream	*ZFAND3*
HP2	4	3,914,509–5,140,071	4890044	rs447889413	0.20	−2.48	5.41e-10	Intergenic	*COBL*
	6	36,679,547–37,179,665	36929541	rs380367705	0.35	−4.02	7.45e-32	Intron	*IBSP*
SBF	4	3,828,821–4,328,675	4078660	rs447073705	0.74	2.15	3.33e-12	Intergenic	*ENSBTAG00000052811*
	6	36,679,547–37,179,665	36929541	rs380367705	0.35	−4.04	1.34e-43	Intron	*IBSP*
	14	22,879,602–23,379,239	23128784	rs109515648	0.20	3.28	3.35e-24	Intergenic	*LYN*
	23	12,830,925–13,332,884	13080865	rs384078502	0.05	−5.42	2.60e-20	Intron	*KCNK5*
SBL	5	105,407,720–106,006,940	105756724	rs137822220	0.44	1.51	1.90e-09	Intergenic	*TIGAR*
	6	37,236,226–38,027,078	37777029	rs109270787	0.09	−7.22	2.95e-35	Intergenic	*LCORL*
	14	22,879,602–23,379,239	23128784	rs384078502	0.20	2.37	1.31e-14	Intergenic	*LYN*
	23	12,830,925–13,332,592	13080865	rs384078502	0.05	−7.50	3.52e-41	Intron	*KCNK5*
	24	788,011–1,334,013	1083788	rs475522581	0.01	−5.71	9.06e-10	Upstream	*ENSBTAG00000053596*

[1]YSS = young stock survival index, BP1 = bull calf survival in the period 2–30 d, BP2 = bull calf survival in the period 31–184 d, HP1 = heifer survival in the period 2–30 d, HP2 = heifer survival in the period 31–458 d, SBF = stillbirth in first lactation, SBL = stillbirth in later lactations. RS ID = reference SNP cluster ID, NA = variants missing the for-reference SNP cluster ID, MAF = minor allele frequency, UTR = untranslated region.

Table 1), BP2 (Figure 3b and Table 1), HP1 (Figure 3c and Table 1), and HP2 (Figure 3d and Table 1). All 4 component traits have the same QTL on BTA6 (Figure 3) as the YSS index (Figure 1). However, HP1 suggested BTA6:37180233 as the lead SNP. The annotation of BTA6:37180233 is a 3′ untranslated region variant of *FAM184B*. There is no linkage disequilibrium (**LD**; r < 0.2) between BTA6:37180233 and BTA6:36929541.

Both BP1 and HP1 detected one more QTL on BTA23 (Figure 3a and 3c) but with different lead SNPs. The lead SNP for BP1 is BTA23:12228376 (rs379326817), which is an intron variant of *BTBD9*. The lead SNP for HP1 is BTA23:12057334, which is a downstream variant of *ZFAND3*. However, these 2 SNPs showed a strong LD, r = 0.6, which suggested that these 2 lead SNPs may be due to the same functional causal locus. Among the YSS component traits, the QTL for YSS index on BTA4 was only identified for HP2.

Association Analysis for Stillbirth

We detected more QTL for both stillbirth traits (Figure 4) than for the YSS index (Figure 1) and its 4 component traits (Figure 3). The most significant signal in SBF was located on BTA6 with the same lead SNPs as YSS, BP1, BP2, and HP2. For SBL, the QTL on BTA6 is the second-strongest signal, but it suggested another lead SNP, BTA6:37777029 (rs109270787). This SNP is an intergenic variant close to *LCORL*. Based on MPD records, mutations in *LCORL* could cause incomplete penetrance preweaning lethality. There is no LD (r < 0.2) between BTA6:37180233 and BTA6:37777029 and low LD (r = 0.29) between BTA6:36929541 and BTA 6:37777029.

The most significant signal in SBL was located at BTA23:13080865 (rs384078502), an intron variant for *KCNK5*. This QTL is shared with SBF, and the record from MPD shows that some mutations in *KCNK5* can cause incomplete penetrance prenatal lethality in mice (Supplemental Table S1; https://doi.org/10.6084/m9.figshare.23822058; Cai, 2023a). The lead SNP has high LD with lead SNPs for BP1 and HP1. BTA 23:13080865 has LD of 0.86 with BTA23:12057334, and of 0.76 with BTA23:12228376.

On BTA4, we identified one QTL for SBF with lead SNP BTA4:4078660 (rs447073705). The annotation of BTA4:4078660 is an intergenic variant close to *ENSBTAG00000052811*. For SBL, one QTL on BTA5 was detected, with lead SNP being an intergenic variant BTA5:105756724 (rs137822220) with *TIGAR* as the nearest gene. On BTA14, SBF and SBL detected one shared QTL. The lead SNP for this QTL is BTA14:23128784 (rs109515648), which is an intergenic vari-

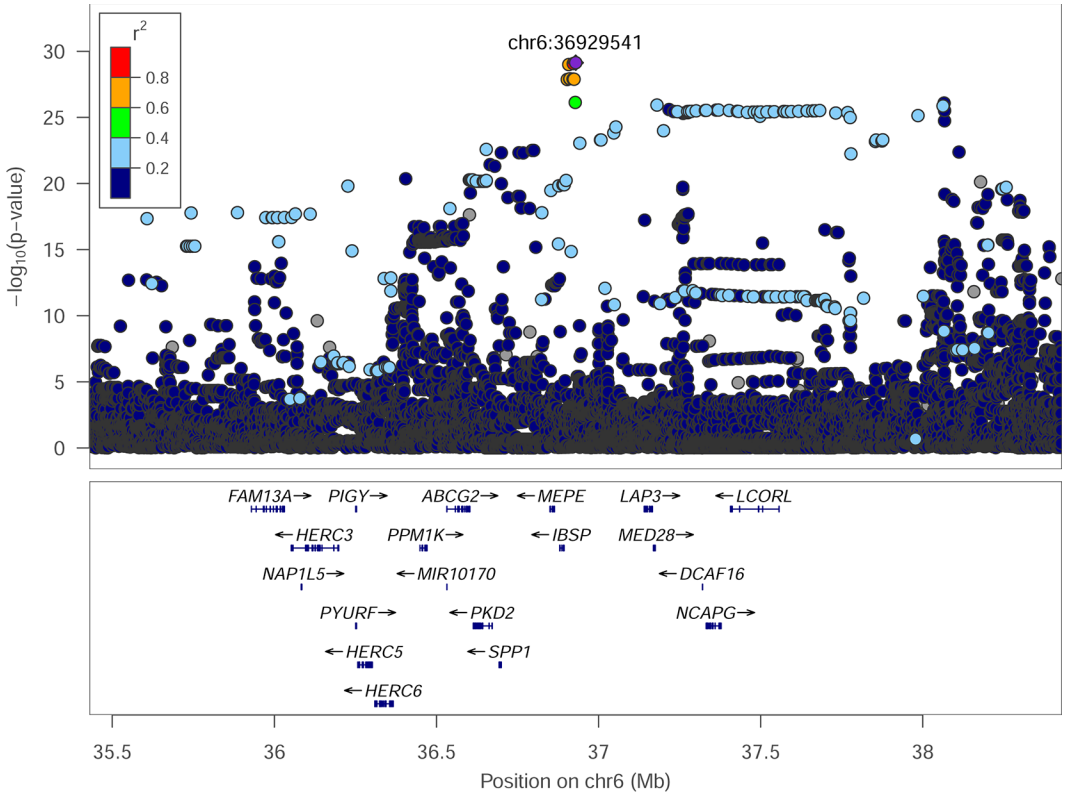

Figure 2. LocusZoom (Pruim et al., 2010) plot for the lead SNPs for young stock survival in Nordic Red Dairy Cattle population. SNPs are shown by their position on the chromosome (chr) against their association ($-\log_{10}$ *P*-value) with young stock survival in Nordic Red population. SNPs are colored to reflect their linkage disequilibrium with the top SNP (6:38065928) with the reference population of Nordic Red Dairy Cattle from the 1000 Bull Genomes Project. The lower panel shows the liver regulatory elements (Kern et al., 2021) of the plot region. This plot was generated using LocusZoom (Pruim et al., 2010).

ant close to *LYN*. The record from MPD showed that some mutations in *LYN* could cause premature death in mice (Supplemental Table S1). A QTL identified only for SBL is located at BTA24 with lead SNP BTA24: 1083788 (rs475522581), which is an upstream variant of *ENSBTAG00000053596*.

Conditional Joint Test in Each Trait

Figure 2 indicated multiple closely located QTL on BTA6. However, the COJO test from GCTA for YSS, BP1, and BP2 indicated only one association signal on BTA6. For HP1, in addition to BTA6:37180233, BTA6: 38074508 (rs133476529) emerges as an independent association signal (Table 2). For SBF, in addition to BTA6: 36929541 (rs380367705), we found another independent association signal with BTA6:37569485 (rs383507085) as lead SNP. For SBL, in addition to BTA6:37777029, we found 2 additional association signals with BTA6: 37776106 and BTA6:38328564 (rs382691096) as lead SNPs (Figure 5). Moreover, an additional QTL for SBL with lead SNP BTA23:23840749 (rs382556458) was detected.

Multi-Trait Meta-Analysis

To identify overlap of YSS and stillbirth QTL, we conducted multi-trait analysis of YSS with SBF and SBL (Figure 6). The multi-trait analysis showed only one QTL detected in YSS located at BTA6, with the lead SNP BTA6:36929541 (rs380367705). For the multi-trait analysis of SBF, we detected 2 QTL located at BTA6 and BTA14. The lead SNPs are BTA6:37369920 (rs714094684) and BTA14:23028072 (rs378444404). For the multi-trait analysis of SBL, we found one QTL located on BTA23. The lead SNPs is BTA23:12057334. The multi-trait analysis did not identify new QTL but suggest 3 new lead SNPs: BTA6:37369920, BTA14: 23028072, and BTA23:12057334. BTA6:37369920 is an intron variant for *NCAPG*. BTA14:23028072 in an intergenic variant near to *TMEM68*. BTA23:12057334 is a downstream variant of *ZFAND3*.

Gene Analysis

We ran gene-based association analysis with the chromosome with QTL in each single-trait analysis

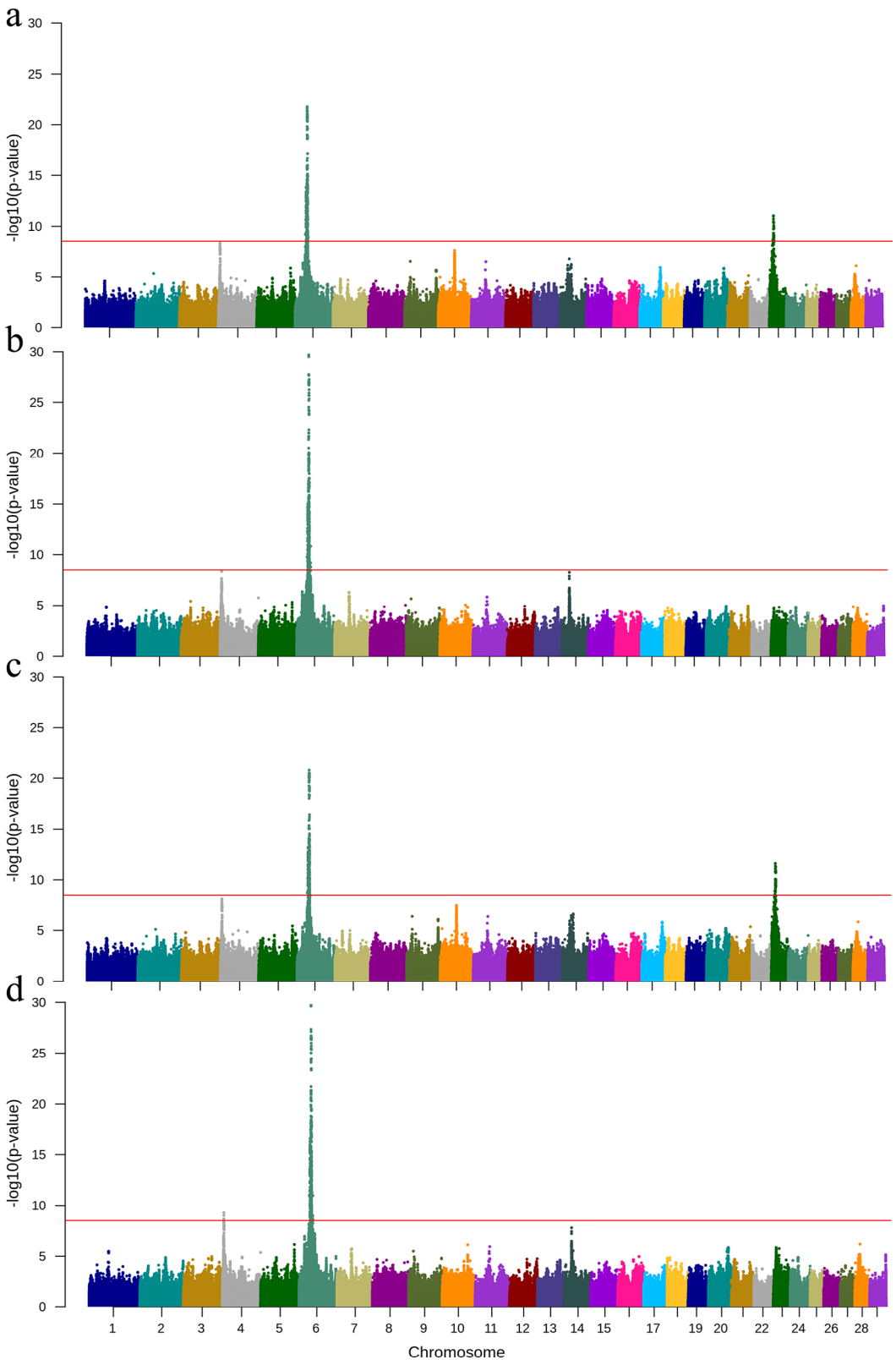

Figure 3. Manhattan plots for the association test for component traits for young stock survival in Nordic Red Dairy Cattle population: (a) bull period 1; (b) bull period 2; (c) heifer period 1; and (d) heifer period 2. The Manhattan plot shows association test ($-\log_{10}$ *P*-value) on the y-axis against physical autosomal location on the x-axis. The standard genome-wide significance cutoff of $-\log_{10}$ *P*-value = 8.5 is shown by the horizontal red line.

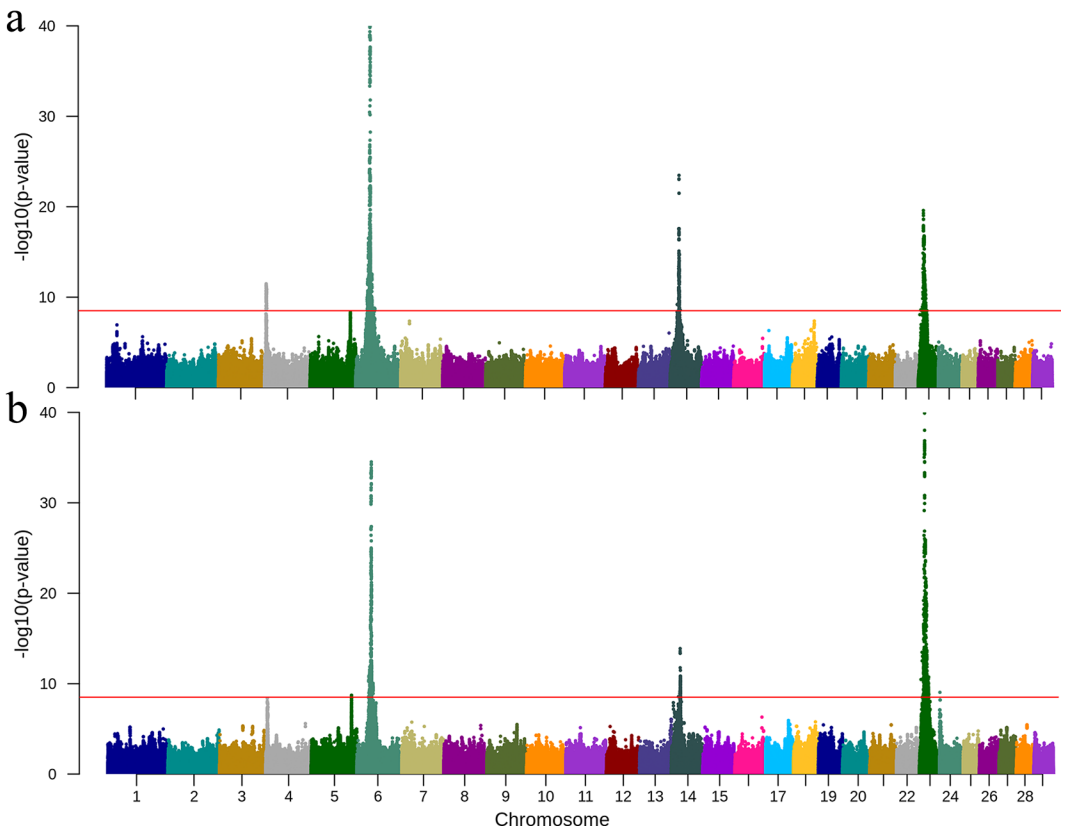

Figure 4. Manhattan plots for the association test for stillbirth in Nordic Red population: (a) stillbirth in first lactation; (b) stillbirth in later lactations. The Manhattan plot shows association test ($-\log_{10}$ *P*-value) on the y-axis against physical autosomal location on the x-axis. The standard genome-wide significance cutoff of $-\log_{10}$ *P*-value = 8.5 is shown by the horizontal red line.

and identified 128 genes above genome-wide significance (Bonferroni correction with *P*-value <1.92e-6, i.e., nominal type I error of 0.05, with total number of 26,071 gene tests). In addition to the nearest genes with biological support, we also found some additional genes with biological support (Supplemental Table S2; https://doi.org/10.6084/m9.figshare.23822067; Cai, 2023b).

On BTA4, we identified 2 candidate genes, *GRB10* and *IKZF1*. From MPD, we know that mutations in *GRB10* can cause complete penetrance preweaning lethality and incomplete penetrance perinatal lethality. Mutations in *IKZF1* can cause premature death and complete penetrance lethality. The records in MPD show that mutations in *NDUFA9* could cause complete penetrance embryonic lethality or complete penetrance preweaning lethality. Mutations in *FGF23* can cause premature death.

On BTA6, we detected a wide chromosomal region with several significant genes with biological support from MPD. Also considering the location of lead SNPs, *PPM1K*, *SPP1*, *LAP3*, and *MED28* could be candidate genes in addition to *LCORL*. The records from MPD show that mutations in *PPM1K* can cause incomplete penetrance postnatal lethality. The gene *SPP1* can be responsible for complete penetrance embryonic lethality between implantation and somite formation, and incomplete penetrance postnatal lethality in mouse mutation lines. Mutations in *LAP3* can cause premature death, complete penetrance neonatal lethality, complete penetrance perinatal lethality, incomplete penetrance perinatal lethality, and incomplete penetrance lethality throughout fetal growth and development. Mutation in *MED28* can be responsible for complete penetrant preweaning lethality in mice.

On BTA14, we identified 5 genes in addition to the nearest genes with support in MPD (Blake et al., 2011). The first is *TGS1*, which could play a role in complete penetrant embryonic lethality between implantation and somite formation. The second gene is *RPS20*, which can be involved in complete penetrant embryonic lethality and complete penetrant preweaning lethality. Then 2 genes, *MOS* and *PLAG1*, showed function in decreased litter size. Finally, mutation of *BPNT2* can cause complete penetrant neonatal lethality. On BTA23, we detected a wide range of significant genes. Combined with the locations of lead SNPs, 2

Table 2. Additional lead SNPs of chromosome 6 suggested by conditional and joint analysis in Nordic Red Dairy Cattle[1]

		Associated region		Lead SNPs					
Trait	BTA	Position (bp)	RS ID	MAF	Effect	*P*-value	Annotation	Nearest gene	LD
HP1	6	38074508	rs133476529	0.10	−2.90	7.04e-11	intergenic	*LCORL*	0.24
SBF	6	37569485	rs383507085	0.09	−7.92	4.18e-38	intergenic	*LCORL*	0.26
SBL	6	37776106	NA	0.21	−4.03	1.60e-26	intergenic	*LCORL*	0.26
	6	38328564	rs382691096	0.09	−3.00	1.48e-18	intergenic	*LCORL*	0.11
	23	23840749	rs382556458	0.05	−3.84	1.82e-11	intergenic	*ENSBTAG00000053324*	0

[1]HP1 = heifer survival in the period 2–30 d, SBF = stillbirth in first lactation, SBL = stillbirth in later lactations, RS ID = reference SNP cluster ID, NA = variants missing the for-reference SNP cluster ID, MAF = minor allele frequency, LD = linkage disequilibrium with the lead SNP in the QTL region.

genes could be listed as candidate genes, *KIF6* and *KCNK5*. Mutations in *KIF6* and *KCNK5* can cause premature death and incomplete penetrance prenatal lethality, respectively.

Variant Effect Prediction

To screen on the potential causal variants, we extracted all significant variants from each analysis and checked the variant annotation by VEP (McLaren et al., 2016). Within the list of 5,863 significant variants, we detected 18 variants with at least moderate VEP predicted consequence (Supplemental Table S3; https://doi.org/10.6084/m9.figshare.23822145; Cai, 2023c). We detected 2 frameshift variants with high impact: BTA4:5296018 in *GRB10*, and BTA6:37401770 (rs384548488) in *LCORL*. In addition to these 2 high-impact variants, we also detected 3 missense variants within *LCORL*. Among them, BTA6:36700131 is predicted to be a deleterious variant with low confidence. On BTA23, we identified 12 missense variants for various genes. Among these 12 variants, BTA23:17008631 (rs520865767) and BTA23:17158250 (rs378906559) are deleterious variants in *ABCC10* and *MAD2L1BP*, respectively. The records in MPD show that mutations in *MAD2L1BP* can cause complete penetrance neonatal lethality.

Detection of Lead SNP Homozygous Status in 1000 Bull Genomes Project Sequence Data

For the variants causing calf mortality, we expected the causal mutation has a recessive lethal effect. Therefore, we should not see individuals that are homozygous for the alternative allele (effect allele) or significantly lower in number than what is expected based on allele frequency. In addition to these criteria, the variant should have a negative effect on the phenotype. So, we estimated the distribution of reference allele in heterozygous and homozygous states. The causal variant should be segregating in RDC where the QTL was detected. A previous study in Nordic Holstein cattle did not report any QTL for YSS on BTA6 (Wu et al., 2017), and, therefore, no individual homozygous for alternative allele is expected. On BTA6, the following lead SNPs fulfilled the criteria: BTA6:36929541 (rs380367705), BTA6:37180233, BTA6:37369920 (rs714094684), and BTA6:38074508 (rs133476529). On other chromosomes, the following lead SNPs fulfilled the criteria: BTA 4:4890044 (rs447889413) and BTA 23:12228376 (rs379326817). Additionally, one of the high-impact variants, BTA4:5296018, also fulfills these criteria (Table 3).

DISCUSSION

The Nordic RDC population comprises 3 cattle populations from Denmark, Sweden, and Finland, and the population structure was earlier presented (Kadri et al., 2015). In this study, the phenotypes (DRP) were adjusted for effect of country, and we considered the population structure in the association analysis through a genomic relationship matrix, which can control false positive association results due to population structure and familial relatedness (Yu et al., 2006; Kadri et al., 2014a). We have identified several QTL for the YSS index and its component traits that have low heritability (range: 0.007–0.034; Pedersen et al., 2015). We used bulls' DRP with high reliability as a response variable in association analysis, and this may be reason to have power to identify QTL for such low heritability traits.

Putative Causal Genes and Variants Underlying the QTL

On BTA6, *LCORL* is associated with prenatal growth and adult height in humans and cattle (Pryce et al., 2011). Bongiorni et al. (2012) suggested that *LAP3* is the most probable gene to affect calving ease in Piedmontese cattle. In this study *LCORL*, *PPM1K*, *SSP1*, *MED28*, and *LAP3* emerged as positional candidate genes in RDC cattle and functionally supported by MPD (Blake et al., 2011) for the calf mortality.

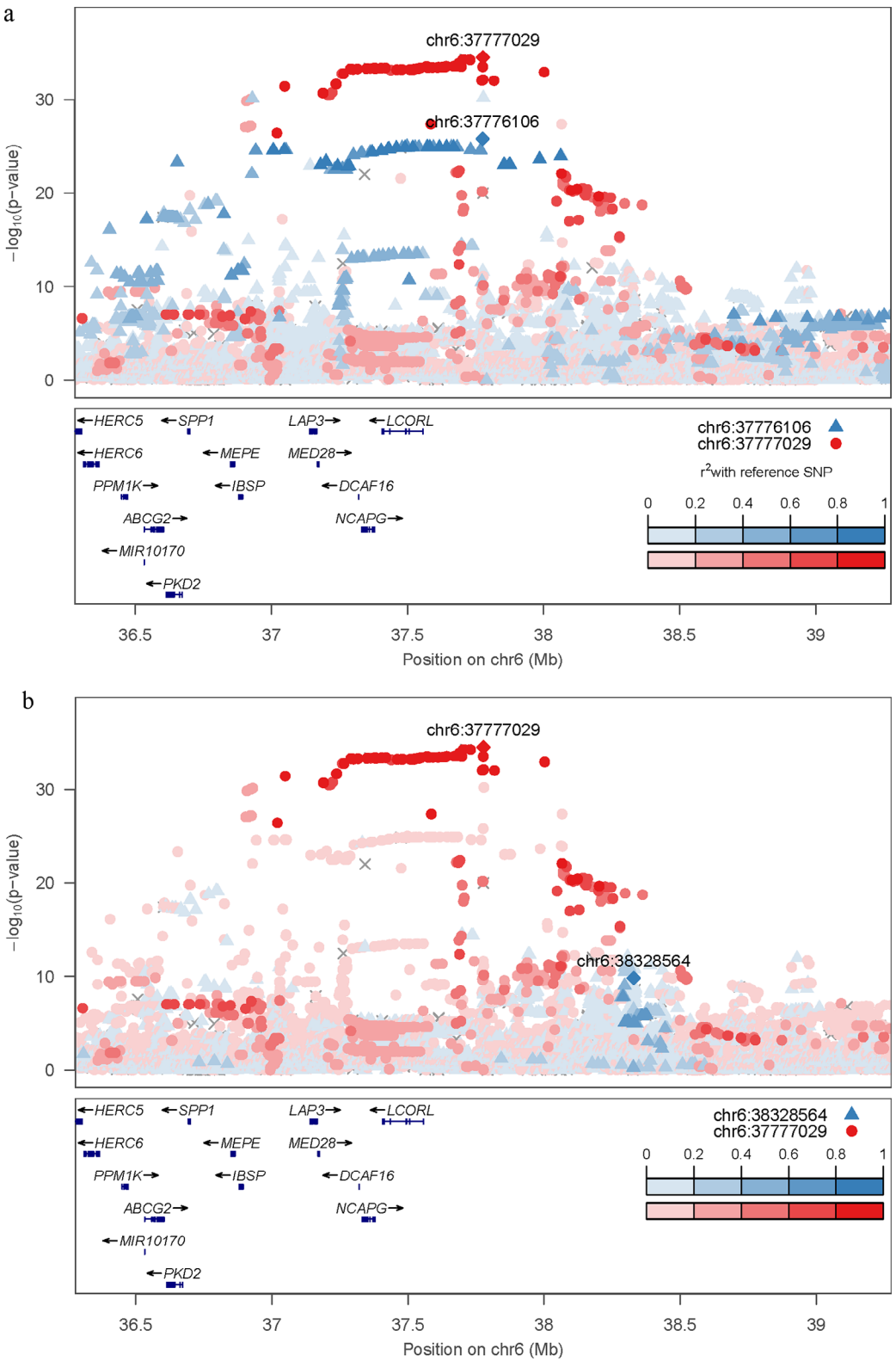

Figure 5. Locus zoom plot for the lead SNPs for stillbirth in later lactations in Nordic Red Dairy Cattle population. SNPs are shown by their position on the chromosome (chr) against their association ($-\log_{10}$ *P*-value) with stillbirth in later lactations in Nordic Red population. SNPs are colored to reflect their linkage disequilibrium with the top SNPs (6:3777729) with the reference population of Nordic Red Dairy Cattle from the 1000 Bull Genomes Project. (a) Locus zoom plot with 2 lead SNPs, 6: 37777029 and 6:37776106 for stillbirth in later lactations; (b) locus zoom plot with 2 lead SNPs, 6: 37777029 and 6:38328564 for stillbirth in later lactations.

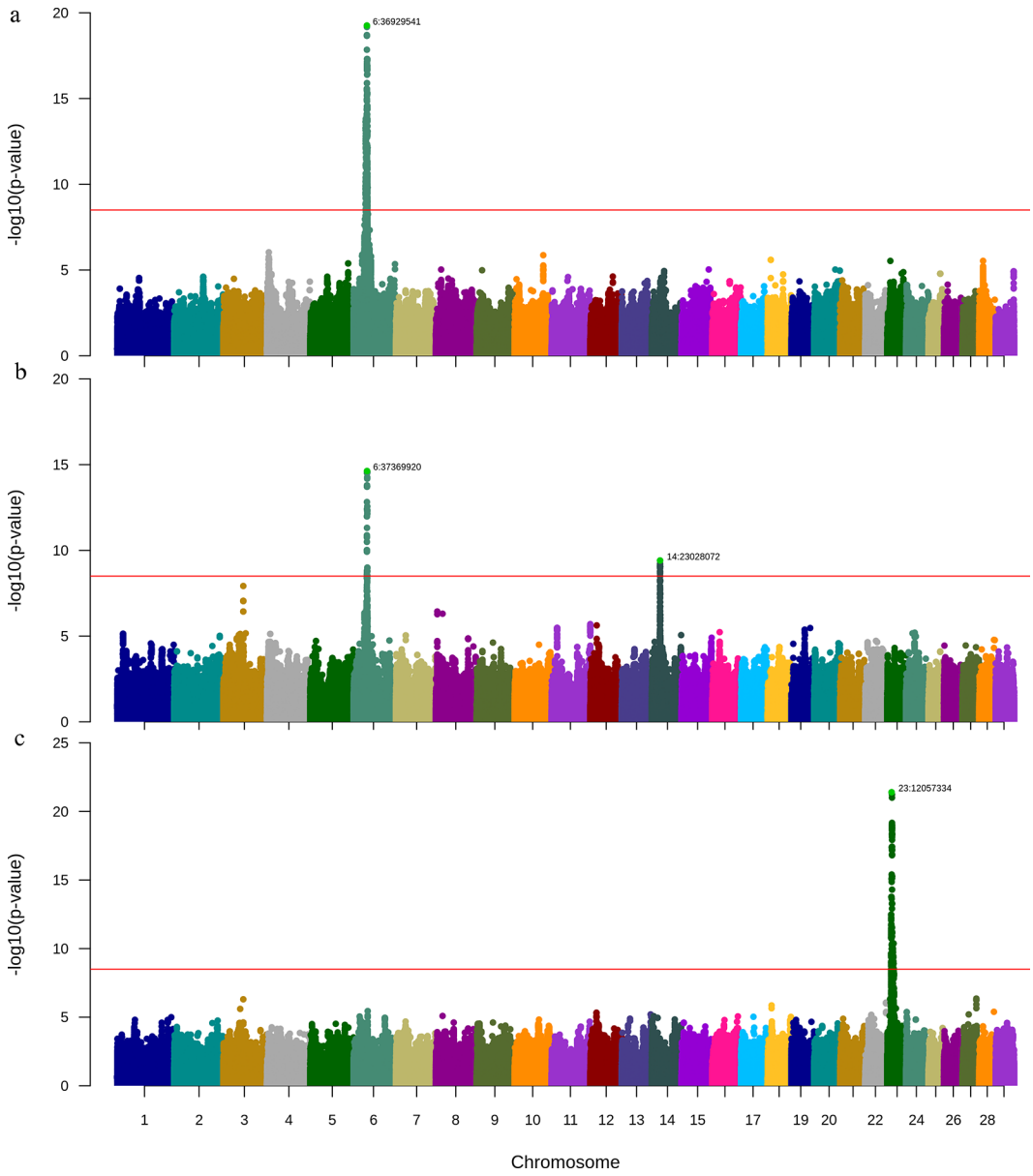

Figure 6. Manhattan plots for the multi-trait analysis between young stock survival index and stillbirth in Nordic Red Dairy Cattle population: (a) young stock survival; (b) stillbirth in first lactation; (c) stillbirth in later lactations. The Manhattan plot shows association test ($-\log_{10}$ *P*-value) on the y-axis against physical autosomal location on the x-axis. The standard genome-wide significance cutoff of $-\log_{10}$ *P*-value = 8.5 is shown by the horizontal red line. The lead SNPs are listed.

For component traits, besides the QTL on BTA6, we also proposed putative causal genes on for other QTL. On BTA4, *GRB10* is the putative causal gene suggested by gene-based analysis and VEP. *NDUFA9* and *FGF23* on BTA5 are putative causal genes suggested by gene-based analysis. Previous study has shown that a mutation in *NDUFA9* can cause neonatally fatal complex I disease (van den Bosch et al., 2012). *FGF23* encodes a bone-derived phosphaturic hormone, which is a useful biomarker for high-risk patients in chronic and acute disease (Schnedl et al., 2015). For stillbirth, we proposed *LYN* on BTA14 as the putative causal gene. *LYN* functions as a signaling intermediary in many cellular signaling processes regulating cell growth, differentiation, apoptosis, migration, immune responses, adhesion, and metabolism (Ingley, 2012). In addition, *KCNK5* on BTA23 is the putative causal gene for SBF based on biological support and the nearest gene crite-

Table 3. The distribution of 3 genotypes of the candidate SNPs in Nordic Red Dairy Cattle (RDC) and Nordic Holstein cattle (HOL)[1]

	Associated region		RDC			HOL		
BTA	Position (bp)	RS ID	AA	AB	BB	AA	AB	BB
4	4890044	rs447889413	3	31	76	0	1	71
4	5296018	NA	5	24	81	0	0	72
6	36929541	rs380367705	3	44	63	0	1	71
6	37180233	NA	1	31	78	0	0	72
6	37369920	rs714094684	2	30	78	0	0	72
6	38074508	rs133476529	3	10	97	0	3	69
23	12228376	rs379326817	2	16	92	0	5	67

[1]AA = homozygous for effect allele, AB = heterozygous, BB = homozygous for the alternative allele. RS ID = reference SNP cluster ID, NA = variants missing the for-reference SNP cluster ID.

ria. *KCNK5* has been repeatedly reported for association with Balkan endemic nephropathy (Toncheva et al., 2014; Reed et al., 2016).

QTL Regions

Calf survival plays an important economic role in cattle breeding and is genetically related to birth index (0.31), which describes a bull's offspring's genetic potential to be born easily and alive, body conformation (−0.30), and leg conformation (0.27; Pedersen et al., 2015). Therefore, we can hypothesize that some of these candidate genes will also affect birth index and conformation traits, which are well documented.

On Chromosome 4. The QTL was detected for YSS, HP2, and SBF. There are 2 independent lead SNPs, and both are intergenic variants. One nearest gene is a novel gene, whereas the other has *COBL* as the nearest gene. In addition, we detected BTA4: 5296018 as a frameshift variant of *GRB10*, which can cause complete penetrant preweaning lethality in mice (Cao et al., 2008). The LD (r) between this SNP with the lead SNP (BTA4:4890044) is only 0.34, and with BTA4:4078660, only 0.21. However, BTA4:5296018 fulfills the criteria of being a recessive lethal.

On Chromosome 5. The QTL on BTA5 was detected only in SBL. Even though the nearest gene is not known for any functional relation to the trait, *NDUFA9* and *FGF23*, which were suggested by gene analysis, could be candidate genes.

On Chromosome 6. The QTL on BTA6 is located around 38 Mb. Previous studies have reported QTL segregating at the same location for feet and leg disorders (Wu et al., 2016), service sire calving index, and body conformation index in RDC (Sahana et al., 2015). The QTL for body weights, calving ease (direct), and weaning weight (maternal) were reported segregating at the same location on BTA6 in 10 U.S. cattle breeds (Saatchi et al., 2014). In this study, this QTL has an effect on stillbirth and calf survival for growing period 1 and 2 for both bull and heifer calves. The QTL region from haplotype analysis was located within *FAM184B* found in Holstein, which is possibly related to feet and leg disorders in RDC (Wu et al., 2016). *FAM184B* is significant in the gene analysis (Supplemental Table S2).

Moreover, the COJO test of SNPs showed at least 2 independent QTL located around BTA6: 38 Mb. The lead SNP of SBL is in a gene desert; however, the upstream area has multiple putative causal genes. The other traits, namely YSS, BP1, HP1, BP2, HP2, and SBF, have lead SNPs embedded in the region of these putative causal genes.

On Chromosome 14. We found one QTL segregating on BTA14 around 23.1 Mb for both stillbirth traits. In this region, several genes are possible targets: *TGS1*, *LYN*, *RPS20*, *MOS*, *PLAG1*, and *BPNT2*. *PLAG1* has an association with early-life body weight, peripubertal body weight, and growth rate in Holstein-Friesian dairy calves (Littlejohn et al., 2012), and its genetic variation influences body weight gain and body size of cattle (Hoshiba et al., 2013). Nishimura et al. (2012) detected an intergenic region of *PLAG1-CHCHD7* for bovine stature in Japanese Black cattle.

On Chromosome 23. A QTL region around 13 Mbp on BTA23 had an effect on bull and heifer period 1 and stillbirth. Two groups of lead SNPs, located around 12 Mb and 13 Mb, are in high LD and close to the 0.5-Mbp deletion for stillbirth (Sahana et al., 2016). Mesbah-Uddin et al. (2018) identified a structural variant, ∼525 KB deletion overlapping *BTBD9*, *GLO1*, and *DNAH8*, causing stillbirth in Nordic Red Cattle. A QTL at the same chromosomal location was detected previously for calving index, calving ease, and stillbirth in Danish and Swedish Holsteins (Höglund et al., 2012).

On Chromosome 24. The QTL on BTA24 was detected only for SBL and was not linked to any gene with biological support.

Different Lead SNPs for the YSS Index, Its Components Traits, and Stillbirth

It is notable that the QTL on BTA6 have effects on all the analyzed survival traits. The lead SNP was BTA6:36929541 (rs380367705) for the YSS index, BP1, BP2, HP2, and SBF; BTA6:37180233 for HP1, and BTA6:37777029 (rs109270787) for SBL. The squared correlation estimates for genotype dosages between these lead SNPs were low. Moreover, the COJO test also suggested at least 2 independent QTL segregating for HP1, SBF, and SBL. Therefore, we concluded that these traits were affected by more than one causal mutation within the BTA6 QTL region.

Similar to Nordic Holsteins (Wu et al., 2017), the association patterns between SNP and component traits were similar in RDC bulls and heifers, but different in growing periods 1 and 2. This is consistent with the phenotypic correlations (DRP), which display higher correlation across sex than across period. This indicated that there is no difference for identified mutations or genes on autosome control calf survival for sex, but a difference does exist for growing periods.

In addition to the QTL on BTA6, some extra QTL regions were detected for stillbirth on BTA5, BTA14, and BTA24. The multi-trait analysis did not reveal any new loci, suggesting that, except for BTA6, YSS and stillbirth have different major causal genes. Therefore, this might indicate that the QTL on these regions have main effects on stillbirth, but if the calves survive 24 h after birth and died later, they are registered as young stock mortality. Although we have listed several candidate genes and putative candidate variants, the causal mutations need to be identified for effective elimination from the population. One way to follow up these candidate mutations is to add them to a custom SNP array used for routine genotyping. This way, large numbers of genotypes for targeted variants could be generated. The animals homozygous for the deleterious allele could be followed to register phenotypes related to survival.

CONCLUSIONS

We performed a genome-wide association study in the Nordic Red Dairy Cattle population and have identified at least 2 independent QTL segregating in a very close region to the YSS index on BTA6. The same pattern is not unique for the YSS index but also appears in its components traits and stillbirth. By conducting post-GWAS, we proposed *LCORL*, *PPM1K*, *SSP1*, *MED28*, and *LAP3* on BTA6, *GRB10* on BTA4, *NDUFA9* and *FGF23* on BTA5, *LYN* on BTA14, and *KCNK5* on BTA23 as the putative causal genes for calf mortality in Nordic Red Dairy Cattle. One frame shift variant located within *LCORL* and another frame shift variant located within *GBR10* could be the putative causal variants.

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