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ORIGINAL ARTICLE

A genome-wide association study for mastitis resistance in phenotypically well-characterized Holstein dairy cattle using a selective genotyping approach

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Abstract

A decrease in the incidence of bovine mastitis, the costliest disease in the dairy industry, can be facilitated through genetic marker-assisted selective breeding programs. Identification of genomic variants associated with mastitis resistance is an ongoing endeavor for which genome-wide association studies (GWAS) using high-density arrays provide a valuable tool. We identified single nucleotide polymorphisms (SNPs) in Holstein dairy cattle associated with mastitis resistance in a GWAS by using a high-density SNP array. Mastitis-resistant (15) and mastitis-susceptible (28) phenotypic extremes were identified from 224 lactating dairy cows on commercial dairy farm located in Utah based on multiple criteria of mastitis resistance over an 8-month period. Twenty-seven quantitative trait loci (QTLs) for mastitis resistance were identified based on 117 SNPs suggestive of genome-wide significance for mastitis resistance ($p \leq 1 \times 10^{-4}$), including 10 novel QTLs. Seventeen QTLs overlapped previously reported QTLs of traits relevant to mastitis, including four QTLs for teat length. One QTL includes the RAS guanyl-releasing protein 1 gene (*RASGRP1*), a candidate gene for mastitis resistance. This GWAS identifies 117 candidate SNPs and 27 QTLs for mastitis resistance using a selective genotyping approach, including 10 novel QTLs. Based on overlap with previously identified QTLs, teat length appears to be an important trait in mastitis resistance. *RASGRP1*, overlapped by one QTL, is a candidate gene for mastitis resistance.

Keywords Genome-wide association study · Bovine mastitis resistance · Selective genotyping · Cattle

Jacqueline P. Kurz and Zhou Yang contributed equally to this work.

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Introduction

Mastitis, defined as inflammation of the mammary gland, is the costliest disease in the dairy industry (Kaneene and Scott Hurd 1990; Seegers et al. 2003). In the USA, the cost of bovine mastitis is estimated at a value of approximately 10% of total milk sales (Nash et al. 2000). Associated costs include loss of milk production, decreased milk quality, discarded milk, labor, veterinary treatments, mastitis-related culls, diagnostics, and preventative measures (Halasa et al. 2007).

Conventional methods to reduce the incidence of mastitis within a herd encompass both management practices and selection for mastitis-resistant phenotypes. Recent technical advancement in cattle genomics, such as genome-wide association studies (GWAS), has led to the identification of quantitative trait loci (QTLs) associated with mastitis traits (Holmbeg and Andersson-Eklund 2004; Meredith et al. 2013; Tiezzil et al. 2015). Genetic marker-assisted selection for mastitis traits provides a valuable tool for decreasing mastitis incidence, as it leads to a higher level of discrimination between phenotypes and a greater uniformity than does conventional selection (Kühn et al. 2008). Genome-wide association studies are well-suited to identifying genetic markers of complex traits such as mastitis, enabling genotyping of large numbers of potential genetic markers, such single nucleotide polymorphisms (SNPs), across the genome (Ziegler et al. 2008; Bush and Moore 2012). Indeed, GWAS carried out over the past several years have identified genetic markers, candidate genes, and QTLs for individual mastitis traits such as somatic cell count (SCC), somatic cells score (SCS) and clinical mastitis (Sodeland et al. 2011; Meredith et al. 2013; Wu et al. 2015). Many of these studies use low- or medium-marker density arrays to detect genetic markers (Sodeland et al. 2011; Sahana et al. 2013; Tiezzil et al. 2015). High-density bovine arrays capable of genotyping close to one million SNPs are available for cattle and offer the advantage of increased genomic coverage and statistical power (Wu et al. 2015). Studies using such high-density arrays have the potential to identify novel genetic markers as well as verify the significance of previously-identified markers.

In this study, we performed a GWAS using a high-density array to identify SNP genetic markers and define QTLs of mastitis resistance in Holstein dairy cows. We used a selective genotyping approach, identifying the most mastitis-resistant and mastitis-susceptible animals within the sample population. This approach facilitated detection of causative alleles due to an enrichment effect of these alleles among phenotypically extreme individuals (Guey et al. 2011). Phenotypic characterization was based on multiple criteria of intramammary infection status in order to achieve more accurate characterization of phenotypic extremes of mastitis resistance and mastitis susceptibility than could be achieved with use of a single measure of mastitis alone.

Methods

Selection of phenotypically extreme cattle

Cattle used in the study were adult lactating Holstein cattle from a single farm, and phenotypically extreme individuals of mastitis resistance and mastitis susceptibility were identified and selected for genotyping. Phenotypic characterization was based on a combination of milk bacterial culture, observation for clinical mastitis, and SCC evaluation over an eight-month period. Subclinical mastitis was defined as cases in which intramammary infection was detected by bacterial culture of milk but no changes were detected in the appearance of the mammary gland or milk. Clinical mastitis was defined as intramammary infection accompanied by clinically detectable inflammatory changes in the mammary gland and/or changes in the consistency or color of the milk.

To detect clinical and subclinical mastitis, bacterial cultures were performed by using aseptically collected milk samples. During one milking per month, composite milk samples were collected from all lactating cows that had no evidence of clinical mastitis. Clinical mastitis was monitored during that milking and, additionally, at bi-monthly evaluations specifically for clinical mastitis during another milking each month. Clinical mastitis examination was carried out by veterinarians and assistants trained by veterinarians along with continuous monitoring by farm personnel. Clinical veterinary examinations consisted of careful visual and tactile inspection of all mammary gland quarters for alterations in color, consistency, and temperature, and visual inspection of milk from all quarters for alterations in color or consistency. When mammary gland or milk abnormality (clinical mastitis) was detected during the monthly sampling of all cows, at the bi-monthly clinical mastitis examinations, or by farm personnel at any other milking time, milk samples were collected from affected quarters for bacterial culture. A composite sample was also collected from the remaining unaffected quarters. Milk microbial culture was carried out according to the guidelines outlined by the National Mastitis Council (1999). Isolation of at least one bacterial colony from a 0.01-ml inoculum of a single quarter or composite milk culture sample was considered sufficient to diagnose intramammary infection, as proposed for individual quarter samples by the Mastitis Research Workers (Dohoo et al. 2011). Composite milk sample cultures have sensitivity of 72%, specificity of 81% (Souza et al. 2016), and positive and negative predictive values of 88.2 to 100% (Reyher and Dohoo 2011) for most mastitis pathogens, when individual quarter samples are considered a "gold standard." Monthly SCCs (< 250,000 cells/ml) were used as supplementary evidence for the absence of intramammary infection in animals from which no bacteria were isolated from milk samples and no clinical mastitis was detected. Monthly SCC measures were obtained from the Dairy Herd Improvement Association.

Criteria for classification as mastitis-resistant included an absence of clinical mastitis, an absence of bacteria cultured from composite milk samples throughout the 8-month period, and consistently low SCCs (< 250,000 cells/ml). The criterion for classification as mastitis-susceptible was the detection of at least four cases of mastitis. Cases of mastitis were defined by isolation of one or more mastitis pathogens from a composite or individual quarter milk sample and/or detection of clinical mastitis. Isolates from more than one quarter on one date could contribute as many mastitis cases as there were culture-positive quarters. Clinical mastitis detected in more than one quarter on one date contributed as many mastitis cases as there were clinically mastitic quarters. All three methods of mastitis detection were applied to all cows in the study. Animals were classified as mastitis-resistant only if no indications of mastitis by any of the three methods were observed, while animals were classified as mastitis-susceptible if four cases of mastitis were detected clinically or by milk bacterial culture alone or in combination.

DNA isolation

Genomic DNA of cows characterized as mastitis-resistant or mastitis-susceptible was isolated from ear notches or hair follicles. Isolation and purification of DNA was carried out using the Gentra Puregene Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

SNP genotyping

Genotype calling was carried out by the Core Facility at the University of Utah for SNP genotyping using the Illumina BovineHD BeadChip (part no. WG-450-1002; Illumina Inc., San Diego, CA), an array with 777,962 SNPs that uniformly span the entire bovine genome. Bead chips were processed according to the Infinium protocol from Illumina, and scanning was carried out by the iScan scanner (Illumina Inc., San Diego, CA). Quality control measures included removal of animals with low call rates (< 96%), SNPs with low call rates (< 0.95), and SNPs with low minor allele frequencies (< 5%). After quality control and allele frequency filtering, a total of 585,949 SNPs were used for association testing.

Statistical analysis

Significant associations between SNPs and mastitis resistance were detected using a single locus mixed model approach as implemented by the SNP and Variation Suite software (SVS version 8.4, Golden Helix, Bozeman, MT). Familial relatedness was corrected for as a random effect by incorporation of a genomic best linear unbiased prediction (gBLUP) kinship matrix (Clark and van der Werf 2013) into the model, constructed from genome-wide SNPs after pruning for linkage disequilibrium (LD). Genome-wide association mapping used a mixed linear model analysis (Segura et al. 2012) based on the gBLUP matrix to correct for cryptic relatedness, with mastitis resistance/susceptibility coded as a binary phenotype. A genome-wide suggestive threshold was set at an uncorrected p value of $p \leq 1 \times 10^{-4}$, with $p \leq 1 \times 10^{-3}$ considered nominal. A genome-wide significance threshold was set at an uncorrected p value of $p \leq 7.65 \times 10^{-7}$ ($-\log_{10}[p \text{ value}] \geq 6.12$), determined empirically using the *simpleM* method (Gao et al. 2010) to calculate the effective number of independent tests (= 65,386) after adjusting for linkage disequilibrium.

Defining QTLs

Quantitative trait loci were defined as described previously (Meredith et al. 2013). A QTL surrounding each SNP detected as significant ($p \leq 1 \times 10^{-4}$) was defined based on local LD structure. Pairwise LD between the target SNP and all individual genotyped SNPs within 1 Mb upstream and downstream was calculated using PLINK (Purcell et al. 2007). Within this region, visualized using the *ggplot* function of the R Studio statistical package (R Core Team 2016), the furthest upstream and downstream SNPs in strong LD with the target SNP ($r^2 \geq 0.8$) were used to define QTLs. Quantitative trait loci comprised of a single SNP only were excluded. Overlapping QTLs were combined into a single QTL, defined by the furthest upstream and downstream SNPs for the combined region. Once defined, QTLs were used to query the bovine genome (Bos_taurus_3.1.1/bosTau8 assembly (Zimin et al. 2009)) using the University of California Santa Cruz Genome Browser tool (https://genome.ucsc.edu/) to identify genes overlapping these regions. These QTLs were checked for overlap with known bovine QTLs using the cattle QTL database (http://animalgenome.org/cgi-bin/QTLdb/BT/index) as of April 2017 (Hu et al. 2013).

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Sample population

Among cows in a commercial dairy herd of 224 lactating Holstein cows, 15 animals were characterized as mastitis-resistant (Table 1) and 28 animals as mastitis-susceptible (Table 2). All mastitis-susceptible cows with the exception of one had four confirmed cases of mastitis. One cow had three cases of mastitis confirmed by isolation of three separate pathogens, and one tentative case where sample contamination precluded definitive pathogen isolation. Cattle within the

Table 1 Lactation number, somatic cell count (SCC), clinical mastitis, and mastitis pathogens isolated from mastitis-resistant cows. Average SCC is calculated from monthly composite SCC measured over 8 months

Lactation number	Average SCC, cells/ml	SCC range, cells/ml	Clinical mastitis cases	Pathogens isolated (number of times)
2	25,000	16,000–33,000	0	None
6	58,000	32,000–84,000	0	None
5	85,000	22,000–155,000	0	None
4	48,000	21,000–125,000	0	None
4	31,000	16,000–50,000	0	None
3	86,000	20,000–150,000	0	None
3	70,000	21,000–123,000	0	None
3	71,000	19,000–107,000	0	None
3	41,000	5000–61,000	0	None
3	21,000	9000–47,000	0	None
2	24,000	20,000–29,000	0	None
2	100,000	35,000–167,000	0	None
2	15,000	5000–27,000	0	None
2	34,000	9000–69,000	0	None
2	123,000	30,000–220,000	0	None

mastitis-resistant group ranged from second to sixth lactation, and cattle within the mastitis-susceptible group ranged from first to sixth lactation. Among the mastitis-resistant group, individual composite milk SCCs over the 8-month period ranged from 5000 to 220,000 cells/ml, with an average of 56,300 cells/ml. Among the mastitis-susceptible group, the number of clinical mastitis cases ranged from none to three. Individual quarter and composite milk SCCs ranged from 6000 to 2,676,000 cells/ml, with an average of 303,000 cells/ml. Commonly isolated bacterial species from composite and individual quarter milk samples from mastitis-susceptible cattle included coagulase-negative staphylococci, *Streptococcus* sp., *Corynebacterium* sp., and *Escherichia coli* (Table 2). Among cows classified as mastitis-susceptible, all individual clinical mastitis cases occurred within a single quarter on a given date; there were no instances of two different quarters with clinical mastitis at the same time.

Genome-wide associations

In order to identify SNP genetic markers and QTLs of mastitis resistance, we carried out a GWAS using a selective genotyping approach on the 15 mastitis-resistant cows and the 28 mastitis-susceptible cows.

Following data quality control measures, 585,949 SNPs remained for association testing using data from 43 animals (28 mastitis-susceptible, 15 mastitis-resistant), represented in Fig. 1a as a Manhattan plot. Based on deviation from a linear relationship between observed and expected p values, as illustrated in Fig. 1b in a quantile-quantile plot, a p value threshold of $p \leq 1 \times 10^{-4}$ was set as suggestive of genome-wide significance and a p value of $p > 1 \times 10^{-4}$, $\leq 1 \times 10^{-3}$ was considered nominal for association. One SNP on chromosome 7, rs43503386 ($-\log_{10}[p \text{ value}] = 6.33$), exceeded the genome-wide significant threshold of $p \leq 7.65 \times 10^{-7}$ ($-\log_{10}[p \text{ value}] \geq 6.12$), and 116 SNPs were suggestive of genome-wide significance (Table 3).

Based on the 116 SNPs suggestive of genome-wide significance, we identified 27 QTLs of mastitis resistance, distributed across 14 chromosomes (2, 3, 7, 8, 10, 11, 12, 15, 16, 17, 18, 26, 27, and 28) and overlapping a total of 29 genes (Table 4). Of these QTLs, 10 have not been reported previously. Although SNP rs43503386 exceeded the genome-wide significance threshold, it was not in strong LD (r^2 was < 0.8) with any other genotyped SNPs in this study and was therefore not considered in the QTL analysis. This SNP is located 72 kb upstream of the casein kinase 1 gamma 3 (*CSNK1G3*) gene, involved in post-translational processing of milk casein and other acidic proteins (Buitenhuis et al. 2016).

The three QTLs most highly suggestive of genome-wide significance ($-\log10(p \text{ value}) \geq 5.41$) are located on *Bos taurus* autosome (BTA) 26 and overlap the sortilin-related VSP10 domain containing receptor 3 (*SORCS3*) gene as well as a previously identified QTL for teat length. The *SORCS3* gene has no known function in bovine mastitis. Another QTL suggestive of genome-wide significance overlaps the RAS guanyl-releasing protein 1 (*RASGRP1*) gene, a candidate gene for mastitis resistance. Seven hundred sixty-three SNPs were nominal for genome-wide significance (Online Resource 1), distributed across all autosomal and the X chromosome.

Seventeen of the QTLs we identified overlap with previously identified QTLs of mastitis traits (somatic cell score, SCC, and clinical mastitis) and/or udder conformation traits (teat length, teat number, udder attachment, and udder depth; Table 5). Our findings reinforce the discovery of these 17

Table 2 Lactation number, somatic cell count (SCC), clinical mastitis, and mastitis pathogens isolated from mastitis-susceptible cows. Average SCC is calculated from monthly composite SCC measured over 8 months. Each case of clinical mastitis listed occurred in a single mammary gland quarter on a given date

Lactation number	Average SCC, cells/ml	SCC range, cells/ml	Clinical mastitis cases	Pathogens isolated (number of times)
2	520,000	21,000–2,406,000	1	CNS (2); CNS and St (2)
6	218,000	146,000–1,327,000	0	CNS (1); St (2): CNS and St (3)
4	136,000	88,000–197,000	0	CNS (1); C (1); Y (1)
4	617,000	8000–1,464,000	3	St (2); CNS and St (1); C and St (3)
3	298,000	120,000–622,000	0	CNS (4); CNS and St (2)
4	477,000	86,000–1,899,000	0	CNS (5); CNS and E (1)
4	391,000	210,000–868,000	2	CNS (2); CNS and St (3)
3	463,000	270,000–591,000	2	St (6)
3	1,293,000	401,000–2,945,000	0	CNS (2); CNS and St (4); E and St (1)
3	144,000	26,000–332,000	0	St (4)
2	49,000	16,000–84,000	0	CNS (5); CNS and C (1)
2	705,000	6000–1,605,000	0	CNS (2); St (5)
2	154,000	14,000–937,000	0	CNS (1); CNS and St (2); C (1)
2	259,000	9000–981,000	3	CNS and St (1); St (1); C (2); E (1)
2	20,000	10,000–30,000	0	CNS (2); C and St (1); C (1)
2	81,000	10,000–248,000	1	CNS (1); CNS and St (2); CNS and C (1)
1	34,000	7000–58,000	0	CNS (2); CNS and St (2); CNS and C (1)
1	109,000	55,000–191,000	0	CNS (5)
1	60,000	48,000–88,000	0	CNS (4); E (1)
1	113,000	76,000–139,000	0	CNS (6)
1	32,000	17,000–58,000	0	CNS (4)
1	97,000	55,000–238,000	0	CNS (5)
1	129,000	79,000–287,000	0	CNS (4)
2	615,000	13,000–2,676,000	1	CNS (4); Y (1)
1	43,000	27,000–74,000	0	CNS (3); St (1)
1	197,000	35,000–568,000	0	CNS (5); CNS and St (1)
1	18,000	7000–33,000	0	CNS (3); C (2)
1	115,000	60,000–175,000	1	CNS (2); St (2); CNS and St (1)

C, *Corynebacterium* sp.; *CNS*, coagulase-negative staphylococci; *E*, *Escherichia coli*; *St*, *Streptococcus* sp.; *Y*, yeast

QTLs and provide supporting evidence that these QTLs may influence mastitis resistance. The top three QTLs overlap with a known QTL for teat length, which may provide the basis of mastitis resistance at these regions.

Discussion

We carried out a GWAS using a selective genotyping approach and a high-density bovine SNP array and identified 117 SNPs

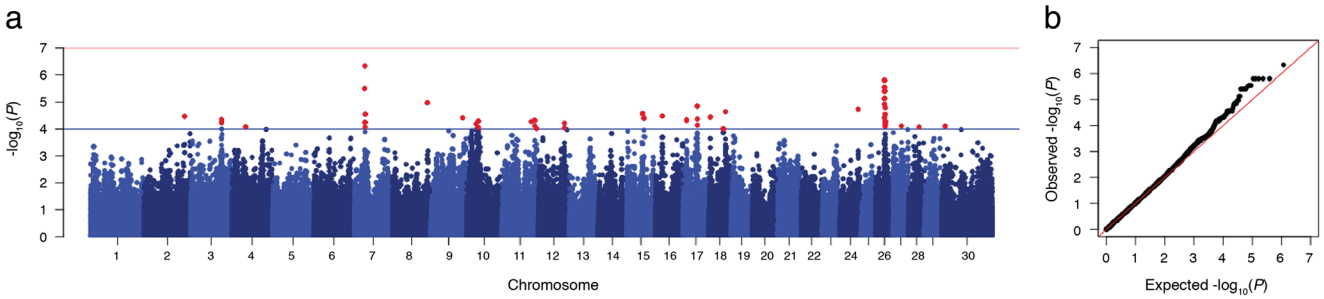

Fig. 1 **a** The genome-wide significance threshold is indicated by the solid line ($p < 0.0001$). Bovine chromosome position is shown on the *x*-axis. Strength of association for a single-locus mixed model GWAS is shown on the *y*-axis. Manhattan plot of genome-wide associations for mastitis resistance in 43 Holstein cows. **b** Quantile-quantile plot of observed and expected *p* values

Table 3 Single nucleotide polymorphisms suggestive for genome-wide significance, with *p* values and the allele associated with mastitis resistance

Marker	Chr	Position	−log10(*p* value)	Protective allele
rs43503386	7	31,648,926	6.331487664	A
rs110130285	26	26,080,988	5.805640748	G
rs110925919	26	26,081,853	5.805640748	T
rs135137805	26	26,083,915	5.805640748	C
rs109051904	26	26,085,037	5.805640748	A
rs134424973	26	26,086,114	5.805640748	G
rs136355517	26	26,202,415	5.551344073	A
rs137057269	26	26,207,987	5.551344073	G
rs109151150	7	31,002,352	5.5056798	T
rs135679846	26	26,213,600	5.416984055	C
rs136832332	26	26,214,187	5.416984055	T
rs135349914	26	26,216,213	5.416984055	C
rs135745332	26	26,170,699	5.410961768	C
rs29026516	26	26,171,235	5.410961768	G
rs133973225	26	26,190,210	5.410961768	A
rs42094305	26	26,078,080	5.135109749	C
rs42094275	26	26,097,110	5.135109749	C
rs110448143	8	103,092,247	4.98000868	G
rs110566862	8	103,096,670	4.98000868	T
rs134258818	26	26,093,838	4.919312092	T
rs109674792	17	41,771,455	4.85167801	G
rs110306521	17	41,773,340	4.85167801	A
rs109747092	17	41,775,569	4.85167801	C
rs110239244	17	41,777,130	4.85167801	C
rs109757388	17	41,785,932	4.85167801	G
rs41837662	26	28,202,019	4.798327141	T
rs109555679	24	53,848,687	4.732826672	C
rs41257394	18	49,690,172	4.643951252	A
rs110711227	15	47,742,405	4.567526846	A
rs109993951	15	47,747,052	4.567526846	A
rs137210653	15	47,752,356	4.567526846	C
rs109366311	15	47,769,743	4.567526846	C
rs110973322	15	47,771,595	4.567526846	A
rs110039012	15	47,774,554	4.567526846	C
rs110259421	15	47,775,426	4.567526846	C
rs136099077	7	32,661,575	4.553826908	G
rs133992636	7	32,662,549	4.553826908	G
rs135340284	7	32,667,624	4.553826908	A
rs29016545	7	32,677,080	4.553826908	T
rs134516100	7	32,678,638	4.553826908	T
rs41836660	26	28,154,738	4.549234149	C
rs41604819	26	28,155,692	4.549234149	T
rs41837669	26	28,204,944	4.549234149	G
rs133596831	16	20,614,247	4.478349849	C
rs133973886	2	118,870,124	4.469694252	T
rs41858359	18	5,268,101	4.44398692	T
rs41858365	18	5,268,998	4.44398692	T
rs109361888	26	26,164,774	4.416956728	T
rs135248266	9	93,691,403	4.416181677	C

Table 3 (continued)

Marker	Chr	Position	−log10(*p* value)	Protective allele
rs135549815	15	51,302,501	4.403968435	C
rs136596564	15	51,303,719	4.403968435	C
rs110825365	17	41,777,826	4.37209264	A
rs137547715	3	91,427,052	4.351966718	T
rs136877205	17	10,393,778	4.345367428	T
rs110090917	11	96,629,841	4.323034288	T
rs381266606	11	96,715,963	4.323034288	T
rs136634740	11	96,720,014	4.323034288	G
rs133879444	11	96,730,359	4.323034288	T
rs134973228	11	96,734,171	4.323034288	T
rs132794203	11	96,739,382	4.323034288	G
rs134297845	11	96,745,000	4.323034288	G
rs109277843	11	96,758,826	4.323034288	C
rs135608670	11	96,767,134	4.323034288	T
rs134694194	17	10,162,372	4.308948211	T
rs109623385	10	34,452,835	4.294182751	A
rs109758936	10	34,455,599	4.294182751	T
rs133303871	11	86,660,988	4.278051212	T
rs42434953	26	28,796,634	4.276743982	T
rs42434958	26	28,799,734	4.276743982	A
rs42434984	26	28,813,937	4.276743982	A
rs42349819	3	91,442,018	4.271571041	A
rs42349795	3	91,449,020	4.271571041	A
rs109782486	7	31,997,138	4.247740682	C
rs109397365	7	31,999,677	4.247740682	T
rs135897745	7	32,000,505	4.247740682	T
rs109305062	7	32,001,031	4.247740682	C
rs135287427	7	32,001,859	4.247740682	A
rs132918628	7	32,002,610	4.247740682	C
rs109653519	7	32,003,545	4.247740682	T
rs109153790	7	32,004,528	4.247740682	C
rs133045718	7	32,005,033	4.247740682	T
rs137193453	7	32,005,542	4.247740682	G
rs133716861	3	91,429,028	4.225508945	A
rs42704013	12	76,820,530	4.217182537	A
rs136350185	10	27,915,567	4.188020335	A
rs41840890	26	27,751,543	4.169793482	G
rs41840882	26	27,754,542	4.169793482	T
rs41840873	26	27,756,172	4.169793482	G
rs41840864	26	27,762,180	4.169793482	A
rs137165178	26	27,763,096	4.169793482	T
rs41840922	26	27,779,611	4.169793482	A
rs41840912	26	27,790,973	4.169793482	G
rs135563166	26	28,157,430	4.16362472	G
rs41636626	26	28,159,060	4.16362472	C
rs133999463	26	28,159,800	4.16362472	C
rs133395250	26	28,161,303	4.16362472	G
rs135413917	26	28,161,892	4.16362472	G
rs133282066	26	28,163,345	4.16362472	A

Table 3 (continued)

Marker	Chr	Position	−log10(*p* value)	Protective allele
rs135170589	26	28,165,109	4.16362472	T
rs136506930	26	28,165,749	4.16362472	G
rs134913097	26	28,167,337	4.16362472	A
rs137741079	26	28,168,568	4.16362472	C
rs133840132	26	28,170,114	4.16362472	A
rs135204195	26	28,172,302	4.16362472	A
rs133679609	17	42,208,979	4.138105354	C
rs41645946	11	96,814,663	4.115264771	C
rs133086162	27	24,405,764	4.108112274	C
rs132797061	X	10,123,521	4.101908826	T
rs110373429	26	27,935,893	4.095697106	C
rs110554155	4	41,207,992	4.081935285	T
rs43506093	7	32,187,800	4.07375602	C
rs134956968	28	30,879,841	4.07061418	G
rs110413607	10	34,258,059	4.055136308	T
rs41668080	12	76,870,470	4.04682745	T
rs110442181	11	102,314,941	4.015306852	C
rs41879775	18	43,568,128	4.003578508	G
rs135753929	18	43,596,859	4.003578508	A

Chr chromosome

suggestive of genome-wide association for mastitis resistance in Holstein dairy cattle. Based on these 117 SNPs, we identified 27 QTLs of mastitis resistance, including 10 novel QTLs.

The *RASGRP1* gene is located within a QTL we identified on BTA10, defined by eight SNPs genotyped in our GWAS. The *RASGRP1* gene is involved in the regulation of lymphocyte development, activation, and function and in T cell receptor signaling (Bonnefont et al. 2011). Differential expression of *RASGRP1* as a result of pathogen challenge occurs in primary bMECs (Brand et al. 2011) and in ovine milk somatic cells (Bonnefont et al. 2011), indicating a potential role in mastitis in ruminants. Overlap of the *RASGRP1* gene by one of the QTLs indicates this gene as a strong candidate for mastitis resistance, warranting further investigation.

In dairy cattle, both immune functions and udder conformation traits are recognized factors affecting mastitis resistance (Ashwell et al. 2005). Udder attachment and udder depth have been associated previously with SCC and clinical mastitis (Seykora and McDaniel 1986; Rupp and Boichard 2003). Teat placement has been associated with SCC (Seykora and McDaniel 1986), and various studies show conflicting results of the association between teat length and SCC and clinical mastitis (Detilleux 2002). The presence of supernumerary teats is considered a risk factor in bovine mastitis, and their surgical removal at an early age may have a protective effect against subclinical mastitis in heifers (Santman-Berends et al. 2012). Seventeen QTLs that overlap with previously identified QTLs of udder conformation traits (teat length, teat number, udder attachment, and udder depth) as well as mastitis traits (somatic cell score, SCC, and clinical mastitis) were identified in this study. Overall, 11 of these 17 QTLs overlap with QTLs for mastitis traits, and 13 overlap with QTLs for udder conformation traits.

Ten of these 17 QTLs overlap with previously identified QTLs for teat length. Six of these, including the top three where the strongest association signals were detected overall, are located on BTA26 and overlap with a single previously identified QTL for teat length (Ashwell et al. 2005). The remaining overlap with QTLs for teat length on BTA16 (Ashwell et al. 2005), BTA18 (Schnabel et al. 2005), and BTA10 (Schnabel et al. 2005). This finding provides strong supportive evidence for an effect of teat length on bovine mastitis resistance, highlighting the importance of udder conformation traits as factors in the pathogenesis of this disease.

In this study, multiple measures were used to determine intramammary infection status over time and identify mastitis-resistant and mastitis-susceptible phenotypic extremes. The effectiveness of selection for mastitis resistance increases when more than a single trait is measured for determination of intramammary infection status. For example, the use of SCC and clinical mastitis together is approximately 20% more effective than the use of either of these traits alone in selecting for mastitis resistance (Philipsson et al. 1995; Odegård et al. 2003). The use of multiple measures to detect mastitis helps to overcome limitations of any one method. For example, patterns of bacterial shedding in milk during the

Table 4 Quantitative trait loci identified for bovine mastitis resistance, with overlapped genes

Chr	QTL start	QTL end	QTL length (bp)	No. SNPs	Tag SNP Illumina ID	Tag SNP reference cluster ID	−log10(*p* value) of tag SNP	No. genes	Genes
26	26,078,080	26,097,110	19,030	7	BovineHD2600006878	rs110130285	5.8056407	1	SORCS3
26	26,202,415	26,216,213	13,798	5	BovineHD2600006926	rs136355517	5.5513441	1	SORCS3
26	26,170,699	26,190,210	19,511	3	BovineHD2600006917	rs135745332	5.4109618	1	SORCS3
8	103,092,247	103,096,670	4423	2	BovineHD0800030647	rs110448143	4.9800087	0	None
17	41,733,436	41,785,932	52,496	10	BovineHD1700011590	rs109674792	4.851678	1	FAM198B
26	28,154,738	28,204,944	50,206	15	BovineHD2600007529	rs41837662	4.79832714	1	SORCS1
18	49,684,020	49,690,172	6152	2	Hapmap54289-ss46526948	rs41257394	4.6439513	2	FBL PSMC4
15	47,742,405	47,775,426	33,021	7	BovineHD1500013678	rs110711227	4.5675268	1	OR52E4
7	32,661,575	32,678,638	17,063	5	BovineHD0700009343	rs136099077	4.5538269	0	None
16	20,608,750	20,623,978	15,228	12	BovineHD1600005705	rs133596831	4.4783498	1	ESRRG
2	118,870,124	118,870,999	875	2	BovineHD0200034302	rs133973886	4.4696943	1	FBXO36
18	5,268,101	5,268,998	897	2	BovineHD1800001643	rs41858359	4.4439869	0	None
15	51,068,247	51,303,719	235,472	3	BovineHD1500014737	rs135549815	4.40396843	1	LOC618050
17	10,393,778	10,411,003	17,225	2	BovineHD1700002963	rs136877205	4.3453674	1	ARHGAP10
11	96,629,841	96,777,054	147,213	11	BovineHD1100028067	rs110090917	4.32303429	1	PBX3
10	34,258,059	34,455,599	197,540	8	BovineHD1000010887	rs109623385	4.2941828	2	RASGRP1 LOC104973119
26	28,796,634	28,813,937	17,303	3	BovineHD2600007717	rs42434953	4.276744	0	None
3	91,429,028	91,852,910	423,882	4	BovineHD0300026302	rs42349819	4.27157104	1	USP24
7	31,997,138	32,005,542	8404	10	BovineHD0700009064	rs109782486	4.2477407	1	PRDM6
10	27,798,183	28,002,566	204,383	31	BovineHD1000009176	rs136350185	4.1880203	3	LOC784925 LOC785050 LOC510112
26	27,751,543	27,790,973	39,430	7	BovineHD2600007366	rs41840890	4.1697935	0	None
17	42,046,346	42,208,979	162,633	7	BovineHD1700011731	rs133679609	4.13810535	0	None
27	24,405,764	24,803,258	397,494	2	BovineHD2700006855	rs133086162	4.1081123	1	TNKS
28	30,834,105	30,879,841	45,736	8	BovineHD2800008169	rs134956968	4.07061418	2	MIR584–3 KAT6B
12	76,826,267	76,870,470	44,203	3	BovineHD1200021698	rs41668080	4.0468275	2	CLDN10 DZIP1
11	102,314,941	102,336,231	21,290	8	BovineHD1100029773	rs110442181	4.0153069	3	NTNG2 SETX LOC101906746
18	43,568,128	43,596,859	28,731	5	BovineHD1800012895	rs135753929	4.00357851	3	FAAP24 RHPN2 CEP89

Chr chromosome, *QTL* quantitative trait locus, *SNP* single nucleotide polymorphism

Table 5 Overlap of QTLs with previously reported QTLs for bovine mastitis and udder conformation traits

Chr	QTL position (bp)	Tag SNP	−log10(*p* value)	Known QTLs	Known QTL ID[1]	Known QTL position (bp)
26	26,078,080–26,097,110	rs110130285	5.8056407	Teat length	1651	25,267,910–30,988,113
26	26,202,415–26,216,213	rs136355517	5.5513441	Teat length	1651	26:25267910–30,988,113
26	26,170,699–26,190,210	rs135745332	5.4109618	Teat length	1651	25,267,910–30,988,113
8	103,092,247–103,096,670	rs110448143	4.9800087	None		
17	41,733,436–41,785,932	rs109674792	4.851678	Teat number	20,841	34,618,653–44,087,629
26	28,154,738–28,204,944	rs41837662	4.79832714	Udder attachment	4995	27,602,977–30,988,113
				Clinical mastitis	4994	27,602,977–30,988,113
				Teat length	1651	25,267,910–30,988,113
				Somatic cell score	2785	27,602,977–30,988,113
				Somatic cell score	2736	27,602,977–30,988,113
18	49,684,020–49,690,172	rs41257394	4.6439513	Somatic cell score	18,471	46,178,647–52,998,234
				Somatic cell score	18,470	46,178,647–52,983,181
				Teat length	1703	44,616,854–55,337,025
15	47,742,405–47,775,426	rs110711227	4.5675268	None		
7	32,661,575–32,678,638	rs136099077	4.5538269	Somatic cell score	2667	27,358,606–42,831,622
16	20,608,750–20,623,978	rs133596831	4.4783498	Teat length	1608	12,209,667–26,166,559
2	118,870,124–118,870,999	rs133973886	4.4696943	None		
18	5,268,101–5,268,998	rs41858359	4.4439869	Somatic cell score	3554	4,992,421–18,045,667
				Udder attachment	1701	1,891,819–7,214,579
15	51,068,247–51,303,719	rs135549815	4.40396843	None		
17	10,393,778–10,411,003	rs136877205	4.3453674	None		
11	96,629,841–96,777,054	rs110090917	4.32303429	None		
10	34,258,059–34,455,599	rs109623385	4.2941828	Udder attachment	10,294	10,323,420–79,980,762
				Teat length	10,296	10,323,420–79,980,762
				Udder attachment	44,454	34,275,633–34,275,673
				Somatic cell score	44,457	34,275,633–34,275,673
				Udder depth	44,459	34,275,633–34,275,673
				Somatic cell count	2701	22,939,631–40,797,089
26	Chr26:28796634–28,813,937	rs42434953	4.276744	Udder attachment	4995	27,602,977–30,988,113
				Clinical mastitis	4994	27,602,977–30,988,113
				Teat length	1651	25,267,910–30,988,113
				Somatic cell score	2785	27,602,977–30,988,113
				Somatic cell score	2736	27,602,977–30,988,113
3	Chr3:91429028–91,852,910	rs42349819	4.27157104	None		
7	Chr7:31997138–32,005,542	rs109782486	4.2477407	Somatic cell score	2667	27,358,606–42,831,622
10	Chr10:27798183–28,002,566	rs136350185	4.1880203	Udder attachment	10,294	10,323,420–79,980,762
				Teat length	10,296	10,323,420–79,980,762
				Somatic cell count	2701	22,939,631–40,797,089
26	27,751,543–27,790,973	rs41840890	4.1697935	Udder attachment	4995	27,602,977–30,988,113
				Clinical mastitis	4994	27,602,977–30,988,113
				Teat length	1651	25,267,910–30,988,113
				Somatic cell score	2785	27,602,977–30,988,113
				Somatic cell score	2736	27,602,977–30,988,113
17	42,046,346–42,208,979	rs133679609	4.13810535	Teat number	20,841	34,618,653–44,087,629
27	24,405,764–24,803,258	rs133086162	4.1081123	Clinical mastitis	2786	24,311,474–24,427,274
28	30,834,105–30,879,841	rs134956968	4.07061418	None		
12	76,826,267–76,870,470	rs41668080	4.0468275	None		
11	102,314,941–102,336,231	rs110442181	4.0153069	None		
18	43,568,128–43,596,859	rs135753929	4.00357851	Somatic cell score	9904	33,939,994–43,945,245
				Somatic cell score	18,469	11,438,802–46,178,647

QTL quantitative trait locus, *SNP* single nucleotide polymorphism

[1] QTLs as listed on the cattle QTL database (http://animalgenome.org/cgi-bin/QTLdb/BT/index)

course of infection may affect the sensitivity of milk bacterial culture to detect intramammary infection (Sears et al. 1990). Examination for clinical mastitis alone by definition excludes cases of subclinical mastitis, potentially excluding a substantial number of intramammary infections from being detected. Indirect measures such as SCC or its derivatives (linear score and estimated breeding values for these traits) can be influenced by a number of management and cow-dependent factors such as immune status, parity, lactation stage, diurnal variation, and sudden changes in feed or water management (Schultz 1977; Reneau et al. 1986; Harmon et al. 1994). Additionally, although low SCC is commonly accepted as indicative of an absence of intramammary infection, some studies have demonstrated low SCC as a risk factor in the subsequent development of clinical mastitis (Waage et al. 1998; Suriyasathaporn et al. 2000). The use of SCC alone to

detect mastitis therefore has the potential to result in false negatives if not supplemented by additional measures.

In consideration of the above limitations, phenotypic characterization in this study was based on multiple criteria in order to accurately identify phenotypic extremes of mastitis resistance and susceptibility. Reliable determination of intramammary infection status is best achieved through a combination of SCC measurement, bacterial culture, and clinical detection (Dohoo et al. 2011), as used in this study. Regular monitoring using these three parameters facilitates detection of clinical and subclinical mastitis, including infections resulting in minor increases in SCC. Additionally, identification of the causative bacteria allows distinction between continuing and new intramammary infections, yielding a more accurate picture of the frequency of intramammary infection in individual cows (i.e., whether increased SCC or clinical mastitis over time represents an ongoing infection or multiple separate infections). As discussed above, the use of SCC alone to detect mastitis may result in false negatives and, thus, potentially misclassification of individual animals as mastitis-resistant or mastitis-susceptible. Therefore, in this study, SCC, milk bacterial culture, and screening for clinical mastitis were used in concert among all cows to minimize false classification of cattle as mastitis-resistant or mastitis-susceptible. Consistently low SCC in the face of multiple cases of mastitis detected by bacterial culture and/or clinical mastitis screening did not exclude an individual cow from being classified as mastitis susceptible, as low SCC may be a predisposing factor to the development of mastitis (Suriyasathaporn et al. 2000). All cows within the current study were within the same herd and were subjected to the same management conditions. Thus, effects of environmental variables on mastitis susceptibility are expected to be low relative to studies in which cattle from different farms and thereby under different environmental and management conditions are included. Direct (milk bacterial culture and evaluation for clinical mastitis) and indirect (SCC) measures for mastitis detection were used in the identification of mastitis-resistant and mastitis-susceptible cows and may have facilitated the identification of 10 novel QTLs of mastitis resistance in this study.

A potential limitation to the current study is the relatively small sample size. Out of 224 lactating cows, 43 were characterized as phenotypic extremes for mastitis resistance or susceptibility. In GWAS, sample size is one of the factors influencing statistical power, and sample sizes in the thousands are often used (Pearson and Manolio 2008). In this study, meticulous phenotypic characterization was chosen at the expense of large sample size in order to identify individual cattle representative of phenotypic extremes. Genotyping only the individuals that represent phenotypic extremes for a trait (no more than 20–25% of the sample population) can be used to detect QTLs for single traits among a small sample size while preserving statistical power in a selective genotyping approach (Lander and Botstein 1989; Darvasi 1997). Out of 224 cows, only the highest and lowest extremes for mastitis resistance of the population at 6.7 and 12.5%, respectively, were genotyped. The use of selective genotyping provides an enrichment effect, as causal and protective variants are more likely to be concentrated in these individuals as compared with individuals sampled randomly from the population. Thus, the power to detect causal and protective variants, particularly rare variants, is increased, although the effect size will be overestimated (Guey et al. 2011). Follow-on studies to replicate results are therefore important (Guey et al. 2011). We believe that, in addition to phenotypic characterization methods, the use of selective genotyping along with a high-density SNP array facilitated identification of the 10 novel QTLs.

Conclusions

One hundred seventeen candidate SNPs and 27 QTLs associated with mastitis resistance within a population of phenotypically well-characterized dairy cattle were identified. The three QTLs most suggestive of genome-wide significance are located on BTA26 and overlap the SORCS3 gene and a previously identified QTL for teat length. Ten of the 27 QTLs have not been reported previously, while 17 overlap previously identified QTLs for mastitis or udder conformation traits relevant to mastitis. One QTL on BTA10 overlaps the RASGRP1 gene, considered a candidate gene of mastitis resistance requiring further study. Validation of these QTLs as genetic markers of mastitis resistance in an expanded population is required.

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Compliance with ethical standards

The use of animals in this study was approved by the Utah State University Institutional Animal Care and Use Committee (protocol IACUC-2282), and permission was obtained from the cattle owner. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practices at which the studies were conducted.

Conflict of interest The authors declare that they have no conflicts of interest.

References

Ashwell MS, Heyen DW, Weller JI, Ron M, Sonstegard TS, van Tassell CP, Lewin HA (2005) Detection of quantitative trait loci influencing conformation traits and calving ease in Holstein-Friesian cattle. J Dairy Sci 88:4111–4119. https://doi.org/10.3168/jds.S0022-0302(05)73095-2

Bonnefont CMD, Toufeer M, Caubet C, Foulon E, Tasca C, Aurel MR, Bergonier D, Boullier S, Robert-Granié C, Foucras G, Rupp R (2011) Transcriptomic analysis of milk somatic cells in mastitis resistant and susceptible sheep upon challenge with Staphylococcus epidermidis and Staphylococcus aureus. BMC Genomics 12:208. https://doi.org/10.1186/1471-2164-12-208

Brand B, Hartmann A, Repsilber D, Griesbeck-Zilch B, Wellnitz O, Kühn C, Ponsuksili S, Meyer HHD, Schwerin M (2011) Comparative expression profiling of E. coli and S. aureus inoculated primary mammary gland cells sampled from cows with different genetic predispositions for somatic cell score. Genet Sel Evol 43:24. https://doi.org/10.1186/1297-9686-43-24

Buitenhuis B, Poulsen NA, Gebreyesus G, Larsen LB (2016) Estimation of genetic parameters and detection of chromosomal regions affecting the major milk proteins and their post translational modifications in Danish Holstein and Danish Jersey cattle. BMC Genet 17:114. https://doi.org/10.1186/s12863-016-0421-2

Bush WS, Moore JH (2012) Chapter 11: genome-wide association studies. https://doi.org/10.1371/journal.pcbi.1002822

Clark SA, van der Werf J (2013) Genomic best linear unbiased prediction (gBLUP) for the estimation of genomic breeding values. pp 321–330

Darvasi A (1997) The effect of selective genotyping on QTL mapping accuracy. Mamm Genome 8:67–68. https://doi.org/10.1007/s003359900353

Detilleux JC (2002) Genetic factors affecting susceptibility of dairy cows to udder pathogens. Vet Immunol Immunopathol 88:103–110. https://doi.org/10.1016/S0165-2427(02)00138-1

Dohoo IR, Smith J, Andersen S, Kelton DF, Godden S, Mastitis Research Workers' Conference (2011) Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. J Dairy Sci 94:250–261. https://doi.org/10.3168/jds.2010-3559

Gao X, Becker LC, Becker DM, Starmer JD, Province MA (2010) Avoiding the high Bonferroni penalty in genome-wide association studies. Genet Epidemiol 34:100–105. https://doi.org/10.1002/gepi.20430

Guey LT, Kravic J, Melander O, Burtt NP, Laramie JM, Lyssenko V, Jonsson A, Lindholm E, Tuomi T, Isomaa B, Nilsson P, Almgren P, Kathiresan S, Groop L, Seymour AB, Altshuler D, Voight BF (2011) Power in the phenotypic extremes: a simulation study of power in discovery and replication of rare variants. Genet Epidemiol Swedish Res Counc (Scania Diabetes Regist) 35:236–246. https://doi.org/10.1002/gepi.20572

Halasa T, Huijps K, Østerås O, Hogeveen H (2007) Economic effects of bovine mastitis and mastitis management: a review. Vet Q 29:18–31. https://doi.org/10.1080/01652176.2007.9695224

Harmon RJ, Anderson KL, Kindahl H et al (1994) Physiology of mastitis and factors affecting somatic cell counts. J Dairy Sci 77:2103–2112. https://doi.org/10.3168/jds.S0022-0302(94)77153-8

Holmbeg M, Andersson-Eklund L (2004) Quantitative trait loci affecting health traits in Swedish dairy cattle. J Dairy Sci 87:2653–2659. https://doi.org/10.3168/jds.S0022-0302(04)73391-3

Hu ZL, Park CA, Wu XL, Reecy JM (2013) Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era. Nucleic Acids Res 41:D871–D879. https://doi.org/10.1093/nar/gks1150

Kaneene JB, Scott Hurd H (1990) The national animal health monitoring system in Michigan. III. Cost estimates of selected dairy cattle diseases. Prev Vet Med 8:127–140. https://doi.org/10.1016/0167-5877(90)90006-4

Kühn C, Reinhardt F, Schwerin M (2008) Marker assisted selection of heifers improved milk somatic cell count compared to selection on conventional pedigree breeding values. Arch Tierz Dummerstorf 51:23–32

National Mastitis Council (1999) Laboratory Handbook on Bovine Mastitis, Revised. National Mastitis Council, Madison, WI

Lander ES, Botstein S (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–674. https://doi.org/10.1038/hdy.2014.4

Meredith BK, Berry DP, Kearney F, Finlay EK, Fahey AG, Bradley DG, Lynn DJ (2013) A genome-wide association study for somatic cell score using the Illumina high-density bovine beadchip identifies several novel QTL potentially related to mastitis susceptibility. Front Genet 4:229. https://doi.org/10.3389/fgene.2013.00229

Nash DL, Rogers GW, Cooper JB, Hargrove GL, Keown JF, Hansen LB (2000) Heritability of clinical mastitis incidence and relationships with sire transmitting abilities for somatic cell score, udder type traits, productive life, and protein yield. J Dairy Sci 83:2350–2360. https://doi.org/10.3168/jds.S0022-0302(00)75123-X

Odegård J, Klemetsdal G, Heringstad B et al (2003) Genetic improvement of mastitis resistance: validation of somatic cell score and clinical mastitis as selection criteria. J Dairy Sci 86:4129–4136. https://doi.org/10.3168/jds.S0022-0302(03)74027-2

Pearson TA, Manolio TA (2008) How to interpret a genome-wide association study. JAMA Stat Res Methods; Genet Genet 29929911:1335–1344. https://doi.org/10.1001/jama.299.11.1335

Philipsson J, Ral G, Berglund B (1995) Somatic cell count as a selection criterion for mastitis resistance in dairy cattle. Livest Prod Sci 41:195–200. https://doi.org/10.1016/0301-6226(94)00067-H

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559–575. https://doi.org/10.1086/519795

R Core Team RF for SC (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Reneau JK, Andrews RJ, Kitchen BJ et al (1986) Effective use of dairy herd improvement somatic cell counts in mastitis control. J Dairy Sci 69:1708–1720. https://doi.org/10.3168/jds.S0022-0302(86)80590-2

Reyher KK, Dohoo IR (2011) Diagnosing intramammary infections: evaluation of composite milk samples to detect intramammary infections. J Dairy Sci 94:3387–3396. https://doi.org/10.3168/jds.2010-3907

Rupp R, Boichard D (2003) Genetics of resistance to mastitis in dairy cattle. 671. Vet Res 34:671–688. https://doi.org/10.1051/vetres:2003020

Sahana G, Guldbrandtsen B, Thomsen B, Lund MS (2013) Confirmation and fine-mapping of clinical mastitis and somatic cell score QTL in Nordic Holstein cattle. Anim Genet 44:620–626. https://doi.org/10.1111/age.12053

Santman-Berends IM, Olde Riekerink RG, Sampimon OC et al (2012) Incidence of subclinical mastitis in Dutch dairy heifers in the first 100 days in lactation and associated risk factors. J Dairy Sci 95:2476–2484. https://doi.org/10.3168/jds.2011-4766

Schnabel RD, Sonstegard TS, Taylor JF, Ashwell MS (2005) Whole-genome scan to detect QTL for milk production, conformation, fertility and functional traits in two US Holstein families. Anim Genet 36:408–416. https://doi.org/10.1111/j.1365-2052.2005.01337.x

Schultz LH (1977) Somatic cells in milk-physiological aspects and relationship to amount and composition of Milk. J of'Food Prot 40:125–131

Sears PM, Smith BS, English PB, Herer PS, Gonzalez RN (1990) Shedding pattern of Staphylococcus aureus from bovine intramammary infections. J Dairy Sci 73:2785–2789. https://doi.org/10.3168/jds.S0022-0302(90)78964-3

Seegers H, Fourichon C, Beaudeau F (2003) Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res 34: 475–491

Segura V, Vilhjálmsson BJ, Platt A, Korte A, Seren Ü, Long Q, Nordborg M (2012) An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nat Genet 44:825–830. https://doi.org/10.1038/ng.2314

Seykora AJ, McDaniel BT (1986) Genetics statistics and relationships of teat and udder traits, somatic cell counts, and milk production. J Dairy Sci 69:2395–2407. https://doi.org/10.3168/jds.S0022-0302(86)80679-8

Sodeland M, Kent MP, Olsen HG, Opsal MA, Svendsen M, Sehested E, Hayes BJ, Lien S (2011) Quantitative trait loci for clinical mastitis on chromosomes 2, 6, 14 and 20 in Norwegian red cattle. Anim Genet 42:457–465. https://doi.org/10.1111/j.1365-2052.2010.02165.x

Souza FN, Cunha AF, Rosa DLSO, Brito MAV, Guimarães AS, Mendonça LC, Souza GN, Lage AP, Blagitz MG, Libera AMMPD, Heinemann MB, Cerqueira MMOP (2016) Somatic cell count and mastitis pathogen detection in composite and single or duplicate quarter milk samples. Pesqui Vet Bras 36:811–818. https://doi.org/10.1590/S0100-736X2016000900004

Suriyasathaporn W, Schukken YH, Nielen M, Brand A (2000) Low somatic cell count: a risk factor for subsequent clinical mastitis in a dairy herd. J Dairy Sci 83:1248–1255. https://doi.org/10.3168/jds.S0022-0302(00)74991-5

Tiezzil F, Parker-Gaddis KL, Cole JB et al (2015) A genome-wide association study for clinical mastitis in first parity US Holstein cows using single-step approach and genomic matrix re-weighting procedure. PLoS One 10:e0114919. https://doi.org/10.1371/journal.pone.0114919

Waage S, Sviland S, Ødegaard SA (1998) Identification of risk factors for clinical mastitis in dairy heifers. J Dairy Sci 81:1275–1284. https://doi.org/10.3168/jds.S0022-0302(98)75689-9

Wu X, Lund MS, Sahana G, Guldbrandtsen B, Sun D, Zhang Q, Su G (2015) Association analysis for udder health based on SNP-panel and sequence data in Danish Holsteins. Genet Sel Evol 47:50. https://doi.org/10.1186/s12711-015-0129-1

Ziegler A, König IR, Thompson JR (2008) Biostatistical aspects of genome-wide association studies. Biom J 50:8–28. https://doi.org/10.1002/bimj.200710398

Zimin AV, Delcher AL, Florea L, Kelley DR, Schatz MC, Puiu D, Hanrahan F, Pertea G, van Tassell CP, Sonstegard TS, Marçais G, Roberts M, Subramanian P, Yorke JA, Salzberg SL (2009) A whole-genome assembly of the domestic cow, Bos taurus. Genome Biol 10:R42. https://doi.org/10.1186/gb-2009-10-4-r42