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Confirmation and fine-mapping of clinical mastitis and somatic cell score QTL in Nordic Holstein cattle

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Summary

A genome-wide association study of 2098 progeny-tested Nordic Holstein bulls genotyped for 36 387 SNPs on 29 autosomes was conducted to confirm and fine-map quantitative trait loci (QTL) for mastitis traits identified earlier using linkage analysis with sparse microsatellite markers in the same population. We used linear mixed model analysis where a polygenic genetic effect was fitted as a random effect and single SNPs were successively included as fixed effects in the model. We detected 143 SNP-by-trait significant associations ($P < 0.0001$) on 20 chromosomes affecting mastitis-related traits. Among them, 21 SNP-by-trait combinations exceeded the genome-wide significant threshold. For 12 chromosomes, both the present association study and the previous linkage study detected QTL, and of these, six were in the same chromosomal locations. Strong associations of SNPs with mastitis traits were observed on bovine autosomes 6, 13, 14 and 20. Possible candidate genes for these QTL were identified. Identification of SNPs in linkage disequilibrium with QTL will enable marker-based selection for mastitis resistance. The candidate genes identified should be further studied to detect candidate polymorphisms underlying these QTL.

Keywords confirmation study, dairy cattle, gene mapping, genome-wide association study

Introduction

Until recently, genome-wide linkage analysis was the method of choice for quantitative trait loci (QTL) detection in cattle, due to the availability of large half-sib family structures, as well as for identifying genes for phenotypes exhibiting Mendelian inheritance (Jimenez-Sanchez *et al.* 2001). Linkage analysis generally results in relatively low mapping resolution, because only few recombination events occur within families and pedigrees with known ancestry. This limits the resolution of candidate polymorphism searches. In contrast, association mapping (linkage disequilibrium mapping) has emerged as a powerful tool to resolve complex trait variation down to the sequence level by exploiting historical recombination events at the population level for high-resolution mapping (Risch & Merikangas 1996; Nordborg & Tavaré 2002). In this approach, markers or haplotypes associated with a trait of interest at the population level are identified. Such markers and haplotypes could be used directly for marker-based selection. Typically, genome scans are used to map QTL for which some test statistic exceeds a pre-defined threshold value. Even when the threshold level is chosen to be very conservative, a risk of the QTL representing a type I error remains. The confirmation of linkage analysis results by an association study will add to the credibility of detected QTL.

Lund *et al.* (2008) mapped QTL for clinical mastitis and somatic cell score in Danish Holstein cattle using linkage analysis. These authors used data on 356 microsatellite markers spread across all autosomes with an average marker spacing of 8.6 cM. Nonetheless, the QTL regions reported were quite long (more than 20 cM for some QTL). Such large QTL regions, along with family specificity of marker–QTL associations, limit the usability of their result for practical animal breeding as well as for candidate polymorphism searches. Thus, a need to confirm these QTL and refine their position estimates remains in order to include QTL information in selection decisions. In the present study, association mapping was carried out for six mastitis traits in cattle using dense SNP markers. The aim of this study was to confirm and fine-map the QTL for mastitis traits previously reported in the Danish Holstein dairy cattle population by Lund *et al.* (2008).

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Materials and methods

Genotyping

A total of 2098 Nordic Holstein bulls with breeding values for mastitis resistance from Denmark, Sweden and Finland were genotyped using the BovineSNP50 BeadChip (Illumina). Only SNPs with minor allele frequency of at least 0.05 and an average GenCall score (an indicator of the reliability of the genotypes called) of at least 0.65 were retained for the analysis. A total of 36 387 SNPs on 29 bovine autosomes (BTAs) were selected for association analyses. Individual SNP typing with a GenCall score less than 0.6 was dropped. The number of SNPs included for analysis varied from 675 on BTA28 to 2320 on BTA1. The details on the genotyping platform and quality control for SNPs are described by Sahana *et al.* (2010a). The SNP positions within a chromosome were based on the *Bos taurus* genome UMD3.1 assembly (Zimin et al. 2009). Missing genotypes were imputed using the software FASTPHASE (Scheet & Stephens 2006).

Phenotypic data

Single-trait breeding values (STBVs) were used as the response variable in this analysis. Six mastitis-related STBVs were analyzed for association with SNPs. Single-trait breeding values were calculated for each animal using best linear unbiased prediction procedures and a sire model by the Nordic Cattle Genetic Evaluation. For definitions and models used in breeding value prediction, see http://www.nordicebv.info, except that the correlation with other traits was set to 0 to avoid information from phenotypes of correlated traits to affect results of any particular trait. Also, only sire–son and son–offspring relationships were included, effectively producing a sire model. The STBVs were adjusted for the same systematic environmental effects as in the official routine evaluations. Treatment for clinical mastitis was recorded by veterinarians. Clinical mastitis was coded as a binary trait: mastitis treatments (one or more in period) were coded as 1, and no mastitis treatment was coded as 0 within each of four time periods: from −15 to 50 days after parturition in first lactation (CM11), 51 to 305 days in first lactation (CM12), −15 to 305 days in second lactation (CM2) and −15 to 305 days in third lactation (CM3). The STBVs for the four mastitis traits are weighted together to form a compound clinical mastitis resistance index (CM): CM = 0.25*CM11 + 0.25*CM12 + 0.3*CM2 + 0.2*CM3 (Johansson *et al.* 2007). All breeding values were standardized to a mean of 100 and a standard deviation of 10. Somatic cell score (SCS) is an important indicator trait for the estimation of breeding values for udder health. The SCS index is an index of the average log somatic cell count from 5 to 170 days from the first three lactations with relative weights of 0.5, 0.3 and 0.2 for first, second and third lactation respectively (Johansson *et al.* 2007). The numbers of STBVs available for analysis among the genotyped animals were 1671, 1668, 1669, 1544, 2098 and 1671 for CM11, CM12, CM2, CM3, CM and SCS respectively. The official publication criterion for the index for mastitis resistance indices is that the reliability of estimated breeding values (EBVs) be at least 40% (http://www.nordicebv.info/News/Joint+Nordic+estimation+of+breeding+values+for+udder+health.htm). The reason for the higher number of observations for CM compared with its subindices is that the EBV for CM for a bull may be published (available) as it had exceeded the accuracy threshold (40%, criterion to publish official breeding value), but some of its subindices may not be published because they did not reach the required minimum accuracy. The distributions of year of birth for the sires and the accuracy of EBVs (only for CM) are presented in Figs. S1 and S2 respectively. Details of the breeding value estimation procedures including the genetic parameters for udder health traits by the Nordic Cattle Genetic Evaluation are available at http://www.nordicebv.info/NR/rdonlyres/5CD2E4DC-F82A-4809-A770-3022E270E205/0/PrinciplesNyeste.pdf. The models to estimate the breeding values for mastitis traits in Nordic countries are described by Negussie *et al.* (2010).

Statistical methods for association analysis

Mixed model

The mixed model analysis as proposed by Yu *et al.* (2006) was used for the association analyses. In this approach, a polygenic genetic effect was fitted as a random effect. Single SNPs were successively included as fixed effects in the model. The model used was:

$$y = 1_n'\mu + S\alpha + Zu + e,$$

where y is a vector of phenotypes (STBV); μ is a shared fixed effect; 1_n is a vector of ones with dimension of number of records (n); α is the allele substitution effect of the SNP; S is an incidence vector with elements 0, 1 or 2 relating α to the individuals with 0, 1 or 2 copies of one of the alleles; Z is a matrix relating records to additive polygenic values; u is a vector of additive polygenic values; and $\mathbf{e}$ is a vector of random residual effects. The random variables u and e are assumed to be multivariate normally distributed. u has a mean of 0 and a covariance matrix $\sigma_g^2 A$, where σ_g^2 is the polygenic genetic variance and A is the additive relationship matrix derived from pedigree. e has a mean of 0 and a covariance matrix $\sigma_e^2 I$, where σ_e^2 is the residual variance and I is the identity matrix. The analysis was carried out using the software package DMU (http://gbi.agrsci.dk/dmu/). Significance of each marker's allele substitution effect (α) was tested using a t-test against a null hypothesis of $\alpha = 0$.

Significance tests

For control of the family-wide error rate (FWER), a Bonferroni correction was applied. The Bonferroni correction controls FWER $(\alpha) = 1-(1-\alpha_i)^m \approx \alpha_i m$, where α_i is the individual test rejection level and m is the number of tests. The 5% genome-wide significance threshold in this study was 1.37e−06 [i.e., $-\log_{10}(P\text{-value}) = 5.86$]. However, the Bonferroni correction is very conservative (Han *et al.* 2009) as it does not take into account correlation (linkage disequilibrium) among SNPs. Therefore, we used a liberal significance threshold of 10^{-4} [i.e., $-\log_{10}(P\text{-value}) = 4.0$] and calculated the false discovery rate (FDR) at this significance threshold. FDR is the ratio of the expected number of significant associations to the actual number of significant associations. FDR was calculated following Benjamini & Hochberg (1995) as FDR = mP/S, where m is the number of markers tested, P is the significant threshold (P-value) and S is the number of markers with significant associations at the pre-defined threshold. In this article, 'genome-wide significant' means $P < 1.37e{-}06$, and 'suggestive significant' means $P < 10^{-4}$.

Results

The present genome-wide association study (GWAS) detected 143 significant SNP-by-trait associations (P-value <0.0001) for clinical mastitis and somatic cell score traits on 20 chromosomes in Nordic Holstein cattle (Table S1). The highest number of significant associations was observed on BTA6 (50), followed by BTA20 (28), BTA16 (15) and BTA14 (14). Only one significant association on a chromosome was observed for each of nine chromosomes. The genome-wide number of false discoveries at the significance threshold ($P < 0.0001$) was 3.6, that is, 2.5% of the associations identified were expected to be false discoveries. Twenty-one associations on four chromosomes (15 on BTA6, two on BTA13, three on BTA14 and one on BTA20) crossed the genome-wide significant threshold [$-\log_{10}$ (P-value) >5.86; Table 1]. Most of the genome-wide significant associations were observed for CM. Only four SNPs showed a genome-wide significant association with CM2. The Manhattan plots (Fig. S3) give an overview of the distribution of SNP associations across the genome. The most significant signal was observed on BTA6, and the same QTL was found for CM2, SCS and CM11. Consistent results across traits for association were observed on BTA16 for SCS, CM11, CM12 and CM and on BTA1 for SCS, CM11, CM12, CM2 and CM. QTL chromosomal locations for CM2 and CM also coincided on BTA14.

Discussion

In this section, we discuss possible candidate genes for the five chromosomal regions on four chromosomes (BTA6, 13, 14 and 20) showing genome-wide significant associations with mastitis-related traits.

Table 1 SNPs showing genome-wide significant association with mastitis traits. SNP positions are based on UMD3.1

Chr.	Marker	rs ID	Position	Trait	−log10 (P-value)	Effect	SE
6	*BTA-92371-no-rs*	41664497	24900639	CM2	7.08	0.581	0.104
6	*ARS-BFGL-NGS-59728*	110927426	26377975	CM	5.9	0.885	0.176
6	*Hapmap52686-rs29017739*	29017739	83374529	CM	8.42	−2.016	0.331
6	*Hapmap60224-rs29001782*	29001782	85178107	CM	8.55	0.949	0.154
6	*BTA-77077-no-rs*	41588957	85527109	CM	6.62	−0.837	0.156
6	*ARS-BFGL-NGS-2418*	110320975	86237705	CM	6.98	−0.883	0.160
6	*ARS-BFGL-NGS-86825*	110289032	86794730	CM	5.9	−0.788	0.156
6	*Hapmap50857-BTA-107920*	41610991	86819633	CM	7.44	−0.880	0.154
6	*Hapmap33210-BTA-144234*	81150388	87838493	CM	7.36	0.974	0.172
6	*ARS-BFGL-NGS-112872*	110707460	88069548	CM	9.53	−1.071	0.164
6	*ARS-BFGL-NGS-118182*	110527224	88592295	CM	9.22	−0.972	0.152
6	*BTB-00133212*	43338539	88656290	CM	6.05	0.917	0.179
6	*ARS-BFGL-NGS-17376*	108988814	88822266	CM	8.18	1.144	0.190
6	*BTB-01654826*	42766480	88891318	CM	6.89	0.916	0.167
6	*UA-IFASA-6709*	41655339	93381472	CM	8.02	−0.987	0.166
13	*ARS-BFGL-NGS-27559*	41694067	57548174	CM	7.65	−0.858	0.148
13	*ARS-BFGL-NGS-30853*	110942493	57570093	CM	7.32	0.846	0.149
14	*ARS-BFGL-NGS-57820*	109146371	1651311	CM2	6.25	−0.282	0.054
14	*ARS-BFGL-NGS-4939*	109421300	1801116	CM2	6.09	−0.278	0.054
14	*ARS-BFGL-NGS-107379*	109350371	2054457	CM2	6.49	−0.279	0.052
20	*BTB-00777979*	41940571	35511396	CM	7.79	1.127	0.193

Chr., chromosome; Effect, allele substitution effect; SE, standard error; CM11, clinical mastitis from −15 to 50 days of parturition in first lactation; CM12, clinical mastitis from 51 to 305 days in first lactation; CM2, clinical mastitis from −15 to 305 days in second lactation; CM3, clinical mastitis from −15 to 305 days in third lactation; CM, clinical mastitis index; SCS, somatic cell score.

The strongest association of SNPs with any mastitis trait in this study was observed on BTA6 at 88.1 Mb (Table 1). This QTL affected CM, CM11 and SCS. Significantly associated SNPs were located between 79.7 and 98.3 Mb. The most significant SNP, *rs110707460*, is located within the gene *deoxycytidine kinase* (*DCK*), which catalyzes the rate-determining step in the deoxyribonucleoside salvage pathway. The highest levels of *DCK* expression are found in thymus and bone marrow, which suggests a role of *DCK* in lymphopoiesis. Indeed, knockout mice lacking enzyme activity revealed a combined immune deficiency phenotype, that is, they produce very low levels of both T and B lymphocytes (Toy *et al.* 2010). Another strong candidate gene in this region is the *IGJ* gene, which encodes the immunoglobulin J polypeptide and is located at 87.75 Mb on BTA6. This protein serves a nucleating function in the formation of the immunoglobulin M (IgM) pentameric complex and in the assembly of IgA dimers and polymers. IgM is the first antibody produced in the primary immune response to microbial infections and therefore plays a crucial role in preventing systemic spread of pathogens (Racine & Winslow 2009). Also, IgA is engaged in the defense against microorganisms, in particular those invading the host through mucosal surfaces. Thus, IgA is the major antibody class found in mucosal secretions, where it combines with microbes to prevent them from attaching to or penetrating the mucosal membranes (Lamm 1997). Supporting evidence for a QTL in this region was reported by Nilsen *et al.* (2009), who located a QTL for clinical mastitis on BTA6 in the Norwegian Red cattle (NRC) breed with the strongest association signals at the casein cluster (87.1 Mb) and immunoglobulin J chain (87.7 Mb). Localization of the QTL in the same chromosomal region in different breeds increases confidence in this QTL. Sodeland *et al.* (2011) studied the haplotype at 86–97 Mb on BTA6 associated with clinical susceptibility in the NRC population. They traced the origin of the haplotype in NRC to a Holstein bull. They reported the highest test scores (LRT = 42 for *rs42766480* at 90.07 Mb) for first lactation clinical mastitis close to a cluster of genes coding for CXC chemokine.

We also have detected another QTL on BTA6 at 24.9–28.8 Mb (Table 1). The most significantly associated SNP was *rs41664497*. A plausible candidate gene in this region is *DAPP1*, also known as Bam32, which is expressed in B-cell lymphocytes. This gene has been implicated in B-cell antigen receptor (BCR) signaling. Thus, antigen binding to BCR involves a chain of signaling processes that are critical to B-cell fate decisions, such as proliferation and differentiation, and BCR-mediated antigen internalization, processing and presentation to T cells (Pierce 2002). Studies of Bam32-deficient mice have shown that Bam32 mediates BCR-induced proliferation of B cells but not survival (Han *et al.* 2003), it regulates B-cell antigen receptor internalization (Niiro *et al.* 2004) and it promotes the formation of stable interactions between B cells and T cells needed for efficient T-cell activation, most likely by promoting adhesion to integrin ligands expressed on T cells (Al-Alwan *et al.* 2010).

On BTA13, we detected a genome-wide significant QTL affecting CM, CM11 and CM12 at 57.54 Mb (Table 1). There were two closely located SNPs (20 kb apart) which showed genome-wide association located very close (located at 57.57 Mb) to the *endothelin 3* (*EDN3*) gene (http://www.ensembl.org/Bos_taurus/). The endothelins ET-1, ET-2 and ET-3 constitute a family of 21 amino acid peptides that are produced by numerous cells and tissues such as macrophages and endothelial and epithelial cells (Giaid *et al.* 1991). In addition to their vasoconstrictive effects, they also influence many different cell types, including activation of neutrophils (Elferink & De Koster 1998). Neutrophiles are blood-borne leukocytes that combat bacterial and fungal infections by phagocytosis or release of antimicrobial peptides (Selsted & Ouellette 2005). Another possible candidate gene located in this region is *Phactr3* (*phosphatase and actin regulator 3*) at 57.20 Mb, which has been shown to stimulate cell spreading and migration through direct interaction with the actin cytoskeleton (Sagara *et al.* 2009). Cell mobility is critically important for cell-mediated immune response (Luster *et al.* 2005).

We detected a genome-wide significant QTL affecting CM and CM2 at the proximal end of BTA14 (0.7 Mb). Three SNPs (between 1.65 and 2.05 Mb on BTA14) showed genome-wide significant association with CM2. On BTA14, a region around the *CYP11B1* (*cytochrome P450, family 11, subfamily B, polypeptide 1*) gene, located at 2.70 Mb on BTA14, harbors a QTL for SCS in German Holstein cattle (Kaupe *et al.* 2007), which may be the same QTL detected in the present study. There are several genes located in the QTL region including *DGAT1* (Fig. S4, Grisart *et al.* 2002), which has a large influence on phenotypic variance in milk fat content and other milk characteristics. The association observed here with mastitis could be an artifact of the QTL affecting milk production traits where the observed association with mastitis is due to the negative genetic relationship between mastitis and milk production traits.

We have identified a genome-wide significant QTL at 35.51 Mb on BTA20 (Table 1). The most significant SNP, *rs41940571*, is located within the *RICTOR* (*RPTOR-independent companion of MTOR complex 2*) gene (35.37–35.51 Mb). There are several other genes located in this QTL region in the cattle genome assembly. Among these is the *C9* gene (35.09–35.15 Mb), encoding the complement component C9 precursor. The complement system is part of the immune response against invading pathogens. Complement C9 is the pore-forming subunit of membrane attack complex gene, and mutations in this gene are associated with increased risk of infections, for example meningococcal meningitis (Zoppi *et al.* 1990; Horiuchi *et al.* 1998; Kira *et al.* 1998).

Comparison with the previously reported linkage analyses from the same cattle population

Lund *et al.* (2008) studied QTL for mastitis traits using linkage analysis in Danish Holstein cattle using part of the data analyzed in the present study. Six chromosomes where overlapping QTL related to mastitis and udder traits were detected in both the present study and the earlier QTL study by Lund *et al.* (2008) are shown in Table S2. However, the definitions of the mastitis traits were slightly different in Lund *et al.* (2008) and the present study, for example, Lund *et al.* (2008) studied clinical mastitis for the first lactation as one trait (CM1). In this section, we discuss the results from the chromosomes not discussed already in the previous section but results from the present study that overlap with the QTL reported by Lund *et al.* (2008). To compare results from the present study with the earlier reports, the previously reported marker positions were taken from cattle QTL database (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index, Hu *et al.* 2010).

We detected two suggestive significant [$-\log_{10}$(*P*-value) >4.0] QTL for CM at 37.4 and 97.0 Mb on BTA5. Lund *et al.* (2008) reported QTL for CM2, CM3 and SCS on BTA5 at 48.2 Mb, 12.3–24.1 Mb and 24.1–27.8 Mb respectively. Although these QTL were reported in different locations, they concluded that it was a single pleiotropic QTL affecting all three mastitis traits by means of comparing pleiotropic and linkage models. Therefore, it could be the same QTL that was detected at 37.4 Mb here.

We found suggestive evidence for a QTL affecting CM at 74.7 Mb on BTA9. Lund *et al.* (2008) reported two QTL, one at 76.8–86.9 Mb for CM1 and another at 10.9–16.2 Mb affecting CM2 in the same population. They concluded that the QTL for CM2 might have a pleiotropic effect on CM1. Lund *et al.* (2007) also reported a QTL for CM in a combined analysis of three Nordic red breeds, which was later fine-mapped at 86.9 Mb by Sahana *et al.* (2008). The SCS QTL reported by Lund *et al.* (2008) on BTA24 and 25 were also confirmed in the present study.

The genetic correlation between clinical mastitis and SCS is greater than 0.70 (Lund *et al.* 1999). Therefore, it was expected that many of the QTL affecting CM would also affect SCS. However, the association signals from SCS were generally weaker compared with clinical mastitis traits (Figure S1). This indicates mapping mastitis QTL using SCS only as a response variable may require a larger sample size than using clinical mastitis data. Of the six mastitis traits analyzed in the present study, the highest number of QTL was observed for mastitis index, which was an index combining clinical mastitis from the first three lactations. In the present study population, the heritability for CM was approximately 0.04; the genetic correlations between the CM and its four subindices were high (0.70–0.95), and the genetic correlation between CM and SCS was 0.60 (http://www.nordicebv.info/News/Joint+Nordic+estimation+of+breeding+values+for+udder+health.htm). The correlated traits increase the reliability of the mastitis resistance index considerably, when a bull gets his first proof. It is important to remember that the mastitis diagnoses are essential for the mastitis resistance index, for example, the reliability of a mastitis resistance index based on SCS alone can never be higher than 36%, given the genetic correlation between CM and SCC of 0.60.

We used STBVs as response variables for the association analysis to avoid information from phenotypes of correlated traits to affect results of any particular trait. A weighted analysis of the model would have been more appropriate, as the residual variances for the STBVs were unequal if some sires had many more daughters than others did. However, the accuracy of STBVs used in the present study was very high, for example, STBV for CM was higher than 0.70 for 89% of the sires (Fig. S2). Pryce *et al.* (2010) conducted a GWAS for milk production and fertility traits in dairy cattle using deregressed proofs as the response variable. They observed that the correlation between SNP effect estimates from weighted and unweighted models was between 0.96 and 0.97. We also have observed similar results for GWAS studies (unpublished data). Guo *et al.* (2010) compared various response variables for genomic prediction and observed similar accuracy using EBVs and weighted daughter yield deviations. Therefore, an unweighted analysis is unlikely to have a sizable effect on the results presented here.

The present study identified strong association between mastitis resistance and SNP markers on four chromosomes. The possible candidate genes for mastitis resistance are identified. The present study also confirmed several mastitis QTL previously detected by Lund *et al.* (2008), who used linkage analyses and sparse microsatellite markers. We used an association study with dense SNP markers in a mixed model analysis, which was observed to perform best for samples from complex pedigreed populations like cattle (Sahana *et al.* 2010b). In the present study, QTL positions were refined to narrow genomic regions, and candidate genes were identified.

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Conflict of interest

The results presented in the manuscript are part of a patent application.

References

Al-Alwan M., Hou S., Zhang T.T., Makondo K. & Marshall A.J. (2010) *Bam32/DAPP1* promotes B cell adhesion and formation of polarized conjugates with T cells. *The Journal of Immunology* **184**, 6961–9.

Benjamini Y. & Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, Series B (Methodological)* **57**, 289–300.

Elferink J.G. & DeKoster B.M. (1998) The involvement of protein kinase G in stimulation of neutrophil migration by endothelins. *European Journal of Pharmacology* **350**, 285–91.

Giaid A., Polak J.M., Gaitonde V. *et al.* (1991) Distribution of endothelin-like immunoreactivity and mRNA in the developing and adult human lung. *American Journal of Respiratory Cell and Molecular Biology* **4**, 50–8.

Grisart B., Coppieters W., Farnir F. *et al.* (2002) Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. *Genome Research* **12**, 222–31.

Guo G., Lund M.S., Zhang Y. & Su G. (2010) Comparison between genomic predictions using daughter yield deviation and conventional estimated breeding value as response variables. *Journal of Animal Breeding and Genetics* **127**, 423–32.

Han A., Saijo K., Mecklenbräuker I., Tarakhovsky A. & Nussenzweig M.C. (2003) *Bam32* links the B cell receptor to *ERK* and *JNK* and mediates B cell proliferation but not survival. *Immunity* **19**, 621–32.

Han B., Kang H.M. & Eskin E. (2009) Rapid and accurate multiple testing correction and power estimation for millions of correlated markers. *PLoS Genetics* **5**, e1000456.

Horiuchi T., Nishizaka H., Kojima T. *et al.* (1998) A non-sense mutation at Arg95 is predominant in complement 9 deficiency in Japanese. *The Journal of Immunology* **160**, 1509–13.

Hu Z.-L., Park C.A., Fritz E.R. & Reecy J.M. (2010) QTLdb: a comprehensive database tool building bridges between genotypes and phenotypes. Invited Lecture with full paper published electronically on The 9th World Congress on Genetics Applied to Livestock Production. August 1–6, 2010 Leipzig, Germany.

Jimenez-Sanchez G., Childs B. & Valle D. (2001) Human disease genes. *Nature* **409**, 853–5.

Johansson K., Eriksson S., Pösö J., Nielsen U.S. & Aamand G.P. (2007) Predictive ability of different models for clinical mastitis in joint genetic evaluation for Sweden, Denmark and Finland. *EAAP Book of Abstracts*, Dublin.

Kaupe B., Brandt H., Prinzenberg E.M. & Erhardt G. (2007) Joint analysis of the influence of *CYP11B1* and *DGAT1* genetic variation on milk production, somatic cell score, conformation, reproduction, and productive lifespan in German Holstein cattle. *Journal of Animal Science* **85**, 11–21.

Kira R., Ihara K., Takada H., Gondo K. & Hara T. (1998) Nonsense mutation in exon 4 of human complement *C9* gene is the major cause of Japanese complement C9 deficiency. *Human Genetics* **102**, 605–10.

Lamm M.E. (1997) Interaction of antigens and antibodies at mucosal surfaces. *Annual Reviews Microbiology* **51**, 311–40.

Lund M.S., Jensen J. & Petersen P.H. (1999) Estimation of genetic and phenotypic parameters for clinical mastitis, somatic cell production deviance, and protein yield in dairy cattle using Gibbs sampling. *Journal of Dairy Science* **82**, 1045–51.

Lund M.S., Sahana G., Andersson-Eklund L. *et al.* (2007) Joint analysis of quantitative trait loci for clinical mastitis and somatic cell score on five chromosomes in three Nordic dairy cattle breeds. *Journal of Dairy Science* **90**, 5282–90.

Lund B., Guldbrandtsen B., Buitenhuis A.J., Thomsen B. & Bendixen C. (2008) Detection of quantitative trait loci in Danish Holstein cattle affecting clinical mastitis, somatic cell score, udder conformation traits, and assessment of associated effects on milk yield. *Journal of Dairy Science* **91**, 4028–36.

Luster A.D., Alon R. & von Andrian U.H. (2005) Immune cell migration in inflammation: present and future therapeutic targets. *Nature Immunology* **6**, 1182–90.

Negussie E., Lidauer M., Mäntysaari E.A., Strandén I., Pösö J., Nielsen U.S., Johansson K., Eriksson J-Å. & Aamand G.P. (2010) Combining test day SCS with clinical mastitis and udder type traits: A random regression model for joint genetic evaluation of udder health in Denmark, Finland and Sweden. 37th ICAR Session and Interbull Open Meeting, 31 May–4 June, 2010, Riga, Latvia.

Niiro H., Allam A., Stoddart A., Brodsky F.M., Marshall A.J. & Clark E.A. (2004) The B lymphocyte adaptor molecule of 32 kilodaltons (*Bam32*) regulates B cell antigen receptor internalization. *The Journal of Immunology* **173**, 5601–9.

Nilsen H., Olsen H.G., Hayes B., Nome T., Sehested E., Svendsen M., Meuwissen T.H.E. & Lien S. (2009) Characterization of a QTL region affecting clinical mastitis and protein yield on BTA6. *Animal Genetics* **40**, 701–12.

Nordborg M. & Tavaré S. (2002) Linkage disequilibrium: what history has to tell us. *Trends in Genetics* **18**, 83–90.

Pierce S.K. (2002) Lipid rafts and B-cell activation. *Nature Reviews Immunology* **2**, 96–105.

Pryce J.E., Bolormaa S., Chamberlain A.J., Bowman P.J., Savin K., Goddard M.E. & Hayes B.J. (2010) A validated genome-wide association study in 2 dairy cattle breeds for milk production and fertility traits using variable length haplotypes. *Journal of Dairy Science* **93**, 3331–45.

Racine R. & Winslow G.M. (2009) IgM in microbial infections: taken for granted? *Immunology Letters* **125**, 79–85.

Risch N. & Merikangas K. (1996) The future of genetic studies of complex human diseases. *Science* **273**, 1516–7.

Sagara J., Arata T. & Taniguchi S. (2009) Scapinin, the protein phosphatase 1 binding protein, enhances cell spreading and motility by interacting with the actin cytoskeleton. *PLoS ONE* **4**, e4247.

Sahana G., Lund M.S., Andersson-Eklund L. *et al.* (2008) Fine-mapping QTL for mastitis resistance on BTA9 in three Nordic red cattle breeds. *Animal Genetics* **39**, 354–62.

Sahana G., Guldbrandtsen B., Bendixen C. & Lund M.S. (2010a) Genome-wide association mapping for female fertility traits in Danish and Swedish Holstein cattle. *Animal Genetics* **41**, 579–88.

Sahana G., Guldbrandtsen B., Janss L. & Lund M.S. (2010b) Comparison of association mapping methods in a complex pedigreed population. *Genetic Epidemiology* **34**, 455–62.

Scheet P. & Stephens M. (2006) A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. *American Journal of Human Genetics* **78**, 629–44.

Selsted M.E. & Ouellette A.J. (2005) Mammalian defensins in the antimicrobial immune response. *Nature Immunology* **6**, 551–7.

Sodeland M., Grove H., Kent M., Taylor S., Svendsen M., Hayes B.J. & Lien S. (2011) Molecular characterization of a long range haplotype affecting protein yield and mastitis susceptibility in Norwegian Red cattle. *BMC Genetics* **12**, 70.

Toy G., Austin W.R., Liao H.I., Cheng D. *et al.* (2010) Requirement for deoxycytidine kinase in T and B lymphocyte development. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 5551–6.

Yu J., Pressoir G. & Briggs W.H. *et al.* (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics* **38**, 203–8.

Zimin A.V., Delcher A.L., Florea L. *et al.* (2009) A whole-genome assembly of the domestic cow, *Bos taurus*. *Genome Biology* **10**, R42.

Zoppi M., Weiss M., Nydegger U.E., Hess T. & Späth P.J. (1990) Recurrent meningitis in a patient with congenital deficiency of the C9 component of complement. First case of c9 deficiency in Europe. *Archives of Internal Medicine* **150**, 2395–9.

Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 The distribution of birth-years of the sire used in the present study

Figure S2 The distribution of accuracy for the single-trait breeding values (STBVs) for the mastitis resistance index (CM) for the bulls used in the present study.

Figure S3 Genome-wide scan for mastitis traits: $-\log_{10}$ of the *P*-value analysis for association with SNPs. Chromosomes are shown in alternating colors for clarity. The dotted line represents suggestive association [−log10 (*P*-value) = 4] as considered in the present study.

Figure S4 Genes located on BTA14 region where significant association between mastitis and SNP markers were observed.

Table S1 SNP showing chromosome-wise significant association with mastitis traits.

Table S2 Confirmation of mastitis quantitative trait loci (QTL) detected earlier from Danish Holstein population by Lund et al. (2008).