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Genome-wide association study using high-density single nucleotide polymorphism arrays and whole-genome sequences for clinical mastitis traits in dairy cattle[1]

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ABSTRACT

Mastitis is a mammary disease that frequently affects dairy cattle. Despite considerable research on the development of effective prevention and treatment strategies, mastitis continues to be a significant issue in bovine veterinary medicine. To identify major genes that affect mastitis in dairy cattle, 6 chromosomal regions on *Bos taurus* autosome (BTA) 6, 13, 16, 19, and 20 were selected from a genome scan for 9 mastitis phenotypes using imputed high-density single nucleotide polymorphism arrays. Association analyses using sequence-level variants for the 6 targeted regions were carried out to map causal variants using whole-genome sequence data from 3 breeds. The quantitative trait loci (QTL) discovery population comprised 4,992 progeny-tested Holstein bulls, and QTL were confirmed in 4,442 Nordic Red and 1,126 Jersey cattle. The targeted regions were imputed to the sequence level. The highest association signal for clinical mastitis was observed on BTA 6 at 88.97 Mb in Holstein cattle and was confirmed in Nordic Red cattle. The peak association region on BTA 6 contained 2 genes: vitamin D-binding protein precursor (*GC*) and neuropeptide FF receptor 2 (*NPFFR2*), which, based on known biological functions, are good candidates for affecting mastitis. However, strong linkage disequilibrium in this region prevented conclusive determination of the causal gene. A different QTL on BTA 6 located at 88.32 Mb in Holstein cattle affected mastitis. In addition, QTL on BTA 13 and 19 were confirmed to segregate in Nordic Red cattle and QTL on BTA 16 and 20 were confirmed in Jersey cattle. Although several candidate genes were identified in these targeted regions, it was not possible to identify a gene or polymorphism as the causal factor for any of these regions.

Key words: dairy cattle, quantitative trait locus, mastitis, NGS

INTRODUCTION

Association mapping aims to identify specific genetic variants (i.e., loci and alleles) associated with phenotypic differences in traits. Genome-wide association studies (**GWAS**) evaluate associations between common genetic variants with phenotypic difference in a trait. Recent advances in our understanding of genetic variation and the technologies that measure such variation have made GWAS more tractable.

Previous mapping studies have identified QTL that affect clinical mastitis (**CM**) and SCC in cattle (e.g., Lund et al., 2007, 2008; Sahana et al., 2008, 2013; Cole et al., 2011; Meredith et al., 2013; Abdel-Shafy et al., 2014). These studies, however, were not able to pinpoint specific candidate gene variants. Today, with increased availability of high-density (**HD**) SNP arrays and whole-genome sequence data, the chances of identifying causative mutations are higher. Identifying causal variants or assigning QTL to one gene or a few genes through association mapping may facilitate identification of genes that control mastitis resistance. However, the linkage disequilibrium (**LD**) in cattle spreads over large regions of the genome (de Roos et al., 2008), which may hinder the identification of one or a few causal variants. Therefore, multiple breed data may be used to provide more precise localization.

Unfavorable genetic correlations have been detected between milk production and CM (from 0.21 to 0.55; Heringstad et al., 2005), which are caused by pleiotropic effects and linkage. Understanding the underlying pleiotropic connections between quantitative traits is important for predicting correlated responses to artificial selection. Therefore, identifying QTL that affect milk production traits and mastitis may increase understanding of the genetic determinants of milk production and CM.

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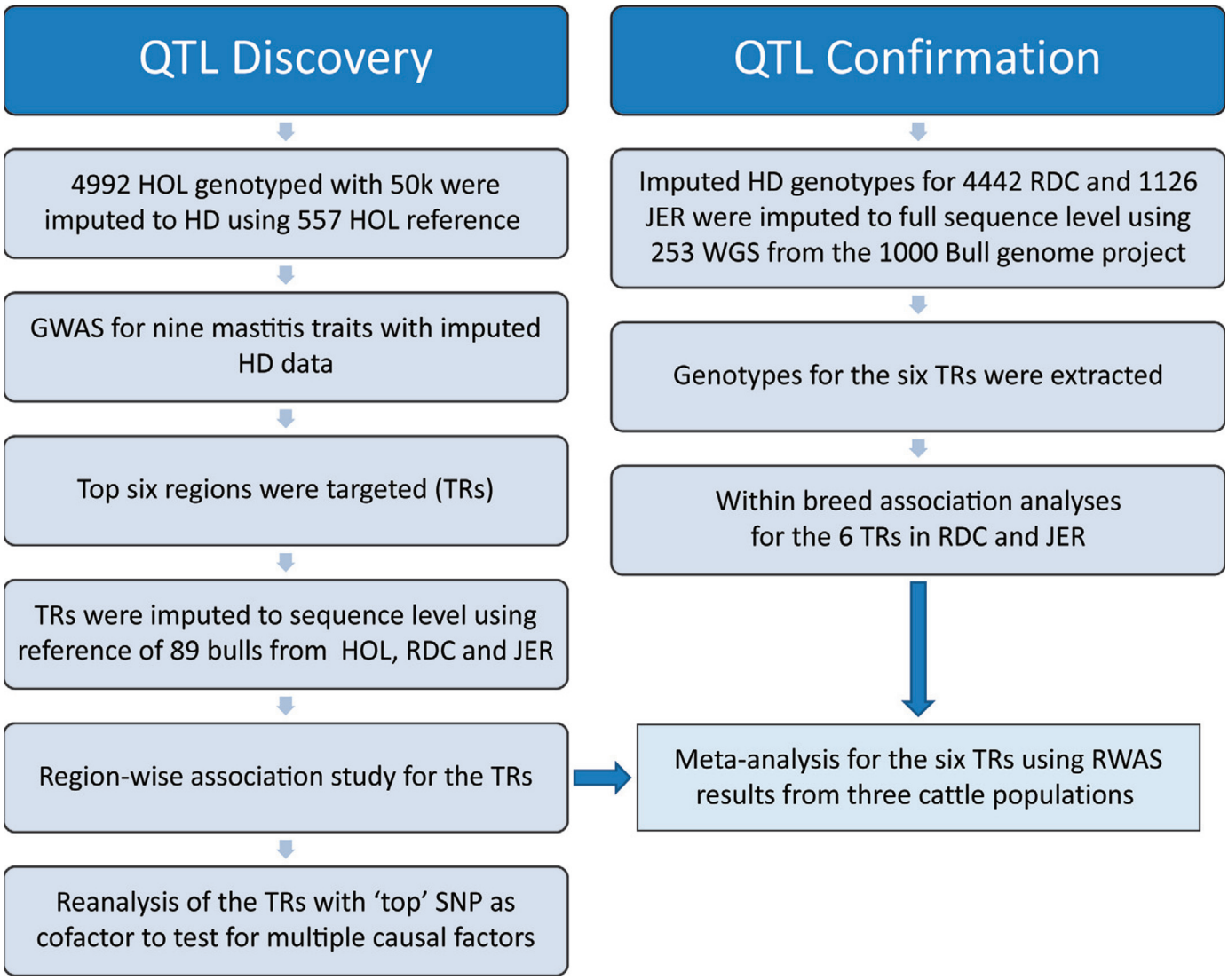

Figure 1. Flowchart of steps for association study for mastitis QTL discovery in Holstein (HOL) cattle and confirmation studies in Nordic Red (RDC) and Danish Jersey (JER) cattle. 50k = 50,000 markers; HD = high density; GWAS = genome-wide association study; WGS = whole-genome sequence; RWAS = region-wise association study. Color version available in the online PDF.

The objectives of the present study were to (1) identify QTL for CM in Nordic Holstein cattle using HD SNP arrays and then fine-map targeted QTL using sequence-level variants, (2) confirm selected QTL in Danish Jersey and Nordic Red cattle to determine whether targeted QTL segregate in these 2 breeds, (3) determine whether multiple-breed data contribute to identification of causative genes or mutations, and (4) examine targeted QTL for association with milk production traits.

MATERIALS AND METHODS

A flowchart of the materials and methods of this study (i.e., populations studied, genotype imputation, and association analyses) is presented in Figure 1.

Nordic Holstein Cattle

Genome scans for mastitis resistance were carried out for 9 mastitis phenotypes in Nordic Holstein cattle. Udder health traits currently evaluated in the Nordic Cattle Genetic Evaluation (http://www.nordicebv.info) included 4 clinical mastitis traits (all coded as binary traits) and 3 SCC traits from the first 3 lactations (Table 1). These indices were standardized to a mean of 100 and a standard deviation of 10 (Johansson et al., 2007). Estimated breeding values were available for 4,992 progeny-tested Nordic Holstein bulls with the 9 mastitis-related phenotypes. Most of these bulls were born between 1986 and 2009; a few older bulls (43) were also included. The SNP