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# Bovine chromosomal regions affecting rheological traits in acid-induced skim milk gels

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## ABSTRACT

The production of fermented milk products has increased worldwide during the last decade and is expected to continue to increase during the coming decade. The quality of these products may be optimized through breeding practices; however, the relations between cow genetics and technological properties of acid milk gels are not fully known. Therefore, the aim of this study was to identify chromosomal regions affecting acid-induced coagulation properties and possible candidate genes. Skim milk samples from 377 Swedish Red cows were rheologically analyzed for acid-induced coagulation properties using low-amplitude oscillation measurements. The resulting traits, including gel strength, coagulation time, and yield stress, were used to conduct a genome-wide association study. Single nucleotide polymorphisms (SNP) were identified using the BovineHD SNPChip (Illumina Inc., San Diego, CA), resulting in almost 621,000 segregating markers. The genome was scanned for putative quantitative trait loci (QTL) regions, haplotypes based on highly associated SNP were inferred, and the additive genetic effects of haplotypes within each QTL region were analyzed using mixed models. A total of 8 genomic regions were identified, with large effects of the significant haplotype explaining between 4.8 and 9.8% of the phenotypic variance of the studied traits. One major QTL was identified to overlap between gel strength and yield stress, the QTL identified with the most significant SNP closest to the gene coding for κ-casein (*CSN3*). In addition, a chromosome-wide significant region affecting yield stress on BTA 11 was identified to be colocated with *PAEP*, coding for β-lactoglobulin. Furthermore, the coagulation properties of the genetic variants within the 2 genes were compared with the coagulation properties identified by the patterns of the haplotypes within the regions, and it was discovered that the haplotypes were more diverse and in one case slightly better at explaining the phenotypic variance. Besides these significant QTL comprising the 2 milk proteins, 3 additional genes are proposed as possible candidates, namely *RAB22A*, *CDH13*, and *STAT1*, and all have previously been found to be expressed in the mammary gland. To our knowledge, this is the first attempt to map QTL regions for acid-induced coagulation properties.
**Key words:** acid-induced coagulation, genome-wide association study, milk protein, dairy cattle

## INTRODUCTION

The production of fermented milk products, such as yogurt, has increased worldwide during the last decade and will most likely increase within the coming decade as well (International Dairy Federation, 2012). Today, more than 25 million tons of fermented products are produced every year throughout the world (International Dairy Federation, 2012), thus making it an economically vital part of the dairy industry. Acidification of milk is the basis in fermented milk production, where a lowering in pH causes insoluble calcium phosphate to dissolve within the CN micelles, and the net negative charge of the CN micelles is neutralized (Lucey, 2009). This results in aggregation as the isoelectric point of the CN micelles (≈pH 4.6) is reached (Lucey, 2009). During fermented milk production, the milk is normally subjected to extensive heat treatment (≈90–95°C, 5–10 min), where whey proteins, mainly β-LG, are denatured (Walstra et al., 2006). The denatured β-LG will, via hydrophobic interactions and disulfide bonds, form either complexes with κ-CN on the surface of the CN micelles or soluble aggregates with themselves, and thereby form a protein gel network (Donato and Guyomarc'h, 2009) with viscoelastic properties (Robinson and Itsa-

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ranuwat, 2006). Studies have shown that heat treatment of milk results in higher gel strength and thereby improved viscoelastic properties of acid gels compared with unheated milk (Donato and Guyomarc'h, 2009; Lucey, 2009).

Several factors have been shown to affect acid milk gels, including milk composition, processing conditions (Donato and Guyomarc'h, 2009; Lucey, 2009), as well as genetic factors (Jakob and Puhan, 1992; Allmere et al., 1998a; Hallén et al., 2009). Despite this, the relations between cow genetics and technological properties of acid milk gels are not fully known. Studies have so far focused on genetic polymorphism of milk proteins; however, the results are somewhat contradictory, mainly regarding the effects of the β-LG variants A and B on acid gel properties (Allmere et al., 1998a; Manderson et al., 1998; Hallén et al., 2009). No significant effects of β-CN (A1, A2, A3, or B; Hallén et al., 2009) or κ-CN [A or B (Allmere et al., 1998a); A, B or E (Hallén et al., 2009)] have been found on acid-induced gel strength or coagulation time (**CT**). On the contrary, $\alpha_{s1}$-CN BB, κ-CN AB, and β-LG BB have been reported to be associated with better consistency and viscosity in fermented milk products (Jakob and Puhan, 1992). These contradictory results suggest that other genes may be of importance for acid milk gels; however, to our knowledge no studies have yet been conducted to explore this.

During the past years, several whole-genome scans and genome-wide association studies (**GWAS**) have been conducted for milk composition traits (e.g., Schopen et al., 2009; Gambra et al., 2013), but such studies are scarce for technological properties of milk. Thus far, only chromosomal regions affecting rennet-induced coagulation important for cheese production have been studied (Gregersen et al., 2015). In the study on rennet-induced coagulation, a total of 21 genomic regions were found for 4 different rennet-induced coagulation traits, and major QTL were identified around the CN gene (*CSN*) cluster on BTA 6. In addition, a candidate gene was identified in relation to O-glycosylation of κ-CN as well as 2 genes in relation to proteolysis of milk proteins. This suggests that chromosomal regions in addition to the regions harboring the milk-protein genes may also be of importance for acid-induced coagulation. The aim of this study was therefore to identify QTL within the bovine genome affecting 3 acid-induced coagulation traits important for viscosity and texture of acidified products, namely gel strength, CT, and yield stress (**YS**). To our knowledge, this is the first study to identify genomic regions related to genes or genomic regions that affect the acidification of milk with the potential to be used in breeding.

## MATERIALS AND METHODS

### Milk and Blood Samples

As part of the Danish–Swedish Milk Genomics Initiative, 377 Swedish Red (**SR**) cows from 21 herds were sampled for morning milk samples and blood samples during the indoor period between April to May 2010 and September 2010 to April 2011. Each herd was sampled in the same day, and a maximum of 24 individual milk samples were collected from each herd. The herds were conventional farms located within the same geographical area in the southern part of Sweden. The cows descended from in total 159 sires and 76 paternal grandsires within the sample population with the number of daughters per sire ranging from 1 to 17. As described in Gregersen et al. (2015), 26 of the bulls had both daughters and granddaughters among the sampled cows. Lactation number ranged from 1 to 4 (1% in lactation number 4) and lactation week from 7 to 40 (2% before lactation wk 7 and 10% after lactation wk 40) for the cows included in the study. The selection of the cows was designed to have a low degree of genetic relatedness between the cows and phenotypic characteristics that represent the current average SR population. None of the cows included in the study showed indication of impaired udder health; all cows had SCC <300,000 cells/mL with mean SCC 74,000 cells/mL. They were fed according to standard practices and milked 2 times (in total 274 of the sampled cows from 16 of the herds) or 3 times (in total 103 of the sampled cows from 5 of the herds) per day. Representative volumes in proportion to milk yield of each cow were sampled and carefully mixed before an aliquot was collected, cooled, and transported to Lund University, Sweden, the day of sampling. The samples were defatted by centrifugation at 2,000 × *g* for 30 min at 4°C, subsampled, and stored at −20°C until rheological analyses. Milk yields were recorded for all cows on each sampling occasion. Fresh milk samples were analyzed for pH as well as SCC by using flow cytometry (CombiFoss 5000, Foss, Hillerød, Denmark) at a certified dairy analysis laboratory (Eurofins Steins Laboratory, Jönköping, Sweden).

### Rheological Analyses

Rheological properties of acid-induced coagulation in individual skim milk samples were determined using low-amplitude oscillation measurements (Kinexus rheometer, Malvern Instruments Ltd., Worcestershire, UK). The milk samples were thawed at 4°C overnight and prewarmed at 30°C for 30 min, timed from the beginning of warming of refrigerated samples to ob-