Genome wide association studies for bovine chromosomal regions affecting rheological parameters in rennet-induced skim milk gels

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Background
Optimizing cheese yield is central to cheese manufacturing. The yield is affected by the time it takes before the gel has an optimal consistency for further processing and it is well known that gel formation differs between individual milk samples. Studies have shown that the presence of both non-coagulating as well as poorly coagulating milk considerably impairs the gelation properties of well-coagulating milk. In the recent years, the effect on milk composition has been analyzed using genome wide association studies (GWAS). Such studies provide substantial information on the chromosomal regions affecting specific milk traits and can be used to generate new hypotheses of genes underlying these traits. The aim of this study was to identify quantitative trait loci (QTL) on the bovine genome affecting rennet-induced gelation. The aim was also to identify genes affecting rennet-induced gelation in general, which in turn could be used within animal breeding to optimize cheese production. This work is part of the Swedish-Danish Milk Genomics Initiative.

Material and methods
Milk and blood samples were collected from 379 Swedish Red (SR) cows in 20 different farms in the southern part of Sweden. The majority of the cows were in lactation number 1-3 and in lactation weeks 7-40. Gel strength (G'), rennet coagulation time (RCT) and yield stress (YS) were determined after rennet-induced gelation on skim milk samples from each cow using low amplitude oscillation measurements (Stressech rheometer, ReoLogica Instruments AB, Sweden). Gelation was induced using chymosin (Chy-Max Plus, Christian Hansen A/S, Denmark). G'0 was defined as the gel strength 30 min. after chymosin addition and G' max was defined as the maximum recorded gel strength in the full run. After 40 min of gelation, a stress sweep was conducted to obtain the YS. A GWAS was performed using the 379 animals of the SR breed. Single nucleotide polymorphisms (SNPs) were identified using the BovineHD BeadChip resulting in almost 621,000 segregating markers. The obtained theological parameters were used to conduct the GWAS. The QTL-regions were screened for SNPs highly associated to the traits, haplotypes were inferred and the additive effect of the most common haplotype within each QTL was analyzed by a mixed model accounting for sire and environmental random effects.

Results

Table 1. Unadjusted means, standard deviation (SD), coefficient of variation (CV%), of rennet coagulation time (RCT), gel strengths (G'0, G' max) and yield stress (YS) in 379 Swedish Red (SR) cows.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCT (min)</td>
<td>19.0</td>
<td>12.0</td>
<td>63</td>
<td>2.7</td>
<td>&gt;40</td>
</tr>
<tr>
<td>G'0 (Pa)</td>
<td>45.1</td>
<td>51.5</td>
<td>114</td>
<td>0</td>
<td>276.1</td>
</tr>
<tr>
<td>G' max (Pa)</td>
<td>64.8</td>
<td>65.8</td>
<td>102</td>
<td>0</td>
<td>312.7</td>
</tr>
<tr>
<td>YS (Pa)</td>
<td>13.0</td>
<td>7.8</td>
<td>60</td>
<td>0</td>
<td>33.4</td>
</tr>
</tbody>
</table>

The rheological measurements performed on individual milk samples from the SR breed indicate a large variation in gelation properties in SR milk (table 1). The coefficient of variation (CV%) is high for RCT and YS and even higher for the G'-measurements. Thus, the large variation indicates that there is a possibility of changing these traits with suitable breeding programs.

The GWAS resulted in 33 unique QTL-regions on 21 different chromosomes, with 10 regions showing association to more than one trait. Within these QTL-regions some candidate genes were identified which might affect rennet induced gelation. A major QTL for all traits were identified around the casein (CN) cluster explaining between 10.9 to 26.6% of the phenotypic variation. In three cases, RCT, G'0 and G' max, the most significant SNP identified was found on CSN1S2 coding for α2-CN. However, SNPs surrounding all the other CN genes were also identified to be significant. For YS, the peak was located on the CSN3 gene coding for k-CN. In addition, other possible candidate genes were identified which concern post translational modifications of the CNs.

Conclusions

• There is a large variation in gelation properties in milk from cows of the SR breed
• The most significant QTL region affected all traits and harbored the CN cluster, giving more detailed information about the important role of CN in rennet-induced gelation.
• This analysis show that even though a large proportion of the phenotypic variance can be explained from haplotypes identified around the CN gene cluster, rennet-induced gelation properties are affected by other genes as well.