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710. Heritability and genomic analysis of coagulation event in sheep milk

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Abstract

Sheep farming plays an important role in the rural economy and about 250 million sheep are reared for dairy purpose worldwide. The whole amount of sheep milk is used for cheese making and the presence of non-coagulating milk affects negatively economics of dairy industry. Beside the effects exerted by the environmental factors, additive genetic individual differences in coagulation exist and allow genetic improvement. The aim of this work was to study differences between coagulating and non-coagulating milk samples, to estimate h^2 and conducting a genomic association study on milk coagulation phenotypes. A total of 8.7% samples did not coagulate within 30 min and a difference in milk composition between normal and non-coagulating samples was observed. Genetic analysis allows both to estimate h^2 for the coagulation event of 0.23 (0.04) and to suggest 45 genes involved in mammary gland metabolism and udder health status.

Introduction

Sheep farming plays an important role in the rural economy, in particular for Mediterranean area, Asian, and developing countries. About 250 million of dairy purpose sheep farmed worldwide produce about 10 million t of ovine milk (Pulina *et al.*, 2018), which is nearly all processed into cheese. Thus, the ability of milk to coagulate properly is crucial. Milk coagulation is assessed by individual laboratory rennet coagulation time (RCT) and curd firmness (A_{30}) (i.e. the time between the addition of rennet to milk and the beginning of the clot formation and the curd consistence, respectively). The combination of low RCT and high A_{30} are usually associated with higher cheese yield (De Marchi *et al.*, 2008). However, a variable proportion of individual milk samples of different species did not coagulate within this time range. The latter aspect is strongly associated with milk composition (e.g. protein, lactose, pH, and somatic cells) (Bittante *et al.*, 2012). In small ruminants, beside the effects exerted by different environment and management, individual differences exist due to the additive animal variability (Puledda *et al.*, 2017). Thus, the coagulation ability could be improved though breeding schemes. Moreover, genome-wide analysis (GWA) of non-coagulating milk allowed to map QTL in cattle (Duchemin *et al.*, 2016) but a few reports are available in dairy ewes (Marina *et al.*, 2021). The aim of this work was to study differences between coagulating and non-coagulating milk samples, to estimate the variance components and heritability and to find regions associated with this trait.

Materials & methods

Data. Individual milk samples of 1,018 ewes from 47 flocks located in Sardinia (Italy) were analyzed. Data came from a project aimed to investigate milk coagulation properties (MCP) in Sarda dairy sheep (Manca *et al.*, 2016; Puledda *et al.*, 2017). RCT was determined by Formagraph instrument (Foss Electric A/S, Hillerød, Denmark) for all samples, that were divided in two classes: (1) COAG, samples coagulating within 30 minutes; (2) NON-COAG, samples that did not coagulate in this time range. Milk composition traits (fat, protein, lactose, SCS, chloride and urea) were recorded for all samples to compare the two groups.

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Moreover, 769 ewes were genotyped with Infinium Ovine SNP50 v1 BeadChip (Illumina Inc., San Diego, CA). After quality control, 44,619 SNPs on 27 OAR chromosomes remained for GWA study.

Heritability estimation. The following threshold animal model was fitted to estimate variance components and heritability (h^2)

$$y = Xb + Z_1f + Z_2a + e \quad (1)$$

where: y is the vector of coagulation binary trait; b is the vector of fixed effects including the month of lambing (4 levels), days in milk in classes of 30d (5 levels) and parity (3 levels); f is the vector of the random effect of flock-test days of sampling combination (69 levels) ~$N(0, I\sigma^2_{ftd})$; a is the vector of the random additive genetic effects ~$N(0, A\sigma^2_a)$ where A is the numerator relationship (5,031 animals) and e is the vector of random residuals ~$N(0, I\sigma^2_e)$ The σ^2_{ftd} σ^2_a σ^2_e are the flock-test day, additive genetic and residual variances. The X, Z_1 and Z_2 are the incidence matrices relating records to effects. Variance components and heritability were estimated using thrgibbs1f90 software (Misztal *et al.*, 2014), which implements a Gibbs sampling method. The following parameters were used: 50,000 samples were generated, with the first initial 5,000 rounds discarded as burn-in, and all were saved. The heritability was estimated as intra-herd h^2. The value on the liability scale, which is a function of h^2 and incidence of coagulation classes, was transformed on the observed scale.

GWA study and gene discovery. The model (1) was modified under the ssGBLUP framework to accommodate H^{-1} matrix in place of A^{-1} and later used for GWA study according to Cesarani *et al.* (2021). Briefly, the GEBV were back-solved into SNP effects and the additive genetic variance explained by each SNP was computed. The SNP exceeding the 99.9th percentile were retained as suggestive of trait association. Using genome browser (https://genome.ucsc.edu/) genes included in the neighbour of suggestive SNP were retrieved, and a gene-by-gene literature review was performed in order to assess previously association with MCP and milk traits.

Results

A total of 89 milk samples (8.7% of the total) did not coagulate within 30 min from the rennet addition. Average RCT for the COAG group was 15.13±6.5 minutes. Samples of the NON-COAG group showed larger values for protein (5.72 vs 5.38%, P<0.001), chloride (194.5 vs 141 mg/100 ml, P<0.001), and SCS (7.50 vs 4.37, P<0.001), whereas they exhibited lower values for lactose (4.44 vs 4.85%, P<0.001) and urea (33.2 vs 38.4 mg/dl, P<0.001). The heritability of the coagulation ability was moderate (0.23±0.04).

As far as GWA is concerned, a total of 458 SNP overcome the threshold defining 40 QTL regions over 18 chromosomes (autosome and sexual); OAR12, 13, 15, 16, 17, 18, 19, 24 and 25 did not show any associated SNP with failure in coagulation phenotype. The distribution of suggestive SNPs and the proportion of additive genetic variance are presented in Figure 1.

Among the genomic regions identified by tag SNPs, nearby 17 of them, 260 genes were retrieved, but only 45 (Table 1) were involved in cellular function associated to dairy traits in ruminant species (i.e. milk coagulation, milk production and composition, udder health, heat stress resistance).

Discussion

Literature reports quite high percentage of non-coagulating milk samples, both in cows and sheep. In Swedish Red cattle breed, about 18% of milk samples did not coagulate within 30-40 minutes (Gustavsson *et al.*, 2014); lower percentages were reported for Holstein (9.7%; Cecchinato *et al.,* 2011) and Brown Swiss

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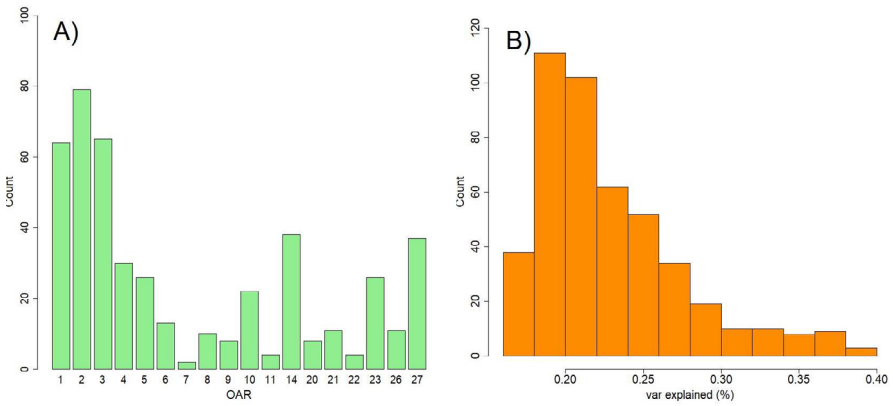

Figure 1. (A) Number of top 0.1% SNP per chromosome and (B) distribution of variance explained by top SNP.

Table 1. QTL regions identified and genes retrieved.

QTL	OAR[1]	Start	End	Peak SNP (max% var)	Mbp	Gene
1	1	19.01	22.59	OAR1_20007545.1	20.03	*AKR1A1,CMPK1,TMEM69, UROD,LRRC41,PIK3R3,TESK2*
2	2	107.07	114.70	OAR2_122611468.1	114.65	*MFAP3L, ARHGEF4, OCA2*
3	2	201.82	202.26	s35200.1	201.86	*BZW1,PPIL3*
4	2	234.72	235.51	s46218.1	235.10	*FABP3, NKAIN, ZCCHC17*
5	3	195.80	198.52	OAR3_211332869.1	196.05	*SLC15A5*
6	3	94.92	95.16	s61740.1	95.16	*CCT7*
7	4	14.35	15.14	OAR4_14557628.1	14.35	*ASNS*
8	5	79.30	79.82	OAR5_87409839_X.1	79.47	*RPS23*
9	5	41.59	42.57	s44617.1	42.16	*CSNK1G2*
10	11	46.99	47.30	OAR11_50094068.1	47.05	*ACE*
11	14	45.38	46.07	OAR14_48226980.1	45.81	*APLP1,CAPNS1,ZNF529, ALKBH6,SYNE4*
12	14	35.07	35.58	s14680.1	35.29	*ZBTB7C, CDH1*
13	20	45.96	46.30	OAR20_50378146.1	46.22	*SLC35B3*
14	21	44.31	45.22	s23338.1	44.96	*CHKA, TCIRG1, CABP2, AIP, CLCF1, NUDT8*
15	22	22.33	22.62	OAR22_26729825.1	22.62	*BORCS7, TRIM8, WBP1L,ARL3*
16	26	6.15	6.75	s41368.1	6.45	*SPCS3, ASB5*
17	X	51.09	52.98	s05480.1	52.39	*TFE3, SLC35A2, AKAP4*

[1] OAR *Ovis aries* chromosomes.

(3.5%; Cecchinato *et al.*, 2011). For sheep milk, about 10% of samples were found to be NON-COAG in studies involving Sarda sheep milk (Manca *et al.*, 2016).

A higher proportion (17.7-19.4%) of NON-COAG samples was reported by Caballeros-Villalbos *et al.* (2017) and Garzón *et al.* (2021) in Manchega sheep breed. In our study, the main differences between the two investigated groups of milk samples seem to be associated with health indicator or involving permeability of mammary gland epithelium, which facilitates the exchanges of molecules from bloodstream to the alveolus of the mammary gland. As far as the heritability was concerned, we did not find reports on coagulation trait as binary trait in sheep. In dairy cattle, threshold model applied on binary outcome rather than linear model on RCT (with the exclusion NON-COAG) halved the h^2 estimates (Cecchinato and

Carnier, 2011). In our case, the h^2 estimate was in agreement with previous report on RCT excluding the NON-COAG samples in different sheep breed (Puledda *et al.*, 2017; Sanchez Mayor *et al.* 2019).

The gene discovery on GWA results allow us to prioritize a set of genes previously associated to milk or cheese related traits in dairy ruminants, such as those affecting somatic cell count (*CHKA*, *TCIRG1*, *PPIL3*), mastitis (*ALKBH6, SPCS3, LRRC41*), either overexpressed in mammary gland in different lactation stage (*CMPK1*) or in different milk ability group (*ZCCHC17*) and involved in lactose synthesis (*SLC35A2*) (Bonnefont *et al.* 2011; Dhorne-Pollet *et al.*, 2012; Ghahramani *et al.*, 2021; Michailidou *et al.*, 2021; Sadovnikova *et al.*, 2021). Many other retrieved genes have been previously associated to milk fat and/or composition in sheep milk. In conclusion, the genes of interest are mostly linked to mammary gland metabolism, udder health status and milk compound known to affect the ability of milk to coagulate. These findings are consistent in explaining the differences in the milk composition observed between COAG and NON-COAG individual milks.

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