

Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep

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Abstract – In this paper, we present recent advances in the molecular dissection of complex traits in dairy sheep and discuss their possible impact on breeding schemes. In the first step, we review the literature data on genetic polymorphisms and the effects of sheep α_{s1} -casein and β -lactoglobulin loci. It is concluded that the results are rather inconsistent and cannot be used in dairy sheep selection. In a second step, we describe the strategy implemented in France, Italy and Spain taking advantage of the genetic maps for QTL detection. These studies were part of a European project, called “genesheepsafety”, which investigated both milk production and functional traits. Preliminary QTL results are presented for production traits.

dairy sheep / milk proteins / genetic polymorphisms / QTL / milk production

1. INTRODUCTION

Dairy sheep have been traditionally farmed in Europe, especially in Mediterranean countries in a wide range of local production systems combined to a great genetic diversity [8]. Most Mediterranean dairy sheep are dual purpose, with incomes originating from both meat (milk-fed lambs) and milk, with milk being processed into high quality cheese. Since milk sales represent 65 to 75% of farm income, increasing milk yield is still the most profitable objective for most breeds [26, 37]. The most frequent breeding strategy corresponds to the selection of the local breeds for milk yield and composition within their specific area and production system [2].

Moreover, due to the evolution of the European consumers demand for typical products, and for higher quality and safety, dairy sheep also now have

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to be improved for new traits of economic importance, which has rapidly increased in recent years. These new traits, such as udder morphology and milkability [15, 19, 36], functional longevity, or resistance to diseases like mastitis [3, 22, 46], are related to the reduction of production costs and the increase of the quality and safety of the products. When compared to cattle, dairy sheep have some important characteristics: (i) the use of artificial insemination is more or less limited and natural mating plays an important role in reproduction; and (ii) the individual recording of any trait is much more costly due to the lower individual income per ewe. Under these conditions, applying marker-assisted selection (MAS) or gene-assisted selection (GAS), either to speed up selection of routinely measured traits or to implement selection for traits costly to record, seems to be attractive in dairy sheep.

Before implementing MAS, or GAS which is economically more realistic in dairy sheep, accurate molecular knowledge is required for traits of interest. In this paper, we review the results on genetic polymorphisms of milk proteins, mainly investigated in the 1990s after the spectacular results observed in goats. Then, we present the strategy implemented in several countries, taking advantage of the genetic marker maps for QTL detection. In the European Union, a series of regional projects started at the end of the 1990s to analyse the genetic variability of economically relevant traits. Most investigations focused on production traits due to the lack of phenotypic information on functional traits. Some of these projects were federated in the EC-funded Genesheepsafety project to investigate both production and functional traits. This paper presents some preliminary QTL results for production traits in three dairy sheep populations involved in this project.

2. GENETIC POLYMORPHISMS OF MILK PROTEINS

Several milk protein polymorphisms have been considered as potential tools for selection of dairy ruminants. DNA-based molecular methods have made genotyping of animals of any age and sex for milk protein genes possible, thus providing a potentially more efficient and flexible selection tool than protein electrophoretic genotyping on milk. Selection efficiency, however, depends on allelic frequencies in the breeds and on the effect of these polymorphisms on dairy traits and technological properties of milk. For dairy sheep, research on the genetic polymorphisms of caseins and whey proteins is still limited to α_{s1} -casein and β -lactoglobulin loci, giving less conclusive results than in goats.

The casein fraction of ruminant milk consists of four caseins, namely α_{s1} , α_{s2} , β and κ , encoded by four clustered genes located on chromosome 6 of

Table I. Allelic frequencies at the α_{s1} -casein and the β -lactoglobulin loci in samples of some European dairy sheep breeds, from [1, 6, 29, 38–41, 45].

Breed	Country	<i>n</i>	Allele frequencies			
			α_{s1} -casein		β -lactoglobulin	
			D allele ⁽¹⁾	Normal	A	B
Lacaune	France	1054 ⁽²⁾	0.007	0.993	0.643	0.357
Lacaune	France	517 ⁽³⁾	0.001	0.999	0.627	0.373
Lacaune	France	992 ⁽²⁾	-	-	0.565	0.435
Manech (blondfaced)	France	222 ⁽²⁾	0.000	1.000	0.473	0.527
Massa	Italy	54	-	-	0.53	0.47
Sarda	Italy	2700 ⁽²⁾	0.027	0.973		
Sarda	Italy	652 ⁽⁴⁾	0.028	0.972		
Sarda	Italy	2957 ⁽²⁾			0.274	0.726
Churra	Spain	901 ⁽²⁾	-	-	0.325	0.675
Merino	Spain	168 ⁽⁴⁾	0.009	0.991	-	-
Merino	Spain	340 ⁽⁴⁾	-	-	0.58	0.41
Manchega	Spain	238 ⁽⁴⁾	0.000	1	0.32	0.68
Segureña	Spain	50 ⁽⁴⁾	0.000	1	0.67	0.33

⁽¹⁾ Known initially as the “Welsh allele”; ⁽²⁾ flocks in official milk recording;

⁽³⁾ experimental flock; ⁽⁴⁾ unrecorded commercial flocks.

cattle, sheep and goats [9, 30]. Two α_{s1} -*cas* variants, “Normal” and “Welsh” were first detected [31] followed by five variants (*A* to *E*) [16]. But only the Welsh, subsequently denoted *D* variant, has been related to a decrease in casein content and renneting properties [41, 42]. This α_{s1} -*cas* unfavourable *D* allele is rare in all analysed breeds (Tab. I), with frequencies ranging from 0 to 0.03, and therefore has little potential for selection.

Three genetic variants (*A*, *B*, and *C*) of ovine β -lactoglobulin have been described [24]. But the β -*lg C* allele has been found only in the Merinos type breeds and at a low frequency. The results reported on the effect of the other *A* and *B* polymorphisms on production traits and cheese-making properties are inconsistent. On the one hand, Caroli *et al.* [10] and Fraghi *et al.* [25] reported a positive effect of the *B* allele on milk yield in the Sarda breed, while Lopez-Galvez *et al.* [34] found that the β -*lg AA* genotype milk had better cheese-making properties than β -*lg BB* or *AB* genotypes in the Manchega breed. Similarly, Gutierrez-Gil *et al.* [29] found that *AA* homozygous animals showed a higher individual cheese yield than *AB* or *BB* ewes in the Churra

breed. On the other hand, Recio *et al.* [43] did not report any difference in renneting properties between the different genotypes. Pietrolà *et al.* [40] did not find any direct effect or linkage between milk yield and β -lactoglobulin genotype using two independent designs: a first sample of 2957 primiparous Sarda ewes in 132 recorded flocks and a granddaughter family (with a heterozygous *AB* sire, 14 sons and 512 daughters). Finally, a trial in the Lacaune breed based on 5 heterozygous β -lg *AB* sire families and 992 daughters did not show any direct effect or association between β -lg alleles and production traits, *i.e.* milk, fat, and protein yields, and fat and protein contents (Barillet, unpublished results). The lack of effects found in the most powerful designs suggests that the significant results reported first could be false positives, due to some confounding with environmental effects and the lack of appropriate adjustments. Indeed, some studies were based on individual milk sample measurements unadjusted for days in milk and parity. To conclude, the relations between β -lg polymorphism and dairy traits or technological properties of milk remain controversial and are generally inconsistent. It is not recommended to use this polymorphism in selection.

3. MAPPING QUANTITATIVE TRAIT LOCI FOR MILK PRODUCTION

3.1. Resource populations of the “Genesheepsafety” project

Two resource populations are involved in a genome scan for primary QTL detection in dairy sheep: (i) a Sarda \times Lacaune backcross population was generated on an experimental farm and recorded for a number of original traits of potential interest in selection; and (ii) a daughter design was used in the Churra breed nucleus flocks to map QTL for routinely recorded traits. Additionally, targeted genome regions or chromosomes known to harbour QTL in dairy cattle or sheep were studied in purebred granddaughter families of French dairy sheep breeds (Basco-Béarnaise, Lacaune and Manech).

3.1.1. Back-cross Sarda \times Lacaune resource population

Crosses between breeds allow the exploitation of linkage disequilibrium for genes differing between breeds in order to detect genome regions controlling the traits of interest. The Sarda and Lacaune breeds are the two most numerous Italian and French dairy sheep breeds, and they differ in many traits [4, 12].

An experimental Sarda \times Lacaune backcross population was created in 1999 by IZCS (Italy) and INRA (France) in order to detect QTL for production and functional traits [11, 13]. In 1998, 14 AI elite Lacaune rams were mated in Italy to Sarda ewes to produce F1 rams. Among these, 10 sons of 10 different Lacaune sires were mated to Sarda ewes to procreate 980 backcross females born in 1999. Family size ranged from 76 to 121. The ewes were bred in an experimental farm of IZCS located in the southern part of Sardinia. They completed their fourth lactation in 2003. During their life, they were recorded for many traits including growth rate, adult body weight and body condition score, reproduction, milk production, conjugated linoleic acid (CLA) content in milk, wool production, udder morphology, milkability, resistance to mastitis, gastrointestinal parasitosis and nasal bot.

3.1.2. Churra breed resource population

In outbred populations, daughter and granddaughter designs are used to identify marker-QTL associations in large dairy half-sib families obtained by artificial insemination [50]. The Churra breed is one of the most important dairy sheep in Spain, and the Churra programme is aimed at mapping QTL affecting milk production and functional traits. It takes advantage of medium to large half-sib families obtained by AI and of genetic evaluations providing accurate estimated breeding values (EBV) used as phenotypes in QTL detection. The daughter design was comprised of 1428 ewes distributed in 11 paternal half-sib families generated by AI in 17 farms. The family size ranged from 69 to 261 with a mean of 130 ewes. All the animals belonged to the nucleus scheme of the Spanish Churra National Breeders (ANCHE) and were routinely on-farm recorded for dairy traits.

3.1.3. French breed resource population

The breeding programmes in the Lacaune, Basco-Béarnaise and Manech breeds are based on a large scale artificial insemination and progeny test (640 AI rams tested per year). EBV are obtained routinely for six on-farm recorded traits: milk, fat, and protein yields, fat and protein contents, and somatic cell score (SCS) for mastitis resistance. Evaluations for udder score, prolificacy, and functional longevity are being implemented.

Seven hundred and eighty-three AI rams were involved in a granddaughter design. They were distributed in 22 families (18 in Lacaune, 1 in Basco-Béarnaise, 3 in Manech breeds). Family size averaged 36 sons per sire and

ranged from 24 to 56. The sons were born between 1992 and 2002 and had 89 daughters on average. A preliminary analysis was performed in 2001 using 419 sons and 13 families [47].

3.2. Microsatellite genotyping of the three resource populations of the “Genesheepsafety” project

A whole genome scan was undertaken for the first two resource populations with 132 and 175 microsatellites compiled from published marker maps [35]. Microsatellite genotypes were identified using Applied Biosystems’ “four dye/one lane” technology on an ABI 377 automatic sequencer.

These panels covered 73% and 83% of the autosomal genome for the Sarda × Lacaune and Churra populations, respectively (Tab. II). The average marker information in backcross Sarda × Lacaune was 0.53, ranging from 0.38 to 0.81. In Churra purebred families, average information content over the whole genome was 0.58, varying from 0.39 to 0.75 according to the chromosome. For the Lacaune and Manech granddaughter designs, 20 microsatellite markers were chosen on seven candidate chromosomes (Tab. II). These regions were selected because their homologous regions in bovines are known to carry QTL affecting dairy traits or SCS.

3.3. Preliminary QTL detection results of the “genesheepsafety” project for production traits

Preliminary results have been recently published for first lactation traits in the Sarda × Lacaune back-cross design [11, 13], for 11 markers of chromosomes OAR9 (complete design) [20] and OAR6 (726 ewes from eight families) in Churra sheep [23], and for 13 of the 22 granddaughter Lacaune and Manech families focusing on seven candidate chromosomal regions [47].

3.3.1. Phenotypes

In the Sarda × Lacaune backcross design, individual first lactations of 975 ewes were analysed considering fixed effects of lactation length, age at lambing, number of lambs born and flock × year interaction. Solutions were obtained from the GLM procedure of SAS[®]. In Churra sheep, the dependent variable was the yield deviation (YD), defined as the weighted average of the ewe’s performances adjusted for fixed environmental effects [20]. Similarly,

Table II. Length and information content (IC) of linkage groups per chromosome, according to resource populations of the “Genesheepsafety” project.

Chrom.	Length cM ¹	Sarda × Lacaune (BC)		Churra (DD)		Lacaune & Manech (GDD)	
		Nb. markers (% Coverage)	IC (average %)	Nb. markers (% Coverage)	IC (average %)	Nb. markers (% Coverage)	IC (average %)
OAR1	347.4	16(90)	60	14(80)	56		
OAR2	308.4	12(97)	50	11(80)	52		
OAR3	313.4	10(78)	52	8(75)	49		
OAR4	151.9	6(87)	52	5(78)	56		
OAR5	156.9	3(61)	64	8(85)	60	4(79)	52
OAR6	156.7	6(76)	58	9(95)	75	2(25)	71
OAR7	145.9	6(68)	38	8(95)	62		
OAR8	132.8	3(54)	59	5(90)	39		
OAR9	126.9	5(65)	61	8(95)	70	2(43)	69
OAR10	105.1	4(87)	67	4(75)	42		
OAR11	112.4	4(49)	44	5(85)	51	3(49)	74
OAR12	113.1	6(81)	58	5(78)	56		
OAR13	128.3	5(90)	48	6(72)	54		
OAR14	119.9	5(79)	81	5(80)	64		
OAR15	124.4	2(40)	65	6(83)	63	3(52)	61
OAR16	86.2	4(81)	62	7(90)	70	4(80)	81
OAR17	130.7	4(80)	59	7(95)	48		
OAR18	131.5	6(70)	51	6(85)	56		
OAR19	73.3	4(70)	58	4(62)	56		
OAR20	89.6	3(43)	62	6(90)	58		
OAR21	74.2	5(79)	66	7(85)	62		
OAR22	82.9	3(72)	51	3(50)	54	2(24)	84
OAR23	95.5	2(38)	66	5(95)	62		
OAR24	89.4	2(15)	53	4(75)	43		
OAR25	69.9	1(-)	46	4(85)	53		
OAR26	71.1	4(97)	55	4(95)	62		

¹sex-average, according to Maddox *et al.* (<http://rubens.its.unimelb.edu.au/~jillm/jill.htm>); IC = information content.

in the French granddaughter designs, the dependent variable for each ram was the DYD [49], *i.e.* the average phenotype of the daughters was adjusted for environmental effects and additive genetic merit of the mates. Milk, fat and protein yields, and fat and protein contents were analysed. YD and DYD were obtained from the Spanish and French dairy sheep evaluation systems.

3.3.2. QTL detection methods

QTL analysis was performed using multi-marker regression [21, 32]. Chromosome-wise significance thresholds were determined through 10 000 phenotype permutations according to Churchill and Doerge [17].

3.3.3. Results

The QTL results for production traits are reported in Table III listing all tests significant at the chromosome-wise $\alpha_c = 0.05$ type I error level. QTL were found for all dairy traits. Each detected QTL usually segregated in only two or three families. Most substitution effect estimates ranged from 0.4 to 1.5 phenotypic standard deviation units but the highest values were likely to have been overestimated due to the limited power of each design.

For the Sarda \times Lacaune backcross ewes, the most significant results related to production were on OAR 3, OAR 16 and OAR 20. These three QTL were found to segregate in the same families and to affect milk, fat and protein yields in the same direction (Tab. III), in agreement with the known high genetic correlations (0.80 to 0.95) between yields [2]. Moreover, QTL for the same traits have been reported on homologous bovine chromosomes [7]: BTA 20 (OAR 16) and BTA 23 (OAR 20). So far, the most significant QTL detected in this resource population was located on OAR 20 for fat content.

In the Churra sheep, a QTL was suggested in the telomeric region of OAR6 in the neighbourhood of the casein region (marker BM4311) affecting both protein percentage and milk yield. Two families were segregating in this region, one for protein percentage and the other for milk yield. Substitution effects clearly revealed distinct effects in the two families. In the first segregating family, the QTL produced a dilution-concentration effect of milk, as described in cattle [27, 48]. On the contrary, the second segregating family showed pleiotropic effects on milk and protein yields as described by Kühn *et al.* [33] in cattle, but in a different chromosome region.

For the Lacaune and Manech breeds, in agreement with dairy cattle results [5, 18, 27, 48, 51], QTL for fat content, protein content and protein yield were found on OAR9, OAR 5 and OAR 6, respectively (Tab. III). The most significant QTL was detected for fat content on OAR 9. This QTL was recently identified as a mutation in the *DGAT1* gene on the homologous bovine chromosome (BTA 14) [28].

Table III. Significant QTL for production traits in the three QTL programmes, from [11, 13, 20, 23, 47].

OAR Programme ¹	Trait ²	Closest marker	Position (cM)	P-value (%)	Informative families	QTL effect (σ_p)	
1	1-BC	Protein content	MCM058	98	0.5	1, 6, 8, 10	0.46/-0.71/-0.45/-0.51
5	3-GDD	Protein content	BMS2258	106	4.3	B, M, N	0.74/1.18/0.79
3	1-BC	Milk yield	BMC1009	168	0.12	4, 6, 10	0.98/0.94/0.68
	1-BC	Fat yield	BMC1009	168	2.3	4, 6, (10)	0.82/0.93/(0.44)
	1-BC	Protein yield	BMC1009	166	0.05	4, 6, (10)	1.06/0.98/(0.54)
6	2-DD	Milk yield	BM4311	128	3.5	4	0.52
	2-DD	Protein content	BM4311	126	3.2	8	0.83
	3-GDD	Protein yield	OARAE101	34	3.9	B, M, N	-1.22/-1.52/-0.85
9	2-DD	Fat content	RHJI	115	5.0	3, 6	-0.43/0.41
	3-GDD	Fat content	CSSM66	12	0.38	G, L, M	-0.68/-1.01/-1.28
16	1-BC	Milk yield	MAF214	34	0.15	1, 7, 9	0.51/-0.57/1.06
	1-BC	Fat yield	MAF214	32	0.37	(1), 7, 9	(0.38)/-0.52/1.06
	1-BC	Protein yield	MAF214	32	0.59	1, (7), 9	0.52/(-0.44)/1.01
20	1-BC	Milk yield	BM1258	2	0.34	6, 9, 10	-0.57/1.27/0.83
	1-BC	Fat yield	BM1258	0	0.68	6, 9, 10	-0.56/1.05/0.69
	1-BC	Protein yield	BM1258	2	1.04	6, 9, 10	-0.49/1.26/0.76
	1-BC	Fat content	OARH56	30	0.05	1, 2, 4, 9	0.74/0.65/-0.64/-0.65

¹1-BC = Sarda \times Lacaune backcross design; 2-DD = Churra daughter design;

3-GDD = Lacaune or Manech granddaughter design.

² First lactation for BC, all lactations for DD and GDD resource populations.

3.4. Preliminary QTL results for conjugated linoleic acid content in milk fat

The fatty acid composition of milk fat was measured twice for the Sarda \times Lacaune backcross population at mid-second (847 records) and -third lactations (795 records). Most attention was paid to conjugated linoleic acid (CLA) content in the milk fat, because of its possible effect on human health. A large part of CLA in milk is produced in the mammary gland where vaccenic acid is desaturated by delta 9-desaturase. Thus QTL detection was carried out

for CLA, and for the CLA/vaccenic acid ratio. In order to account for repeated records, a mixed model was used including the fixed effect of year*group of management and the random effects of ewes and sires. The effects of the 727 ewes with two records were used as phenotypes for QTL analysis. Significant QTL at the 5% chromosome-wide level were found on OAR 4, OAR 14 and OAR 19 for CLA content in milk fat, and on OAR 4, OAR 6, OAR 14 and OAR 22 for the CLA/vaccenic acid ratio [14]. One QTL was found for the ratio on OAR 22 where the SCD gene encoding for delta 9-desaturase is located. A candidate gene approach for five genes, including SCD, is carried out on the same resource population.

3.5. Other resource populations

3.5.1. In Spain

There are two other projects involving the Latxa and the Manchega dairy sheep breeds. The Latxa QTL-mapping project uses a daughter design and “selective DNA pooling” in order to reduce the genotyping effort [44]. Preliminary results in the Latxa breed pinpointed two different regions of OAR 6 affecting milk traits: one coinciding with the region described in Churra, the other one in the telomeric end near the marker CSR293 [44]. The Manchega QTL-mapping project uses a candidate-gene approach rather than a genome scan strategy (Serrano, personal communication).

3.5.2. In Australia

Another QTL mapping project, based on crossbreeding between Awassi and Merino sheep, started some years ago in Australia. It is linked to a dairy cattle research programme and the first results are expected soon (Raadsma, personal communication).

4. CONCLUSION

Milk protein polymorphisms have been considered as potential tools for selection of dairy ruminants. In dairy sheep, research on this topic is limited to α_{s1} -casein and β -lactoglobulin loci, so far. For α_{s1} -casein, only the *D* (or Welsh) variant has been related to a decrease in casein content and renneting

properties. Nevertheless, its low frequency makes it nearly useless for selection. Similarly, the effects of β -lg polymorphism on dairy traits or milk technological properties are too inconsistent for implementing selection.

As a consequence, efforts have concentrated on QTL mapping projects. To our knowledge, most of them are regional projects in Mediterranean countries. Three of them in France, Italy, and Spain are part of the “Genesheepsafety” European project to investigate both production and functional traits. QTL have already been found for all dairy traits, the most significant being presently reported for fat content on OAR 9 and OAR 20. They are only preliminary results, based on the first lactation in the Sarda \times Lacaune backcross population, on two chromosomes (OAR 6 and OAR 9) in the Churra sheep, and seven targeted chromosomal regions in the Lacaune and Manech granddaughter families. This project is continuing in three directions: the complete results of the genome scan of the Sarda \times Lacaune backcross and Churra resource populations for all lactations will be published, the genome scan of the Lacaune and Manech resource populations will be completed, and the fine mapping of some QTL will begin. Nevertheless, the present preliminary results are promising and show that cattle QTL results can be partly transposed to sheep. Finally, QTL fine mapping will be a crucial step for dairy sheep. Due to the economical constraints in this species, only MAS based on population-wide linkage disequilibrium or, even better, GAS based on causal mutations, will be profitable.

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