QTL Detection For Milk Traits On Ovine Chromosome 7 Through A Sire Design Using Marker Genotypes And Production Records Of Daughters And Their Progeny

Sara Casu[*], M.G. Usai[*], S. Sechi[*], G. Mulas[*], T. Sechi[*], S. Miari[*], and A. Carta[*]

Introduction

So far QTL mapping experiments in dairy sheep have been based on whole-genome linkage analysis of daughter or granddaughters families (Barillet et al. 2005; Barillet et al., 2006). The mapping resolution of most of these experiments was limited by the low density of the used microsatellite maps and number of informative meioses. One of these experiments involved a population of Sarda X Lacaune (BC) backcross ewes organized in a daughter design of 10 half-sibs families of around 100 ewes per F1 sire. The genome scan analysis performed on this population allowed to identify several chromosomal regions affecting traits of economic interest (Barillet et al. 2006; Carta et al. 2008). With the aim of confirming and locating more precisely the identified QTL, the regions of interest were densified by adding further microsatellite markers. Among these, chromosome 7 was chosen since there was evidence for a segregating QTL which affected milk fat and protein content (Barillet et al. 2006). Furthermore, in order to validate the segregation of the found QTL in the pure Sarda breed a new resource population (BCD) was procreated between 2002 and 2004 by mating Sarda rams with BC ewes. This population was measured for milk traits and genotyped at the same loci on OAR7. Thus, combining marker genotype data of BC and BCD ewes, it is possible to calculate the probability of inheriting one defined QTL allele from the grandsire. A similar approach was proposed by Coppieters et al. (1999) in the great-grand-daughter design.
The objective of this work was refining the previous sire design analysis on OAR7 by using a denser genetic map and exploiting additional information provided by marker genotypes and production records of granddaughters.

Material and methods

Data from 887 BC and 756 BCD ewes were considered. The latter were daughters of 475 BC ewes and 18 Sarda rams. Milk yield and contents were measured twice a month during each lactation. The number of lactation records per ewe ranged from 2 to 4. Finally five milk traits were analyzed on a lactation basis: milk yield (MY, Kg), fat yield (FY, Kg), protein yield (PY, Kg), fat content (FC, %) and protein content (PC, %). Breeding values were estimated

[*] Agris Sardegna DIRPA Research Unit: Genetics and Biotechnology 07040 Olmedo Italy

including in the analysis also lactation records of the BC ewes' dams. In a first step, records of the three populations were analyzed separately to adjust for the specific environmental effects. Random residuals of these models were analyzed jointly with a penta-trait repeatability animal model (16.437 records per trait). The pedigree file included 11,927 individuals, born between 1960 and 2004. EBV of BC and BCD ewes were then adjusted for ½ the EBV of their Sarda dams and sires respectively.

Twelve microsatellites were added to the six originally used in the BC population (Barillet et al., 2005). The 18 analyzed microsatellites are reported in Figure 1 and located according to the Sheep Best Position Linkage Map V4.7 (sex averaged, cM), available at the Australian Sheep Gene Mapping web site. Only the five BC families showing significant contrasts in the previous analysis were genotyped for the new microsatellites, while BCD ewes were genotyped for all microsatellites except DB2, which had amplification problems. Genotyping of 18 Sarda rams were also performed. Genotyping was carried out in multiplexes using an ABI PRISM® 3130 - Avant Genetic Analyzer (Applera, Foster City, CA).

An across family single trait QTL analysis was carried out, by within-sire linear regression (Knott et al., 1996; Elsen et al., 1999) using the model: $Y_{ij} = s_i + (p_{ij1} - p_{ij2})*a_i + e_{ij}$, where Y_{ij} is the individual phenotype, s_i is the F1 sire (grandsire), p_{ijk} is the probability of inheriting one defined QTL allele from the sire (grandsire) i for daughter (granddaughter) j given the marker information, a_i is half the substitution effect of the putative QTL carried by sire (grandsire) i, and e_{ij} is the residual assumed to be normally distributed with zero expectation and heterogeneous variance σ^2_{ei}. The probability for each possible phase of the F1 sires was estimated from daughters' marker information. The most likely phase was retained and the probability that each progeny received the k chromosomal segment was estimated at every position given this phase, using a 1 cM step. For granddaughter j of sire i this probability was calculated as the probability of granddaughter inheriting a given chromosomal segment from her dam multiplied by the probability the dam inheriting this chromosome segment from the F1 sire.

The rejection thresholds were estimated by 100,000 within-family permutations for each trait, as proposed by Churchill and Doerge (1994). Permutations were performed within family separately for BC and BCD ewes. The sire was considered to be heterozygous for the QTL at the 5% chromosome –wise level when the family contribution to the overall likelihood ratio test at the most probable QTL location exceeded the critical value of 3.84 (= $\chi^2_{(1,0.05)}$). The confidence interval of the QTL location was estimated by 10,000 bootstrapings. Each bootstrap sample was generated by drawing at random only BC ewes' data from informative families and maintaining the corresponding progeny records.

Results and discussion

Average distance between microsatellites was 8.3 ± 5.7 cM. Including the granddaughters' marker information allowed to increase the informative content calculated as $\Sigma_{ij}|p_{ij1} - p_{ij2}|$ of 46% on average (Figure 1). Chromosome –wise significant (P-value < 0.01) LRT values were found in the interval between 120.5 and 126.5 cM for all analyzed milk traits (Table 1). The most significant QTL was found for FC with 5 significant families. Two of them showed LRT values greater than 18 at the most probable QTL location. For this trait the chromosome –wise probability of no QTL resulted lower than 0.00001. The QTL substitution effects

ranged from 0.35 to 0.94 residual standard deviation units (r.s.d.u.). Four families were also informative for PC. The allelic substitution effects ranged from 0.46 to 0.87 r.s.d.u. The QTL affecting MY segregates in 4 families with the highest allelic effect estimated in family 5 (1.21 r.s.d.u). Maximum LRT values for FY and PY mapped the same location (120.5 cM) with a chromosome-wise significant level of 0.00132 and 0.0937 respectively and allelic substitution effects ranging from 0.41 to 0.85.

Table 1 – Likelihood ratio test (LRT), Chromosome Wise significance level (CWP), most probable location from the origin of the chromosome (POS), Informative Families (IF) and QTL allelic substitution effects (a)

Trait	LRT	POS (cM)	CWP	IF	Range of a (r.d.s.u)
MY	37.30	123.5	0.00655	1,5*,8,9*	0.39-1.21
FY	42.38	120.5	0.00132	1*,4,6*	0.41-0.68
PY	36.17	120.5	0.00937	1*,2,9	0.51-0.85
FC	60.30	121.5	>0.00001	1,4,6*,8*,9*	0.35-0.94
PC	48.96	126.5	0.00026	4*,5*,6*,8,9	0.46-0.87

MY: milk yield; FY: fat yield; PY: protein yield, FC: fat content; PC: protein content; r.d.s.u.: residual standard deviation units; *families with LRT >6.64 in the interval between 120.5 e 126.5 cM

Available data only gave limited resolution of the QTL locations with 90% bootstrap confidence interval spanning 77 cM for the most significant one.
Previous analysis of BC phenotypes using the sparser OAR7 map suggested the presence of a QTL affecting FC (Pvalue=0.003) and PC (Pvalue=0.04) located at 101.3 cM. When the denser map was used on BC phenotypes, FC and PC showed a more significant peak (Casu *et al.* 2009). Thus, in this specific case, an increase of the effective population size of 46% was more powerful than lowering the average marker distance from 20 to 8.5 cM on average.

Conclusion

The inclusion of information derived from marker genotypes and production records of granddaughters and the use of a denser microsatellite map allowed to confirm the presence of a chromosome-wise significant QTL affecting milk contents, previously detected with a sparse map and a classical daughter design, on ovine chromosome 7. Furthermore, this approach led to detect QTL for milk, fat and protein yields which had not been found with the previous analysis.
As a whole, increasing the number of informative meioses seems more powerful than adding microsatellite markers, which do not allow to saturate the genetic maps. However, the genotyping of the backcross population and their progeny with the ovine 50K SNP is ongoing and it is expected to increase the contribution of molecular information. Moreover, a further generation of BC descendants has been created. Finally, the availability of an additional number of informative meioses coupled to high resolution SNP genetic maps and more sophisticated methods of analysis is expected to strongly increase the overall power of our experimental design.

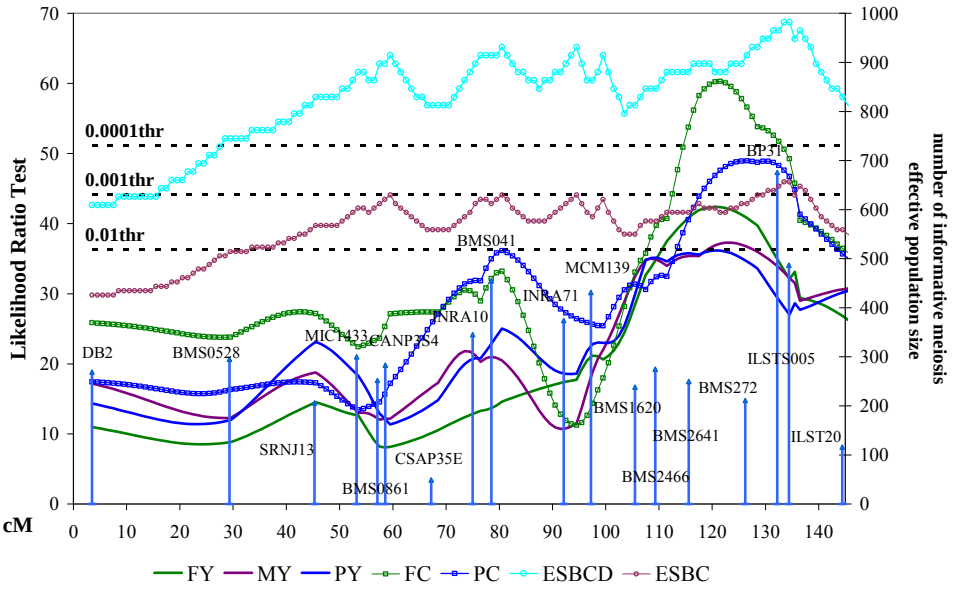

Figure 1 OAR7 map; likelihood ratio test profile (LRT) for milk (MY), fat (FY) and protein (PY) yields, fat (FC) and protein (PC) contents; n. informative meioses (height of the marker flashes), effective population size considering (ESBCD) or excluding (ESBC) marker information of granddaughters, chromosome wise significance thresholds (thr)

Acknowledgments This work was funded by MIPAF in the framework of the « SelMol » project.

References

Barillet F., Arranz J.J. & Carta A. (2005). *Genet. Sel. Evol.*, 37: S109–23.

Barillet, F., Arranz, J.J., Carta A., *et al.* (2006) Final Consolidated Report of the European Union Contract, QTLK5-CT-2000-00656, p. 145.

Carta, A., Casu, Sara, Usai, M.G., *et al.* (2008) *Small Rumin. Res.* 79: 22–28

Casu, Sara, Colombino, M., Mulas, G., *et al.* (2009). *Ital. J. Anim. Sci.* 8: 45-47

Churchill, G.A. and Doerge, R.W. (1994) *Genetics*, **138**: 963-971.

Coppieters, W., Kvasz, A., Arranz, J.J., *et al.* (1999). *Genet. Res.* 74: 189-199.

Elsen, J.M., Mangin, B., Goffinet, B., *et al.* (1999) *Genet. Sel. Evol*, **31**: 213-224

Knott S.A., Elsen J.M., and Haley C.S. (1996). Theor. Appl. Genet, **93**: 71-80.