Identification of milk protein percentage QTLs in Italian Friesian cattle by selective genotyping two GDD families with AFLP and SSR markers

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ABSTRACT - In this work we evaluated the combined use of AFLP markers and selective genotyping in a Grand Daughter experimental design (GDD), to identify chromosomal regions candidate to contain QTL for milk protein percentage (PP) in Italian Friesian cattle. We verified the approach by microsatellite analysis of 4 candidate chromosomal regions. Twenty-five AFLP primer combinations, assayed on the extreme minus and plus-variant half-sibs and on the grandsires of 2 GDD families, produced 180 polymorphic bands of paternal origin. Association analysis resulted in 16 AFLPs significantly associated to PP-EBVs (P≤0.05), that mapped on 8 chromosomes, in regions where PP-QTLs were identified in previous studies. Microsatellite analysis confirmed association for 2 regions of BTA10 and BTA28.

Key words: Quantitative trait loci, Molecular markers, Cattle, Milk quality.

INTRODUCTION - In dairy cattle, most studies carried out to identify QTL for productive and functional traits have used the Daughter (DD) or the Grand Daughter (GDD) experimental design and a few hundred microsatellite markers distributed over the genome (see Khatkar et al., 2004 for a review). To reduce genotyping effort, maintaining the statistical power of QTL identification, the strategy of typing individuals in the tails of the phenotypic distribution (Selective Genotyping, Darvasi and Soller, 1992) has been proposed. AFLP technology (Vos et al., 1995) is a cost-effective, PCR-based technique for the rapid identification of hundreds of reproducible polymorphisms in any species. Here we evaluate the combined use of AFLP markers and selective genotyping in a GDD to identify chromosomal regions candidate to contain QTL for milk protein percentage in Italian Friesian cattle. The approach is verified by microsatellite (SSR) analysis of 4 candidate regions.

MATERIAL AND METHODS - Samples: two GDD families having at least 15 half-sibs sires per tail with extreme EBVs for PP-EBV (difference in mean PP-EBV between tails was 0.22% and 0.20% in the two families) were selected for molecular analyses. A total of 64 sires, 31 of family D (16 extreme minus and 15 extreme plus-variant) and 33 of family S (16 extreme minus and 17 extreme plus-variant) and the 2 grandsires were assayed with AFLP markers. A total of 122 half-sib sires from family D were assayed by SSRs in 4 target regions.

DNA extraction and molecular analyses: genomic DNA was extracted from 50 ml of semen, using the Puregene™ DNA purification system (Gentra, Minneapolis MN 55441). AFLPs were produced according to
Ajmone-Marsan et al. (1997). A total of 25 primer combinations were assayed on the half-sibs and grandsires. AFLPs were binary scored on individuals as dominant markers.

**Association between AFLPs and PP-EBV:** analysis of marker-trait association was performed on all AFLP markers of paternal origin according to the following model:

\[
y_{ijk} = \mu + \text{fami} + \text{band}_j + (\text{fami} \times \text{band})_{ij} + e_{ijk}
\]

where \(y_{ijk}\) is the \(k^{th}\) sires' PP-EBV, \(\mu\) is the factor common to all observations, \(\text{fami}\) is the \(i^{th}\) family, \(\text{band}\) is the presence/absence of the \(j^{th}\) band and \(e\) the random residual (N, 0, \(I_\sigma^2e\)). Significant threshold was set at \(P \leq 0.05\), without and with Bonferroni correction for multiple comparisons.

**Sequencing and Mapping of AFLPs:** AFLP bands significantly associated to PP-EBVs were excised from the gel, cloned and sequenced. Primers were designed on band sequences to convert AFLP markers into Sequence Tagged Sites (STS), that were mapped on the TM-112-3000 Rad bovine/hamster Radiation Hybrids (RHs) panel (Williams et al., 2002). AFLP-derived STSs were assigned to linkage groups and included in the BovGen_RH 2006 map (Jann et al., 2006; www.thearkdb.org). Sequences were also mapped *in silico* on the assembled 7.1X bovine sequence assembly (Btau_3.1, Baylor College of Medicine; http://www.hgsc.bcm.tmc.edu/projects/bovine/) by *in silico* PCR.

**Microsatellite Markers:** to validate the AFLP-based strategy, SSR analysis was run in 4 chromosomal regions in which we mapped AFLP-derived STSs suggestive to be associated to PP in family D (proximal BTA10, proximal and distal BTA18 and proximal BTA28). A total of 16 SSRs heterozygous in the grandsire were used to genotype 122 sires of the D family.

**Microsatellite Association Testing:** to assess the presence of QTLs in the candidate regions one way ANOVA weighted by trait reliability was employed, with a 5% threshold as significance criterion. Marker-trait association was investigated with EBVs calculated with the lactation animal model (L2001), the test-day random

### Table 1. Association between SSRs and PP-EBVs.

| BTA | Marker  | cM  | Num df | df | PP   |
|-----|---------|-----|--------|----|------|
|     |         |     |        |    | L2001| P2006| A2006|
| 10  | BMS528  | 24.0| 100    |    | 0.3092| 0.1186| 0.157 |
|     | BM1237  | 24.7| 90     |    | 0.1286| 0.0298*| 0.0424*|
|     | BMS2349 | 27.0| 84     |    | 0.0329*| 0.0051**| 0.0055**|
|     | BL1035  | 31.6| 103    |    | 0.1382| 0.0725| 0.0779|
| 18  | BMS2213 | 24.5| 91     |    | 0.8696| 0.6778| 0.7106|
|     | INRA121 | 30.2| 70     |    | 0.9772| 0.6898| 0.8586|
|     | BM8151  | 40.2| 72     |    | 0.2569| 0.1477| 0.2044|
|     | IDGVA55 | 67.7| 51     |    | 0.1695| 0.2339| 0.157 |
|     | BMS2785 | 72.0| 61     |    | 0.7649| 0.9304| 0.9232|
|     | BM2078  | 76.8| 103    |    | 0.9833| 0.604  | 0.618 |
|     | DIK5235 | 79.5| 69     |    | 0.5363| 0.9333| 0.9673|
|     | TGLA227 | 84.1| 71     |    | 0.2882| 0.4354| 0.5663|
| 28  | BL25    | 24.8| 64     |    | 0.0238*| 0.085 | 0.0762|
|     | ILST5078| 26.7| 67     |    | 0.012*| 0.023*| 0.0329*|
|     | BMS510  | 29.2| 81     |    | 0.0048**| 0.007**| 0.0103*|
| ø   | BMS2079 | 33.8| 106    |    | 0.1923| 0.108  | 0.2 |

*significant at \(P \leq 0.05\); **significant at \(P \leq 0.01\).*
regression model with only the first lactation (P2006) and the test-day random regression model with the first three lactations (A2006; Canavesi et al. 2004), to evaluate the influence of the method for EBV calculation on results obtained.

RESULTS AND CONCLUSIONS - AFLP Analysis: twenty-five primer pairs amplified approximately 2000 loci out of which 347 were polymorphic in one or both GDD families. Association was run on 129 segregating paternal bands in family D and 136 in family S. Sixteen markers resulted significantly associated to PP-EBV at a 5% significance value (10 in family D and 6 in family S). The application of the Bonferroni correction resulted in a value of P<0.0004. No marker was significant under these conditions. However, with no correction for multiple comparison, in family D the number of suggestive markers exceeded the number expected significantly by chance (expected values: 6.5) and deserved independent confirmation by SSR analysis.

Mapping of Candidate AFLP Markers: the combined in silico and RH mapping approaches permitted the localization of 11 AFLPs on BTA1, BTA2, BTA8, BTA10 BTA12, BTA18 and BTA28. All 11 AFLP markers mapped in the region of QTLs identified in the Israeli Holstein (Mosig et al., 2001; Ron et al, 2004), French Holstein-Friesian (Boichard et al., 2003) and North American Holstein-Friesian populations (Heyen et al., 1999; Georges et al., 1995; Zhang et al., 1998). With one exception on BTA18, AFLPs mapped within 10cM from the PP QTL peaks. Microsatellite Analysis: Association with PP EBVs suggested by AFLP analysis was confirmed by single marker analysis in two regions on BTA10 and BTA28 (Table 1). On BTA18, none of the 2 regions in which suggestive AFLPs were found was confirmed by SSR analysis.

Considering the low stringency significance threshold used, AFLP data alone can only be suggestive of the presence of PP QTLs. However, confirmation from SSRs and agreeable data from independent investigations reinforce one-other, and provide circumstantial evidence of the presence of significant PP effects in at least two of the chromosomal locations identified in BTA10 and BTA28.

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