

Bovine chromosome 20: milk production QTL and candidate gene analysis in the Italian Holstein-Friesian breed

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ABSTRACT: Bovine chromosome 20 (BTA20) was studied to identify QTL for milk yield and protein percentage in the Italian Holstein-Friesian breed using a selective milk DNA pooling strategy in a daughter design with sire haplotype analysis. Several QTL were identified. The effect of the known *GHR F279Y* and *PRLR S18N* mutations were in for the most part confirmed. However, it was also shown that these markers cannot explain all significant effects observed on BTA20 for the investigated traits.

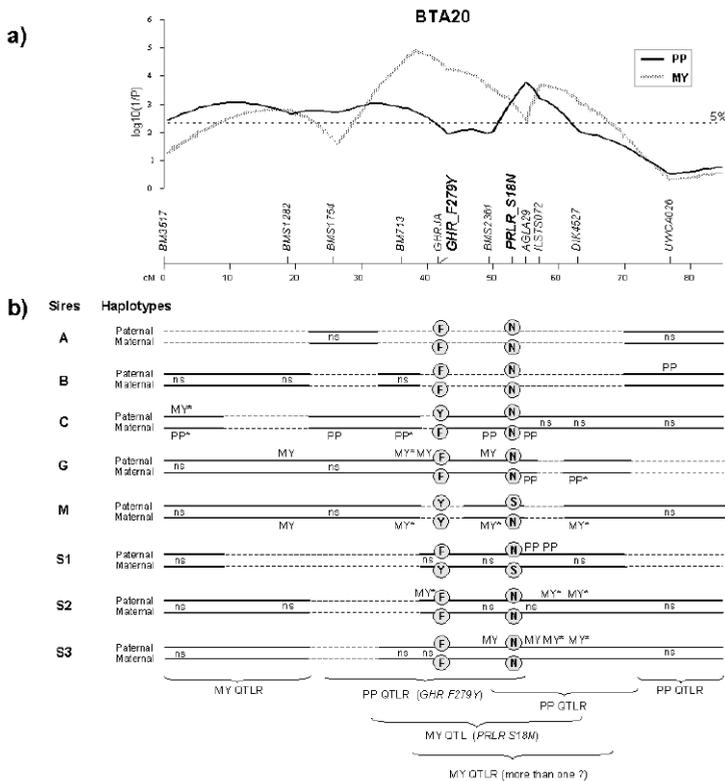
Key words: QTL, Candidate genes, Milk production traits, Dairy cattle.

INTRODUCTION – Several studies have shown that BTA20 harbours QTL for milk production traits (e.g.: Blott *et al.*, 2003; Fontanesi *et al.*, 2006). Then a subsequent combined linkage and linkage disequilibrium approach fine mapped a major QTL containing region (QTLR) for milk yield and composition (Blott *et al.*, 2003) close to the map position of two plausible candidate genes: growth hormone receptor (*GHR*) and prolactin receptor (*PRLR*), that both play major roles in the mammary gland and lactation physiology. A missense mutation in the *GHR* gene (*F279Y*) was associated with strong effects mainly on protein and fat percentage, and but also on milk yield in a few dairy cattle populations (Blott *et al.*, 2003; Viitala *et al.*, 2006). Allele *F* was indicated to increase milk protein and fat percentages while allele *Y* may increase milk yield. A missense mutation (*S18N*) in the *PRLR* gene was found to be associated with milk, protein and fat yield in the Finnish Ayrshire breed, with allele *N* having a positive and allele *S* having a negative effect on these traits affecting positively these traits (Viitala *et al.*, 2006). In order to verify these effects and to investigate BTA20 in more detail BTA20, we herein scanned this chromosome for milk production QTL segregating in the Italian Holstein-Friesian breed applying a selective milk DNA pooling strategy in a daughter design and with sire haplotype analysis.

MATERIAL AND METHODS – Eight Holstein-Friesian sires (*A, B, C, G, M, S1, S2* and *S3*), each with at least 3000 lactating daughters, were chosen for the daughter design. Milk pools were constructed using milk samples collected from daughters in the extreme divergent tails (high and low) of the estimated breeding value distribution for protein percentage (PP) or according to the daughter yield deviation for milk yield (MY). The sires and pools were genotyped for microsatellites distributed on BTA20 (Fontanesi *et al.*, 2006). Peak heights in the pools were used to estimate allele frequencies after shadow band correction, as described in Lipkin *et al.* (1998). Comparison-wise linkage tests were calculated as indicated by Lipkin *et al.* (1998) and Mosig *et al.* (2001) for each sire by marker and trait combination. The proportion of false positives (PFP; Fernando *et al.*, 2004) calculated by Fontanesi *et al.* (2006) at the chromosome-wise level was used as significance threshold in the single marker analysis. The single marker

linkage tests were used in consecutive approximate interval mapping (AIM) analyses according to Dolezal *et al.* (2005) applying a 5% chromosome-wise Bonferroni corrected threshold. In addition, sires only were genotyped for the *GHR F279Y* and *PRLR S18N* sites (Fontanesi *et al.*, 2007; Scotti *et al.*, 2007). Marker haplotypes of the eight sires were obtained based on paternal genotypes or when these were uninformative, on individually genotyped daughters. The direction of each significant sire-marker-trait effect along a haplotype was determined on the marker alleles which were found more frequent in the phenotypic high or low tails. The putative QTLs were identified according to Tchourzyna *et al.* (2002).

Figure 1. a) AIM analysis for MY and PP on BTA20. b) Sire haplotype analysis for BTA20. with the phase (above or below the paternal or maternal haplotype segments, respectively) for the positive MY and PP QTL alleles Dashed or continuous lines: chromosome segments in which sire markers were homozygous or heterozygous, respectively; circled letters: alleles of the *GHR* and *PRLR* genes; PP or MY above the paternal haplotype or below the maternal haplotype: the corresponding markers above of the paternal or below the maternal haplotype have a positive effect on PP or MY (with *: PFP<5%; without *: 5%<PFP<10%;). ns,: markers not significant). The brackets in the lower part of the figure indicate the putative QTLs.



RESULTS AND CONCLUSIONS – Figure 1a shows the AIM plots for MY and PP across BTA20. Significant peaks were found at about 40 cM for MY and 55 cM for PP, which could be consistent with a single QTL affecting both traits. However, both traits present a very broad region of significance, suggesting that more than one QTL may be present in this region. In this context however, it should be noted that in all cases effects on a given trait along a given haplotype were consistent in direction (Fig. 1b). There were no instances in which significant effects of opposite sign for the same trait were present on the same haplotype. Thus, haplotype analysis does not provide evidence supporting the presence of multiple QTL for MY or PP. Considering haplotype analysis by sires, Sires B, G, S2 and S3, although homozygous at both candidate genes, showed putative QTLR affecting PP in the distal portion of BTA20 (Sires B and G) and affecting MY in the central portion of BTA20 (Sires G, S2 and S3). Sire C (heterozygous at *GHR*) showed a putative QTLR affecting PP in the central part of BTA20; the haplotype carrying the *F* allele, known to favourably affect PP, indeed had a large positive effect on PP. Sire M (heterozygous at *PRLR*) showed a putative QTLR affecting MY in the same region; the haplotype carrying the *N* allele, known to have a positive effect on this trait, indeed had a positive effect on MY. In contrast, Sire S1 (heterozygous for both candidate genes) showed only a weak effect on PP. This is to be expected, however, since the alleles with positive effects on MY and PP were in opposite phase in the two candidate genes. Thus, the results of this study in sires C, M, and indirectly S1, tend to confirm the effects of the mutations already reported in the *GHR* and *PRLR* genes. However, from the presence of significant marker-associated effects on BTA20 in sires B, G, S2 and S3, all homozygous at the candidate genes, it is evident that at least in the Italian Holstein population, additional QTL, that are not explained by these mutations, also segregate on BTA20. Thus marker assisted selection based on the analysed markers of the *GHR* and *PRLR* genes may need to wait for a more complete characterization of the QTL on BTA20; a matter that now appears more complex than expected.

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