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The BovMAS Consortium: investigation of bovine chromosome 14 for quantitative trait loci affecting milk production and quality traits in the Italian Holstein Friesian breed

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RIASSUNTO – Studio del cromosoma 14 di bovino per l'identificazione di QTL per la produzione e la qualità del latte nella razza Frisona Italiana. *Nel presente studio, utilizzando le metodologie del* daughter design *e del* selective DNA pooling, *abbiamo verificato la presenza di un importante QTL per la produzione di latte nella regione prossimale del cromosoma 14 nella razza Frisona Italiana. Inoltre, abbiamo effettuato la ricerca di altri QTL per la percentuale di proteine, la percentuale di grasso e la produzione di latte su questo cromosoma. I risultati mettono in evidenza che il QTL nella regione prossimale è dovuto solo in parte alla mutazione A232K del gene DGAT1 e che probabilmente un altro o altri QTL segregano nella regione centrale-distale del cromosoma. Ulteriori studi sono necessari per confermare e chiarire questi risultati.*

KEY WORDS: QTL, DGAT1, bovine chromosome 14, Italian Holstein-Friesian

INTRODUCTION – Many studies have demonstrated that quantitative trait loci (QTL) can be identified and mapped in commercial dairy cattle populations using genetic markers in daughter and granddaughter designs. The final objective of these studies is to identify genes or markers that can be used in breeding schemes via marker assisted selection (MAS).

A major QTL affecting several milk production traits (fat percentage, fat yield, protein percentage, protein yield and milk yield) has been mapped in the centromeric end of bovine chromosome 14 (BTA14; i.e: Coppieters *et al.*, 1998). Using a positional candidate cloning strategy, Grisart *et al.* (2002) and Winter *et al.* (2002) identified the acyl-CoA: diacylglycerol acyltranferase 1 (DGAT1), that encodes an enzyme that play a central role in the synthesis of triglycerides, as a strong candidate gene for this QTL. A nonconservative amino acid substitution in this gene at position 232 (alanine>lysine; A>K) was first suggested as the causal mutation of the QTL. However, other evidence indicated the presence of more mutations at the *DGAT1* locus or other loci on this chromosome that can contribute to explain the QTL effects on milk content and production traits (Bennewitz *et al.*, 2004; Kühn *et al.*, 2004). Close to the *DGAT1* position, Barendse (1999) identified another gene that play a role in lipid metabolism, the thyroglobulin (*TG*) gene, as a positional candidate for carcass traits in cattle.

So far, BTA14 has not been the subject of extensive studies in the Italian Holstein-Friesian population. In order to verify the effect of the QTL described in the proximal region of BTA14 and to have a more complete overview of other QTL segregating in this breed, we scanned this chromosome for QTL affecting milk yield, protein percentage and fat percentage. The scan was carried out applying a selective milk DNA pooling strategy in a daughter design.

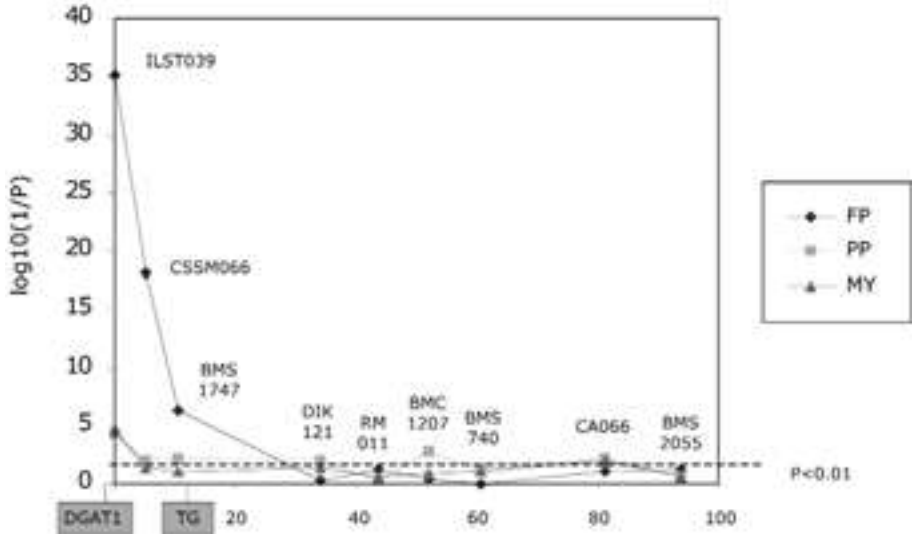
MATERIAL AND METHODS – Eight Holstein-Friesian sires (A, B, C, G, M, S1, S2 and S3) each with at least 3500 lactating daughters were chosen. Among these cows, about 200 daughters with high and 200 with low daughter yield deviation (DYD) values for milk yield (MY) and about 200 daughters with high and 200 with low estimated breeding value (EBV) for protein percentage (PP) and fat percentage (FP) were selected. Two replicate pools were constructed within each tail, each with milk of about 100 randomly chosen daughters. Each pool was prepared in two independent duplicates. Based on the cell count, each daughter contributed with the same number of milk somatic cells to the pool. Sire DNA was extracted from semen using a Chelex protocol. DNA was extracted from the milk pools using an adapted QIAamp DNA Blood Midi Kit (Qiagen). PCR products were electrophoresed on an ABI3100 Avant sequencer (Applied Biosystems).
The sires were genotyped for the DGAT1 A232K polymorphism as described by Winter et al. (2002), for the TG mutation that distinguish allele 2 from allele 3 as reported by Barendse (1999) and for eleven dinucleotide microsatellites chosen in the USDA/MARC map of BTA14 (Table 1 and Figure 1). TG is included in this map at 11.95 cM. DGAT1 is not included in the USDA/MARC map, thus its position was inferred from data reported in literature (-3 cM from the origin of the map; Grisart et al., 2002). The pools were genotyped for the heterozygous microsatellites. Peak heights in the pools were scored using GeneScan and Genotyper software (Applied Biosystems). Sire allele frequencies were obtained after correction for shadow bands, as described in Lipkin et al. (1998). Comparison-wise (P) linkage tests were computed as reported by Lipkin et al. (1998) and Mosig et al. (2001) for each sire by marker and trait combinations and for each marker/trait. An adjusted false discovery rate (aFDR) was applied to calculate chromosome-wise significant levels (Mosig et al., 2001).

RESULTS AND CONCLUSIONS – Only two sires (*S1* and *S2*) were heterozygous for the A>K polymorphism of *DGAT1* and three sires (*A, B* and *S3*) were heterozygous for the *TG* mutation. Microsatellite heterozygosity ranged from 36 (sires *G* and *M*) to 73% (sires *B* and *S1*). Two microsatellites (*BM1508* and *DIK4681*) were homozygous in all sires. Table 1 reports the significant sire by markers by trait tests. Figure 1 shows the significance at the marker level across the chromosome for the three considered traits.
The results clearly confirm the presence of a major segregating QTL affecting mainly FP, but also PP and MY, in the proximal region of BTA14. The strong effects for sires *S1* and *S2* can be attributed to the A>K polymorphism at the *DGAT1* locus. These effects seem to involve a region that spans 15-35 cM. Extensive linkage disequilibrium between markers in the proximal end of this chromosome may be the reason of these results even if it is not possible to exclude the presence of another close QTL. Some the other sires that are homozygous for this mutation presented a significant association between markers in the 0.0-10.5 cM region and FP (*A, B, G* and *S3*) and MY (*S3*). The variable number of tandem repeat (VNTR) alleles in the 5' regulatory region

Table 1. Results of the sire by marker tests for FP, MY and PP. Bolded trait, aFDR<0.01; non-bold trait, aFDR<0.05; ns, not significant; nt, not tested; --, homozygous sire.

Markers (cM)	*A*	*B*	*C*	*G*	*M*	*S1*	*S2*	*S3*
ILSTS039 (0.0)	**FP**	FP	ns	**FP**	ns	**FP**,**MY**,PP	**FP**,MY,**PP**	--
CSSM066 (5.1)	ns	ns	ns	ns	--	**FP**	**FP**,PP	**FP**,MY
BMS1747 (10.5)	ns	ns	ns	--	ns	**FP**	**FP**,**PP**	MY
BM1508 (17.8)	--	--	--	--	--	--	--	--
DIK4681 (25.7)	--	--	--	--	--	--	--	--
DIK121 (34.2)	ns	ns	ns	--	--	ns	**PP**	ns
RM011 (43.6)	ns	--	ns	-	--	FP	--	ns
BMC1207 (51.9)	--	**PP**	ns	--	ns	ns	ns	ns
BMS740 (60.7)	PP	ns	--	MY	ns	--	ns	ns
CA066 (81.3)	--	ns	--	--	--	PP	--	FP,MY,PP
BMS2055 (93.7)	ns	ns	ns	ns	--	ns	nt	--

Figure 1. Marker tests for FP, MY and PP across BTA14.



of this gene may be responsible for these effects as recently reported by Kühn *et al.* (2004). These sires will be genotyped for the VNTR to evaluate its role in the observed significant results. The *TG* polymorphism does not seem to be associated with QTL, at least in sires *A* and *B*, while for sire S3 this gene might be affecting MY as a significant association was observed for *BMS1747*, the closest genotyped microsatellite to *TG*. Some significant results from markers in the middle-distal end of BTA14 may suggest the presence of one (or more) QTL, affecting mainly PP, localized in this chromosome region. More microsatellites will be genotyped in this region in order to clarify this hypothesis and, eventually, to fine map this putative QTL.
Further studies are currently underway to investigate BTA14 for QTL affecting other production traits. Once these data are available it will be possible to evaluate whether this information can be implemented in MAS programs in the Italian Holstein-Friesian population.

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