Mapping QTL affecting milk somatic Cell count in the Italian Brown Swiss dairy Cattle – the QuaLAT Project

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ABSTRACT - A selective DNA pooling approach using milk samples was employed to map QTL affecting milk somatic cells count (MSCC) in the Italian Brown Swiss dairy cattle population. The mapping population consisted of five half-sib daughter families of Brown Swiss bulls, sires of 1000 to 3600 daughters. Two hundred highest and 200 lowest daughters, ranked by dam-corrected EBV, were selected from the high and the low tail. Four independent replicate pools, each made of 50 randomly chosen daughters, were prepared for each sire-tail combination. Di-nucleotide microsatellite markers were used to scan the genome. Sire marker allele frequencies were estimated by densitometry and shadow correction analysis. Significance threshold of 10% aFDR was used at the marker level, and resulted in a critical CWER P-value of 0.054. A threshold of 20% aFDR within the significant markers was used at the sire-marker level and resulted in a critical P-values of 0.058. Out of 145 markers, 41 were significant. Out of 122 sire-marker tests, at the significant markers, 58 resulted significant. QTL regions will be selected for further intensive study. This is the first complete genome scan for MSCC in the Brown Swiss breed.

Key words: Milk somatic cell count, QTL, Selective DNA pooling, Brown swiss.

INTRODUCTION - The recent development in molecular genetics has paved the way to a genomic approach of selection in livestock, as an integration of the ongoing phenotypic methodology (Dekkers, 2004). The basic needed information for the genomic approach is the knowledge of the association between markers and QTL affecting economical important traits. Numerous studies have found a large number of QTL associated with productive and functional traits in dairy cattle (http://www.vetsci.usyd.edu.au/reprogen/QTL_Map/; http://bovineqtl.tamu.edu; /http://www.animalgenome.org/QTLdb/cattle.html). The most studied breed is the Holstein, while, very little has been investigated in other populations, if at all. We are not aware of any previous mapping study on milk somatic cell count (MSCC) in the Brown Swiss breed.

The use of selective DNA pooling in a daughter design (DD) (Darvasi and Soller, 1994; Lipkin et al., 1998) is an efficient method for an initial whole genome screening for marker-QTL associations. It maintains the same statistical power of the DD (Weller et al., 1990) while saving about 90% of the number of the required genotypes (Darvasi and Soller, 1994; Lipkin et al., 1998; Mosig et al., 2001). This design has made the application of large scale QTL mapping in dairy population economically feasible, enlarged the number of traits that can be mapped, and reduced costs and time for completing the study. Additionally, the use of milk somatic cell as a source of DNA (Lipkin et al., 1993; 1998), allows collection of large number of samples through the routine milk recording testing, performed every 4-6 weeks by the Breeders Associations.

The aim of this study is to perform a whole genome scan for milk somatic cell count in the Italian Brown Swiss breed population using a Selective DNA Pooling approach.
MATERIAL AND METHODS - Five large sire families were identified, and a milk aliquot was collected from all lactating daughters during the routine milk recording test and frozen at -20°C. Semen samples of all sires were obtained from the Italian Brown Cattle Breeders' Association semen bank or at their reference lab (LGS-Cremona). To optimize the grouping of individuals based on the sire genetic contribution and the contribution of the dam, daughter's EBV were corrected (cEBV) by half of the mother's EBV value, according to Dolezal et al. (2005). The best and the worst 200 daughters from each sire family, according to the cEBV distribution for MSCC, were included in the pools. Four pools of 50 randomly chosen daughters were formed for each tail (8 pools per sire family, a total of 40 pools). Based on MSCC information provided by milk processing agencies, the same number of cell was taken to the pool from each individual milk sample. Cell lysate was used in PCR reaction instead of purified DNA according to Lipkin et al. (1993, 1998). A panel of 145 microsatellite markers covering all autosomes were chosen (www.marc.usda.gov/genome/genome.html), at an average distance of 20 cM. Pools have been genotyped only at heterozygous sire-marker combinations. PCR products were separated by electrophoresis on an automatic sequencer ABI377 of Applied Biosystems. Densitometric values (peak height) of each detectable fragment were obtained from the genotypes of sires and pool using Genescan® and Genotyper® softwares. Allele frequencies were estimated after shadow band correction as in Lipkin et al. (1998). Adjusted false discovery rate (aFDR) to account for the multiple tests was performed as proposed by Mosig et al. (2001). Markers significance threshold was set at aFDR of 10%, and significant sire-by-marker at an aFDR of 20% within the significant markers. For each heterozygous sire-marker combination, the comparison-wise error rate (CWER), $P_{ij}$, for the $i$th sire-$j$th marker combination, was obtained as twice the area of the normal curve from $Z(D_{ij})$ to $+\infty$, where

$$Z_{ij} = \frac{D_{ij}}{SE(D_{ij})} = Z_{1-\alpha/2}$$

where $D_{ij}$ is the difference in sire-allele frequencies between the high and low daughter pools of the $i$th sire with respect to the $j$th marker, SED is the standard error of $D_{ij}$, and $Z_{1-\alpha/2}$ is the ordinate of the standard normal distribution. The CWER, $P_{jk}$, for the $j$th marker combination was obtained as the area of the Chi-square distribution from $\chi^2_j$ to $+\infty$, where

$$\sum_{j=1}^{m} \chi^2_j = \chi^2_m$$

and $d.f. = m$

where $m$ is the number of heterozygous sires tested at that marker (i.e. degrees of freedom).

RESULTS AND CONCLUSIONS - A total of 376 sire-by-marker tests, over a total of 435 combinations genotyped, were used in the analysis, representing 86% of all the sire-by-marker combinations analysed. Proportion of pools used in the analysis was evenly distributed across family (18% to 22%). Critical P value at marker level was 0.054 (aFDR of 10%). Within the significant markers, critical value for the sire-by-marker level was 0.058 (aFDR of 20%).

A total of 41 markers distributed over 23 chromosomes were found significant. One chromosome showed 4 significant markers (BTA 7), five showed three significant markers (BTA 1, 6, 8, 17, 19), five two significant markers (BTA 2, 12, 13, 26, 28), and twelve only one significant marker (BTA 3, 5, 11, 16, 18, 20, 22, 23, 24, 25, 27, 29).

Compared with results presented in the three web-based QTL maps mentioned above, five chromosomes - BTA 6, 17, 24, 25 and 28 - were not reported previously as harbor QTL affecting MSCC, and thus may indicate novel QTL regions specific for the Italian Brown Swiss breed.

Significant association on chromosome 6 could be influenced by the genetic correlation between MSCC and milk yield and protein percent that in the Italian Brown Swiss is 0.18 and -0.22 respectively (unpublished, 2006). Markers in other chromosomes were already reported in at least one web-based QTL database.

At sire-by-marker level 58 tests were significant at aFDR of 20% within the markers significant at aFDR of 10%.

In the follow up of the study some chromosome regions will be tested with additional markers to substitute markers that were homozygous for all sires (6 markers) or did not amplify well.

The results of the genome scan on MSCC, jointly with the results from the BovMAS project (Bagnato et al., 2005) on productive traits (milk yield and protein percent), are furnishing the basic knowledge to perform Marker Assisted Selection (MAS) in the Brown Swiss population.

The QuaLAT project is mapping two populations with a genome scan for MSCC, the Italian Brown Swiss breed, and several other populations.
and the Israel Holstein. Comparison of the analysis of the genome scan in the two populations will allow the identification of QTL regions common for both populations, candidate for investigation with higher resolution.

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