

ORIGINAL ARTICLE

A whole genome scan to map QTL for milk production traits and somatic cell score in Canadian Holstein bulls

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Keywords

Linkage disequilibrium; Milk traits; SNP; QTL.

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Received: 10 March 2008;
accepted: 2 November 2008

Summary

The detection and mapping of genetic markers linked to quantitative trait loci (QTL) can be utilized to enhance genetic improvement of live-stock populations. With the completion of the bovine genome sequence assembly, single nucleotide polymorphisms (SNP) assays spanning the whole bovine genome and research work on large scale identification, validation and analysis of genotypic variation in cattle has become possible. The objective of the present study was to perform a whole genome scan to identify and map QTL affecting milk production traits and somatic cell scores using linkage disequilibrium (LD) regression and 1536 SNP markers. Three and 18 SNP were found to be associated with only milk yield (MY) at a genome and chromosome wise significance ($p < 0.05$) level respectively. Among the 21 significant SNP, 16 were in a region reported to have QTL for MY in other dairy cattle populations and while the rest five were new QTL finding. Four SNP out of 21 are significant for the milk production traits (MY, fat yield, protein yield (PY), and milk contents) in the present study. Six and nine SNP were associated with PY at a genome and chromosome wise significant ($p < 0.05$) level respectively. Three and 17 SNP were found to be associated with FY at a genome and chromosome wise significant ($p < 0.05$) level. Five and seven SNP were mapped with somatic cell score at a genome and chromosome wise significant ($p < 0.05$) level respectively. The results of this study have revealed QTL for MY, PY, protein percentage, FY, fat percentage, somatic cell score and persistency of milk in the Canadian dairy cattle population. The chromosome regions identified in this study should be further investigated to potentially identify the causative mutations underlying the QTL.

Introduction

Recent advances in molecular biotechnology provide great opportunities to incorporate molecular information into the traditional genetic evaluation models and to improve selection accuracies in livestock populations. These advancements have enabled the detection of some of the genes that contribute to genetic variation in economically important quantitative traits. Because DNA can be obtained at any

age and from both genders, molecular genetics can overcome some of the limitations of quantitative genetic selection (Dekkers & Hospital 2002). Classical detection and mapping of genes, genetic markers and QTL can be used to enhance genetic improvement of breeding stock through marker or gene-assisted selection.

The first whole genome scans in dairy cattle were initiated by Georges *et al.* (1995). Since that time, several genome scans and fine mapping

projects have been undertaken to identify the genomic regions harboring genes that underlie phenotypic variation in dairy cattle production traits (Smaragdov *et al.* 2006). These three reviews reported on QTL in dairy cattle, from which it is evident that there are many loci influencing the quantitative traits. The QTL mapping to regions containing potential candidate genes are of special interest. Several QTL and candidate genes for milk production, reproduction, functional, and conformation traits have been described for several *Bos taurus* autosomes in previous studies and most of these regions have been mapped in multiple studies (Boichard *et al.* 2003; Viitala *et al.* 2003; Ashwell *et al.* 2004; Schrooten *et al.* 2004; Schnabel *et al.* 2005).

Single nucleotide polymorphisms (SNP) are the most abundant form of DNA variation in the genome and are becoming preferred over other genetic markers because of their relatively low mutation rate as well as the ease and low cost of genotyping (Hinds *et al.* 2005; Snelling *et al.* 2005). SNP have been used for the detection and localization of QTL for complex traits in many species (Daw *et al.* 2005). In cattle, a 7.1 X sequence assembly has been produced with accompanying information on over 2300 000 SNP genome wide (Bovine Genome Project: <http://www.hgsc.bcm.tmc.edu/projects/bovine>). From this data, a set of SNP markers spanning the whole bovine genome can be used in the large scale identification, validation and analysis of genotypic variation in cattle.

Several statistical approaches have been developed for whole genome scans and QTL mapping projects, including the least square method, based on regression of phenotype on marker genotypes or haplotypes, and random effects models based on identity by descent (IBD) approaches (George *et al.* 2000; Kolbehdari *et al.* 2005; Gautier *et al.* 2006). High density SNP marker genotypes have increased the feasibility of QTL detection and mapping using historical population-wide linkage disequilibrium (LD). LD mapping method requires a marker allele to be in LD with the QTL allele across the entire population. LD can be a result of migration, mutation, selection, small finite population size or other genetic events experienced by a population. In livestock populations, finite population size is generally implicated as the key cause of LD, as effective population sizes for most livestock population are relatively small (Meuwissen & Goddard 2000). In previous studies, extensive LD has been observed in dairy cattle, sheep, and pig

populations (Farnir *et al.* 2000; Dekkers & Hospital 2002).

The linkage disequilibrium method regresses the phenotypes of a quantitative trait on marker genotypes. Regression on single SNP marker genotypes does not require knowledge of SNP position and linkage phase and is easier to implement; therefore, it is the recommended method for large scale genome wide genotyping analysis (Grapes *et al.* 2004; Zhao *et al.* 2007). Studies have shown that single marker tests based on the LD regression model provide similar or greater power than haplotype based and IBD-based models and QTL can be detected and mapped by LD regression approach. Further, this method offers flexibility to include dominance and epistatic effects. In addition, the random polygenic effects accounting for the relationship can be added to this model.

The objective of this study was to perform a whole genome scan to identify and map QTL affecting milk production traits such as MY, PY, fat yield (FY), protein percentage (PP), fat percentage (FP), somatic cell scores (SCS) and persistency of milk using LD regression and SNP genotype markers.

Methods

Genotyping assay design and platform

Altogether 1536 SNP markers were selected to represent introns and exons of potential candidate genes across the bovine genome sequence assembly (Btau_2.0). A subset of bovine SNP already characterized by the Alberta Bovine Genomics Laboratory was combined into a single multiplex assay of 1536 SNP for analysis on the Illumina Beadstation 500G SNP genotyping platforms (Oliphant *et al.* 2002). Among these SNP, 139 poorly amplified and 56 monomorphic SNP during the genotyping were removed from the analysis. The genotyping assay was designed based on Bovine Genome Sequence Assembly version 2 (Btau_2.0). In the original design, these SNP were selected to be distributing as evenly as possible based on the physical locations mapped on the Bovine Genome Sequence Assembly (Btau 2.0). The SNP known to be in putative candidate genes for milk production and functional traits were strategically added to the assay. However, after the assay was manufactured, a new updated version of the Bovine Genome Sequence Assembly (Btau 3.1) was released. New chromosome assignments and SNP positions

in the designed assay were recalculated based on the updated version of Bovine Genome Sequence Assembly.

A local database was developed that contains over 1.8 million bovine SNP and almost 30 000 genes gathered from the databases at National Centre for Biotechnology Information (NCBI). Querying the database provided details on each SNP, including its location and functional class. SNP that are present in a gene locus can have one of the following NCBI-defined functional classes: locus-region, coding, coding-synonymous, coding-non-synonymous, mRNA-UTR, intron, and splice-site. If a SNP was not located in a gene, the nearest gene which is cover the regulatory factors was determined by querying the database for a list of genes on the same chromosome as the SNP and identifying the gene closest to the location of the SNP.

Phenotypic data

A total of 462 Canadian Holstein bulls from Semex Canada (Guelph, ON, Canada), 319 of them originating from 10 core sire families, and the rest of the bulls (143) from the general pedigree were used in the study. DNA was extracted from bull semen and genotyped. Complete general pedigree information along with the most up to date national genetic evaluation results (EBV) of these bulls were extracted from the national dairy recording database. The SNP genotypes were used to test the association with measured economically important traits, such as MY, PY, FY, PP, FP, persistency of milk (PM) and health traits such as SCS. SCS is the linear score ranging from 0 to 10 which are transformed from the actual cell counts of milk samples. These SCS are measured by the national milk recording system and used by Canadian Dairy Network (Guelph, ON, Canada) to compute genetic evaluations for bulls and cows. SCS are useful for monitoring the level of the sub clinical mastitis. Persistency of milk or lactation persistency is expressed in terms of a percentage based on the bull's genetic ability of MY at 280 day in milk compared with 60 days. Since each bull's estimated breeding value is based on the records of many daughters and is a much more accurate estimate of the genetic potential of each animal than a single cow phenotype, it was used in the gene association study. The descriptive statistics of these traits that were estimated from the 462 bulls that have been genotyped in current study are given in Table 1.

Table 1 Descriptive statistics EBV of the milk production and functional traits for the studied 462 bulls

Traits	Mean	Standard deviation	Minimum	Maximum
Milk yield	594.66	797.05	-1575.00	3386.00
Protein yield	21.12	22.87	-57.00	82.00
Fat yield	18.49	29.77	-61.00	111.00
Protein%	0.017	0.12	-0.52	0.35
Fat%	-0.021	0.29	-0.81	0.74
SCS	3.033	0.28	2.34	4.21
Persistency	67.22	3.05	57.00	75.00

Statistical analysis

Single locus LD regression model

To test the association between SNP and the QTL, a single locus LD regression model was implemented in this study. The markers were assumed to be in LD with the QTL over the entire genome. The simple regression model based on LD has been shown using simulated data to have the acceptable level of power and accuracy for fine mapping QTL (Grapes *et al.* 2004; Zhao *et al.* 2007). The following mixed linear model was fitted in this study by using the ASReml package (Gilmour *et al.* 2006).

Mixed linear LD regression model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \quad (1)$$

where \mathbf{y} is the vector of phenotypes (EBV), \mathbf{X} is the design matrix, \mathbf{b} is the vector of coefficients of the regression on recoded SNP genotypes, \mathbf{Z} is the incidence matrix for animal effects, $\mathbf{a} \sim (0, A\sigma_a^2)$ is a vector of the polygenic animal effects and $\mathbf{e} \sim (0, I\sigma_e^2)$ is the vector of residuals, in which A is an additive genetic relationship matrix of bulls and I is a identity matrix, σ_a^2 and σ_e^2 are the animal's additive polygenic variance and residual error variance respectively.

Quantitative trait loci allele substitution effects and additive genetic effects were evaluated in this model. The design matrix, \mathbf{X} , were coded as 2, 1, 0 for the SNP genotypes 1-1, 1-2 and 2-2 allele combination respectively. F -statistic, type I error (p-value) and allele substitution effects were estimated for all of the SNP genotypes in the whole genome.

False discovery rate

A major issue in multiple testing is the setting of significance thresholds. A useful statistic is the false discovery rate (FDR) which is the expected proportion of falsely detected QTL. FDR was developed by Benjamini & Hochberg (1995) and it takes into

account the number of tests that are performed as well as how significant one test is relative to the others in multiple comparison procedures. Family wise error rate (FWER) controls the probability of committing any type I error in families of comparison. FDR was used to establish the statistical significance critical value in this study. Two levels of significant controls were used in this study based on genome wise and chromosome wise type I error which was computed for all SNP (Benjamini & Hochberg 1995; Fernando *et al.* 2004). The genome wise level is a very conservative approach for large number of markers in whole genome scan study. Therefore, when those SNP that are significant with the trait and exceed the genome wise Type I error that means are highly associated with the trait.

False discovery rate was calculated for a QTL mapping on a genome or a chromosome wise level by assuming n number of tests performed on a genome or a chromosome, and the p-values were ranked from lowest to highest. The following equation was used to calculate the FDR.

$$\text{FDR} = \frac{n \times P(k)}{k} \quad (2)$$

Where k is the individual relative test position.

Results and discussion

SNP analysis

Among 1536 SNP that design on platform, 139 poorly amplified and 56 monomorphic SNP during the genotyping were removed from the analysis. The SNP with minor allele frequency (MAF) <0.05 was also removed from the analysis. The SNP used in this study were distributed throughout the whole bovine genome. The total number of SNP on each chromosome varied from 14 on BTA12 to 78 on BTA3. The average interval between SNP on different chromosomes varied from 668.9 kilobase-pairs (Kbp) on BTA 29 to 5530.7 kbp on BTA12. The X chromosome was not included in this study. The average SNP polymorphism information content (PIC) was 0.287 (0–0.375) and the average observed SNP heterozygosity was 0.389. The details of the number of SNP per chromosome and their average interval in kbp per chromosome are given in Table 2. The genotyping assay was originally designed based on Bovine Genome Sequence Assembly version 2 (Btau_2.0). In the original platform, SNP were evenly distributed through out the bovine genome.

Table 2 Frequency and the average intervals between SNP (Kbp) by chromosome based on bovine genome sequence assembly (Btau_3.1)

BTA	Frequency	Intervals (kbp)
1	64	2193.3
2	64	1935.3
3	78	1492.4
4	75	1424.5
5	59	1828.6
6	40	2749.2
7	71	1392.8
8	36	2788.1
9	33	2656.7
10	44	2058.6
11	56	1722.1
12	14	5530.7
13	54	1452.9
14	27	2690
15	67	1115.6
16	48	1432.7
17	39	1809.7
18	44	1380.9
19	64	991.7
20	29	2223.1
21	32	1950.3
22	42	1425.7
23	44	1075.7
24	19	2877.4
25	40	1058.5
26	41	1142.9
27	16	2123.7
28	34	1135.6
29	67	668.9
Total	1341	

New chromosome assignments and SNP positions were calculated (Table 2) based on the updated version of Bovine Genome Sequence Assembly (Btau_3.1). This recalculation resulted in a less evenly distribution of SNP than originally intended. On average 21% of the SNP have changed physical position on the genome. The linear interpolation approach has been used to obtain an approximate linkage map position in cM from their known base-pair (bp) position of the SNP on the genome. The approximate linkage map position of a marker was calculated based on linear genetic distance across the closest adjacent flanking genetic markers on the bovine genome database home page contains links to composite map (<http://www.bovinegenome.org>).

Milk yield

The whole genome scan to map QTL for MY identified three SNP significantly associated with MY at the genome wise level and 18 at the chromosome

wise level ($p < 0.05$) on BTA 1, 3, 5, 8, 9, 11, 14, 18, 19, 21 and 23. SNP (rs41643632, rs41585246, rs41631818, rs41569023) that showed larger estimated allele substitution effects compared to other significant SNP were found to have highly significant ($p < 0.001$) association with MY. A large F -test statistic showed strong evidence of associations between these chromosome regions of the genome with MY. For instance, SNP rs41643632 on BTA23 (5 984 251 bp) has a F -test statistic of 21.15 and allele substitution effects of 337.98 kg. Out of the 21 significant ($p < 0.05$) chromosome wise level SNP, 16 of them correspond to regions that have been reported in previous studies on chromosomes 1, 5, 9, 11, 14, 18, 21 and 23 (Boichard *et al.* 2003; Viitala *et al.* 2003; Ashwell *et al.* 2004; Schrooten *et al.* 2004; Schnabel *et al.* 2005) while five of them are newly detected QTL in this study and map to BTA 3, 5, 8 and 19. Twelve of these significant SNP are located in the introns of predicted genes, and four of them cause synonymous mutations in coding regions, while five are not located in genes. The details of the SNP showing association with MY, including their heterozygosity, position, F -test statistic value, allele substitution effects and p -value are shown in Table 3. The 16 genes that contain significant SNP maybe considered as possible candidate genes for milk production in dairy cattle populations.

Milk yield traits are known to be highly correlated with other yield (positively) and percentage (negatively) traits. As expected, some of the SNP associated with MY identified in this study were also significant for protein and FY. Five of these MY significant SNP (rs41631818, rs41662488, rs41569023, rs41581694, rs41643632) show significant ($p < 0.001$) association with PY. Two of these regions (51 612 980, 50 655 113 bp) on BTA1 and BTA18 that were associated with milk and PY have been reported in previous studies (Ashwell *et al.* 2004). Three of these QTL mapped regions (63 811 517, 82 453 396, 5 984 251 bp) on BTA9, BTA11 and BTA23 are new regions mapped in this study. The SNP on BTA23 (rs41643632) showed association with milk and PY at genome wise significant level ($p < 0.01$) and is a synonymous coding SNP in the gene LOC534225. Two SNP (rs41631818, rs41643632) on BTA1 and BTA21 were associated with milk and FY. The region on BTA1 (51 612 980 bp) has been associated with MY around the 60 cM in previous studies (Smaragdov *et al.* 2006). This region is significant with FY at genome wise level in the current study. This QTL has

negative effects on milk and FY production in dairy cattle population. The SNP (rs41644615) on BTA21 is a newly detected QTL region and has a negative association with milk and FY at chromosome and genome wise significant level, respectively.

Protein yield

The whole genome scan to map QTL for PY identified six SNP significantly associated with PY at genome wise level and nine at the chromosome wise level ($p < 0.05$) on BTA 1, 4, 7, 8, 9, 11, 14, 18, 21, 23, 26 and 28. SNP (rs41591535, rs41643632) on BTA 4 and 23 show highly significant ($p < 0.001$) association with PY. These two regions (1 883 884, 5 984 251 bp) were newly mapped to the bovine genome for PY with large effects 6.44 and 10.02 kg respectively on PY EBV. The SNP on BTA9 (rs41662488) is showing association with PY at genome wise level ($p < 0.05$), and MAF of this SNP is 0.07 and estimated effects could be due to very few genotypes and might lead to biased effects. The details of the SNP showing association with PY, including their heterozygosity, position, F -test statistic value, allele substitution effects and p -value are shown in Table 4. Ten of these regions have been reported in previous studies on chromosomes 1, 7, 9, 11, 14, 21 and 26 (Boichard *et al.* 2003; Viitala *et al.* 2003; Ashwell *et al.* 2004; Schrooten *et al.* 2004; Schnabel *et al.* 2005) and five of them are newly detected QTL in this study and are located on BTA 4, 8, 18, 23 and 28. Ten of these significant SNP are located in the introns of predicted genes, and three of them cause synonymous mutations in coding regions, while two are not located in genes (Table 4). These 13 genes that contain significant SNP associated with PY may be considered as possible candidate genes for PY in dairy cattle populations.

Eight of the regions that were significantly associated with PY were also associated with MY on BTA 1, 9, 11, 18 and 23 and FY on BTA 1, 7 and 21. Five significant regions associated with PY were also associated with MY which was discussed in previous section. Three QTL regions have significant associations with PY and FY and there were on BTA1 (63 326 365 bp), BTA 7 (3 671 878 bp) and BTA21 (52 391 240 bp). A QTL for PY and FY has been previously reported on BTA1 of around 60 cm (Smaragdov *et al.* 2006), which would correspond to the QTL identified in this study by SNP (rs41631818) at 63 326 365 bp. Our study confirmed the regions on BTA7 (36 718 783 bp) and BTA21

Table 3 Significant genome and chromosome wise ($p < 0.05$) SNP with milk yield

SNP NCBI ID	BTA	Position (bp)	MAF	F-test	Effect (kg)	p-value	Functional Class	GeneName
rs41631818	1	51612980	0.49	13.59	210.94	0.00013*	CS ^a	MGC128242
rs41629125	1	63326365	0.31	11.39	200.07	0.00042	Intron	ITGB5
rs41633664	1	64810574	0.20	8.68	198.73	0.0018	Nearest_gene	LOC785291
rs41643471	1	131477966	0.19	7.71	191.46	0.0031	CS ^a	LOC511740
rs43709850	3	17940226	0.18	11.22	224.13	0.00046	Intron	LOC511740
rs41655901	5	28108890	0.43	10.87	168.78	0.00055	Intron	GALNT6
rs41656714	5	31798764	0.47	9.78	161.99	0.00099	Nearest_gene	LOC407194
rs41592943	5	92164299	0.27	9.04	178.27	0.0014	Intron	GUCY2C
rs41658330	8	76799436	0.43	8.66	148.00	0.0018	Intron	FANCC
rs41662488	9	63811517	0.07	11.20	350.24	0.00046	Nearest_gene	LOC785678
rs41569023	11	82453396	0.24	12.58	216.80	0.00022	Intron	ROCK2
rs41633631	14	7969609	0.46	5.92	129.09	0.0086	Intron	TG
rs41628862	14	31527726	0.31	9.09	167.42	0.0014	Intron	BIG1
rs41587081	14	40196357	0.45	8.68	152.55	0.0018	CS ^a	ZFHX4
rs41632222	14	63167981	0.29	5.73	141.90	0.0096	Nearest_gene	LOC512656
rs41581694	18	50655113	0.33	8.91	176.82	0.0016	Intron	FOXA3
rs41577598	19	51230833	0.36	10.52	182.29	0.00067	Intron	BAIAP2
rs41608371	21	50355809	0.36	7.49	166.37	0.0035	Intron	FBLN5
rs41644615	21	52391240	0.49	9.23	157.95	0.0013	Intron	SERPINA5
rs41585246	21	54289063	0.48	14.62	216.80	0.000077*	Nearest_gene	SERPINA3-5
rs41643632	23	5984251	0.14	21.15	337.98	0.0000027*	CS ^a	LOC534225

^aCoding synonymous SNP (CS).

*Significant genome wise ($p < 0.05$) SNP.

(52 391 240 bp) that were reported to be associated with PY and FY of around the 39 and 56 cM respectively (Smaragdov *et al.* 2006).

Fat yield

The whole genome scan to map QTL for FY identified 13 SNP significantly associated with FY at the genome wise level and 7 at the chromosome wise level ($p < 0.05$) on BTA 1, 4, 5, 7, 10, 11, 14, 21, 23, 24 and 28. Out of the 20 significant genome and chromosome wise level ($p < 0.05$) SNP, nine of these were in regions that have been reported in previous studies on chromosomes 5, 10, 14 and 21 (Boichard *et al.* 2003; Viitala *et al.* 2003; Ashwell *et al.* 2004; Schrooten *et al.* 2004; Schnabel *et al.* 2005) and 11 of them are newly detected QTL in this study and are located on BTA 1, 4, 5, 7, 23, 24 and 28. Fourteen of these significant SNP were located in the introns of predicted genes, and four of them caused synonymous mutations in coding regions, while two were not located in genes (Table 5). The SNP showing association with FY at significant genome and chromosome wise ($p < 0.05$) level, including their heterozygosity, position, F-test statistic value, allele substitution effects and p-value's are shown in Table 5. These 18 genes that contain significant SNP associated with FY maybe considered as possible

candidate genes for fat production in dairy cattle populations.

The effect of the previously identified DGAT1 gene on BTA 14 (Kuhn *et al.* 2004; Olsen *et al.* 2005; Gautier *et al.* 2006) is supported by this study. The QTL that are in close proximity to this gene should account for a high proportion of FY genetic variance in dairy cattle populations. This region of chromosome 14 (0–10 Mbp) have been showed significant association with MY, FY and FP in this study.

Three regions on BTA1 (51 612 980, 63 326 365 bp) and BTA21 (52 391 240 bp) were associated with MY, PY and FY. The two SNP on BTA1 (rs41631818, rs41629125) have been reported to be associated with MY and PY in previous studies (Khatkar *et al.* 2004; Smaragdov *et al.* 2006). The results of the current study showed that these three SNP significantly associated with FY. The SNP on BTA21 (rs41644615) have been reported with negative association with FY which supported with the result of the current association test.

Protein percentage

One and 9 significant SNP were identified on BTA 3, 4, 5, 10, 13, 17, 22 and 23 for PP at genome and chromosome wise levels ($p < 0.05$) respectively. The details of the SNP showing association with PP at

Table 4 Significant genome and chromosome wise ($p < 0.05$) SNP with protein yield

SNP NCBI ID	BTA	Position (bp)	MAF	F-test	Effect (kg)	p-value	Functional class	GeneName
rs41631818	1	51612980	0.49	12.14	5.75	0.00028*	CS ^a	MGC128242
rs41629125	1	63326365	0.31	9.02	5.14	0.0015	Intron	ITGB5
rs41591535	4	1883884	0.45	13.76	6.44	0.00012*	Intron	LOC781748
rs41578761	7	36718783	0.49	12.37	5.45	0.00025	Intron	LOC529633
rs29011990	8	29415533	0.31	9.52	4.99	0.0012	Intron	MGC155155
rs41662488	9	63811517	0.07	11.94	10.39	0.00031*	Nearest_gene	LOC785678
rs41569023	11	82453396	0.24	12.33	6.19	0.00025*	Intron	ROCK2
rs41628862	14	31527726	0.31	9.59	4.95	0.0011	Intron	BIG1
rs41581694	18	50655113	0.33	8.72	5.05	0.0018	Intron	FOXA3
rs41636749	18	52852134	0.23	8.44	5.39	0.0021	CS ^a	LOC538513
rs41644615	21	52391240	0.49	12.75	5.33	0.00020*	Intron	SERPINA5
rs41585246	21	54289063	0.48	9.50	5.05	0.0012	Nearest_gene	SERPINA3-5
rs41643632	23	5984251	0.14	22.34	10.02	0.0000016*	CS ^a	LOC534225
rs41648723	26	40422508	0.14	22.34	10.02	0.0000015*	Intron	CTBP2
rs41606880	28	21540955	0.49	5.90	3.72	0.0087	Intron	JDP1

^aCoding synonymous SNP (CS).

*Significant genome wise ($p < 0.05$) SNP.

significant genome and chromosome wise ($p < 0.05$) level, including their heterozygosity, position, *F*-test statistic value, allele substitution effects and p-value are given in Table 6. Out of the 10 significant genome and chromosome wise level ($p < 0.05$) SNP, six of these regions have been reported in previous studies on chromosomes 3, 5, 10 and 13 (Boichard *et al.* 2003; Viitala *et al.* 2003; Ashwell *et al.* 2004;

Schrooten *et al.* 2004; Schnabel *et al.* 2005) and four of them are newly detected QTL in this study mapped on BTA 4, 13, 17 and 22. Six of these significant SNP are located in the introns of predicted genes, and two of them cause synonymous mutations in coding regions, and one of them cause non-synonymous mutations in coding regions resulted in the change of protein sequence, while one is not

Table 5 Significant genome and chromosome wise ($p < 0.05$) SNP with fat yield

SNP NCBI ID	BTA	Position (bp)	MAF	F-test	Effect (kg)	p-value	Functional class	GeneName
rs29020642	1	8361453	0.16	10.98	8.58	0.00052*	CS ^a	LOC512171
rs41637121	1	9253442	0.30	14.15	7.35	0.000098*	Intron	APP
rs41631818	1	51612980	0.49	9.12	6.34	0.0014	CS ^a	MGC128242
rs41629125	1	63326365	0.31	15.63	8.53	0.000047*	Intron	ITGB5
rs41634488	1	74402543	0.33	6.97	5.06	0.0047	Intron	LOC786403
rs41588659	4	11865677	0.33	11.02	6.55	0.00051*	Intron	COL1A2
rs41652648	5	81674580	0.34	10.22	6.64	0.00079	Intron	ITPR2
rs41591894	5	82057218	0.48	9.27	5.48	0.0013	Intron	ITPR2
rs41592942	5	92143685	0.27	13.70	7.99	0.00012*	Intron	GUCY2C
rs41578761	7	36718783	0.49	16.72	8.00	0.000026*	Intron	LOC529633
rs41653025	10	49942146	0.13	12.90	10.56	0.00019*	CS ^a	LOC540856
rs43703342	11	63461383	0.31	13.42	7.61	0.00014*	CS ^a	LOC514626
rs41579049	14	486841	0.27	19.13	9.15	0.0000076	Intron	5-OPase
rs41580517	14	8663254	0.27	16.25	9.00	0.000033*	Intron	KCNQ3
rs41644615	21	52391240	0.49	11.47	6.43	0.00040*	Intron	SERPINA5
rs41640789	23	15280862	0.37	16.68	7.77	0.000026*	Nearest_gene	POLR1C
rs41645253	24	20917926	0.27	14.18	8.29	0.000096*	Intron	MGC139244
rs41653440	28	25280138	0.35	9.06	6.24	0.0015	Intron	PSAP
rs41569649	28	26685127	0.12	6.81	7.74	0.0052	Intron	CAMK2G
rs41653491	28	27153960	0.35	9.76	6.70	0.0010	Nearest_gene	LOC514949

*Significant genome wise ($p < 0.05$) SNP.

^aCoding synonymous SNP (CS).

Table 6 Significant genome and chromosome wise ($p < 0.05$) SNP with protein percentage

SNP NCBI ID	BTA	Position (bp)	MAF	F-test	Effect (%)	p-value	Functional class	GeneName
rs41587408	3	21510186	0.36	10.79	0.03	0.00058	Nearest_gene	PDZK1
rs41650658	4	40561214	0.41	12.78	0.03	0.00020	Intron	NRCAM
rs29014633	5	74016631	0.31	10.39	0.03	0.00071	Intron	CACNG2
rs41590827	5	75905624	0.27	13.53	0.03	0.00013	Intron	RAC2
rs41593881	10	69314163	0.32	9.91	0.03	0.00093	Intron	HIF1A
rs41566192	13	43058415	0.43	10.84	0.03	0.00056	CNS ^b	MGC127374
rs29021058	13	68711263	0.07	8.72	0.05	0.0018	CS ^a	PLCG1
rs41570561	17	47419827	0.19	22.22	0.05	0.0000016*	Intron	SCARB1
rs41637636	22	48159856	0.30	9.95	0.03	0.00091	Intron	SLC38A3
rs29016156	23	13617161	0.07	9.79	0.05	0.00099	CS ^a	LOC517805

^aCoding synonymous SNP (CS).

^bCoding non-synonymous SNP (CNS).

*Significant genome wise ($p < 0.05$) SNP.

located in any gene (Table 6). Two SNP on BTA 13 and 23 (rs29021058 and rs29016156) are showing association with PP at chromosome wise level ($p < 0.05$), and MAF of these two SNP is 0.07. The estimates could be due to very few genotypes and might lead to biased estimated effects. These nine genes that contain significant associated SNP that are associated with PP maybe considered as possible candidate genes for protein production in dairy cattle populations.

We have identified two SNP (rs29014633, rs41590827) on chromosome 5 that show significant chromosome wise association with PP. This region of chromosome 5 (74–75 Mbp) has QTL that account for a high proportion of genetic variance in production traits in dairy cattle. Further studies using additional markers are recommended to map these QTL.

The SNP on BTA17 (rs41570561) is highly significant ($p < 0.001$) associated with negative effect on PP and this is the new QTL region (47 419 827 bp) which is identified in this study. This SNP is in the intron of SCARB1 gene. This causative mutation in this gene would be a good candidate for PP in further investigation in Canadian dairy cattle population.

Fat percentage

The whole genome scan to map QTL for FP identified 6 and 12 SNP that were significantly associated with FP at genome and chromosome wise level ($p < 0.05$) respectively. There were located on BTA 3, 6, 9, 10, 14, 17, 21, 23 and 26 (Table 7). Large allele substitution effects, 0.12% for fat EBV were found for SNP (rs41579049) on BTA14 with a large *F*-test statistic (35.91). Compared with all these traits this is the highest value for the test statistic and most

significant ($p < 0.001$) SNP. Out of the 18 significant genome and chromosome wise level SNP, 14 of these regions have been reported in previous studies on chromosomes 3, 6, 9, 10, 14, 17, 21, and 26 (Boichard *et al.* 2003; Viitala *et al.* 2003; Ashwell *et al.* 2004; Schrooten *et al.* 2004; Schnabel *et al.* 2005) and four of them are newly detected QTL in this study mapped on BTA 9, 14, 17 and 23. Eight of these significant SNP are located in the introns of predicted genes, and three of them cause synonymous mutations in coding regions, while seven are not located in genes (Table 7). The SNP showing association with FP at significant genome and chromosome wise ($p < 0.05$) level, including their heterozygosity, their position, *F*-test statistic value, allele substitution effects and p-value are shown in Table 7. These 11 genes that contain significant associated SNP with FP maybe considered as possible candidate genes fat production in dairy cattle populations.

The results of this study are consistent with the previously identified importance of the DGAT1 gene, which is located on BTA14. The QTL that are in close proximity of this gene controls the high proportion of FP genetic variance in dairy cattle populations. Two significant regions on BTA14 were identified in this study: one region close to the DGAT1 gene, and the second approximately 40 Mbp away (Kuhn *et al.* 2004; Olsen *et al.* 2005; Weikard *et al.* 2005; Gautier *et al.* 2006; Khatib *et al.* 2006). We found these QTL to be significantly associated with MY, FY, PY, PP and FP.

The SNP on BTA14 (rs41580517) is located at intron of the gene KCNQ3 and has been shown to have a significant association with FP and FY. This region of BTA14 (7–8 Mbp) is significant for FY at genome wise level increasing the EBV of MY by 9 kg

and significant with FP at the chromosome wise level with an effect of 0.08%. This QTL is located at the beginning of the BTA14 (0–10 Mbp) and this finding supported by previous studies (Gautier *et al.* 2006). The SNP on BTA23 (rs41640789) is highly associated ($p < 0.001$) with FY and FP with a negative effect on FY of 7.77 kg and FP of 0.07% respectively. This significant QTL region on BTA23 (1 528 086 bp) at genome wise level with FY and FP traits might be good candidate regions for further investigation.

Somatic cell score

The SNP showing association with SCS at significant genome and chromosome wise ($p < 0.05$) level, including their heterozygosity, their position, *F*-test statistic value, allele substitution effects and *p*-value are shown in Table 8. The whole genome scan identified 5 and 7 significant SNP associated for somatic cell score (SCS) at genome and chromosome wise level ($p < 0.05$) respectively on BTA 1, 13, 18, 23, 26, 28 and 29. Out of the 12 significant SNP, five of these regions have been reported in previous studies on chromosomes 1, 23, 26 and 29 (Klungland *et al.* 2001; Ashwell *et al.* 2004; Schnabel *et al.* 2005; Khatib *et al.* 2006; Smaragdov *et al.* 2006) and seven of them are newly detected QTL in this study mapped on BTA 13, 18, 23 and 28. Six of these significant SNP were located in the introns of predicted

genes, and three of them cause synonymous mutations in coding regions, and one in the untranslated region of mRNA, while one is not located in any gene (Table 8). These nine genes that contain significant associated SNP with SCS maybe considered as possible candidate genes for mastitis in dairy cattle populations.

Four SNP on BTA 1, 13, 18 and 26 were highly significant ($p < 0.001$) at genome wise level with SCS. Two of these QTL regions in BTA13 (54 cM) and BTA26 (14 cM) have been reported in the previous studies (Ashwell *et al.* 2004; Khatib *et al.* 2006) and these are supported by our finding. The two new QTL regions on BTA1 (9 253 498 bp) and BTA18 (807 748 bp) were recognised in our study with significant allelic effects on SCS. The SNP on BTA1 (rs41637212) was located on the intron of the APP gene while the SNP on BTA18 (rs29014958) was not located on the coding synonymous region of the LOC51093 gene. These two regions are recognized good QTL candidates for further investigation for SCS in dairy cattle population.

Persistency of milk

The whole genome scan to map QTL for persistency of MY (PM) identified one and 11 SNP significantly associated with this PM at genome and chromosome wise level ($p < 0.05$) on BTA 1, 8, 11, 12, 15, 19, 23 and 27. These 12 significant SNP, are newly detected

Table 7 Significant genome and chromosome wise ($p < 0.05$) SNP with fat percentage

SNP NCBI ID	BTA	Position (bp)	MAF	<i>F</i> -test	Effect (%)	<i>p</i> -value	Functional Class	GeneName
rs41587408	3	21510186	0.36	9.33	0.06	0.0013	Nearest_gene	PDZK1
rs43709929	3	25148396	0.27	9.03	0.06	0.0015	CS ^a	LOC514870
rs29018853	6	71355225	0.49	18.05	0.08	0.000013*	Intron	LEC3
rs41657163	9	12369884	0.48	7.88	0.05	0.0028	CS ^a	LOC535127
rs41592660	9	29069399	0.12	7.87	0.09	0.0028	Nearest_gene	LOC616136
rs43710950	10	41992224	0.23	16.20	0.09	0.000034*	Nearest_gene	TPM1
rs41579049	14	486841	0.27	35.91	0.12	0.000000002*	Intron	5-OPase
rs41633631	14	7969609	0.46	22.05	0.09	0.0000017*	Intron	TG
rs41567322	14	8091343	0.33	5.24	0.04	0.013	Intron	TG
rs41580517	14	8663254	0.27	11.97	0.08	0.00031	Intron	KCNQ3
rs41579063	14	31427291	0.31	6.14	0.05	0.0075	Intron	BIG1
rs41587081	14	40196357	0.45	27.72	0.10	0.00000011*	CS ^a	ZFHX4
rs41639879	17	3814195	0.29	8.67	0.06	0.0018	Intron	LOC505156
rs41570561	17	47419827	0.19	10.42	0.08	0.00070	Intron	SCARB1
rs41641678	21	8943787	0.09	8.19	0.09	0.0024	Nearest_gene	LOC514186
rs41643783	21	45716987	0.27	11.04	0.07	0.00051	Nearest_gene	MGC139789
rs41640789	23	15280862	0.37	13.68	0.07	0.00012*	Nearest_gene	POLR1C
rs41648176	26	33887202	0.31	8.67	0.06	0.0018	Nearest_gene	LOC515764

^aCoding synonymous SNP (CS).

*Significant genome wise ($p < 0.05$) SNP.

Table 8 Significant genome and chromosome wise ($p < 0.05$) SNP with somatic cell score

SNP NCBI ID	BTA	Position (bp)	MAF	F-test	Effect (SCS)	p-value	Functional Class	GeneName
rs41637122	1	9253498	0.30	17.27	0.09	0.000020*	Intron	APP
rs41628293	13	37973625	0.13	10.42	0.10	0.00071	Intron	BFSP1
rs41601522	13	60045226	0.49	9.63	0.06	0.0011	CS ^a	MGC142355
rs41576572	13	61716889	0.09	15.08	0.14	0.000061*	CS ^a	MGC142850
rs29014958	18	807748	0.45	14.54	0.08	0.000080*	CS ^a	LOC510933
rs41578926	18	1414404	0.33	9.86	0.07	0.00096	Nearest_gene	MGC139689
rs41654340	23	27834645	0.14	11.18	0.10	0.00047	mrna-utr	HIST1H2BK
rs41650611	26	15147856	0.35	14.24	0.08	0.000094*	Intron	DNTT
rs41606777	26	34495734	0.28	7.88	0.07	0.0028	Intron	SLC18A2
rs41648482	28	6866108	0.13	13.19	0.13	0.00016*	Intron	CHS1
rs41608052	29	28544795	0.14	10.44	0.09	0.00070	CNS ^b	LOC517432

^aCoding synonymous SNP (CS).

^bCoding non-synonymous SNP (CNS).

*Significant genome wise ($p < 0.05$) SNP.

QTL in this study mapped for this trait and some regions have been reported in previous studies in different regions that are not detected in this study (Ashwell *et al.* 2004; Harder *et al.* 2006; and Smaragdov *et al.* 2006). Eight of these significant SNP were located in the introns of predicted genes, and three of them cause synonymous mutations in coding regions, while one was not located in any gene (Table 9). The details of the SNP showing association with PM at significant genome and chromosome wise ($p < 0.05$) levels, including their heterozygosity, position, F-test statistic value, allele substitution effects and p-value are shown in Table 9. The SNP on BTA 15 (rs41634436) is showing association with PM at significant genome wise ($p < 0.05$) levels, MAF of this SNP is 0.06 and estimated effect could be due to very few genotypes and might lead to biased effect. These 11 genes maybe considered as

possible candidate genes as for persistency of milk in dairy cattle populations.

The SNP on BTA15 (rs41634436) is highly significant at genome wise level with persistency of milk production. This SNP is located in an intron of CD44 gene. This QTL region is a good candidate for persistency of MY in dairy cattle population.

Conclusion

This study has used a medium-density genome-wide scan that markers distributed over the genome and using SNP markers located in or nearby known genes. Considering the marker density and the limitations (single marker LD regression) of the statistical model used in this study, the variances expressed by the QTL detected in this study may include partial of the polygenic variance. Therefore, it is unlikely that

Table 9 Significant genome and chromosome wise ($p < 0.05$) SNP with persistency of milk yield

SNP NCBI ID	BTA	Position (bp)	MAF	F-test	Effect (kg)	p-value	Functional Class	GeneName
rs41629125	1	63326365	0.31	9.46	1.09	0.00119	Intron	ITGB5
rs41607547	8	16683360	0.14	13.46	1.60	0.00014	Intron	LOC786714
rs29024299	8	34331637	0.13	11.22	1.29	0.00046	Intron	LOC613759
rs41591742	8	97443379	0.09	8.78	1.59	0.0017	Intron	PAPPA
rs43703342	11	63461383	0.31	9.27	1.05	0.0013	CS ^a	LOC514626
rs29010310	12	31526216	0.48	11.82	1.14	0.00033	CS ^a	LOC539378
rs41626908	12	32355128	0.15	11.45	1.49	0.00040	CS ^a	MGC139047
rs41634436	15	57653376	0.06	14.19	2.36	0.000096*	Intron	CD44
rs41641482	19	12477719	0.42	8.30	0.96	0.0022	Intron	AP2B1
rs41603088	19	12499139	0.49	8.37	0.94	0.0021	Intron	AP2B1
rs41589741	23	41842783	0.47	12.64	1.06	0.00022	Nearest_gene	LOC529115
rs41607485	27	12842179	0.05	10.00	2.25	0.00089	Intron	IRF2

^aCoding synonymous SNP (CS).

*Significant genome wise ($p < 0.05$) SNP.

any of the SNP found associated with milk production or functional traits represent causal mutations. The assumption of SNP are in LD with QTL may suffer from sampling errors with this density marker map. In addition, the phenotypic traits used in this study are highly accurate EBVs, the heritability for the traits analysed are expected very high. It does, however, provide a starting point for uncovering quantitative trait nucleotides, particularly where the results of this study are backed up by other independent studies. This study has revealed QTL for MY, PY, PP, FY, FP, somatic cell score and persistency of milk in the Canadian dairy cattle population. Several of the significantly associated SNP are located in genes that are known to be components of the fat and protein metabolism pathways. More detailed investigations of these genes may lead to identification of the causative mutations underlying the QTL. This study has also shown that LD regression using SNP markers is an effective methodology for detecting and mapping QTL in dairy cattle populations. Use of much denser marker sets recently developed for cattle will no doubt aid in moving from the marker associations, such as those reported in this study, toward revealing the causal mutations underlying many of economically important traits in dairy cattle.

Acknowledgements

The authors acknowledge the financial contributions of Semex Alliance, the Institute for Food and Agricultural Science Alberta (IFASA), and Natural Sciences and Engineering Research council (NSERC) of Canada.

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