SCUOLA DI DOTTORATO IN SANITÀ E PRODUZIONI ANIMALI: SCIENZA, TECNOLOGIA E BIOTECNOLOGIE

DOTTORATO DI RICERCA IN PRODUZIONI ANIMALI
XXVII CICLO

Genome-Wide detection of QTL and CNVs in dairy cattle population

Tesi di: Dott.ssa Maria Giuseppina Strillacci
Docente guida: Prof. Alessandro Bagnato


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To my little
Family

CONTENTS

ABSTRACT

The QTL involved in susceptibility/resistance of infectious diseases and in the productive traits variations, are characterized by genetic heterogeneity and multifactorial inheritance, involving gene polymorphisms from different alternative pathways. With the availavility of single nucleotide polymorphism (SNP) genotyping arrays, the genome-wide association studies (GWAS) have been frequently used to determine the genetic component of complex trait. The Copy Number Variations (CNVs) are another genomic marker that can be possibly used in GWAS and that can be identified from SNP chips themselves.

The aims and related discussions for each of the studies presented in this thesis were grouped into three different chapters.

- Chapter I described the QTL mapping analysis to identify the existence of genetic variability associated to the CLA, VA and D9D contents in milk of the Italian Brown Swiss dairy cattle breed. For this study a selective DNA pooling in a daughter design was adopted, using the Illumina Bovine SNP50 Bead Chip to genotype the pools. Milk samples from 60 animals with higher values (after correction for environmental factors) and 60 animals with lower values for each of these traits from each of five half-sib families were pooled separately. Allele frequencies were compared between pools of high and low value at the sire and marker level for each SNPs for which the sires were heterozygous. An R procedure was implemented to perform data analysis. A correction for multiple tests was applied using the proportion of false positives approach. BTA 19 showed the largest number of markers in association with CLA. Associations between SNPs and the VA and D9-desaturase traits were found on several chromosomes. A bioinformatics survey identified genes with an important role in pathways for milk fat and fatty acids metabolism within 1 Mb distance from SNP markers associated with fatty acids contents. This is the first available mapping for fatty acid content in the Brown Swiss population.

- Chapter 2 described a genome-wide association study for somatic cell score (SCS) in the Valdostana Red Pied cattle, with a selective DNA pooling analysis, using the Illumina BovineHD BeadChip. The phenotypes of 275 sires for SCS were expressed as Deregressed Proofs (DP-EBVs) for SCS. The sires were ranked according to DP-EBVs for SCS and the 20% high and 20% low sires included in the pools. The multiple marker test was performed in R software. On BTAs 1, 2, 3, 4, 9, 13, 15, 17, 21 and 22 the largest number of markers in association to the trait was found identifying novel genomic regions related to mastitis (1-Mb SNP windows) and confirming others already mapped. The largest number of significant SNPs exceeding the threshold for genome-wide significant signal was found on BTA 15, located at 50.43-51.63 Mb. The genomic regions identified in this study contribute to a better understanding of the genetic control of the mastitis immune response in cattle and may allow the inclusion of more detailed QTL information in selection programs.
- Chapter 3 described a genome wide CNVs discovery in 651 bulls of the Italian Brown Swiss breed using the Illumina Bovine SNP50 BeadChip data. Hidden Markov Model (HMM) of PennCNV and SVS7 software (Golden Helix) were used for the identification of the CNVs and Copy Number Variation Regions (CNVRs). A total of 5,099 and 1,289 CNVs were identified using PennCNV and SVS7 software, respectively. These were grouped at the population level into 1,101 (220 losses, 774 gains, 107 complex) and 277 (185 losses, 56 gains and 36 complex) CNVRs, covering 682 Mb (27.14%) and 33.7 Mb (1.35%) of the autosome, respectively. Ten of the selected CNVRs were experimentally validated with qPCR and the proportions of confirmed positive samples for each region varied from 50% to 100%. The GO and pathway analyses identified genes (false discovery rate corrected) in the CNVRs related to biological processes, cellular component, molecular function and metabolic pathways. Although there is variability in the CNVRs detection across methods, platforms, this study allowed the identification CNVRs in Italian Brown Swiss,

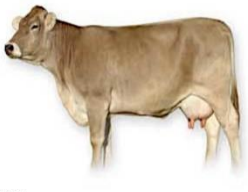
overlapping those already detected in other breeds and finding additional ones.

ABREVIATIONS

aCGH	(Comparative genomic hybridization array)
BAF	(B allele frequency)
CLA	(conjugated linoleic acid)
CNV	(copy number variant)
CNVR	(copy number variant region)
DP	(deregressed proofs)
DLRS	(derivative log ratio spread)
D9D	(Δ9-Desaturase)
EBV	(estimated breeding value)
FDR	(false discovery rate)
FISH	(Fluorescent in situ hybridization technique)
DGV	(direct genomic values)
GS	(genomic selection)
GWAS	(genome-wide association study)
HMM	(hidden markov model)
Kb	(kilobase)
KEGG	(Kyoto Encyclopedia of Genes and Genomes)
LD	(Linkage disequilibrium)
LRR-LR	(log R ratio)
MAS	(marker-assisted selection)
Mb	(megabase)
NCBI	(National Center for Biotechnology Information)
NGS	(next generation sequencing).
PFP	(proportion of false positives)
PCA	(Principal component analysis)
PFP	(proportion of false positives)
qPCR	(quantitative polymerase chain reaction)
QTL	(quantitative trait loci)
SCS	(somatic cell score)
SCC	(somatic cell count)
SDP	(Selective DNA pooling)
SNP	(single nucleotide polymorphism)

PART I

Genome scan for the QTL identification involved in the phenotypic variation of the economic traits in dairy cattle



A.1 Qantitative trait loci (QTL)

Goddard and Hayes (2009) have defined Quantitative trait loci (QTL) as: "*A measurable trait that depends on the cumulative action of many genes and the environment, and that can vary among individuals over a given range to produce a continuous distribution of phenotypes*".

For livestock, as well as for human and for other classes of organism online database[1] grouping all the openly accessible trait mapping data, (e.g. including QTL, candidate gene, association data from GWAS and copy number variations mapped on genomes) are available. These databases make it feasible to facilitate the location of genes responsible for quantitative traits, confirming and comparing QTL within and between species. Among the various databases, the Cattle QTLdb reports about 9,180 QTL for 472 different traits[2].

The identification of the genes involved in the phenotypic variation of one trait can be approached mainly into two ways: the "candidate gene" "and genome wide scanning" approaches. The "candidate gene" is an approach that requires, for its application, the knowledge of the biological and biochemical pathways (physiology) involved in the phenotypic variation of the traits. The candidate gene is a gene in which functional mutations (including *e.g.* single nucleotide and CNVs variation), may be causative of extreme phenotypes. In animals, this method can be based on comparative human genomics. Instead, the "genome wide scanning" (GWS) allows to detect the chromosomal regions of QTL at base-pair level with the use of DNA markers in population-based experimental designs (Zhu and Zhao (2007)).

The general principle of the identification of QTL is based on the presence of LD Linkage disequilibrium (LD) among QTL alleles and marker loci.

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[1] Examples of Livestock animal QTL databases: AnimalQTLdb (http://www.animalgenome.org); Bovine QTL Viewer (http://genomes.sapac.edu.au/bovineqtl/); cgQTL database: QTL for milk production traits in cattle identified from expression experiments (http://cowry.agri.huji.ac.il/QTLMAP/qtlmap.htm).

[2] (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index).

In particular, QTL can be mapped on chromosomal regions using linkage and association mapping analyses.

The *linkage analysis* allow to map the chromosome regions location by identifying the genetic markers that are co-inherited with a QTL involved in the expression of a phenotype of interest, within pedigree and on the same chromosome. Due to the restricted number of meiotic events that are captured in a bi-parental mapping population, the genetic resolution of QTL maps often remains confined, to a range of 10-30 centimorgan (cM). Moreover, *linkage analysis* can only sample a small fraction of all possible alleles in a population from which the parents originated. (Pasam K. et al, 2012). Instead, the *association mapping or LD analysis* is mapping QTL on different chromosomes and in not related individuals (natural/designed population). LD is the non-random association of alleles in haplotypes[3] at different loci within a population. LD mapping exploits ancestral recombination events that occurred in the population and takes into account all present alleles in the population to identify significant marker-phenotype associations (Pasam K. et al, 2012). LD always exists if there is physical linkage between QTL and the marker: if two genes segregate together, they are told to be in LD (Goddard and Hayes, 2009).

One of the available LD mapping strategies is based on genome-wide association (GWA), which exploits marker polymorphisms across all chromosomes. In a GWA study (GWAS), samples are recorded for a trait of interest and tested for a genome-wide panel of markers (high-throughput genotyping), to detect possible associations between the trait and the markers (Goddard and Hayes, 2009). GWAS has the ability to detect smaller chromosomal regions affecting a trait in respect to linkage analysis, thus providing more precise evaluations of the size and direction of the effects of the alleles at identified loci (Abdel-Shafy et al. 2014). The power to establish a relationship between genetic polymorphisms (allele at loci) and phenotypic

[3] Haplotype consists of two or more SNP in close proximity, which tend to be inherited together with high probability (Abdel-Shafy et al, 2014)

variation is dependent on the accuracy with which these can be measured.

AI.2 QTL detection for milk fatty acid content in dairy cattle

Cow's milk is one of major components of human diet and it is a great source of balanced nutrients, with a range of biological activities that influence metabolic processes and disease resistance. Among the microcomponents in milk, the conjugated linoleic acids (CLA) is one of the most significant. CLA is a collective term for isomers of linoleic acid with conjugated double bonds in several positions and conformations. The major precursor of CLA in milk fat is the vaccenic acid (11-*trans*-octadecenoic acid; VA). Desaturation of VA to CLA (C18:2 cis-9, trans-11) occuring in the mammary gland (75-90%) and other tissues, is catalyzed by Δ9-Desaturase (D9D).

The heritability value (h^2) of CLA reported in literature ranged from 0.12 to 0.21 (Mele et al., 2009; Stoop et al., 2008). Also, Mele et al., (2009) reported the h^2 values of 0.19 and 0.15 for VA and D9D (as CLA/VA), respectively and highlighted the negative correlation between CLA, VA, D9D with milk fat (%) (−0.55, −0.69, −0.52).

The detail regarding the studies reported in literature including the QTL detection associated to CLA, VA and D9D are reported in chapter 2. Some of them are graphically summarized in Figure A.1 (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index)

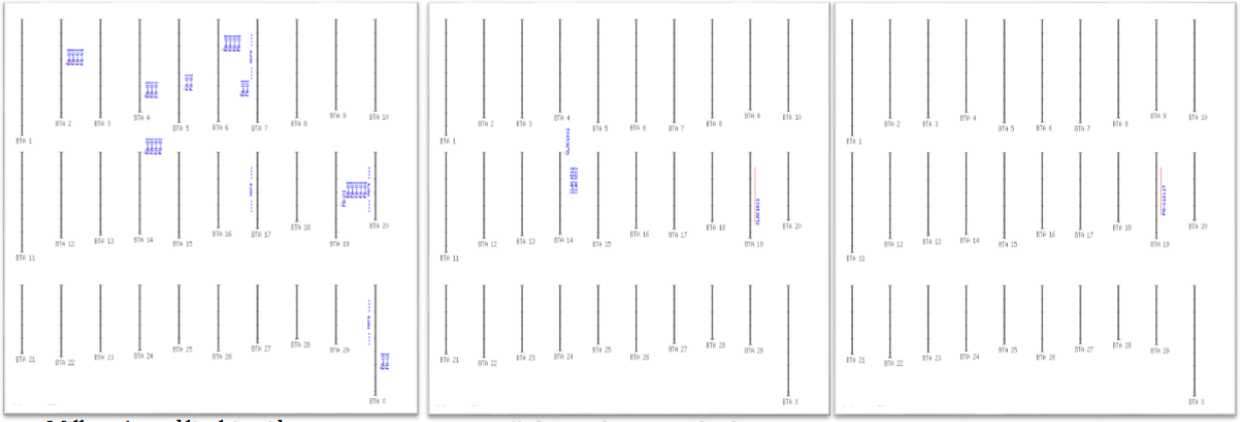

Figure A.1 Graphical representation of QTL associated with milk conjcated linoleic acid percentage, milk fatty acid unsaturated index and milk trans-vaccenic acid percentage on all bovine chromosomes.

A.I.3 QTL detection for somatic cell score in dairy cattle

Mastitis is an inflammation of bovine mammary gland that occurs in response to physical damages or infection and is one of the most costly production-related diseases in dairy farms.

The difference in "mastitis state" (progress and resolution) are mainly due to how the responsible factors of the disease (animal, environment and pathogens) interact each others. Individuals can differentially be susceptible/resistant to mastitis depending on their genetic that is responsible for the udder conformation and for the physiological and immunological responses to the infections.

The commonly phenotypes used to investigate the resistance to mastitis are the milk somatic cell count (SCC), its log transformation in somatic cell score (SCS) for positive correlation with clinical mastitis (0.50-0.80) (Rupp and Boichard 2003) and the clinical mastitis occurrence.

The genetic correlations among SCC and milk traits were investigated by several authors and seem to be different for parity and stage of lactation. Samorè et al. (2008) showed that genetic correlations for lactation measures (305-d protein yield and lactation SCS) were positive in the first parity (0.31) and close to 0 in the second (0.01) and third (0.09) parities. In addition, the genetic correlation among SCC and milk traits was positive in the first lactation, and near zero in the second lactation (Koivula et al., 2005).

Several authors have investigated the mastitis resistance in cattle and the existence of QTL for this trait has been reported on almost all bovine chromosomes (www.animalgenome.org/QTLdb/)(Figure A.2).

(http://www.animalgenome.org/cgi-bin/QTLdb/BT/index).

The detail regarding the studies reported in literature, including the QTL detection for mastitis resistance are reported in chapter 3.

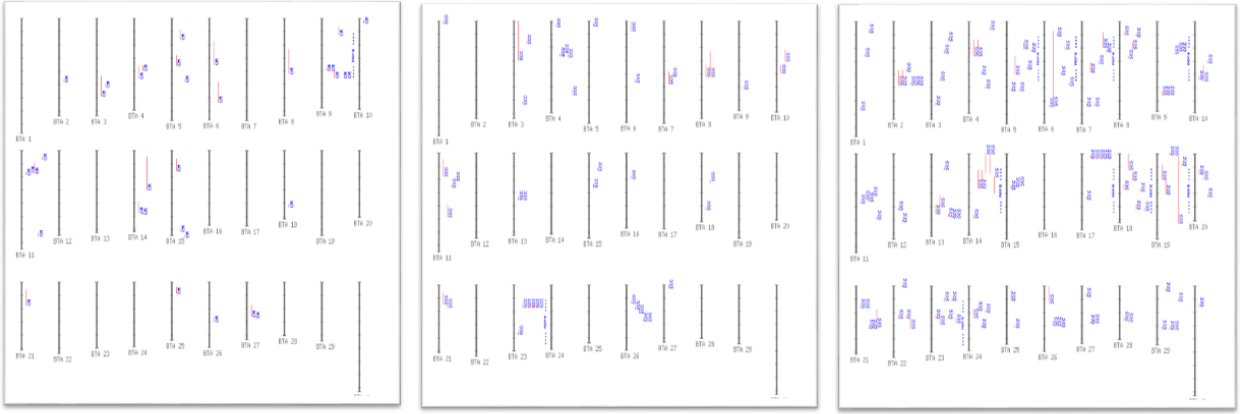

Figure A.2 Graphical representation of QTL on all bovine chromosomes associated to clinical mastitis (CM), somatic cell count (SCC) and somatic cell count (SCS).

A.2 Selective DNA pooling

The selective DNA pooling experimental design is an efficient method to detect the association between markers and QTL by comparing marker allele frequencies in pooled DNA from phenotypically extreme individuals (Darvasi and Soller, 1994). This approach is based on the theoretical demonstration that almost all the mapping information for a trait are linked to the allele frequency of the marker in the best and the worst 25% of the population phenotypic distribution for the trait.

Pools construction requires equal amount of DNA from individual samples, and the differences in allele frequencies in pools are estimated based on the intensity of the signal for each allele in the pool. Experimental error can be occur during the pool constitution and genotyping; these can be reduced by averaging allele frequency estimates over repeated measurements of the pools.

Within a selective DNA pooling design, the major disadvantage occurs with the study of different traits of interest, for which it will be necessary to genotype different samples for each tail (Sham et al., 2002).

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I

QUANTITATIVE TRAIT LOCI MAPPING FOR CONJUGATED LINOLEIC ACID, VACCENIC ACID AND Δ^9-DESATURASE IN ITALIAN BROWN SWISS DAIRY CATTLE USING SELECTIVE DNA POOLING

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QUANTITATIVE TRAIT LOCI MAPPING FOR CONJUGATED LINOLEIC ACID, VACCENIC ACID AND Δ^9–DESATURASE IN ITALIAN BROWN SWISS DAIRY CATTLE USING SELECTIVE DNA POOLING

M.G. Strillacci*I, E. Frigo*I, F. Canavesi*I, Y. Ungar§I, F. Schiavini*#, L. Zaniboni*, L. Reghenzani*, M.C. Cozzi*, A.B. Samoré*, Y. Kashi§, E. Shimoni§, R. Tal-Stein†, M. Soller†, E. Lipkin†, A. Bagnato*#

*Department of Health, Animal Science and Food Safety (VESPA), University of Milan, Via Celoria 10, 20133, Milan, Italy.
#Genomic and Bioinformatics Platform, University of Milan, c/o Fondazione Filarete, Viale Ortles 20, 20100 Milano, Italy.
†Department of Genetics. The Hebrew University of Jerusalem, 91904 Jerusalem, Israel.
§Israel Institute of Technology (Technion), Department of Biotechnology and Food Engineering, Technion City, Haifa 3200003, Israel

I.I. ABSTRACT

A selective DNA pooling approach was applied to identify QTLs for conjugated linoleic acid, vaccenic acid and Δ^9-Desaturase milk content in Italian Brown Swiss dairy cattle. Milk samples of 60 animals with higher values (after correction for environmental factors) and 60 animals with lower values for each of these traits from each of five half-sib families were pooled separately. The pools were genotyped using the Illumina Bovine SNP50 BeadChip. Sire allele frequencies were compared between high and low tails at sire and marker level for SNPs for which the sires were heterozygous. An R procedure was implemented to perform data analysis in a selective DNA pooling design. A correction for multiple tests was applied using the proportion of false positives among all test results. BTA 19 showed the largest number of markers in association to CLA. Associations among between SNPs and the traits VA and Δ9-Desaturase were found on several chromosomes. A bioinformatics survey identified

genes with an important role in pathways for milk fat and fatty acids metabolism within 1 Mbp of SNP markers associated with fatty acids contents.

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1.2. INTRODUCTION

The detection of genomic regions affecting complex traits has led the interest in using dense panels of single nucleotide polymorphisms (SNPs) to identify quantitative trait loci (QTL) (Goddard & Hayes, 2009).

Selective DNA pooling (SDP) is an experimental design that is able to reduce costs in genomic studies by genotyping pooled DNA samples from selected individuals at each of the two phenotypic extremes of a sample (Darvasi & Soller, 1994). The test to identify markers in association with a QTL is based on the difference of marker allele frequencies between the pools of individuals at the two tails of the phenotypic distribution. Theoretical analysis shows that for experiments involving backcross, F2 and half-sib designs, SDP power to detect genes with large effect, is comparable to individual selective genotyping (Darvasi & Soller, 1994).

Milk contains a number of micro-components having nutraceutical properties with beneficial effect on human health. Among these compounds, conjugated linoleic acid (CLA) is one of the most relevant. Bauman & Lock (2006), Benjamin & Spener (2009) reported that studies with animal models have demonstrated a variety of beneficial health effects from CLA, including anti-carcinogenic, anti-atherogenic, anti-obesity, immune system enhancement and anti-diabetic effect. Although in recent studies the biological effects of CLA results are controversial, many of its benefits related to the diet supplementation were confirmed (Oleszczuk et al., 2012). CLA represents a heterogeneous group of positional and geometric isomers of linoleic acid with a conjugated double bond system. These are

produced as transient intermediates in a rumen enzymatic biohydrogenation of unsaturated fatty acids consumed in the diet. The vaccenic acid (VA) (C18:1 trans-11) is the major biohydrogenation intermediate produced in the rumen and 75-90% of it is converted into CLA (C18:2 cis-9, trans-11) by Δ9-Desaturase (D9D) in the mammary gland and other tissues (Bauman & Lock, 2006). The role of rumen biohydrogenation and tissue D9D in the production of CLA in milk fat and in other tissues is represented in Figure 1 adapted from Bauman & Lock (2006).

Genetic analyses of bovine milk fatty acids in several populations have shown heritability of 0.12, 0.12 to 0.21, and 0.15 for VA, CLA and D9D, respectively (Stoop et al, 2008; Mele et al., 2009). The identification of genomic regions that may be responsible for genetic variation in milk fat composition could help in understanding the genetic basis of the biological pathways involved in fatty acid synthesis and thus, may create opportunities for selection for milk nutraceutical components. A number of studies identified QTLs affecting bovine milk fatty acids composition. Morris et al. (2007) identified QTLs for VA and CLA on BTA19 in a linkage analysis using microsatellite markers. Schennink et al. (2009) in a GWAS analysis using SNP markers, found QTLs for CLA (BTA11, BTA14 and BTA17), VA (BTA1, BTA11, BTA18 and BTA27) and D9D (BTA1, BTA6, BTA14, BTA16 and BTA19). Moreover, Bouwman *et al.* (2011) identified QTLs for CLA on BTA6, BTA7, BTA14, BTA17, BTA19, BTA26, BTA27 and BTA28. All of these results are reported in AnimalQTLdb (http://www.animalgenome.org).

The purpose of the present study was to verify the existence of genetic variability related to the major actors involved in the CLA synthesis in the mammary gland in cattle.

Hence, the variation of VA level as substrate for the D9D activity, of D9D as the indicator of the efficiency of the enzymatic activity and of CLA as the product of the efficiency of the endogenous synthesis of VA by the D9D, were studied biometrically. In addition, a QTL

mapping analysis for CLA, VA and D9D in Italian Brown Swiss dairy cattle, with a selective DNA pooling in a daughter design (Lipkin et al. 1998) using the Illumina Bovine SNP50 BeadChip was performed.

I.3. MATERIALS AND METHODS

I.3.1. Sampling of families

Five large Italian Brown Swiss half-sib sire families (denoted B, C, E, F, G as per Bagnato et al. 2008) were used in this study. Milk samples were collected and stored from previous studies (Bagnato et al. 2008) and were available for further analyses. The number of milk samples available for each of the 5 families is reported in Table 1.
Semen samples of the sires for genotyping were provided by the Italian Brown Cattle Breeders Association semen bank. Milk samples of about 500 available daughters for each family (total 2,601 samples) were used for milk fatty acids determination and for D9D calculation, and as a source of DNA from Somatic Cells.

I.3.2. Fatty acids determination

Milk fat was extracted and transmethylated according to Chouinard et al. (1999). Fatty acid methyl esters were analyzed by gas chromatography (GC-FID) with a highly polar 100 m SP-2560 column, using GLC-60. An indirect measurement of D9D was used, calculated as the ratio of milk CLA to the sum of milk CLA and VA, as described by Bauman & Lock (2006), Conte et al. (2010) and Schennink et al. (2008).

I.3.3. Variance components analysis

(Co)variance components for fatty acids were estimated using the VCE 6.0 package (Groeneveld et al., 2010; Neumaier & Groeneveld, 1998). Environmental factors included in the model of analysis for variance components estimation were previously tested for their significance with the GLM procedure of SAS®.

Pedigree information were provided by the Herd Book of ANARB and included all known ancestors for a total of 8,604 animals.
The following single trait animal model was used to obtain estimates of heritabilities for VA, CLA and D9D:

yijklmnp= μ + Pi + AGj + YMk + Sl + DIMm + PRn + ap + eijklmnp
where:

- ✓ yijklmnp is the value of VA, CLA or D9D determined for each daughter milk sample;
- ✓ μ is the factor common to all observations;
- ✓ Pi (i=I,…4) is the fixed effect of the class of parity;
- ✓ AGj (j=I,…4) is the fixed effect of class age at calving;
- ✓ YMk (k=I, ….16) is the fixed effect of the interaction between year and month of calving;
- ✓ Sl (l=I,…4) is the fixed effect of the season of calving;
- ✓ DIMm (m=I,…15) is the fixed effect of the class of days in milk;
- ✓ PRn (n=92) is the fixed effect of province;
- ✓ ap is the random additive genetic effect of the animal p (0, Aσ2a);
- ✓ eijklmnp is a random residual (0, Iσ2e).

Parity was classified into 4 classes for first, second, third and later parities. Age at calving was classified into 4 classes (class I: from 18 to 42 months, class 2: from 42 to 54 months, class 3: from 54 to 66 months, class 4: ≥ 67 months). Season effect was classified in 3-mo classes (I=spring; 2=summer; 3=autumn; 4=winter). Days in milk were grouped in 30-days classes (15 classes).

1.3.4. Pools constitution

The residual values (phenotypes adjusted for all environmental factors) for the three traits VA, CLA and D9D, obtained from a

GLM analysis with the same fixed effect of the variance component estimation model described above (no additive genetic effect), were used to identify the 60 more extreme daughters in the high and low tails of the trait distribution within each family/trait combination. Thus, for each trait, a total of 120 daughters were identified for each family. The selected samples for each tail (high and low values respectively for VA, CLA and D9D) were divided (even and odd sample numbers) into 2 sub-pools of 30 individuals each, in order to have two sub-pools with comparable phenotypic value. Hence, a total of 4 pools were constructed for each family/trait combination, for a total of 20 pools per trait (60 pools across all the three traits). The milk of each individual was included in the pools in different volumes according to Somatic Cell Count (SCC), ensuring that DNA of all individuals was equally represented within each pool. SCC were available from routine milk sampling, or determined by Somacount 150 (Bentley instrument, Chaska, MN). Each sub-pools contained a total of 40,000 cells.

1.3.5. DNA extraction and genotyping

Milk pools were treated according to Murphy et al. (2002) to obtain a clear pellet of cells; genomic DNA was then extracted utilizing NucleoSpin® Blood kit (Macherey-Nagel, GmbH & Co. KG). Genomic DNA was also extracted from semen using the ZR Genomic DNA TM Tissue MiniPrep (Zymo Research, Irvine, CA).

DNA samples were quantified using NanoQuant Infinite m200 (Tecan) and diluted to 50 ng/ul. A Quality Control (QC) was performed on each sample to verify the DNA integrity on Invitrogen E-Gel 1% Agarose Gel. DNA samples were genotyped using Illumina Bovine SNP50 BeadChip interrogating 54,001 SNPs.

1.3.6. Statistical analyses

Statistical analysis of pools.

Pools were analyzed according to the SDP approach in a daughter design (Darvasi & Soller, 1994; Bagnato et al., 2008). Statistical analyses were performed with respect to SNP markers for which the sires were heterozygous, as these were the only ones that could segregate alternative sire alleles within family linked to a QTL for the trait of interest.

Frequency estimates.
The estimation of allele frequency in DNA pools is one of the critical steps in DNA pooling analysis, especially with SNP chips (Janicki & Liu, 2009). These authors demonstrated the validity of the B-allele frequency, calculated by the BeadStudio software from Illumina, as a good estimator of the allele frequency of the individuals that are part of a pool. In the present study, the generation of B-allele frequency was performed using the self-normalization algorithm of Illumina BeadStudio software (Genotyping Module v3.2) as suggested by Janicki & Liu (2009).
The marker-sire-trait test. A pipeline in R software (http://www.r-project.org/) was programmed to perform a single-marker sire test. In SDP, significance of marker j for a single sire i, heterozygous at marker j, was determined for each trait by the single-sire test statistic (Darvasi & Soller, 1994; Lipkin et al., 2008; Bagnato et al., 2008). Briefly, a test statistic for the ijth sire × marker combination was calculated as:

$$Zij=Dtestij/SD(Dnullij)$$

where $Dtestij = [(H1 + H2) - (L1 + L2)]/2$ is the difference in sire allele frequencies between the high and low daughters pools of the ith sire with respect to the jth marker, averaged over the two subpools of the same tail.

Dnullij = [(H1 − H2) + (L1 − L2)]/2 is the difference in allele frequencies between the 2 subpools of the same tail of the ith sire with respect to the jth marker, averaged over the high and low pools. Dtestij and Dnullij were calculated only for markers for which the sire was heterozygous. Because Dnullij is calculated within tails, it has expectation of 0 and thus should distribute as the D under the null hypothesis of no QTL effecting linkage to the marker. Thus, the standard deviation (SD) of the Dnullij values obtained across all markers [SD(Dnullij)] is an empirical estimate of the standard error of Dtestij under the null hypothesis.

Under the null hypothesis, Zij values distribute as a standardized normal variable and P-values for the individual sire-marker combinations were obtained accordingly. The test statistic (TSj) for the jth marker was then calculated by summing the Zij2 across all heterozygous sires:

$$TSj=\sum(Z2ij)$$

Under the null hypothesis, TSj distributes as chi-square with degrees of freedom (df) equal to k, where k is the number of sires heterozygous at the marker (Lipkin et al., 1998). The comparison wise error rate P-values for the jth marker (CWER-P) were obtained accordingly.

Quality control.

Dnull for each pool was computed as the difference in allele frequency estimates between replicate pools in the same tail. As such, it should represent the distribution of D under the null hypothesis. Anderson-Darling, Shapiro-Wilk and Kolmogorov-Smirnov normality tests were performed on Dnull distribution within and across sires (Stephens, 1986; Royston, 1995; Marsaglia et al., 2003). The quantiles of the observed p-values corresponding to the Dnull values were compared with the quantiles of the standard normal

distribution using a quantile-quantile plot (Q-Q plot) to visually assess the quality of data distribution.

The distribution of actual allele frequency differences within and across tails was analyzed in order to identify SNPs with unexpected variability within tail (Bagnato et al., 2008; Huang et al, 2010) and possible outlier pools whose estimated allele frequencies deviated within tails over many markers. All SNPs that showed a significant allele frequency difference at p-value ≤0.01 within tail of the tested distribution (2.33 SD), were excluded from the analysis. These represented SNPs whose allele frequency estimations could be linked to errors of various sources (Bagnato et al., 2008; Huang et al., 2010). One pool for the high CLA tail in family B was entirely excluded from the analysis. An additional quality control step was to identify SNPs with at least 10 bead score reads that are the base for the estimation of pooling allele frequency (PAF) used to compute B-allele frequencies by Illumina BeadStudio (McGregor et al,. 2008). All SNPs that did not have at least 10 PAF within pool were removed from the analysis.

Correction for multiple tests.

A second Q-Q plot was used to assess the number and magnitude of observed linkage between SNPs and the traits under study, comparing the linkage statistics expected under the null hypothesis of no linkage and the observed -log10(p-value).

A multiple-test correction was applied using the proportion of false positives (PFP). As illustrated by Fernando et al. (2004), PFP was computed as:

$$\widehat{PFP_\alpha} = \frac{\alpha K \widehat{p_0}}{R_\alpha}$$

where P_0, the proportion of true null hypothesis among all hypotheses tested, is estimated as proposed by Mosig et al. (2001), using a R routine developed by Nettleton et a,. (2006), α is the set significance

level (0.05; 0.10 or 0.20), K is the number of tests and Rα denotes the observed number of rejected null hypothesis at the set significance level. As reported by Fernando et al. (2004), PFP is the estimator that Mosig et al. (2001) called "adjusted false discovery rate (FDR)". The corresponding threshold for PFP levels of 5, 10 and 20% were determined.
Using the -log10 of the linkage test p-values for each SNP, Manhattan plots were created for each trait.

Bioinformatics.
A list of genes with an important role in pathways for milk fat and fatty acids metabolism was generated using Kyoto Encyclopedia of Genes and Genomes (KEGG)
(http://www.genome.ad.jp/kegg/pathway.html).
Bos_taurus_UMD_3.1 assembly in NCBI, ENSEMBL and UCSC databases were used in order to verify which of the significant SNPs were close (within 1 Mb) to one of these genes.

1.4. RESULTS AND DISCUSSION

A total of 1,482 milk samples were successfully analysed by gas chromatography GC-FID. Descriptive statistics are reported in Table 2 where means, residual values for each trait within each family, are reported in high and low tails for the sub-pools. Means for CLA were higher than those reported by Kelsey et al. (2003) and De Marchi et al. (2011) in US and Italian Brown Swiss cattle, while values for VA and D9D were similar to those reported by Kelsey et al. (2003).
Moderate heritability values were estimated for VA (0.33) and CLA (0.37). CLA heritability was similar to that reported by Stoop et al. (2008). Because CLA is a recognized bioactive food component of milk fat, the existence of genetic variability of this fatty acid shows that the nutritional properties of milk fat can be improved by selective breeding. D9D heritability was 0.38, confirming a genetic variability related to the enzyme activity.

A total of 13,533, 14,560, 14,389, 13,447 and 13,325 SNPs, for which the sires were heterozygous, were analysed for family B, C, E, F, G, respectively.

Figure 2 shows the Q-Q plots of the observed and expected p-values of sire markers for CLA, VA and D9D. Data appeared to follow approximately a normal distribution, deviating from it only at the two extremes of the regression line. Across all traits, the mean value of Dnull at sire level was equal to zero as expected. Observations at the extreme of the observed distribution showed values that were slightly smaller than expected. Thus, Dnull distributes as a standard normal distribution with mean zero, confirming that Dnull indeed represents D under the null hypothesis. The values of SD of Dnull were 0.149, 0.163 and 0.147 for CLA, D9D and VA, respectively.

Figure 3 shows the Q-Q plots, comparing, for each trait, the number and magnitude of observed linkage test p-values across all sires x heterozygous-marker combinations, and the test distribution expected under the null hypothesis of no QTL linkage. Marked deviations from the identity line suggest that the samples contain many values arising from truly falsified null-hypothesis tests.

According to PFP corrections adopted, different thresholds levels of –log10(p-values) significance were obtained and applied to Manhattan plots (Figure 4) for each trait. The PFP thresholds (5%, 10%, 20%) were different in the Manhattan plots for CLA, VA and D9D. In particular, p-values corresponding to 5% PFP were 1.5E-4, 1.0E-5, and 1.2E-5 respectively for CLA, VA and D9D; values corresponding to 10% PFP were 1.1E-3, 1.0E-4, and 5.0E-5 respectively; and values corresponding to 20% PFP were 6.6E-3, 7.2E-4, and 1.7E-3 respectively. Each point in the Manhattan plots is a SNP set out across the chromosome from left to right, and the heights correspond to the strength of the association to the analysed trait. Figure 5, Figure 6 and Figure 7 illustrate significant markers at different PFP for CLA, VA and D9D, showing the region in each chromosome where the markers were associated with putative QTL.

I.4.1. Association tests of significant SNPs considering PFP threshold of 5%

Table 3 shows for each trait, the significant SNPs located above the 5% PFP threshold, their chromosomal positions and p-values. A total of 73, 6, and 7 SNPs were significant for CLA, VA and D9D at 5% PFP. These significant markers were distributed over BTAs 5, 7 and 21. Only a single marker, BTA-38242-no-rs on BTA16, was significant for more than 1 trait (CLA and VA).

On BTA19 no less than 21 markers were significant for CLA (the next significant chromosome was 17 with only 10 significant markers).

Also shown in Table 3 is whether the significant marker is intragenic, within 1 Mbp of an annotated gene (independently of is function), or not close to a known gene. We will not discuss in detail in this paper all of the chromosomal regions associated with the traits considered. What follows are some selected regions that showed associations with the most studied metabolic pathways in literature. Most of the regions included in Table 3 have significant effects on predisposition to cancer in humans (e.g.: *PCDH10, MYC, AATF*) (Wang et al., 2009; Kaul & Mehrotra, 2007). Also, several SNPs on different BTAs are significantly associated with genes involved in human neurodegenerative diseases (e.g.: *ATXN10, NSF, RIMBP2*) (Wardle et al., 2009; Liu et al., 2011; Hollingworth et al., 2012) and hypertension (e.g.: *KCNA5*) (Wipff et al., 2010).

Table 4 shows significant SNPs located above the 5% PFP threshold line of the Manhattan plots within 1 Mbp from genes encoding for enzymes with an important role in fat and fatty acid metabolism. Genes near significant SNPs for the three traits are here commented separately.

- *CLA:* On BTA 2, at 98.4 Mbp, ACADL gene was found close to the ARS-BFGL-NGS-3990 SNP (98.2 Mbp) that was significantly associated to CLA amount in milk. This gene encodes for acyl-CoA dehydrogenase long-chain that is involved in several metabolic

pathways, including fatty acid metabolism and the peroxisome proliferation-activated receptors (*PPARs*) signalling pathway that has a strategic role in increased adipogenesis and fatty acid storage. On BTA 4, at 101.8 Mbp, *DGKI* gene encodes for diacylglycerol kinase-iota, which is involved in glycerophospholipid and glycerolipids metabolism. The major chromosomal regions that showed highly significant associations with CLA were on BTA 19: SNPs close to the *ACACA* gene (13.7 Mbp, involved in fatty acid biosynthesis) were found significantly associated to CLA phenotypic variation. In the region located at 30-44 Mbp, where SNPs were found associated to the trait, genes involved in the biosynthesis of milk fat, including sterol regulatory element binding transcription factor 1 (*SREBF1* at 35.7 Mbp), citrate lyase (*ACLY*, at 43.4 Mbp) and signal transducer and activator of transcription 5A (*STAT5A*, at 43.7 Mbp), are annotated and are reported by Bouwman et al. (2011). Additionally, within the same region on BTA 19, three SNPs (BTB-01316060, BTB-01315978, ARS-BFGL-NGS-42430) close to *ADPRM* gene at 30.30 Mbp (ADP-ribose/CDP-alcohol diphosphatase, manganese-dependent) involved in glycerophospholipid metabolism. At 35.3 Mbp, ARS-BFGL-NGS-112923 SNP is mapped into the *PEMT* gene (phosphatidylethanolamine N-methyltransferase) that is involved in glycerophospholipid metabolism. Finally, two SNPs (Hapmap58303-ss46526468 and Hapmap49617-BTA-45355) were associated to the *PHOSPHO1* gene at 37.9 Mbp (phosphatase, orphan 1) involved in glycerophospholipid, phospholipid, lipids and lipoproteins metabolism and biosynthesis.

- *VA :* No SNPs were found "significant" above the 5% PFP threshold line of the Manhattan plots for this trait.
- *D9D:* The region at 3.37 Mbp on BTA 28 is the region harboring *GNPAT* (glyceronephosphate O-acyltransferase) gene that is involved in lipids, lipoproteins and glycerophospholipid metabolism. The region at 66.1 Mbp on BTA 17 showing an

association with D9D, is the region harboring *ACACB* (acetyl-CoA carboxylase beta) gene that is involved in lipids and lipoproteins metabolism.

1.4.2. Association tests of significant SNPs located below the 5% PFP threshold line of the Manhattan plots

Tables 5 and 6 show significant SNPs located below the 5% PFP threshold line of the Manhattan plots, associated with genes (< 1 Mb) encoding for enzymes with an important role in fat and fatty acid metabolism. There was an overlap in the list of chromosomes that had the largest number of SNPs associated with CLA and D9D below the 5 % PFP threshold line of the Manhattan plots, and some chromosomal regions showed associations with VA.

- *CLA:* The major regions that showed significant associations with CLA were on BTA 19, where most of the SNPs were close to *PEMT, SREBF1, STAT5A, PHOSPHO1* and *ADPRM* genes. Moreover, located at 51.38 Mb, mapped *FASN* gene that encodes for fatty acid synthase which is a multifunctional enzyme that catalyses de novo fatty acid synthesis. The region at 64.8 Mbp on BTA 13 is the region harbouring *ACSS2* gene (acyl-CoA synthetase short-chain family member 2), one of the most abundant enzymes in bovine mammary tissue whose expression increased during lactation and is responsible for the activation of acetate for de novo fatty acid synthesis (Bionaz & Loor, 2008). The region on BTA 14 includes the *DGAT1* gene (diacylglycerol O-acyltransferase 1), which is known to influence milk production traits and milk fat composition (Bouwman et al., 2011). On BTA 15, the region located at 78.3 Mbp encodes the NR1H3 (nuclear receptor subfamily 1, group H, member 3), alias *LXRalpha*, a nuclear hormone receptor whose activation (alone or in conjunction with SREBP gene), promotes the SCD stearoyl-CoA desaturase (*D9D*) gene expression in a wide range of tissue (Hebbachi et al., 2008). On BTA 26, the glycerol-3-phosphate acyltransferase mitochondrial

(*GPAM*), is the enzyme that catalyses the initial and committed step of glycerolipids synthesis and, therefore, it is a potential site for triacylglycerol synthesis regulation (Roy et al., 2006)

- *VA:* The region at 64.95 Mbp on BTA 17 showing an association with VA, is the region harboring *ALDH2* (aldehyde dehydrogenase 2 family) gene that is involved in lipids and lipoproteins metabolism. On BTA 27 the region located at 37.1 Mbp showed association with 1-acylglycerol-3-phosphate-O-acyltransferase (*AGPAT6*), that has been recognized as microsomal glycerol-3-phosphate acyltransferase (*GPAT*), which catalyzes the glycerolipids biosynthesis pathway (Bionaz & Loor, 2008). Also, *AGPAT6* isoform expression is under the control of the above mentioned *PPAR* signalling pathway in several tissues.
- *D9D:* The major region that showed significant associations with D9D were on BTA 17. The region between 64.95-66.79 Mbp on BTA 17 is the region harboring *ALDH2* (aldehyde dehydrogenase 2 family) and ACACB (acetyl-CoA carboxylase beta) genes which are involved in lipids and lipoproteins metabolism. On BTA 19, the region located at 55.7 Mbp encodes for acyl-CoA oxidase (*ACOXI*) that catalyse the first step of peroxisomal fatty acid β-oxidation. On BTA 26, as described for CLA, the region encoding for *GPAM* was associated with D9D.

1.4.3. Pathways

Several metabolic lipid pathways, according to KEGG database, were identified for the genes associated with SNPs located within 5-20% PFP threshold (Tables 3-4-5), and they are represented in Figure 8. For VA, D9D and CLA, the most frequent pathways were the metabolism of lipids and lipoproteins, the glycerophospholipid and fatty acid metabolism and the triacylglycerol biosynthesis.

I.5. CONCLUSIONS

Using a selective DNA pooling approach a QTL mapping was performed for CLA, VA and D9D, resulting in various genomic associated regions. In particularly, on BTA 19 there were several genes involved in CLA synthesis, while for VA and D9D the significant SNPs were distributed over all the chromosomes.

This is the first mapping for fatty acids contents in Italian Brown Swiss cattle.

The results may allow improving milk fat composition using breeding selection based on genomic merit of cows for milk fat composition. The identification of genomic regions that may be responsible for genetic variation in milk fat composition will help understanding the biological pathways involved in fatty acid synthesis and relevant markers can be added to SNP prediction equations.

The possibility to calculate prediction equations for fatty acid is enhanced and made possible by the NIR technology able to phenotype milk samples from the routine milk recording system for fatty acids. The interest of farmers in enhancing the nutraceutical value of milk is growing, as the Bleu-Blanc-Coeur consortium has been successful in marketing Omega 3 naturally enriched milk.

The Italian Brown Swiss breed is currently having a specific consortium for marketing cheese produced only from Brown Swiss milk. An additional specialized product may be attractive to consumers, especially in short production to consumer chains, as often found in alpine areas.

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Table 1. Number of daughters (family size) for each of the 5 families used in the analysis

Family	Size
B	1,413
C	1,782
E	2,568
F	947
G	1,255

Table 2. Residual mean values and their STD for CLA, VA and D9D for each of the 5 selected Italian Brown Swiss families in the sub-pools in the low (L) and high (H) tails.

		CLA		D9D		VA	
FAMILY	TAIL	MEAN	STD	MEAN	STD	MEAN	STD
B	L1	-0.49390	0.10852	-0.14094	0.05296	-0.98427	0.17217
B	L2	-0.48696	0.10104	-0.13735	0.04867	-0.96798	0.14856
B	H1	0.74772	0.70436	0.20718	0.069	0.99531	0.72585
B	H2	0.70672	0.64998	0.20341	0.06599	0.92021	0.53167
C	L1	-0.43098	0.09008	-0.13594	0.05934	-0.65494	0.17082
C	L2	-0.44452	0.11882	-0.12992	0.04603	-0.64364	0.16760
C	H1	0.66386	0.52609	0.08092	0.05768	133.338	0.62860
C	H2	0.63265	0.49619	0.07627	0.05099	126.801	0.49839
E	L1	-0.47744	0.13375	-0.14867	0.04537	-0.79669	0.30011
E	L2	-0.48485	0.13695	-0.14591	0.04201	-0.77258	0.27312
E	H1	130.968	0.44947	0.22401	0.15114	193.435	282.528
E	H2	127.321	0.40445	0.2116	0.13075	144.863	0.60471
F	L1	-0.50308	0.10199	-0.07378	0.07427	-103.623	0.16730
F	L2	-0.49592	0.09206	-0.06773	0.06253	-102.954	0.16530
F	H1	0.61353	0.56768	0.21271	0.07876	0.20082	0.44377
F	H2	0.55806	0.43636	0.20518	0.0693	0.16536	0.38295
G	L1	-0.52235	0.15092	-0.18892	0.07339	-0.63733	0.33431
G	L2	-0.53004	0.15388	-0.19254	0.07874	-0.6152	0.31305
G	H1	0.20165	0.23559	0.02299	0.0692	126.227	0.85484
G	H2	0.18913	0.22741	0.01712	0.05935	120.646	0.79237

Table 3. SNPs significant above 5% PFP threshold with their chromosomal positions, p-values, along with symbols of genes located and Genbank used to identify the gene-SNP association. Gene and SNPs location (Mbp) as in the Bos_taurus_UMD_3.1 assembly; gene symbol as in Genbank; (*) SNP designated as in a gene; (-) not near a gene; () near gene.

Illumina SNP name	Genbank SNP code	Bta	SNP position	P-value	Gene symbol
CLA					
DPI-50	*rs43006866*	1	21693513	8.7E-05	*NRIP1*
Hapmap55498-ss46527080	*rs41255623*	1	57752344	1.2E-04	*ATG3**
Hapmap44172-BTA-96950	*rs41616212*	1	58985342	9.1E-05	*GRAMD1C**
Hapmap48236-BTA-17964	*rs41623976*	1	90714916	4.4E-06	*TBL1XR1*
Hapmap47178-BTA-111157	*rs41566432*	1	92461552	3.2E-06	*NAALADL2**
ARS-BFGL-NGS-3990	*rs110539904*	2	98217598	6.2E-05	*UNC80**
Hapmap44637-BTA-17098	*rs41579391*	2	117151265	7.2E-05	*A7E352_BOVIN*
BTB-01240408	*rs41701446*	3	86180854	5.6E-05	*C1orf87*
ARS-BFGL-NGS-113196	*rs111010813*	4	103231866	1.0E-04	*ATP6V0A4**
BTB-00218031	*rs43425225*	5	6528982	1.0E-05	*E2F7*
ARS-BFGL-NGS-12094	*rs108986373*	5	22053661	1.3E-05	*BTG1*
BTA-111858-no-rs	*rs41611289*	5	91494429	7.1E-05	*PLCZ1*
ARS-BFGL-NGS-8730	*rs43442824*	5	105449703	5.8E-05	*KCNA5*
BTB-01105737	*rs42260933*	7	66550122	6.2E-05	*GRIA1*
Hapmap44668-BTA-119022	*rs41622993*	7	87556048	1.3E-04	*EDIL3*
Hapmap45685-BTA-80525	*rs41597368*	7	109970008	2.2E-05	*LOC523504**
ARS-BFGL-NGS-61077	*rs110932603*	8	10140515	4.6E-05	*FBX016*
ARS-BFGL-NGS-103122	*rs110608572*	10	12870180	1.0E-04	*MEGF11**
Hapmap41480-BTA-20737	*rs41624135*	10	50462356	6.0E-05	*FOXB1*
Hapmap59000-rs29026853	*rs29026853*	10	53560658	5.8E-05	*TCF12*
BTA-106955-no-rs	*rs41615197*	11	35897464	9.8E-05	*ASB3*
ARS-BFGL-NGS-106479	*rs110205996*	11	79852413	8.3E-05	*OSR1*
ARS-BFGL-NGS-5267	*rs42256240*	12	9925695	1.4E-04	*LOC786945*
ARS-BFGL-NGS-1426	*rs109605584*	12	33576827	1.0E-04	*SHISA2*
ARS-BFGL-NGS-55763	*rs109152570*	12	34635671	1.2E-04	*MIPEP**
Hapmap40222-BTA-65450	*rs41651027*	12	56055676	7.9E-06	*OR10P1*
Hapmap50611-BTA-19865	*rs41628446*	12	57684714	3.5E-05	*IL23A*
BTB-00505587	*rs41680023*	12	76896007	3.7E-06	*DZIP1**
Hapmap25446-BTC-054694	*rs110267284*	14	26003598	1.3E-04	*FAM110B*

Hapmap51149-BTA-113410	*rs41571939*	15	5400560	2.9E-07	*DYNC2H1 **
BTB-00862781	*rs42022714*	15	5825778	4.3E-05	*MMP13*
BTA-38242-no-rs	*rs41578757*	16	27164390	3.5E-07	*DISP1**
ARS-BFGL-NGS-27682	*rs109893602*	16	30186769	1.3E-04	*PARP1*
ARS-BFGL-NGS-19358	*rs109405104*	16	70754436	1.3E-04	*SMYD2*
ARS-BFGL-NGS-17251	*rs109024372*	16	72114575	9.1E-06	*RPS6KC1*
ARS-BFGL-NGS-14591	*rs108956519*	17	20892863	1.4E-04	*PCDH18*
BTA-46636-no-rs	*rs41572972*	17	26418537	9.5E-09	*PCDH10*
BTA-40721-no-rs	*rs41604816*	17	27826675	9.3E-05	-
ARS-BFGL-NGS-61134	*rs109438470*	17	30676454	1.6E-06	*INTU*
BTB-01870009	*rs42982163*	17	30925441	1.4E-05	*INTU*
ARS-BFGL-BAC-34666	*rs110202120*	17	38467666	6.9E-06	-
ARS-BFGL-NGS-32208	*rs110602266*	17	41066514	2.3E-05	*C17H4orf45*
ARS-BFGL-NGS-73072	*rs110459320*	17	44822427	6.5E-05	*GUCY1A3*
BTA-27953-no-rs	*rs41633195*	17	47700237	3.0E-05	*RIMBP2**
Hapmap41801-BTA-21911	*rs41627925*	17	50429878	4.3E-05	*HSFY2*
ARS-BFGL-NGS-116497	*rs109230481*	19	13720853	2.6E-05	*AATF**
ARS-BFGL-NGS-114182	*rs110697583*	19	14008574	7.9E-06	*C19H17orf78 **
ARS-BFGL-NGS-6298	*rs109209050*	19	16779459	6.9E-07	*ACCN1**
Hapmap51231-BTA-44563	*rs41584865*	19	17118867	5.6E-07	*ACCN1**
UA-IFASA-5746	*rs41617418*	19	18384729	1.1E-04	*ADAP2**
ARS-BFGL-NGS-73727	*rs109876252*	19	19587050	3.7E-06	*KSR1 **
Hapmap41541-BTA-44653	*rs41640976*	19	20293612	1.3E-05	*NLK**
ARS-BFGL-NGS-32894	*rs109057891*	19	20974167	2.9E-05	*PIPOX **
ARS-BFGL-NGS-81462	*rs41598054*	19	24917540	1.0E-04	*SHPK*
ARS-BFGL-NGS-3281	*rs110386214*	19	25047166	1.9E-08	*ITGAE**
BTB-01316060	*rs42442741*	19	30340650	5.1E-07	*TMEM220*
BTB-01315978	*rs42441962*	19	30446351	8.9E-06	*PIRT*
ARS-BFGL-NGS-42430	*rs109099212*	19	31087581	7.8E-05	*DNAH9**
ARS-BFGL-NGS-4759	*rs109182853*	19	35253851	4.1E-05	*RAI1**
ARS-BFGL-NGS-112923	*rs41909659*	19	35419429	9.8E-07	*PEMT**
Hapmap58303-ss46526468	*rs41256931*	19	37552530	1.1E-04	*SLC35B1**
Hapmap49617-BTA-45355	*rs41576388*	19	38466576	9.8E-04	*HOXB9*
Hapmap56957-ss46526454	*rs41256918*	19	42902904	9.5E-05	*RAB5C**
ARS-BFGL-NGS-24479	*rs41916457*	19	45109206	3.1E-05	*ADAM11**
UA-IFASA-6117	*rs41636123*	19	46075773	4.3E-05	*WNT3*
BTA-45655-no-rs	*rs41577559*	19	46202442	1.0E-04	*NSF**
BTA-50728-no-rs	*rs41581533*	20	48749320	4.0E-08	*CDH10*
ARS-BFGL-NGS-107424	*rs111020323*	21	63708710	4.6E-08	*VRK1*
ARS-BFGL-NGS-79806	*rs109898853*	23	16625327	1.4E-04	*PPP2R5D**

UA-IFASA-7925	*rs41604928*	24	679380	9.5E-05	*ADNP2*
Hapmap50827-BTA-94026	*rs41668379*	24	2166631	4.9E-05	*GALR1*
ARS-BFGL-NGS-20502	*rs108990458*	25	42097688	8.3E-05	*MICALL2*
Hapmap48141-BTA-98457	*rs41566027*	27	42751177	1.2E-05	*UBE2E2*
VA					
ARS-BFGL-NGS-19301	*rs110847444*	4	89017584	3.8E-06	*SPAM1*
Hapmap38391-BTA-18545	*rs41575963*	12	26810556	1.0E-05	*RFC3*
ARS-BFGL-NGS-97051	*rs42357017*	15	20500282	6.3E-06	*ZC3H12C**
BTA-38242-no-rs	*rs41578757*	16	27164390	2.8E-06	*DISP1**
ARS-BFGL-NGS-108496	*rs109178989*	17	25441346	4.7E-06	*PCDH10*
BTB-01017247	*rs42176310*	29	28809817	4.8E-06	*CCDC15**
D²D					
ARS-BFGL-NGS-98565	*rs109886869*	5	116861955	1.2E-05	*ATXN10**
Hapmap43748-BTA-103824	*rs41609745*	7	65358446	2.7E-06	*GLRA1*
Hapmap56398-rs29010937	*rs29010937*	14	13949095	8.7E-06	*MYC*
ARS-BFGL-NGS-62454	*rs41851087*	17	66751217	3.8E-06	*ISCU*
ARS-BFGL-NGS-42947	*rs42703571*	28	2313753	7.1E-06	*RHOU*
Hapmap47516-BTA-116004	*rs41566730*	28	2902778	3.2E-06	*OR4P4*
ARS-BFGL-NGS-16913	*rs109873278*	28	8346709	4.0E-06	*GNG4**

Table 4. SNPs located above PFP 5% threshold within 1 Mbp distance from genes encoding for enzymes with an important role in fat and fatty acid metabolism. Gene and SNPs location (near gene) as in the Bos_taurus_UMD_3.1 assembly; gene symbol as in GenBank.

Illumina SNP name	Genbank SNP code	Bta	SNP position	P-value	Gene symbol
CLA					
ARS-BFGL-NGS-3990	*rs110539904*	2	98217598	6.2E-05	*ACADL*
ARS-BFGL-NGS-113196	*rs111010813*	4	103231866	1.1E-04	*DGKI*
ARS-BFGL-NGS-116497	*rs109230481*	19	13720853	2.6E-05	ACACA
BTB-01316060	*rs42442741*	19	30340650	5.1E-07	*ADPRM*
BTB-01315978	*rs42441962*	19	30446351	8.9E-06	*ADPRM*
ARS-BFGL-NGS-42430	*rs109099212*	19	31087581	7.8E-05	ADPRM
ARS-BFGL-NGS-4759	*rs109182853*	19	35253851	4.1E-05	*PEMT/SREBF1*
ARS-BFGL-NGS-112923	*rs41909659*	19	35419429	9.8E-07	*PEMT/SREBF1*
Hapmap58303-ss46526468	*rs41256931*	19	37552530	1.2E-04	PHOSPHO1
Hapmap49617-BTA-45355	*rs41576388*	19	38466576	1.1E-04	*PHOSPHO1*
Hapmap56957-ss46526454	*rs41256918*	19	42902904	1.0E-04	*STAT5A/ACLY*
D'D					
ARS-BFGL-NGS-62454	*rs41851087*	17	66751217	3.8E-06	*ACACB*
Hapmap47516-BTA-116004	*rs41566730*	28	2902778	3.3E-06	*GNPAT*

Table 5. SNPs significant between 5 and 10% PFP threshold located within 1 Mbp from genes encoding for enzymes with an important role in fat and fatty acid metabolism. Gene and SNPs location (near gene) as in the Bos_taurus_UMD_3.1 assembly; gene symbol as in GenBank.

Illumina SNP name	Genbank SNP code	Bta	SNP position	P-value	Gene symbol
CLA					
BTA-114831-no-rs	*rs41574370*	2	39709004	3.3E-04	*GPD2*
ARS-BFGL-BAC-2813	*rs42208635*	2	39086521	3.0E-04	*GPD2*
ARS-BFGL-NGS-17824	*rs41664795*	4	118169220	4.2E-04	*INSIG1*
ARS-BFGL-NGS-116551	*rs110675288*	12	12446625	9.8E-04	*DGKH*
ARS-BFGL-NGS-119102	*rs109324940*	14	70003286	3.0E-04	*PTDSS1*
ARS-BFGL-NGS-44706	*rs41781118*	15	76438547	4.8E-04	*DGKZ*
Hapmap42977-BTA-55653	*rs41640777*	16	1784252	7.2E-04	*ETNK2*
BTB-01631910	*rs42743382*	18	64045527	1.0E-03	*MBOAT7*
ARS-BFGL-NGS-100532	*rs109873397*	18	63878550	7.8E-04	*PLA2G15*
UA-IFASA-7338	*rs41636041*	19	8200102	1.7E-04	*DGKE*
ARS-BFGL-NGS-14867	*rs110036994*	19	7940557	6.9E-04	*DGKE*
ARS-BFGL-NGS-114182	*rs110697583*	19	14008574	3.0E-04	*ACACA*
ARS-BFGL-NGS-101807	*rs109477972*	19	30413271	2.5E-04	*ADPRM*
ARS-BFGL-NGS-101953	*rs41913537*	19	35191657	5.6E-04	*PEMT/SREBF1*
ARS-BFGL-NGS-28121	*rs43729464*	19	42227236	5.0E-04	*STAT5A*
ARS-BFGL-NGS-2725	*rs110970486*	23	24904300	7.4E-04	*ELOVL5*
UA-IFASA-6229	*rs41626402*	23	31485437	1.1E-03	*BTN1A1*
ARS-BFGL-NGS-35579	*rs110035524*	26	26058953	6.9E-04	*ECHS1*
VA					
BTA-13765-no-rs	*rs29018723*	15	56548395	3.1E-05	*MOGAT2/DGAT2*
ARS-BFGL-NGS-94026	*rs111010211*	27	37145353	6.0E-05	*AGPAT6*
D'D					
ARS-BFGL-NGS-104914	*rs109526874*	5	119512385	1.7E-05	*CPT1B/CHKB*

Table 6. SNPs significant between 10 and 20% PFP threshold located within 1 Mbp from genes encoding for enzymes with an important role in fat and fatty acid metabolism. Gene and SNPs location (near gene) as in the Bos_taurus_UMD_3.1 assembly; gene symbol as in GenBank.

Illumina SNP name	Genbank SNP code	Bta	SNP position	P-value	Gene symbol
CLA					
ARS-BFGL-NGS-19572	*rs110857438*	1	97420045	1.7E-03	*PLD1*
BTA-31262-no-rs	*rs41629000*	2	128889674	3.9E-03	*LYPLA2*
BTA-72579-no-rs	*rs41591617*	4	22706540	4.9E-03	*DGK4*
Hapmap27013-BTA-158242	*rs108938799*	4	23543662	3.8E-03	*DGK4*
ARS-BFGL-NGS-112658	*rs109311371*	4	98486908	1.8E-03	*DGKI*
ARS-BFGL-NGS-7597	*rs110528559*	4	101758469	3.5E-03	*DGKI*
ARS-BFGL-NGS-30174	*rs110294118*	4	117100753	1.5E-03	*INSIG1*
ARS-BFGL-NGS-15520	*rs109546807*	5	65986618	2.1E-03	*CHPT1*
ARS-BFGL-NGS-24122	*rs110183937*	5	65228699	4.0E-03	*CHPT1*
Hapmap59389-rs29023212	*rs29023212*	5	88978964	6.5E-03	*ETNK1*
BTA-107103-no-rs	*rs41615970*	5	88659509	3.6E-03	*ETNK2*
ARS-BFGL-NGS-115195	*rs109444154*	5	119235517	1.7E-03	*CHKB/CPT1B*
Hapmap48480-BTA-80747	*rs41568613*	7	13526016	6.0E-03	*GCDH*
ARS-BFGL-NGS-70183	*rs109815065*	7	17913294	4.1E-03	*PNPLA6*
ARS-BFGL-NGS-52642	*rs109522117*	8	25958375	2.6E-03	*PLIN2*
BTB-00415258	*rs43621939*	10	28680745	6.2E-03	*LPCAT4*
BTB-00424771	*rs43626465*	10	52558914	2.3E-03	*LIPC*
Hapmap41972-BTA-79298	*rs41654582*	10	85547284	5.4E-03	*ACOT4/ACOT2*
ARS-BFGL-NGS-116336	*rs110826199*	10	86126108	5.0E-03	*ACOT4/ACOT2*
ARS-BFGL-NGS-108846	*rs110842319*	10	86155673	2.5E-03	*ACOT4/ACOT2*
ARS-BFGL-NGS-113057	*rs110103846*	11	2603799	5.9E-03	*GPAT2*
Hapmap38795-BTA-97039	*rs41616215*	11	48179532	6.6E-03	*FABP1*
ARS-BFGL-NGS-22048	*rs109927983*	11	69884769	3.5E-03	*LPCAT1*
Hapmap53580-rs29012667	*rs29012667*	12	12041734	1.5E-03	*DGKH*
ARS-BFGL-NGS-114368	*rs111008377*	13	65817864	3.8E-03	*ACSS2*
ARS-BFGL-NGS-101653	*rs109661298*	14	2319504	5.9E-03	*DGAT1*
ARS-BFGL-NGS-108612	*rs109758686*	14	45945108	3.2E-03	*FABP5*
Hapmap44329-BTA-98197	*rs41664749*	15	18924675	5.0E-03	*ACAT1*
ARS-BFGL-NGS-118149	*s109438582*	15	28646485	2.4E-03	*APOA1*
Hapmap42192-BTA-37799	*rs41632633*	15	78966608	5.0E-03	*NR1H3*
Hapmap52389-rs29027509	*rs29027509*	16	68785131	1.7E-03	*PLA2G4A*
Hapmap46938-BTA-114095	*rs41565443*	16	69795545	6.0E-03	*PLA2G4A*

Hapmap23161-BTA-162019	*rs110655056*	18	27281676	5.2E-03	*GOT2*
Hapmap38205-BTA-17257	*rs41574731*	18	23559322	4.2E-03	*LPCAT2*
ARS-BFGL-NGS-100532	*rs109873397*	18	63878550	1.7E-03	*PLA2G15*
ARS-BFGL-NGS-113896	*rs109284305*	18	64231273	3.6E-03	*MBOAT7*
ARS-BFGL-NGS-31543	*rs110960592*	19	28545943	4.1E-03	*ACADVL*
ARS-BFGL-NGS-119468	*rs42882121*	19	28342107	1.3E-03	*ACADVL*
ARS-BFGL-NGS-31404	*rs110660541*	19	30783257	5.4E-03	*ADPRM*
ARS-BFGL-NGS-105181	*rs109678934*	19	37670702	2.0E-03	*PHOSPHO1*
BTA-45324-no-rs	*rs41644849*	19	37817322	2.7E-03	*PHOSPHO1*
ARS-BFGL-NGS-112209	*rs110497942*	19	37994541	4.4E-03	*PHOSPHO1*
ARS-BFGL-NGS-39738	*rs110510166*	19	38059659	4.7E-03	*PHOSPHO1*
ARS-BFGL-NGS-22409	*rs109036118*	19	43295532	4.6E-03	*STAT5A*
BTA-108326-no-rs	*rs41569897*	19	43804606	5.4E-03	*STAT5A*
ARS-BFGL-NGS-109613	*rs109581848*	19	51299813	4.2E-03	*FASN/PCYT2*
Hapmap49546-BTA-25249	*rs41574666*	23	49260004	4.1E-03	*ECI2*
BTB-00938770	*rs42099589*	26	32821171	3.9E-03	*GPAM/ACSL5*
ARS-BFGL-NGS-62648	*rs110039409*	26	42807171	5.9E-03	*ACADSB*
ARS-BFGL-NGS-72832	*rs42116262*	27	14615571	4.2E-03	*ACSL1*
VA				1.0E+00	
Hapmap61072-rs29024053	*rs29024053*	4	23915993	3.3E-04	*DGK4*
ARS-BFGL-NGS-87919	*rs109197682*	8	63383924	5.9E-04	*ALDH1B1*
Hapmap57042-rs29016514	*rs29016514*	17	64950742	2.2E-04	*PLA2G1B/ALDH2*
BTB-00750203	*rs41911936*	19	38268968	4.2E-04	*PHOSPHO1*
ARS-BFGL-NGS-35579	*rs110035524*	26	26058953	1.4E-04	*ECHS1*
D[a]*D*					
BTA-85566-no-rs	*rs43743037*	5	66040455	1.4E-03	*CHPT1*
BTB-01858480	*rs42971522*	5	88249394	1.7E-03	*ETNK1*
ARS-BFGL-NGS-99043	*rs110908109*	13	71301458	3.6E-04	*LIPIN3*
ARB-BFGL-NGS-50023		14	18597213	8.1E-04	*ACAT1*
ARS-BFGL-NGS-119102	*rs109324940*	14	70003286	6.5E-05	*PTDSS1*
Hapmap57042-rs29016514	*rs29016514*	17	64950742	8.9E-05	*ALDH2/PLA2G1B*
ARS-BFGL-BAC-36625	*rs110325149*	17	64982245	7.8E-04	*ALDH2/PLA2G1B*
ARS-BFGL-NGS-112123	*rs41852678*	17	65771136	1.0E-03	*ACACB*
ARS-BFGL-NGS-102695	*rs41852077*	17	66790999	4.0E-04	*ACACB*
ARS-BFGL-NGS-112916	*rs109578063*	19	26398385	5.9E-04	*PLD2/ACADVL*
ARS-BFGL-NGS-46832	*rs41921756*	19	55721945	1.4E-03	*ACOX1*
ARS-BFGL-NGS-721	*rs109731156*	23	49061686	9.8E-04	*ECI2*
BTA-91041-no-rs	*rs41659095*	26	32792279	9.8E-04	*GPAM/ASCL5*
BTA-116005-no-rs	*rs41613328*	28	2869287	5.6E-04	*GNPAT*
Hapmap49856-BTA-108815	*rs41615922*	28	3998395	6.9E-04	*GNPAT*

Figure 1. Role of rumen biohydrogenation and tissue D9D in the production of cis-9 trans-11 conjugated linoleic acid in milk fat and in different tissues. Adapted from Bauman & Lock (2006).

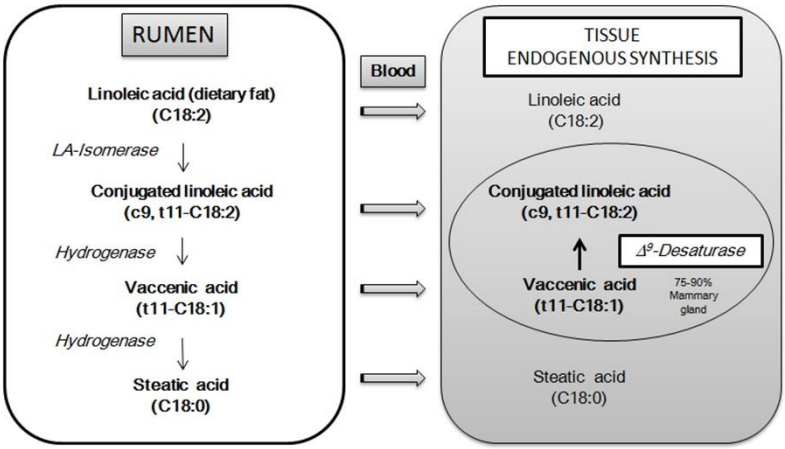


Figure 2. Quantile-quantile plots of the observed distribution of the p-value at marker level for CLA, VA and D9D

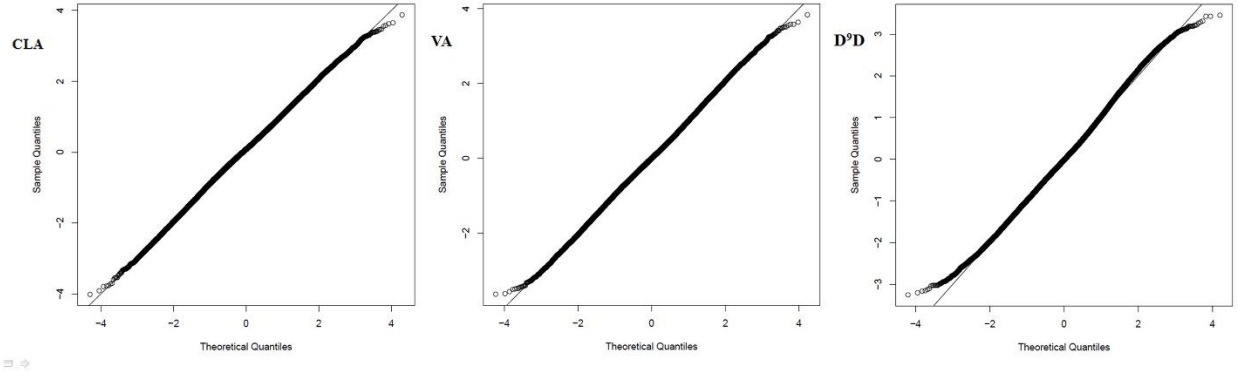


Figure 3. Quantile-quantile plots of SNPs at marker level for CLA, VA and D9D comparing the association statistics expected under the null hypothesis of no association.

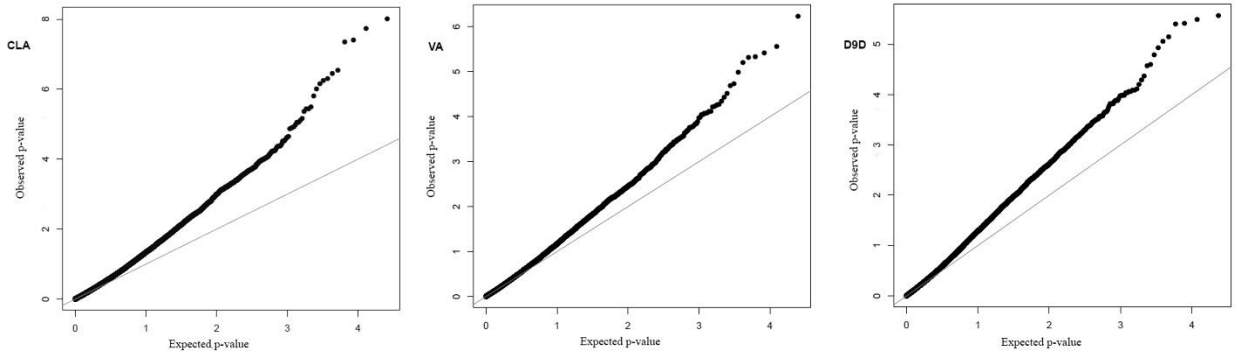


Figure 4. The location of SNPs associated with CLA VA and D9D shown as a Manhattan plot. Odd-numbered chromosomes are shown in orange; even-numbered chromosomes are shown in black. The horizontal blue and dashed line represent the 5% proportion of false positives (PFP) threshold; the horizontal red and dotted line represent the 10% PFP threshold and the horizontal green and solid line represent the 20% PFP threshold.

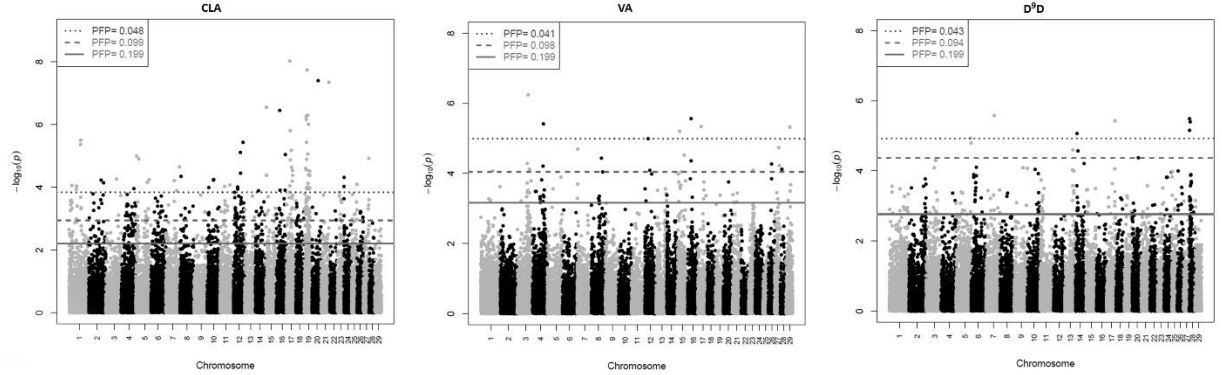


Figure 5. Association regions for CLA in all chromosomes

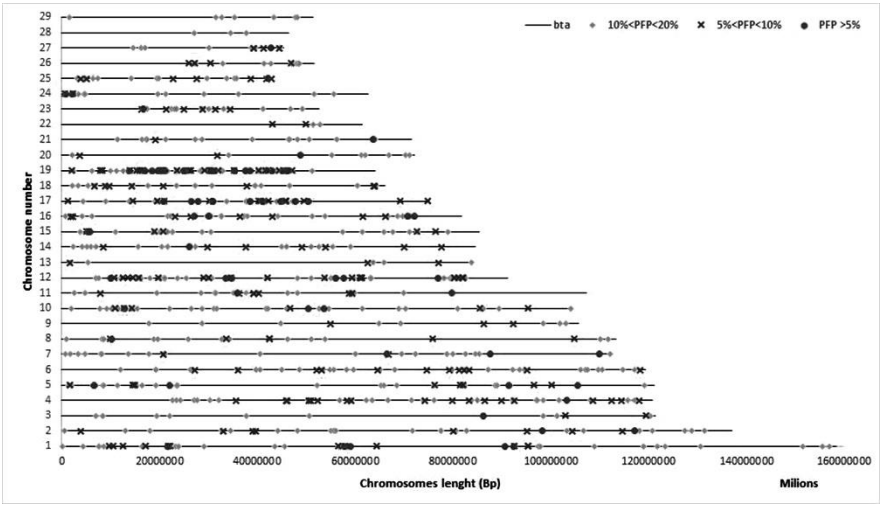


Figure 6. Association regions for VA in all chromosomes

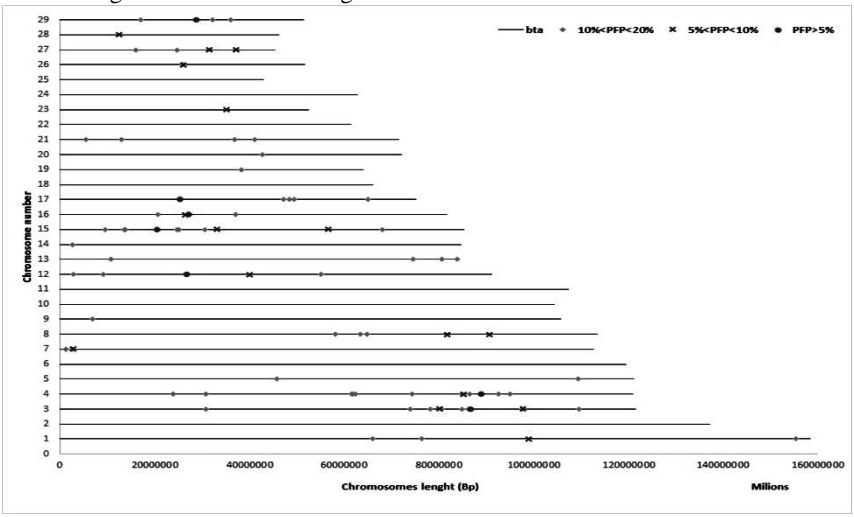


Figure 7. Association regions for D9D in all chromosomes

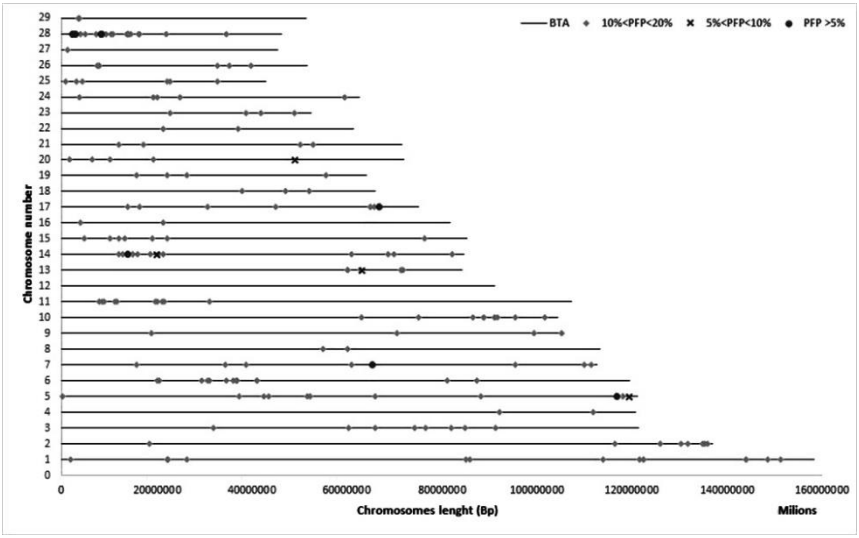
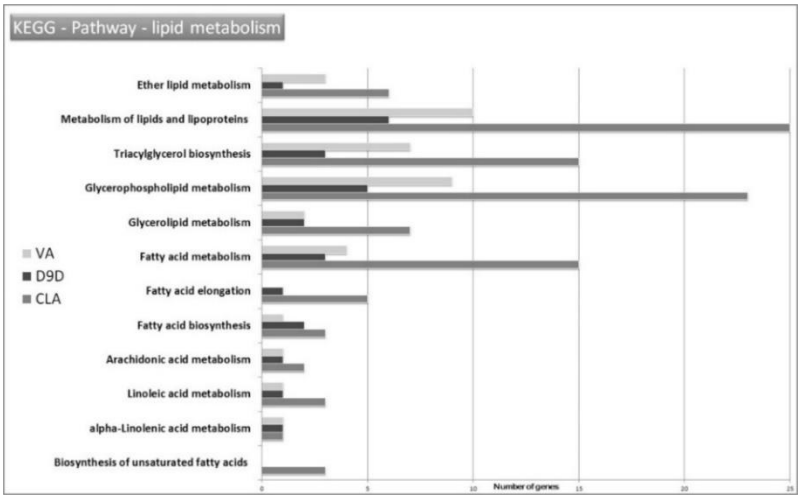


Figure 8. Metabolic lipid pathways in which genes are involved according to KEGG database



2

GENOME-WIDE ASSOCIATION STUDY FOR SOMATIC CELL SCORE IN VALDOSTANA RED PIE CATTLE BREED USING POOLED DNA

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GENOME-WIDE ASSOCIATION STUDY FOR SOMATIC CELL SCORE IN VALDOSTANA RED PIED CATTLE BREED USING POOLED DNA

Maria G. Strillacci*, Erika Frigo*, Fausta Schiavini*#, Antonia B. Samoré*, Fabiola Canavesi*, Mario Vevey§, Maria C. Cozzi*, Morris Soller†, Ehud Lipkin†, Alessandro Bagnato*#

*Department of Health, Animal Science and Food Safety (VESPA), University of Milan, Via Celoria 10, 20133, Milan, Italy.
#Genomic and Bioinformatics Platform, University of Milan, c/o Fondazione Filarete, Viale Ortles 20, 20100 Milano, Italy
†Department of Genetics. The Hebrew University of Jerusalem, 91904 Jerusalem, Israel
§Associazione Nazionale Allevatori Bovini di Razza Valdostana (A.N.A.Bo.Ra.Va.), Fraz. Favret, 5 11020 Gressan (AO), Italy

2.1. ABSTRACT

Background: Mastitis is a major disease of dairy cattle occurring in response to environmental exposure to infective agents with a great economic impact on dairy industry. Somatic cell count (SCC) and its log transformation in somatic cell score (SCS) are traits that have been used as indirect measures of resistance to mastitis for decades in selective breeding. A selective DNA pooling (SDP) approach was applied to identify Quantitative Trait Loci (QTL) for SCS in Valdostana Red Pied cattle using the Illumina Bovine HD BeadChip.
Results: A total of 171 SNPs reached the genome-wide significance for association with SCS. Fifty-two SNPs were annotated within genes, some of those involved in the immune response to mastitis. On BTAs 1, 2, 3, 4, 9, 13, 15, 17, 21 and 22 the largest number of markers in association to the trait was found. These regions identified novel genomic regions related to mastitis (1-Mb SNP windows) and confirmed those already mapped. The largest number of significant SNPs exceeding the threshold for genome-wide significant signal was found on BTA 15, located at 50.43-51.63 Mb.

Conclusions: The genomic regions identified in this study contribute to a better understanding of the genetic control of the mastitis immune response in cattle and may allow the inclusion of more detailed QTL information in selection programs.

2.2. BACKGROUND

Mastitis is one of the most frequent inflammatory disease with a significant economic implication for the dairy herds and the resistance to this pathology may be improved by breeding.

The development of mastitis is the result of the interaction among three components: the individual genotype, the pathogens (ordinarily classified in contagious and environmental bacteria) and the environment (hygiene, housing, climate, milking machines, feeding) [1].

The resistance to an infection disease or the absence of susceptibility may be defined as the immune response ability (immuno-competence capability) of an animal, to avoid the pathogens replication after the establishment of an infection. This implies that animals tend to vary in their genetic potential for immuno-competence [2]. The genetic resistance or the genetic susceptibility to mastitis involves interlinked biological mechanisms that activate and regulate the different levels of the immune response, as a consequence of the differences existing in the response to mastitis involving several pathogens [3]. A better understanding of the immune system and of the metabolic pathways involved in the response to various pathogens of resistant and susceptible animals may be used as complementary approach for the disease control.

The discovery of millions of SNP markers in animal genomes forming dense marker panels, and the concomitant decrease in genotyping costs have allowed the performing of genome-wide association studies (GWAS) [4]. The availability of SNP dense genotypes have increased the power of the identification of QTL related to the traits of interest [5], allowing more accurate breeding values estimation with the use of genomic selection methodology and helping the understanding of the genetic control of the traits of

interest [6]. Because of the established knowledge of the positive genetic correlation between clinical mastitis and SCS ranging from 0.6 to 0.8 [1], SCC is one of the traits used as an indirect measure of mastitis resistance/susceptibility in breeding programs in cattle and sheep. Many GWAS have detected QTL for SCC in cattle on BTAs 5, 6, 8, 11, 17, 18, 20 and 23 in cosmopolite improved dairy cattle breeds [1-7].

The high costs of screening large populations for marker allele frequencies can be decreased using the SDP approach, genotyping pooled DNA samples from selected individuals at each of the two phenotypic extremes of the trait distribution [8]. Equal amounts of DNA are pooled from individuals in the extreme tails, and pools are then genotyped to estimate allele frequency differences for each SNP among high and low tail pools. The significant identified candidate SNPs are then used for confirmatory association studies [9].

The aim of this study was to identify QTL associated with SCS as an indicator of mastitis. We performed a GWA study for SCS in the Valdostana Red Pied cattle, with a selective DNA pooling analysis, using the Illumina BovineHD Bead chip.

2.3. RESULTS AND DISCUSSION

Among the 2,417 bulls with DP-EBV values, 275 had semen samples available in the Valdostana Red Pied bio-bank that encompassed in total 373 sires samples spanning across generations.

The Valdostana Red Pied population counting at present about 11,000 milking cows did not undergo focussed selection for milk production only and no gene introgression from other populations have ever occurred. The breed is strongly adapted to harsh alpine environment because breed natural adaptation and because has been selected to maintain pasture capability (summer pasture is the common farming system), longevity, functionality and fertility. Thus, the population is somehow a unique genetic resource to map mastitis resistance, a trait related to adaptation, functionality and longevity. The study used all the sire samples available in the Valdostana Red Pied bio-bank thus highlighting the overall observable variability for

productive and functional traits in this breed. The smaller number of sire available for the study respect to mapping in cosmopolitan population, may limit the capacity to disclose QTL for mastitis resistance. Nevertheless the experimental design here used and the genetic makeup of the population allowed to identify several new QTL and confirm regions identified in the Italian and Swiss Brown population [10], another breed originating from alpine region, now strongly selected for milk production.

Descriptive statistics for the DP-EBVs and the size of the pools for each tail are reported in Table 1.

The initial dataset included 721,644 SNPs. After editing, the association analysis were performed with 655,665 SNPs for SCS DP-EBV.

Figure 1 shows the Q-Q plot of SNPs at marker level (p-values). Deviations from the identity line showed the amount of false positive tests resulted from the analysis of the data. Figure 2 showed the Manhattan plot of genome-wide associations for SCS trait.

A total of 171 significant SNPs in 24 chromosomes were identified above the Bonferroni genome-wide threshold of 0.05. The Additional file 1 showed the list of the 171 significant SNPs identified. The SNPs location and the gene annotation were reported for both the UMD3.1 and Btau4.6.1 assembly. Table in Additional file 1 included the indication of QTL, amongst the ones here disclosed, reported in the online AnimalQTLdb (http://www.animalgenome.org/cgi-bin/QTLdb/index) for clinical mastitis, SCC and SCS.

Intragenic SNPs

Among the 171 significant markers, 52 SNPs were annotated within 36 genes (Table 2). In Table 2 the significant intragenic SNPs and their corresponding annotated genes in the Btau 4.6.1 assembly are reported.

The BovineHD0900019961 (rs136413030) SNP was associated to the VNN1 (vanin 1) on BTA9, the BovineHD1500008135 (rs134980659) SNP was associated to the THY1 (Thy-1 cell surface antigen) located on BTA 15 and the BovineHD2100001405 (rs133992914) SNP was associated to the IGF1R (insulin-like

growth factor I receptor), located on BTA 21.
Also the BovineHD1500008366 (rs41754552) and the BovineHD1500008367 (rs110269361) SNPs were located respectively at 594,104bp and 601,630bp from THY1 on BTA15.
THY1 is one of the genes differentially expressed between control quarters from cows infected with *E. coli* and *S. aureus* pathogens [11]. Also Moyes et al., 2009 [12] reported the THY1 upregulation in *S. uberis* intramammary infections.
Sugimoto and Sugimoto, 2012 [13] provided evidence that the IGF1R is involved in innate immunity through autophagy (general term for the degradation of cytoplasmic components within lysosomes, [14]) in bovine. In *Bos taurus*, in fact a polymorphism in the 5'UTR region of IGF1R (BTA 21) was associated to mastitis incidence, determining the inhibition of autophagy in response to S. Agalactiae invasion.
Nearby Genes SNPs
The BovineHD0900019716 (rs109049649), the BovineHD4100007550 (rs41662465) and the Hapmap49339-BTA-84110 (rs41662464) SNPs were mapped near the VNN1 (vanin 1) and the VNN2 (vanin 2) located on BTA 9 respectively at 73.37Mb and 73.39Mb. On the same BTA 9, the BovineHD0900019961 (rs136413030) SNP were close to VNN2. Jiang et al., 2012 [15] reported that VNN1 and VNN2 are related to resistance to bovine mastitis, being ranked among the 160 most mastitis relevant genes.
On BTA 19, at 55 Mb, SOCS3 (suppressor of cytokine signalling 3) was found at 673,863 bp upstream the BovineHD1900015066 (rs132720248) SNP. This gene, important for the mammary tissue homeostasis, encodes an intracellular inhibitor of cytokine signaling, thus playing an important role in the initial steps of the recognition of pathogen-associated molecular pattern (PAMP) of the innate immune cells. This leads to the activation and initiation of the innate and the adaptive immune responses. Heeg and Dalpke, 2003 [16] and Brenaut et al., 2014 [17] found the SOCS3 gene among the 39 differentially expressed genes in milk fat globules of goats in response

to an experimental intramammary infection with *S. aureus*.

The gene encoding for the serine dehydratase (SDS) on BTA17 was located 416,619 bp upstream of the BovineHD1700018352 (rs135157738) SNP. This gene is included in the glycine, serine and threonine metabolism, as reported by [18]. These authors demonstrated that the serine dehydratase is one of the enzymes that changed significantly in bovine affected to mastitis.

Four SNPs on BTA9 (BovineHD0900019961 (rs136413030), BovineHD0900019716 (rs109049649), BovineHD4100007550 (rs41662465) and Hapmap49339-BTA-84110 (rs41662464)) mapped near CTGF (connective tissue growth factor). The ZNFX1 (X1-type zinc finger-containing) on BTA13 was close to four SNPs (BovineHD4100010442 (rs41634068), BovineHD1300022626 (rs137320993), BovineHD1300022630 (rs109123247) and BovineHD1300022672 (rs41710487)). The TRIM21 (tripartite motif containing 21) was located 444,354 Mb upstream the strongest association chromosome region identified in BTA 15 (Table 3). The CXCL2 (Chemokine (C-X-C motif) ligand 2) and the CXCL10 (Chemokine (C-X-C motif) ligand 10) on BTA6 were significantly associated to the BovineHD0600025253 (rs42615160) SNP.

The genes above mentioned near to significant SNPs (ZNFX1, CTGF, TRIM21, CXCL2 and CXCL10) are significantly differentially expressed by the bovine mammary epithelial cells stimulated with *E. coli* crude lipopolysaccharide [19].

Jensen et al., 2013 [10] studied and compared the transcriptional responses of uninfected mammary gland quarters adjacent to quarters infected with *E. coli* and *S. aureus* in Holstein cows. The CXCL2 resulted to be one of the genes differentially expressed between control quarters infected with both the pathogens, while the CXCL10 resulted to be one of the genes differentially expressed in control quarters from animals infected with *S. aureus* for 24 and 72 hours.

The BovineHD2200003506 (rs110821186) SNP on BTA 22 mapped close to the MYD88 (myeloid differentiation primary-response gene 88) at 11.72Mb which plays a functional role in transducing pro-inflammatory molecule lipopolysaccharide (LPS) that

are responsible for the majority of acute clinical cases of mastitis [20].

Chromosome regions associated to SCS and clinical mastitis

Table 3 reported a list of the chromosome regions defined by at least three SNPs that were strongly associated to SCS. The highest number of significant SNPs (14) exceeding the significant threshold for genome-wide significance signal was found on BTA 15 (located at 50.43-51.63 Mb). On the same BTA15, also two smaller peaks consisting of three SNPs located at 28.39-28.99 and 5 SNPs located at 31.28-32.02 Mb were identified. These regions are located in QTL that were mapped, respectively, for clinical mastitis using a linkage analysis [21] and for SCS [22]. The region located at 50.43-51.63 Mb on BTA15 has not been reported before in cattle breeds (http://www.animalgenome.org/cgi-bin/QTLdb/index), thus identifying a supposed candidate chromosome region associated to SCS. The chromosome region on BTA9 (72.78-72.80 Mb) mapped in a QTL region previously identified for the general disease resistance (including clinical mastitis) and for SCS [23]

Lund et al., 2008 [21] found a QTL region associated to SCS located at 32.62-43.31 Mb on BTA 22. In our study, three significant SNPs were in this region.

Sahana et al., 2013 [24] in a study on the confirmation and fine-mapping of clinical mastitis and SCS QTL in Nordic Holstein cattle using BovineSNP50 BeadChip found the highest number of significant associations on BTA6 identifying a QTL region for clinical mastitis at 83.37-88.89 Mb (UMD3.1 assembly). This result was also confirmed in a recent study in German Holstein cattle [25]. In our study, two significant SNPs (BovineHD0600023179 (rs133319155) and BovineHD0600023185 (rs136907262)) were found respectively at 84.25 and 84.26 Mb on BTA6 (UMD3.1 assembly; Btau4.6.1 assembly position was not available), being mapped within the QTL region described by the authors previously cited (see Additional file 1).

Annotation

Among the 36 genes listed in Table 2, the annotation data were available for 23 genes reported in the Additional file 2. This lists the

biological processes (BP), the cellular components (CC), the molecular function (MF) and the metabolic pathways (KEGG) obtained with the annotation analyses performed with DAVID online Database.
The literature brings evidence that some of the genes reported in Table 2 map in QTL associated to traits of economic importance in bovine (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index) as showed in Additional file 3. Those mapping in QTL already associated to clinical mastitis and SCS reported in the QTLdb were only 4: the PLXNA4 (plexin A4) on BTA4, the THY1 (Thy-1 cell surface antigen) on BTA15 and the SHISA9 (known as CKAMP44, shisa homolog 9) on BTA 25, the FAM19A1 (family with sequence similarity 19 (chemokine (C-C motif)-like), member A1) on BTA22 associated with SCS. This study thus highlighted possible QTL related to mastitis resistance in the other 19 genes annotated and considered in the GO analysis.

2.4. CONCLUSIONS

This is the first mapping for SCS in Valdostana Red Pied population, an autochthonous alpine dual purpose cattle breed whose selection is mainly focused on milk quality, meat production and functionality.

This study brings evidence of significant associations between SCS and SNP markers on several chromosomes in known and newly disclosed QTL regions. Some genes involved in mastitis resistance or variation of SCS content were in QTL on BTAs 9, 13, 15, 17, 19, 21, 22. In particular, the strongest associations were highlighted on BTA 15 with a total of 24 significant SNPs distributed in three regions.
The detection of genomic regions will help to understand which potential candidate genes may be responsible for the genetic variation in mastitis resistance/susceptibility, a trait of primary importance in dairy cattle breeding and farming.

2.5. METHODS

Sampling

The Valdostana Red Pied cattle is the most common autochthonous dual purpose breed in the region Val d'Aosta (13,000 animals in 2013, almost all of them registered in the Herd Book), coming from the red pied cattle and dating back to the end of the fifth century. The National Association of Valdostana Breeders (A.N.A.Bo.Ra.Va.) provided semen samples for 373 bulls and 725,337 test day records from milk routine recording from 45,410 cows.

The daily SCC were transformed into SCS [26]. Genetic parameters and estimated breeding values (EBVs) were calculated with a test day repeatability model on first parity cows. The model of analysis considered the fixed effects of days in milk (10 classes of 30 days each), herd-test day effect (32,870 levels), month of calving and age at calving (12 classes). Additive genetic and permanent environmental effects were considered as random. Three generations of ancestors were used for each individual extracting information from the National Herd Book for a total of 35,803 animals. Variance component estimations were calculated based on 258,680 test day records with the software VCE [27] and individual EBVs were obtained with the package BLUPF90 [28]. Deregressed proofs (DP-EBV) were calculated for 2,417 bulls according to [29].

Pool constitution

The bull families structure was verified in terms of number of sons per bull, in order to avoid overrepresentation of a single sire. Only 1 bull had 6 sons, 4 bulls had 5 sons, 3 bulls had 4 sons and the rest of bulls had 3 or less sons. The sires were ranked according to DP-EBVs for SCS: the top 20% and bottom 20% sires were identified for the constitution of independent pools within tail of the DP-EBV distribution. In order to obtain two independent groups of different animals within tail with comparable phenotypic value, the selected samples for each tail were clustered (even and odds numbers) into 2 sub-pools.

A total of 79 samples were selected for the pools constitution as follows: 2 independent pools of 20 individuals each in the high tail and 2 independent pools of 20 and 19 individuals each in the low tail. Furthermore, for each pool, 2 DNA duplicate-pools were independently constructed from identical samples. Thus, a total of 4 pools per tail were produced.

DNA extraction and genotyping

Bulls DNA was extracted from semen samples using the ZR Genomic DNA TM Tissue MiniPrep (Zymo). The quality control was performed on each sample to verify the DNA integrity on Invitrogen E-Gel 1% Agarose Gel. The GloMax®-Multi Detection System instrument using the Quant-iT™ dsDNA Broad-Range (BR) Assay Kit (Life Technologies), determined the initial DNA concentrations. The DNA concentration for a single sample was evaluated three times and each read was verified twice (e.g. 2 instrument runs). Samples having concentration diverging ±1 SD from the mean value were not included in the pools. Samples of DNA were normalized to a concentration of 10 ng/ul which was reconfirmed with the same methods above described. DNA pools were constructed by taking equivalent amounts of DNA from each sample.

The final pools were concentrated to 50 ng/ul, as required for the Illumina array protocol. Each sub-pool was genotyped 3 times on different chips (array replicates). In all, 24 different chip positions on 3 microarrays were used for the pooled genotyping. Genotyping was performed using the Illumina BovineHD BeadChip (777,962 SNPs) according to the Infinium protocol. SNPs positions were accordingly to the UMB 3.1 bovine assembly.

Statistical analysis of pools

Pools were analysed according to the SDP approach. The B-allele frequencies being a good estimator of the allele frequency of the individuals in a pool for each array replicate [30], were used in the analyses after obtaining them from the self-normalization algorithm of Illumina BeadStudio software®.

The multiple marker test

A pipeline in R software (http://www.r-project.org/) was adapted from [31] and [32] to perform a multiple marker test. The test statistic used for each SNP was:

$$Ztest=Dtest/SD(Dnull)$$

where Dtest is the difference of the B-allele frequencies means among tails; Dnull is the difference of the B-allele frequencies means within tails. The test statistic was distributed as $\chi 2$ with one degree of freedom under the null hypothesis of equal allele frequencies.

Quality control

We performed the analysis after excluding the 1% of SNPs that showed the highest variability as indicated by the size of the mean measures from the replicate array within tail [9]. In addition, the monomorphic SNPs were deleted from the dataset. Anderson-Darling, Shapiro-Wilk and Kolmogorov-Smirnov normality tests were performed on the Dnull distribution [33-34-35]

The distribution of the p-values using the quantile-quantile (Q-Q) plot was examined to estimate the number and the magnitude of the observed associations between genotyped SNPs and DP-EBVs, compared to the statistics expected under the null hypothesis of no association.

Using the -log10 of the linkage test p-values for each SNP, a Manhattan plot was created. Manhattan plot is a SNP set out across the chromosomes for left to the right, and the heights correspond to the strength of the associations of the trait.

Bonferroni correction for multiple testing was applied in the analysis. The genome-wide significance threshold was set as a corrected p-value ≤ 0.05, which equated to a nominal p-value of approximately 7.62 x 10-8.

Annotation

The annotation analysis of significant SNPs was performed using UCSC, NCBI ENSEMBL and the Bovine SNP Annotation Tool

(Snat) (http://animalgenetics.cau.edu.cn/snat/dbSNP.html), integrating the information from a variety of public bioinformatics databases (NCBI Entrez Gene, UniProt, Gene Ontology (GO), KEGG PATHWAY and AnimalQTLdb [36]). The Illumina BovineHD SNPs positions were converted from Bos_taurus_UMD_3.1 to Btau_4.6.1 assembly using the Batch Coordinate Conversion option in UCSC database as required by Snat tools. UCSC and NCBI databases were used to annotate those SNPs not included in Snat and to verify which of the significant SNPs were close (within 1 Mb [31], [37]) to functional genes. GO and pathway analyses were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7.

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Table 1. Details for DP-EBVs mean and SD values for low and high tail pools.

POOL	N° OF SAMPLES	DP-EBV MEAN	MEAN SD	DP-EBV REL MEAN	POOL	N° OF SAMPLES	DP-EBV MEAN	MEAN SD	DP-EBV REL MEAN
Low tail_1	20	-1.151	0.324	0.535	High tail_1	20	1.257	0.395	0.493
Low tail_2	19	-1.080	0.251	0.600	High tail_2	20	1.134	0.285	0.574

Table 2. Significant intragenic SNPs above the Bonferroni genome-wide threshold of 0.05.

ILLUMINA SNP NAME	GENBANK SNP CODE	P-Value	BTA	SNP LOCATION	GENE SYMBOL
BovineHD0100007623	*rs137585939*	4.43E-08	1	26254309	*ROBO1*
BovineHD0100040084	*rs43273786*	5.41E-08	1	141228619	*NEK11*
BovineHD0200004154	*rs110997154*	3.76E-08	2	15142189	*SSFA2*
BovineHD0300000560	*rs110459674*	inf	3	2612333	*TADA1*
BovineHD0300002104	*rs110093914*	1.87E-08	3	7276675	*DDR2*
BovineHD0300018913	*rs42371455*	1.87E-09	3	66866123	*LPHN2*
BovineHD0300023699	*rs135870054*	8.96E-10	3	87296944	*ALG6*
BovineHD0400026934	*rs109307332*	4.69E-08	4	98543003	*PLXNA4*
BovineHD0500003126	*rs134685896*	3.26E-10	5	12834395	*ACSS3*
BovineHD0700010213	*rs133885406*	4.73E-08	7	33526558	*HSD17B4*
BovineHD0900019961	*rs136413030*	3.70E-08	9	73355572	*VNN1*
BovineHD1000004333	*rs43612234*	5.32E-10	10	12722576	*MEGF11*
BovineHD1000009424	*rs43623003*	6.65E-08	10	28079552	*MIR2284Z-1*
BovineHD1000009428	*rs110034517*	5.56E-09	10	28102288	*MIR2284Z-1*
BovineHD1000017503	*rs42486408*	1.16E-09	10	60793897	*TRPM7*
BovineHD1100003814	*rs109489659*	5.53E-12	11	11771322	*CCT7*
BovineHD1300006368	*rs109943824*	1.12E-08	13	20845530	*PLXDC2*
BovineHD1300022672	*rs41710487*	4.53E-10	13	78416778	*KCNB1*
BovineHD1500008135	*rs134980659*	6.49E-08	15	28399876	*THY1*
Hapmap40064-BTA-36665	*rs41631137*	4.65E-12	15	33953859	*PIK3C2A*
BovineHD1500015036	*rs41769292*	5.15E-09	15	50730325	*NUP98*
BovineHD1500015037	*rs134338365*	2.50E-10	15	50733648	*NUP98*
BTB-00604170	*rs41769258*	5.84E-10	15	50753778	*NUP98*
BovineHD1500015042	*rs41769237*	3.41E-10	15	50765770	*NUP98*
BovineHD1500015044	*rs109649273*	6.55E-08	15	50769861	*NUP98*
BovineHD1500015047	*rs41768429*	7.66E-12	15	50774198	*NUP98*
BovineHD1500015049	*rs41768423*	1.42E-08	15	50780537	*NUP98*
BovineHD1500015051	*rs41768414*	6.94E-11	15	50784307	*NUP98*
BovineHD1500015054	*rs41768364*	7.12E-08	15	50792403	*NUP98*
BovineHD1500015055	*rs109966062*	1.99E-08	15	50795681	*NUP98*
BovineHD1500015056	*rs41768379*	9.17E-13	15	50799229	*NUP98*
BovineHD4100012071	*rs136525289*	7.93E-09	15	51638163	*PDE2A*
BTA-18105-no-rs	*rs109715014*	4.33E-09	15	62952170	*CCDC73*
BovineHD1600009946	*rs41798963*	4.62E-08	16	31290905	*CEP170*
BovineHD1600021693	*rs41819133*	7.33E-08	16	71743691	*CAMK1G*
BovineHD1700002750	*rs110828704*	1.11E-09	17	10472292	*NR3C2*

BovineHD1700018352	*rs135157738*	2.29E-09	17	64466089	*RPH3A*
BovineHD1700019237	*rs110644998*	2.43E-10	17	67344705	*CORO1C*
BovineHD1700019238	*rs134453171*	1.90E-09	17	67347843	*CORO1C*
BovineHD1700020721	*rs109085689*	4.61E-08	17	72364594	*MTMR3*
BovineHD1700021131	*rs135044766*	4.88E-08	17	73638738	*DEPDC5*
BovineHD1700021132	*rs135814317*	7.10E-08	17	73640453	*DEPDC5*
BovineHD1900006167	*rs134967563*	7.01E-10	19	20731168	*SSH2*
BovineHD4100014346	*rs29017164*	2.41E-13	19	57590345	*ATP5H*
BovineHD2100001405	*rs133992914*	6.47E-08	21	6826694	*IGF1R*
ARS-BFGL-NGS-10830	*rs109014211*	1.74E-09	21	14303664	*SLCO3A1*
BovineHD2200009526	*rs110064285*	8.84E-09	22	33753508	*FAM19A1*
BovineHD2200009645	*rs135018045*	3.20E-08	22	34006051	*FAM19A1*
BovineHD2200009658	*rs133223316*	5.38E-09	22	34051778	*FAM19A1*
BovineHD2300014695	*rs110724706*	4.60E-09	23	50469508	*TUBB2B*
BovineHD2500003334	*rs42064606*	1.77E-08	25	13011549	*SHISA9*
BovineHD2500003336	*rs109087355*	2.45E-09	25	13017281	*SHISA9*

Genes and SNPs location as in the Btau4.6.1 assembly; gene symbol as in GenBank.

Table 3. List of chromosome regions strongly associated to SCS.

BTA	START*	END*	LENGHT (BP)	N. SNPs	GENBANK SNP CODE
1	21625461	21632949	7488	3	*rs110141424; rs42365792; rs42367069*
1	27814460	28017039	202579	7	*rs135454183; rs110174548; rs134436790; rs136371716; rs111001290; rs41586446; rs110002182*
2	117668432	118739748	1071316	9	*rs134103593; rs109545959; rs133621389; rs135143470; rs136343471; rs109908642; rs133815275; rs135205101; rs43320680*
3	6388643	6396280	7637	3	*rs110787209; rs42458782; rs132773940*
4	117852857	118898784	1045927	4	*rs133335423; rs43417362; rs133867064; rs136879377*
9	72784616	72804256	19640	4	*rs41662464; rs109049649; rs41662465; rs136413030*
13	78273095	78416778	143683	4	*rs41634068; rs137320993; rs109123247; rs41710487*
15	28399876	28999494	599618	3	*rs134980659; rs41754552; rs110269361*
15	31285729	32027462	741733	5	*rs135835073; rs29018094; rs110325464; rs43299708; rs43299703*
15	50438721	51638163	1199442	14	*rs137687321; rs108941833; rs41769292; rs134338365; rs41769258; rs41769237; rs109649273; rs41768429; rs41768423; rs41768414; rs41768364; rs109966062; rs41768379; rs136525289*
17	67344705	67375670	30965	3	*rs110644998; rs134453171; rs41850009*
21[#]	60154246	60175026	20780	4	*rs29018575; rs42236250; rs42236274; rs109897238*
22	33753508	34051778	298270	3	*rs110064285; rs135018045; rs133223316*

Start. End*: candidate region start and end (bp)

[#] Start and End position referred to Btau4.6.1 assembl

FRigure 1. Q-Q plot of SNPs at marker level (p-values).

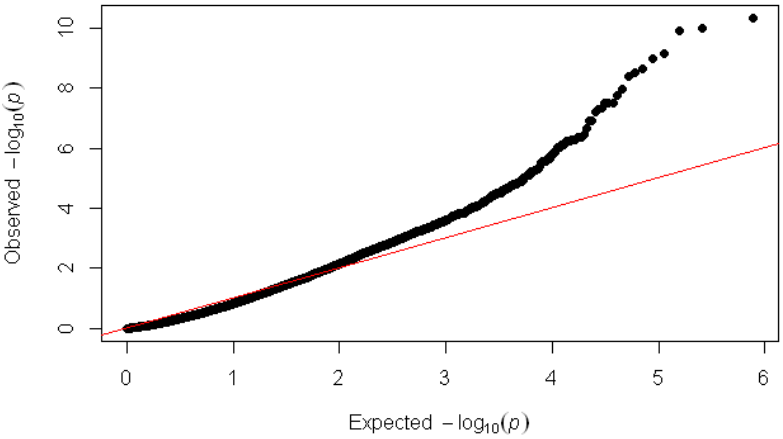
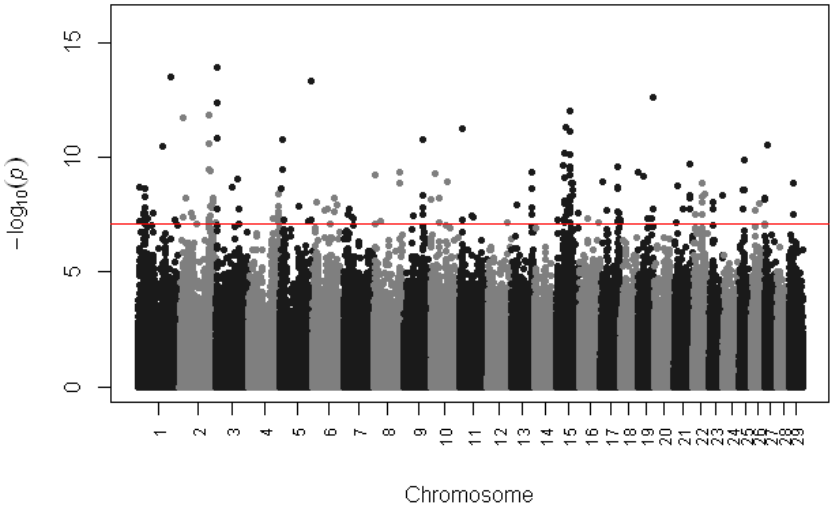


Figure 2. Manhattan plot of genome-wide associations for SCS in Valdostana Red Pied breed. The red line represents the Bonferroni correction threshold.



Additional file

Additional file 1. List of the significant SNPs identified in the Valdostana Red Pied breed.

ILLUMINA SNP NAME	GENBANK SNP CODE	P-VALUE	BTA	SNP LOCATION UMD3.1	SNP LOCATION Btau4.6.1	GENE SYMBOL (UMD3.1)	GENE SYMBOL (Btau4.6.1)	QTL ID	QTL TRAIT	QTL REGION
BovineHD0100002474	*rs137725625*	5,70E-08	1	7719210	7578168					
BovineHD0100002486	*rs134021632*	1,85E-09	1	7758076	7617863					
BovineHD0100006355	*rs110141424*	3,18E-08	1	21392476	21625461					
BTB-01210076	*rs42365792*	6,15E-08	1	21393283	21626268					
BovineHD0100006362	*rs42367069*	2,47E-08	1	21400228	21632949					
BovineHD0100007623	*rs137585939*	4,43E-08	1	25854635	26254309	*ROBO1*	*ROBO1*			
BovineHD0100008100	*rs135454183*	2,27E-09	1	27267091	27814460					
BovineHD0100008178	*rs110174548*	3,08E-08	1	27457470	28007458					
BovineHD0100008181	*rs134436790*	2,71E-08	1	27461010	28011000					
BovineHD0100008182	*rs136371716*	4,69E-08	1	27462684	28012675					
BovineHD0100008183	*rs111001290*	5,40E-09	1	27463446	28013437					
Hapmap50048-BTA-5926.3	*rs41586446*	5,03E-08	1	27465349	28015340					
BovineHD0100008185	*rs110002182*	1,44E-08	1	27467048	28017039					
BovineHD0100010452	*rs134558019*	4,74E-08	1	36519141	37345318					
BovineHD0100016492	*rs110467395*	2,67E-08	1	58292531	58471459					
BovineHD0100047111	*rs137083739*	3,35E-11	1	93793831	NA					
BovineHD0100035049	*rs137041533*	3,21E-14	1	124142939	125505033					
BovineHD0100040084	*rs43273786*	5,41E-08	1	140263787	141228619	*NEK11*	*NEK11*			
BovineHD0200003441	*rs133588497*	1,94E-12	2	12207127	12523892					
BovineHD0200004154	*rs110997154*	3,76E-08	2	14690055	15142189		*SSFA2*			
BovineHD0200006189	*rs136280214*	6,02E-09	2	21674287	22305417					
BovineHD0200012099	*rs43305393*	2,78E-08	2	41699309	42800586	*SP140L*				
BovineHD0200014323	*rs135901001*	3,93E-08	2	49678936	51067581					
BovineHD0200032375	*rs134103593*	6,77E-08	2	112544408	117668432					
BovineHD0200032591	*rs109545959*	4,53E-08	2	113184258	118307083					
BovineHD0200032593	*rs133621389*	2,45E-11	2	113188017	118311005					
BovineHD0200032594	*rs135143470*	3,26E-10	2	113188626	118311614					
BovineHD0200032600	*rs136343471*	4,86E-08	2	113193549	118316537					
BovineHD0200032602	*rs109908642*	1,32E-12	2	113195734	118318722					
BovineHD0200032671	*rs133815275*	3,72E-10	2	113394404	118520597					
BovineHD0200032675	*rs135205101*	3,25E-08	2	113406371	118532566					
BovineHD0200032735	*rs43320680*	1,42E-08	2	113610357	118739748					

BovineHD0200034409	*rs135912109*	1,54E-08	2	119182414	123878559					
BovineHD0200036972	*rs109385272*	8,78E-09	2	127361853	132282060	*ZNF683*				
BovineHD0200037360	*rs111014754*	6,28E-09	2	128626101	133678668					
BovineHD0200039687	*rs132819804*	6,52E-08	2	135577686	140889824					
BovineHD0300000560	*rs110459674*	inf	3	2093487	2612333	*TADA1*	*TADA1*			
BovineHD0300001752	*rs110787209*	3,92E-13	3	5676318	6388643					
BovineHD0300001754	*rs42458782*	1,39E-11	3	5680627	6392715					
BovineHD0300001755	*rs132773940*	1,17E-14	3	5684191	6396280					
BovineHD0300002104	*rs110093914*	1,87E-08	3	6699584	7276675	*DDR2*	*DDR2*			
BovineHD0300018913	*rs42371455*	1,87E-09	3	63185254	66866123	*LPHN2*	*LPHN2*			
BovineHD0300023699	*rs135870054*	8,96E-10	3	82527720	87296944	*ALG6*	*ALG6*			
BovineHD0300024738	*rs43353415*	1,69E-08	3	86546042	NA					
BovineHD0400026934	*rs109307332*	4,69E-08	4	96635633	98543003	*PLXNA4*	*PLXNA4*	2491	clinical mastitis	75421999-99603227
BovineHD0400032670	*rs43422436*	3,65E-08	4	113410262	116268808					
BovineHD0400032690	*rs43415893*	1,33E-08	4	113491345	116347881					
BovineHD0400033263	*rs133335423*	2,23E-08	4	114993038	117852857					
BovineHD0400033469	*rs43417362*	3,67E-09	4	115498539	118340933					
BovineHD0400033488	*rs133867064*	6,78E-08	4	115563215	118405576					
BovineHD0400033665	*rs136879377*	4,03E-08	4	116067636	118898784					
BovineHD0500001585	*rs109553703*	2,37E-09	5	5913578	6327862			1742	SCC	6023104-8023104
BovineHD0500003106	*rs136127388*	1,68E-11	5	10759058	NA	*ACSS3*				
BovineHD0500003126	*rs134685896*	3,26E-10	5	10792875	12834395	*ACSS3*	*ACSS3*			
BovineHD0500003925	*rs134449483*	5,04E-08	5	13065161	15164797					
BovineHD0500020666	*rs137104148*	1,29E-08	5	72963752	77528326					
BovineHD0500031184	*rs132800543*	5,80E-08	5	108300221	114122159					
BovineHD0500034148	*rs134479470*	4,52E-14	5	117557538	NA					
BovineHD0500034150	*rs136956586*	4,93E-08	5	117568421	NA					
BovineHD0600005426	*rs137467024*	9,44E-09	6	19542495	19696350					
BovineHD0600013116	*rs133682920*	2,15E-08	6	47890814	47615237					
BovineHD0600015748	*rs135342305*	1,72E-08	6	57562332	58114205	*C6H4orf19*				
BovineHD0600023179	*rs133319155*	2,20E-08	6	84252180	NA					
BovineHD0600023185	*rs136907262*	5,88E-09	6	84265468	NA					
BovineHD0600025253	*rs42615160*	1,14E-08	6	91964609	93410115					
BovineHD0700003681	*rs109949034*	3,03E-08	7	14010306	11160093					
ARS-BFGL-NGS-112444	*NA*	1,75E-08	7	23275178	NA					
BovineHD0700008448	*rs137545102*	3,92E-08	7	30002652	27548813					
BovineHD0700010213	*rs133885406*	4,73E-08	7	35679243	33526558	*HSD17B4*	*HSD17B4*			
BovineHD0800002755	*rs135037740*	5,95E-10	8	8560601	8576004					

BovineHD0800007500	*rs42215668*	6,20E-08	8	24782491	26295529					
BovineHD0800029893	*rs136410732*	1,27E-09	8	101005762	104278477					
BovineHD0800029896	*rs42501093*	4,11E-10	8	101009106	104281820					
BovineHD0900010437	*rs42575049*	2,86E-08	9	37514911	39325613					
Hapmap49339-BTA-84110	*rs41662464*	1,58E-08	9	71162153	72804256	*VNN1*		1745	clinical mastitis	49477988-76680782
BovineHD0900019716	*rs109049649*	4,62E-09	9	71163463	72784616			1745	clinical mastitis	49477988-76680782
BovineHD4100007550	*rs41662465*	1,60E-11	9	71181789	72802946			1745	clinical mastitis	49477988-76680782
BovineHD0900019961	*rs136413030*	3,70E-08	9	71844127	73355572		*VNN1*	1745	clinical mastitis	49477988-76680782
BovineHD1000000101	*rs135481686*	6,36E-09	10	461041	NA					
BovineHD1000004333	*rs43612234*	5,32E-10	10	12893029	12722576	*MEGF11*	*MEGF11*			
BovineHD1000009424	*rs43623003*	6,65E-08	10	28732407	28079552		*MIR2284Z-1*			
BovineHD1000009428	*rs110034517*	5,56E-09	10	28741867	28102288		*MIR2284Z-1*			
BovineHD1000017503	*rs42486408*	1,16E-09	10	59867344	60793897	*TRPM7*	*TRPM7*			
BovineHD1100003814	*rs109489659*	5,53E-12	11	11292682	11771322		*CCT7*	1693	SCC	5506264-25860118
BovineHD1100003818	*rs134575850*	5,33E-08	11	11300974	11871895			1693	SCC	5506264-25860118
BovineHD1100004129	*rs109878012*	5,46E-08	11	12535496	13127338			1693	SCC	5506264-25860118
BovineHD1100014224	*rs134822269*	3,49E-08	11	48432001	50274524	*REEP1*				
BovineHD1100016327	*rs134809352*	3,75E-08	11	55905582	57584171					
BovineHD1200020095	*rs134063113*	6,89E-08	12	72740699	NA					
BovineHD1300006368	*rs109943824*	1,12E-08	13	21852047	20845530	*PLXDC2*	*PLXDC2*			
BovineHD4100010442	*rs41634068*	3,19E-08	13	78137874	78273095					
BovineHD1300022626	*rs137320993*	2,38E-09	13	78145838	78280335					
BovineHD1300022630	*rs109123247*	1,54E-08	13	78163033	78297531					
BovineHD1300022672	*rs41710487*	4,53E-10	13	78282460	78416778	*KCNB1*	*KCNB1*			
BovineHD1500001239	*rs42595411*	5,49E-08	15	5153883	3849717					
BovineHD1500007318	*rs136596272*	2,37E-10	15	27339214	25223587			4985	clinical mastitis	13868104-29490317
BovineHD1500007427	*rs134799988*	4,88E-08	15	27670811	25558182			4985	clinical mastitis	13868104-29490317
BovineHD1500008135	*rs134980659*	6,49E-08	15	30514604	28399876	*THY1*	*THY1*	4985	clinical mastitis	13868104-29490317
BovineHD1500008366	*rs41754552*	4,32E-08	15	31105101	28993968			4985	clinical mastitis	13868104-29490317
BovineHD1500008367	*rs110269361*	1,02E-08	15	31110621	28999494			4985	clinical mastitis	13868104-29490317
BovineHD1500009024	*rs135835073*	7,58E-10	15	33313379	31285729					
BovineHD4100011940	*rs29018094*	7,59E-09	15	33419454	31391827			2778	SCS	31515378-33515378
BovineHD1500009068	*rs110325464*	6,90E-11	15	33465218	31437551			2778	SCS	31515378-33515378
BovineHD1500009221	*rs43299708*	8,37E-09	15	34029055	32026910			2778	SCS	31515378-33515378
BovineHD1500009222	*rs43299703*	3,60E-08	15	34029604	32027462			2778	SCS	31515378-33515378
Hapmap40064-BTA-36665	*rs41631137*	4,65E-12	15	35873422	33953859	*PIK3C2A*	*PIK3C2A*			
BovineHD1500011688	*rs137114551*	2,10E-08	15	42159894	40426557					
BovineHD1500014997	*rs137687321*	6,46E-08	15	51975113	50438721	*STIM1*				

BovineHD1500025874	*rs108941833*	2,54E-08	15	52094874	50717333					
BovineHD1500015036	*rs41769292*	5,15E-09	15	52107961	50730325	*NUP98*	*NUP98*			
BovineHD1500015037	*rs134338365*	2,50E-10	15	52111223	50733648	*NUP98*	*NUP98*			
BTB-00604170	*rs41769258*	5,84E-10	15	52131353	50753778	*NUP98*	*NUP98*			
BovineHD1500015042	*rs41769237*	3,41E-10	15	52143337	50765770	*NUP98*	*NUP98*			
BovineHD1500015044	*rs109649273*	6,55E-08	15	52147428	50769861	*NUP98*	*NUP98*			
BovineHD1500015047	*rs41768429*	7,66E-12	15	52151765	50774198	*NUP98*	*NUP98*			
BovineHD1500015049	*rs41768423*	1,42E-08	15	52158101	50780537	*NUP98*	*NUP98*			
BovineHD1500015051	*rs41768414*	6,94E-11	15	52161871	50784307	*NUP98*	*NUP98*			
BovineHD1500015054	*rs41768364*	7,12E-08	15	52169973	50792403	*NUP98*	*NUP98*			
BovineHD1500015055	*rs109966062*	1,99E-08	15	52173251	50795681	*NUP98*	*NUP98*			
BovineHD1500015056	*rs41768379*	9,17E-13	15	52176798	50799229	*NUP98*	*NUP98*			
BovineHD4100012071	*rs136525289*	7,93E-09	15	52993385	51638163		*PDE2A*			
BovineHD1500015732	*rs41769333*	1,36E-09	15	54462811	53189677					
BovineHD1500017243	*rs42595490*	2,68E-09	15	59908603	58736392					
BovineHD1500017244	*rs42595494*	1,23E-09	15	59913013	58740802					
BTA-18105-no-rs	*rs109715014*	4,33E-09	15	64168198	62952170	*CCDC73*	*CCDC73*			
BovineHD1600009946	*rs41798963*	4,62E-08	16	34743597	31290905	*CEP170*	*CEP170*			
BovineHD1600021693	*rs41819133*	7,33E-08	16	75621260	71743691	*CAMK1G*	*CAMK1G*			
BovineHD1700002750	*rs110828704*	1,11E-09	17	9808520	10472292	*NR3C2*	*NR3C2*			
BovineHD1700007360	*rs134414083*	2,01E-08	17	26106136	27406155					
BovineHD4100013230	*NA*	5,71E-08	17	62982492	62982492	*RBM19*				
BovineHD1700018352	*rs135157738*	2,29E-09	17	63725088	64466089	*RPH3A*	*RPH3A*			
BovineHD1700019237	*rs110644998*	2,43E-10	17	66513466	67344705	*CORO1C*	*CORO1C*			
BovineHD1700019238	*rs134453171*	1,90E-09	17	66516604	67347843	*CORO1C*	*CORO1C*			
BovineHD1700019246	*rs41850009*	2,31E-08	17	66544435	67375670					
BovineHD1700020540	*rs41851405*	2,73E-08	17	70548656	71645885					
BovineHD1700020721	*rs109085689*	4,61E-08	17	71240044	72364594	*MTMR3*	*MTMR3*			
BovineHD1700021131	*rs135044766*	4,88E-08	17	72513026	73638738	*DEPDC5*	*DEPDC5*			
BovineHD1700021132	*rs135814317*	7,10E-08	17	72514741	73640453	*DEPDC5*	*DEPDC5*			
BovineHD1900000597	*rs132787142*	4,23E-10	19	2604934	1497119					
BovineHD1900006167	*rs134967563*	7,01E-10	19	21492405	20731168		*SSH2*			
BovineHD1900010531	*rs41916837*	4,57E-08	19	36316508	36523730					
BovineHD1900015066	*rs132720248*	4,52E-08	19	53803085	54329700					
BovineHD1900015929	*rs133890886*	1,85E-08	19	56365338	57026445					
BovineHD4100014346	*rs29017164*	2,41E-13	19	57020892	57590345		*ATP5H*			
BovineHD2100001405	*rs133992914*	6,47E-08	21	6826694	6826694		*IGF1R*			
ARS-BFGL-NGS-10830	*rs109014211*	1,74E-09	21	15345488	14303664	*SLCO3A1*	*SLCO3A1*	5451	clinical mastitis	8909340-26179970

								1708	SCC	8978647-20054369
BovineHD2100010844	*rs110071682*	1,85E-08	21	36898862	36616455					
BovineHD4100015306	*rs29018575*	1,84E-08	21	60154246	NA					
BovineHD2100017446	*rs42236250*	1,88E-10	21	60156822	NA					
BovineHD2100017451	*rs42236274*	5,70E-09	21	60173163	NA					
ARS-BFGL-NGS-42178	*rs109897238*	4,46E-09	21	60175026	NA					
BovineHD2200003506	*rs110821186*	2,89E-08	22	11932652	12014326					
BovineHD2200009526	*rs110064285*	8,84E-09	22	33187687	33753508	*FAM19A1*	*FAM19A1*	4987	SCS	32628727-43319438
BovineHD2200009645	*rs135018045*	3,20E-08	22	33440399	34006051	*FAM19A1*	*FAM19A1*	4987	SCS	32628727-43319438
BovineHD2200009658	*rs133223316*	5,38E-09	22	33487012	34051778	*FAM19A1*	*FAM19A1*	4987	SCS	32628727-43319438
BovineHD2200010551	*rs110495093*	1,27E-09	22	37118913	37824479			4987	SCS	32628727-43319438
BovineHD2200017991	*rs133442856*	4,16E-09	22	47632083	47969909					
BovineHD2300003999	*rs109020826*	1,85E-08	23	15651922	16146305					
BTA-55613-no-rs	*rs41640755*	8,70E-09	23	15656140	16150523					
BovineHD2300014695	*rs110724706*	4,60E-09	23	50379740	50469508	*TUBB2B*	*TUBB2B*			
BovineHD2500003334	*rs42064606*	1,77E-08	25	11926465	13011549	*SHISA9*	*SHISA9*	1751	clinical mastitis	0-17024171
BovineHD2500003336	*rs109087355*	2,45E-09	25	11932191	13017281	*SHISA9*	*SHISA9*	1751	clinical mastitis	0-17024171
BovineHD2500005013	*rs110718749*	2,72E-09	25	17747440	18806275					
BovineHD2500005088	*rs110865743*	1,24E-10	25	18059161	19115600					
BovineHD2600004216	*rs109614481*	2,00E-08	26	16809329	17451400	*SORBS1*		2689	SCS	0-21729091
BovineHD2600009267	*rs132886180*	9,62E-09	26	34258305	34514978					
BovineHD2700001072	*rs110001968*	6,84E-09	27	3211214	4392694			2712	SCC	4223574-11982501
BovineHD2700001075	*rs109557235*	5,64E-09	27	3215100	4396580			2712	SCC	4223574-11982501
BovineHD2700002962	*rs110992741*	2,77E-11	27	9887995	11837558			2712	SCC	4223574-11982501
BovineHD2900004558	*rs133088106*	3,21E-08	29	15410280	16151571					
ARS-BFGL-NGS-97397	*rs110652594*	1,31E-09	29	16353986	17429138			13249	SCS	16253597-18253597

Additional file 2. List biological processes, cellular components, molecular function and metabolic pathways obtained with the annotation analyses performed with DAVID on line Database

GENE SYMBOL (GENE FULL NAME)	GO and KEGG ANNOTATION	LIST OF BIOLOGICAL PROCESSES (BP), CELLULAR COMPONENTS (CC), MOLECULAR FUNCTION (MF) AND METABOLIC PATHWAYS (KEGG)
ATP5H (ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d)	GOTERM_BP_FAT	generation of precursor metabolites and energy, oxidative phosphorylation, purine nucleotide metabolic, purine nucleotide biosynthetic, ATP biosynthetic, phosphorus metabolic, phosphate metabolic, ion transport, cation transport, hydrogen transport, nucleoside triphosphate metabolic, nucleoside triphosphate biosynthetic, purine nucleoside triphosphate metabolic, purine nucleoside triphosphate biosynthetic, purine ribonucleotide metabolic, purine ribonucleotide biosynthetic, nucleotide biosynthetic, ribonucleoside triphosphate metabolic, ribonucleoside triphosphate biosynthetic, purine ribonucleoside triphosphate metabolic, purine ribonucleoside triphosphate biosynthetic, ribonucleotide metabolic, ribonucleotide biosynthetic, monovalent inorganic cation transport, energy coupled proton transport, down electrochemical gradient, ATP synthesis coupled proton transport, proton transport, phosphorylation, ion transmembrane transport, nucleobase, nucleoside and nucleotide biosynthetic, nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic, nitrogen compound biosynthetic, ATP metabolic, transmembrane transport
	GOTERM_CC_FAT	mitochondrial proton-transporting ATP synthase complex, coupling factor F(o), mitochondrion, mitochondrial envelope, mitochondrial inner membrane, mitochondrial proton-transporting ATP synthase complex, proton-transporting two-sector ATPase complex, organelle inner membrane, organelle membrane, mitochondrial membrane, organelle envelope, envelope, proton-transporting two-sector ATPase complex, proton-transporting domain, mitochondrial part, mitochondrial membrane part, proton-transporting ATP synthase complex, proton-transporting ATP synthase complex, coupling factor F(o)
	GOTERM_MF_FAT	monovalent inorganic cation transmembrane transporter activity, hydrogen ion transmembrane transporter activity, inorganic cation transmembrane transporter activity
	KEGG_PATHWAY	Oxidative phosphorylation, Alzheimer's disease, Parkinson's disease, Huntington's disease
DEPDC5 (DEP domain containing 5)	GOTERM_BP_FAT	intracellular signaling cascade

NEK11 (NIMA (never in mitosis gene a)-related kinase 11)	GOTERM_BP_FAT	protein amino acid phosphorylation, phosphorus metabolic, phosphate metabolic, phosphorylation
	GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, protein kinase activity, protein serine/threonine kinase activity, ATP binding, purine nucleotide binding, adenyl nucleotide binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding
THY1 (Thy-1 cell surface antigen)	GOTERM_BP_FAT	angiogenesis, blood vessel development, eye development, eye photoreceptor cell differentiation, vasculature development, activation of immune response, immune response-activating cell surface receptor signaling pathway, negative regulation of immune system, positive regulation of immune system, regulation of leukocyte activation, positive regulation of leukocyte activation, immune response-activating signal transduction, immune response-regulating signal transduction, immune response-regulating cell surface receptor signaling pathway, negative regulation of protein kinase activity, cytoskeleton organization, cell-substrate junction assembly, cell adhesion, cell-matrix adhesion, cell surface receptor linked signal transduction, sensory organ development, negative regulation of signal transduction, regulation of calcium ion transport into cytosol, positive regulation of calcium ion transport into cytosol, negative regulation of cell communication, negative regulation of cell development, regulation of cell morphogenesis involved in differentiation, regulation of metal ion transport, regulation of neuron projection development, cell-cell adhesion, regulation of phosphate metabolic, regulation of cell morphogenesis, biological adhesion, neuron differentiation, regulation of cell migration, negative regulation of cell migration, regulation of cell projection organization, negative regulation of cell projection organization, cell-substrate adhesion, regulation of homeostatic, positive regulation of homeostatic, negative regulation of kinase activity, cell junction assembly, cell junction organization, regulation of locomotion, negative regulation of locomotion, regulation of phosphorylation, photoreceptor cell development, eye photoreceptor cell development, retinal cone cell differentiation, camera-type eye development, positive regulation of catalytic activity, negative regulation of catalytic activity, regulation of GTPase activity, regulation of ion transport, positive regulation of ion transport, positive regulation of GTPase activity, regulation of kinase activity, negative regulation of molecular function, positive regulation of molecular function, negative regulation of cell differentiation, regulation of neuron differentiation, regulation of protein kinase activity,

		photoreceptor cell differentiation, retinal cone cell development, focal adhesion formation, blood vessel morphogenesis, positive regulation of response to stimulus, eye morphogenesis, camera-type eye morphogenesis, neuron development, regulation of neurogenesis, negative regulation of neurogenesis, regulation of axonogenesis, negative regulation of axonogenesis, positive regulation of immune response, antigen receptor-mediated signaling pathway, T cell receptor signaling pathway, regulation of antigen receptor-mediated signaling pathway, regulation of T cell receptor signaling pathway, negative regulation of antigen receptor-mediated signaling pathway, negative regulation of T cell receptor signaling pathway, regulation of T cell activation, regulation of cell activation, positive regulation of cell activation, positive regulation of T cell activation, positive regulation of transport, negative regulation of cellular component organization, regulation of phosphorus metabolic, regulation of lymphocyte activation, positive regulation of lymphocyte activation, regulation of cell motion, negative regulation of cell motion, regulation of release of sequestered calcium ion into cytosol, positive regulation of release of sequestered calcium ion into cytosol, regulation of hydrolase activity, regulation of transferase activity, positive regulation of hydrolase activity, negative regulation of transferase activity, regulation of calcium ion transport, positive regulation of calcium ion transport, regulation of nervous system development, retina development in camera-type eye, retina morphogenesis in camera-type eye, camera-type eye photoreceptor cell differentiation, regulation of cell development
	GOTERM_CC_FAT	endoplasmic reticulum, plasma membrane, external side of plasma membrane, cell surface, dendrite, growth cone, site of polarized growth, intrinsic to membrane, anchored to membrane, intrinsic to plasma membrane, intrinsic to external side of plasma membrane, anchored to external side of plasma membrane, cell projection, neuron projection, plasma membrane part, membrane raft, anchored to plasma membrane
	GOTERM_MF_FAT	small GTPase regulator activity, GTPase activator activity, Ras GTPase activator activity, Rho GTPase activator activity, integrin binding, phospholipid binding, enzyme activator activity, lipid binding, GTPase regulator activity, protein complex binding, GPI anchor binding, phosphoinositide binding, nucleoside-triphosphatase regulator activity
	KEGG_PATHWAY	Leukocyte transendothelial migration
ACSS3 (acyl-CoA	GOTERM_CC_FAT	mitochondrion

synthetase short-chain family member 3)	GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, acetate-CoA ligase activity, ATP binding, CoA-ligase activity, ligase activity, forming carbon-sulfur bonds, acid-thiol ligase activity, purine nucleotide binding, adenyl nucleotide binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding
	KEGG_PATHWAY	Propanoate metabolism
ALG6 (asparagine-linked glycosylation 6, alpha-1,3-glucosyltransferase homolog (S. cerevisiae))	GOTERM_CC_FAT	endoplasmic reticulum, endoplasmic reticulum membrane, endomembrane system, organelle membrane, nuclear envelope-endoplasmic reticulum network, endoplasmic reticulum part
	KEGG_PATHWAY	N-Glycan biosynthesis
CAMK1G (calcium/calmodulin-dependent protein kinase IG)	GOTERM_BP_FAT	protein amino acid phosphorylation, phosphorus metabolic, phosphate metabolic, phosphorylation
	GOTERM_CC_FAT	plasma membrane, calcium- and calmodulin-dependent protein kinase complex, endomembrane system
	GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, protein kinase activity, protein serine/threonine kinase activity, calmodulin-dependent protein kinase activity, ATP binding, purine nucleotide binding, adenyl nucleotide binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding
CCT7 (chaperonin containing TCP1, subunit 7 (eta))	GOTERM_BP_FAT	protein folding
	GOTERM_CC_FAT	cytosol, chaperonin-containing T-complex, cytosolic part
	GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, ATP binding, purine nucleotide binding, adenyl nucleotide binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding, identical protein binding, unfolded protein binding
DDR2 (discoidin domain receptor tyrosine kinase 2)	GOTERM_BP_FAT	protein amino acid phosphorylation, phosphorus metabolic, phosphate metabolic, cell adhesion, cell surface receptor linked signal transduction, enzyme linked receptor protein signaling pathway, transmembrane receptor protein tyrosine kinase signaling pathway, positive regulation of cell proliferation, phosphorylation, biological adhesion, regulation of cell proliferation
	GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, protein kinase activity, protein tyrosine kinase activity, transmembrane receptor protein tyrosine kinase activity, ATP binding, purine nucleotide binding, adenyl nucleotide binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding

HSD17B4 (hydroxysteroid (17-beta) dehydrogenase 4)	GOTERM_BP_FAT	very-long-chain fatty acid metabolic, reproductive developmental, fatty acid metabolic, fatty acid beta-oxidation, sex differentiation, gonad development, male gonad development, fatty acid catabolic, lipid catabolic, organic acid catabolic, fatty acid oxidation, lipid modification, lipid oxidation, cellular lipid catabolic, development of primary sexual characteristics, carboxylic acid catabolic, development of primary male sexual characteristics, male sex differentiation, reproductive structure development, reproductive cellular, oxidation reduction, Sertoli cell differentiation, Sertoli cell development
	GOTERM_CC_FAT	mitochondrion, peroxisome, microbody
	KEGG_PATHWAY	Primary bile acid biosynthesis
IGF1R (insulin-like growth factor I receptor)	GOTERM_BP_FAT	reproductive developmental, regulation of DNA replication, protein complex assembly, protein amino acid phosphorylation, phosphorus metabolic, phosphate metabolic, immune response, cell surface receptor linked signal transduction, enzyme linked receptor protein signaling pathway, transmembrane receptor protein tyrosine kinase signaling pathway, intracellular signaling cascade, sex determination, positive regulation of biosynthetic, positive regulation of macromolecule biosynthetic, positive regulation of macromolecule metabolic, phosphorylation, second-messenger-mediated signaling, male sex determination, regulation of cell migration, positive regulation of cell migration, mammary gland development, positive regulation of cellular biosynthetic, regulation of locomotion, positive regulation of locomotion, macromolecular complex subunit organization, positive regulation of DNA replication, positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic, protein amino acid autophosphorylation, insulin-like growth factor receptor signaling pathway, phosphoinositide-mediated signaling, gland development, regulation of DNA metabolic, positive regulation of DNA metabolic, positive regulation of nitrogen compound metabolic, protein oligomerization, protein tetramerization, regulation of cell motion, positive regulation of cell motion, macromolecular complex assembly, protein complex biogenesis
	GOTERM_CC_FAT	cell fraction, membrane fraction, insoluble fraction, microsome, integral to membrane, intrinsic to membrane, vesicular fraction
	GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, protein kinase activity, protein tyrosine kinase activity, transmembrane receptor protein tyrosine kinase activity, insulin receptor binding, insulin-like growth factor binding, ATP binding, peptide hormone binding, purine

		nucleotide binding, growth factor binding, enzyme binding, kinase binding, adenyl nucleotide binding, insulin-like growth factor I binding, protein complex binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding, peptide binding, hormone binding, identical protein binding, phosphoinositide 3-kinase binding, insulin binding, insulin receptor substrate binding
	KEGG_PATHWAY	Oocyte meiosis, Endocytosis, Focal adhesion, Adherens junction, Long-term depression, Progesterone-mediated oocyte maturation, Pathways in cancer, Colorectal cancer, Glioma, Prostate cancer, Melanoma
LPHN2 (latrophilin 2)	GOTERM_BP_FAT	cell surface receptor linked signal transduction, G-protein coupled receptor protein signaling pathway, neuropeptide signaling pathway
	GOTERM_CC_FAT	plasma membrane, integral to membrane, intrinsic to membrane
	GOTERM_MF_FAT	sugar binding, carbohydrate binding
MTMR3 (myotubularin related protein 3)	GOTERM_BP_FAT	phosphorus metabolic, phosphate metabolic, dephosphorylation
	GOTERM_MF_FAT	phosphatase activity
PDE2A (phosphodiesterase 2A, cGMP-stimulated)	GOTERM_BP_FAT	regulation of nucleotide metabolic, purine nucleotide metabolic, purine nucleotide catabolic, nucleoside monophosphate metabolic, nucleoside monophosphate catabolic, purine nucleoside monophosphate metabolic, purine nucleoside monophosphate catabolic, purine ribonucleotide metabolic, purine ribonucleotide catabolic, ribonucleoside monophosphate catabolic, ribonucleoside monophosphate metabolic, nucleotide catabolic, purine ribonucleoside monophosphate metabolic, purine ribonucleoside monophosphate catabolic, ribonucleotide metabolic, ribonucleotide catabolic, regulation of cyclic nucleotide metabolic, regulation of cAMP metabolic, nucleobase, nucleoside, nucleotide and nucleic acid catabolic, nucleobase, nucleoside and nucleotide catabolic, nitrogen compound catabolic, GMP metabolic, GMP catabolic, heterocycle catabolic,
	GOTERM_CC_FAT	extrinsic to membrane
	GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, cyclic-nucleotide phosphodiesterase activity, 3',5'-cyclic-nucleotide phosphodiesterase activity, phosphoric diester hydrolase activity, purine nucleotide binding, guanyl nucleotide binding, GMP binding, cyclic nucleotide binding, cGMP binding, ribonucleotide binding, purine ribonucleotide binding, guanyl

		ribonucleotide binding
	KEGG_PATHWAY	Purine metabolism
PLXNA4 (plexin A4)	KEGG_PATHWAY	Axon guidance
KCNB1 (potassium voltage-gated channel, Shab-related subfamily, member 1)	KEGG_PATHWAY	Taste transduction
RPH3A (rabphilin 3A homolog (mouse))	GOTERM_BP_FAT	intracellular protein transport, protein localization, protein transport, cellular protein localization, establishment of protein localization, intracellular transport, cellular macromolecule localization
	GOTERM_CC_FAT	plasma membrane, synaptic vesicle, cytoplasmic membrane-bounded vesicle, cell junction, coated vesicle, clathrin-coated vesicle, cytoplasmic vesicle, vesicle, membrane-bounded vesicle, synapse part, plasma membrane part, synapse
	GOTERM_MF_FAT	small GTPase regulator activity, zinc ion binding, Ras GTPase binding, Rab GTPase binding, enzyme binding, GTPase regulator activity, small GTPase binding, ion binding, cation binding, metal ion binding, transition metal ion binding, GTPase binding, nucleoside-triphosphatase regulator activity
ROBO1 (roundabout, axon guidance receptor, homolog 1 (Drosophila))	KEGG_PATHWAY	Axon guidance
PIK3C2A (similar to Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing alpha polypeptide (Phosphoinositide 3-)	KEGG_PATHWAY	Inositol phosphate metabolism, Phosphatidylinositol signaling system
SSH2 (slingshot homolog 2 (Drosophila))	GOTERM_BP_FAT	protein amino acid dephosphorylation, phosphorus metabolic, phosphate metabolic, dephosphorylation
	GOTERM_MF_FAT	phosphoprotein phosphatase activity, protein tyrosine phosphatase activity, protein tyrosine/serine/threonine phosphatase activity, phosphatase activity
	KEGG_PATHWAY	Regulation of actin cytoskeleton

SLCO3A1 (solute carrier organic anion transporter family, member 3A1)	GOTERM_BP_FAT	ion transport
	GOTERM_CC_FAT	integral to membrane, intrinsic to membrane
TRPM7 (transient receptor potential cation channel, subfamily M, member 7)	GOTERM_BP_FAT	protein amino acid phosphorylation, phosphorus metabolic, phosphate metabolic, ion transport, phosphorylation, transmembrane transport
	GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, protein kinase activity, protein serine/threonine kinase activity, ion channel activity, ATP binding, channel activity, purine nucleotide binding, passive transmembrane transporter activity, substrate specific channel activity, adenyl nucleotide binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding
VNN1 (vanin 1)	GOTERM_BP_FAT	acute inflammatory response, chronic inflammatory response, positive regulation of immune system, regulation of leukocyte activation, positive regulation of leukocyte activation, cellular amino acid derivative metabolic, coenzyme metabolic, anti-apoptosis, defense response, inflammatory response, immune response, cell adhesion, response to wounding, regulation of cell death, pantothenate metabolic, cell-cell adhesion, biological adhesion, regulation of T cell differentiation in the thymus, positive regulation of T cell differentiation in the thymus, regulation of apoptosis, negative regulation of apoptosis, regulation of programmed cell death, negative regulation of programmed cell death, innate immune response, regulation of T cell differentiation, positive regulation of T cell differentiation, positive regulation of cell differentiation, regulation of lymphocyte differentiation, positive regulation of lymphocyte differentiation, regulation of T cell activation, regulation of cell activation, positive regulation of cell activation, positive regulation of T cell activation, positive regulation of developmental, cofactor metabolic, regulation of lymphocyte activation, positive regulation of lymphocyte activation, negative regulation of cell death
	GOTERM_CC_FAT	plasma membrane, intrinsic to membrane, anchored to membrane
	GOTERM_MF_FAT	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides, pantetheine hydrolase activity
	KEGG_PATHWAY	Pantothenate and CoA biosynthesis

Additional file 3. List of the genes mapping in QTL associated to traits of economic importance in bovine

GENE SYMBOL	FULL GENE NAME	QTL REGIONS AND ASSOCIATED TRAITS		
		ID	TRAIT	QTL region
NR3C2	nuclear receptor subfamily 3, group C, member 2	1356	Fat percentage	Chr17:0-28901254
		10534	Final packed red blood cell volume	Chr17:6750519-32301061
		10533	PCV variance	Chr17:6750519-32301061
		10532	PCVF minus PCVM	Chr17:6750519-32301061
		10531	PCVI minus PCVF	Chr17:6750519-32301061
		10536	Percentage decrease in PCV up to day 100 after challenge	Chr17:6750519-32301061
		10535	Percentage decrease in PCV up to day 150 after challenge	Chr17:6750519-32301061
		4484	Post-weaning average daily gain	Chr17:6750519-23354294
		4376	Residual feed intake	Chr17:10037387-12037387
CORO1C	coronin, actin binding protein, 1C	11051	Calving ease (maternal)	Chr17:54265266-72227102
		11386	Dystocia (maternal)	Chr17:63940959-72227102
		11052	Fat thickness at the 12th rib	Chr17:63940959-72227102
		2561	Milk fat percentage	Chr17:42508177-72227102
		11326	Milk fat yield (EBV)	Chr17:63940959-72227102
		2556	Milk protein yield	Chr17:42508177-72227102
		2679	Milk protein yield	Chr17:64476592-72227102
		2560	Milk yield	Chr17:42508177-72227102
		11327	Milk yield (EBV)	Chr17:63940959-72227102
		11360	Stillbirth (maternal)	Chr17:63940959-72227102
		5014	Veterinary treatments	Chr17:63940959-72227102
NEK11	NIMA (never in mitosis gene a)-related kinase 11	10647	Body weight (weaning)	Chr1:133500304-156647138
		1450	Chest width	Chr1:122708689-151468516
PLXDC2	plexin domain containing 2	10937	Body weight (weaning)	Chr13:4537163-28146135
		10938	Carcass weight	Chr13:15494818-28146135
		10936	Fat thickness at the 12th rib	Chr13:4537163-28146135
		2720	Milk protein percentage	Chr13:15832550-28146135
		10939	Weaning weight-maternal milk	Chr13:15494818-28146135

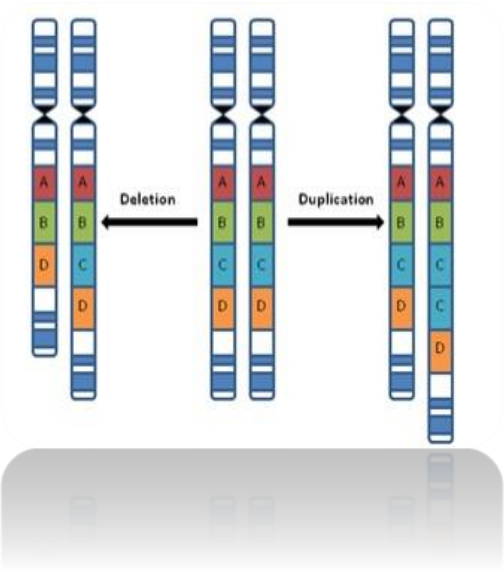
TADA1L	Transcriptional adapter 1-like protein	1300	Body weight (birth)	Chr3:0-7545339
		10678-79-81	Body weight (birth)	Chr3:0-15421599
		12143	Body weight (slaughter)	Chr3:0-5753088
		10685	Body weight (weaning)	Chr3:0-19119323
		10677	Carcass weight	Chr3:0-23673607
		12144	Carcass weight	Chr3:0-5753088
		1325	Fat thickness	Chr3:0-24894464
		13158	Fat thickness at the 12th rib	Chr3:0-16258580
		6053	Interval to first estrus after calving (EBV)	Chr3:0-21939455
		13157	Longissimus muscle area	Chr3:0-16258580
		2442-3	Milk fat percentage	Chr3:0-19119323
		2655	Milk fat yield	Chr3:0-15320965
		2656	Milk protein percentage	Chr3:0-15320965
		13224-5-6	Milk protein yield	Chr3:0-11862259
		13222-3	Milk yield	Chr3:0-11862259
		5663	Non-return rate (direct)	Chr3:974625-30143223
		5325	Residual feed intake	Chr3:0-19119323
		5331	Residual feed intake	Chr3:0-14976489
		10680	Ribeye area	Chr3:0-15421599
LPHN2	Latrophilin-2 Precursor	10693	Body weight (birth)	Chr3:57075548-73870850
		10692	Body weight (yearling)	Chr3:57075548-73870850
		10691	Height (mature)	Chr3:57075548-73870850
PLXNA4	plexin A4	491	**Clinical mastitis**	Chr4:75421999-99603227
		10515	Parasites mean of natural logarithm	Chr4:98555994-119913949
		4485	Post-weaning average daily gain	Chr4:98555994-108527288
		4972	Udder depth	Chr4:77210987-99603227
THY1	Thy-1 cell surface antigen	13502-3-4	Body length	Chr15:27792116-29792116
		13504	Body length	Chr15:27792116-29792116
		13498	Body weight (6 months)	Chr15:27792116-29792116
		10986	Body weight (birth)	Chr15:13868104-29490317
		10989	Body weight (yearling)	Chr15:13868104-29490317
		13505	Chest girth	Chr15:27792116-29792116

		4985	**Clinical mastitis**	Chr15:13868104-29490317
		10984	Fat thickness at the 12th rib	Chr15:13868104-29490317
		10987	Ribeye area	Chr15:11545436-29490317
		13499-500-501	Withers height	Chr15:27792116-29792116
ATP5H	ATP synthase subunit d, mitochondrial	1391	Body weight (birth)	Chr19:56095743-64812771
		12175	Stearic acid content	Chr19:56090170-58090170
FAM19A1	family with sequence similarity 19 (chemokine (C-C motif)-like), member A1	11140	Body weight (birth)	Chr22:12171103-35097286
		11142	Body weight (weaning)	Chr22:12171103-35097286
		11149	Body weight (yearling)	Chr22:32628727-43319438
		4672	Calf size (maternal)	Chr22:32628727-43319438
		11141	Calving ease (maternal)	Chr22:12171103-35097286
		7105	Fat percentage	Chr22:32628727-47692569
		11147	Height (mature)	Chr22:32628727-43319438
		11144	Height (yearling)	Chr22:12171103-35097286
		2487	Milk protein percentage	Chr22:32539717-34539717
		11143	Scrotal circumference	Chr22:12171103-35097286
		4987	**Somatic cell score**	Chr22:32628727-43319438
		4988	Udder depth	Chr22:32628727-43319438
MEGF11	multiple EGF-like-domains 11	4692	Angularity	Chr10:8292053-15297220
		10864	Body weight (birth)	Chr10:8965571-14961698
		4524	Carcass weight	Chr10:0-20877327
		7091	Fat percentage	Chr10:0-14961698
		7118	Meat percentage	Chr10:0-19995511
		5007	Veterinary treatments	Chr10:8292053-14961698
KCNB1	potassium voltage-gated channel, Shab-related subfamily, member 1	1306	Body weight (yearling)	Chr13:78375827-82517204
		10950	Marbling score (EBV)	Chr13:77615929-82517204
		2671	Milk protein yield	Chr13:59914941-80170380
		2670	Milk yield	Chr13:59914941-80170380
		1585	PTA type	Chr13:59914941-80170380
		1584	Udder attachment	Chr13:59914941-80170380
		1589	Udder composite index	Chr13:59914941-80170380
		1588	Udder depth	Chr13:59914941-80170380

		1586	Udder height	Chr13:59914941-80170380
		1587	Udder width	Chr13:59914941-80170380
TRPM7	transient receptor potential cation channel, subfamily M, member 7	10875	Height (yearling)	Chr10:56559463-78264482
		10219	Milk fat yield (daughter deviation)	Chr10:47964403-76680782
		4826	Tenderness score	Chr10:55424742-78024227
PIK3C2A	phosphoinositide-3-kinase, class 2, alpha polypeptide	4836	Myofibrillar fragmentation index	Chr15:29490317-40147387
ROBO1	roundabout, axon guidance receptor, homolog 1 (Drosophila)	10635	Body weight (birth)	Chr1:11538282-31377557
		10633	Calving ease (direct)	Chr1:11538282-31377557
		10634	Calving ease (maternal)	Chr1:11538282-31377557
		10637	Height (mature)	Chr1:11538282-31377557
		10636	Marbling score (EBV)	Chr1:11538282-31377557
		2500	Milk protein yield	Chr1:26284167-33876150
		2501	Milk yield	Chr1:26284167-33876150
		1674	Udder cleft	Chr1:20124367-33876150
SHISA9	(CKAMP44) shisa homolog 9	11201	Body weight (birth)	Chr25:792146-13184868
		1307	Body weight (yearling)	Chr25:792146-13449134
		11207	Body weight (yearling)	Chr25:0-25073950
		1751	**Clinical mastitis**	Chr25:0-17024171
		11377	Dystocia (maternal)	Chr25:0-13184868
		2607	Milk protein percentage	Chr25:792146-13449134
		6134	Milk protein yield (EBV)	Chr25:0-23241144

PART B

Genome scan for the CNVs discovery in dairy cattle



B.I Copy number variation

Copy number variation (CNV) is a class of genetic variation where DNA segments of 0.5 or more kilobase (kb) are present at a variable copy number in comparison with a reference genome. Classes of CNVs include insertions, deletions and duplications (Feuk et al., 2006). Different phenotypic features can occur by genetic variants (for size and form) and can be explained by modification of gene expression (by action on transcription, splicing, or translation and stability) and/or by the alteration of protein structure. Usually, CNVs may encompass parts of genes or, in the case of larger variants, include several known genes (Wain et al., 2009).

Because genomic disorders can be caused by de-novo deletions, insertions, or other chromosomal rearrangements, the CNVs contribute to the non-heritable components of a disease risk, although evidence suggests that common CNVs are inherited and therefore caused by ancestral structural mutations (McCarroll et al., 2008).

B.I.I Biological impact of copy number variation in bovine

The determination of CNVs is very important for the evaluation of genomic traits in several species because they are among the major sources for the genetic variation (mainly in complex traits), influencing gene expression (gene dosage), phenotypic variation, adaptation and the development of diseases (Metzger et al. 2013).

The improvement of the SNP array permitted to detect CNVs by high-throughput genotyping on different bovine species (Bos taurus and Bos indicus).

The Table B.1 shows the list of CNV studies in bovine population.

Table B.2 Reports the list of diseases and development of abnormalities identified in bovine population caused by CNV presence.

Table B.1 List of CNVs studies in bovine population (total breeds sampling, platform and technologies applied, total number of CNVRs identified, total CNVRs length (Mb) and % of CNVRs coverage on the genome).

Number of samples	Breed	Number of CNVR	Size range CNVR (kb)	CNRV in Mb (% coverage)	Methods employed to detect CNVR	References
265	1 breed (*Bos taurus*)	368	25.350-967.178	63.10 (2.078)	BovineSNP50 BeadChip	Bae et al. (2010)
20	4 breeds (*Bos taurus*)	304	1.7–2,031	22 (0,68)	Bovine 2.1 M aCGH arrays	Fadista et al. (2010)
90	14 breeds (*Bos taurus-Bos indicus*)	177	18–1260	28.1 (1.07)	Bovine 385k aCGH arrays	Liu et al. (2010)
539	27 breeds (*Bos taurus-Bos indicus*)	682	32.5–5569	139.8 (4.60)	BovineSNP50 BeadChip	Hou et al. (2011)
674	27 breeds (*Bos taurus-Bos indicus*)	3,346	1.018-5,552.622	142.7 (4.89)	BovineHD BeadChip	Hou et al. (2012)
96	1 breed (*Bos taurus*)	367	10.76-2,806.42	42.74 (1.61)	BovineHD BeadChip	Jiang et al. (2013)

Table B.2 Examples of phenotypes produced by CNVs in domestic animals (modified from Clop et al., 2012)

Phenotype	Type of mutation	References
Anhidrotic ectodermaldysplasia	A deletion encompassingexon 3 of the *EDA* gene	Drögemüller et al. (2001)
Renal tubular dysplasia	Deletion of 37 kb (exons 1–4) or 56 kb (exons 1–4 and 21 bp of exon 5) in the *CLDN16* gene	Ohba *et al.* (2000) and Hirano *et al.* (2000)
Osteopetrosis	A 2.8-kb deletion at the *SLC4A2* gene that encompasses exon 2	Meyers et al. (2010)
Abortions and stillbirths	A 110-kb deletion involving the loss of exons 3 and 4 of the *MIMT1* gene	Flisikowski et al. (2010)

B.2 Identification methods of Copy Number Variants

The methods used for CNVs detection can be summarized as following:

- Fluorescent in situ hybridization technique (FISH). The FISH experiment allows to identify CNVs as microscopically visible alterations (Wain et al., 2009). The technique consists in the use of labeled probes of specific DNA sequences that are hybridized to the complementary sequences on the DNA target. The hybridized and tagged with fluorochromes probes are directly visualized under a microscope[4].
- Comparative genomic hybridization array (aCGH). Using this technique, test and reference DNA samples are labeled differentially with fluorochromes Cy3 (green) and Cy5 (red) respectively, and together hybridized to a set of probes which are imprinted on a microarray (long oligonucleotides or BAC clones).
  The amount of red and green fluorescence on each probe are measured and the ratio of intensities of the two fluorochromes is analyzed with an analytical computer software, to infer the relative copy numbers of each specific DNA sequence[5].
- Quantitative PCR (qPCR). Using this approach, the quantity of a DNA segment is directly measured using locus-specific PCR primers. The copy numbers of target segments are estimated on the measured quantities from a test and a reference sample.
  The qPCR technique is locus-specific and therefore cannot be applied to genome-wide detection. A drawback of the qPCR is the low throughput when great amounts of CNVs need to be validated (Wain et al., 2009).
- Next Generation Sequencing (NGS). NGS allows the detection of multiple types of structural variation with a single sequencing trial.

[4] http://www.cs.cmu.edu/~sssykim/teaching/s13/slides/Lecture_SVI.pdf
[5] http://cgimatba.com/array-cgh-technology/

NGS based CNV detection methods can be categorized into five different strategies[6].

- SNP Microarray. Similarly to CGH platform, the SNP microarray platforms are based on hybridization.

B.2.1 Illumina Infinium II Whole Genome Genotyping Assay

The Infinium II Whole Genome Genotyping Assay is able to analyse a huge number of SNPs across the whole genome for each sample, using a single bead type and double color channel approach. The Figure B.1 shows the Infinium assay protocol[7].

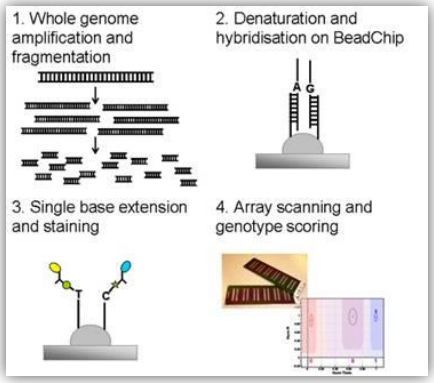


Figure B.1. Principle of Infinium II assay for whole genome genotyping

B.2.2 GenomeStudio Genotyping module

The GenomeStudio Genotyping module[8] is used to analyze data generated by the Illumina Infinium II Genotyping Assay. This module uses algorithms to execute primary data analysis, e.g. raw data normalization (which is needed in order to compare different samples), genotype calling and clustering. With the normalization step the signal intensities from A and B alleles for a specific locus are transformed into X norm and Y norm. Normalized allele specific intensities are

[6] Five approaches to detect CNVs from NGS short reads. A. **Paired-end mapping (PEM)** strategy detects CNVs through discordantly mapped reads. A discordant mapping is produced if the distance between two ends of a read pair is significantly different from the average insert size. B. **Split read (SR)-based** methods use incompletely mapped read from each read pair to identify small CNVs. C. **Read depth (RD)-based** approach detects CNV by counting the number of reads mapped to each genomic region. D. **Assembly (AS)-based** approach detects CNVs by mapping contigs to the reference genome. E. **Combinatorial** approach combines RD and PEM information to detect CNVs (Zhao et al. 2013)

[7] http://www.molmed.medsci.uu.se/SNP+SEQ+Technology+Platform/Genotyping/SNP_methods_and_references/Illumina_Infinium_assay/

[8] http://res.illumina.com/documents/products/datasheets/datasheet_genomestudio_software.pdf

transformed in a coordinate plot of combined overall SNP intensity value (R) (R = X norm + Y norm), and in an allelic intensity ratio, (theta) ($\theta = 2/\pi$ * arctan (Y norm/X norm)) (Peiffer *et al.*, 2006).

Canonical clusters of normalized transformed signal intensities are established for each SNP during the array development preferentially on a large number of individual representing several population or species. The canonical (circular) clusters are three and colored in red, purple and blue, representing the AA, AB, and BB genotypes, respectively. To create these canonical clusters, R and θ values of each SNP marker, per sample, are used (Figure B.2)[9]. In a genotyping assay, a genotype for a SNP is successfully called when the signal intensities of the sample fall within one of the three clusters for the specific SNP. Instead, when signal intensities fall outside the clusters, no genotype or allele can be associated.

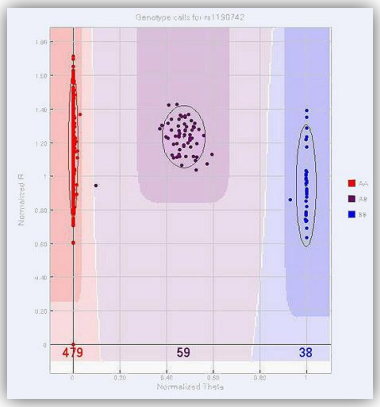


Figure B.2 SNP graph example illustrating the typical red, purple, and blue clusters

To detect the CNVs, the log R ratio (LRR) and the B allele frequency (BAF) from GenomeStudio software are used.

The LRR value represents the total signal intensity of the probe and BAF value is the allelic balance. LRR of signal intensities is calculated as:

$$\log2\ (Rsubject/Rexpected)$$

where *Rsubject* is the observed total signal intensity for SNP for each individual and *Rexpected* is the interpolation of the midpoints of two neighbouring canonical clusters.

[9] Figure from: http://dnatech.genomecenter.ucdavis.edu/illumina.html

BAF is an estimate of the relative frequency of allele B at a locus for an individual, ranging from zero to one ("0.5" indicates a heterozygous genotype, whereas "0" and "1" corresponds to the homozygous genotype AA and BB, respectively).
The allele frequency is obtained by linear interpolation with D_1 and D_2 lines for an observed θ value of a sample that is localized between two clusters (Figure B.3). In particular, BAF is calculated with respect to known allele frequencies assigned to each canonical cluster (BAFcc: 0, 0.5, 1) as:

$$BAF = \frac{D_1}{D_2} * BAF_{cc}$$

where D_1 is the distance of an observed theta value to the midpoint of the closest cluster solution. θ values between neighbouring canonical cluster solutions are interpolated and the distance between them is measured as D_2 (Pfeiffer et al. 2006).

B.3 CNV detection Algorithms based on SNP array data

Several CNV detection algorithms aim to identify genomic CNVs comparing the LRR of a reference genome to the LRRs of the genotyped samples. PennCNV and Golden Helix (variation Suite 7.6.4) are some of the software that can be used for the detection of the CNVs.

B.3.1 PennCNV software

PennCNV is an open source software for CNV detection from SNP genotyping arrays. PennCNV uses a hidden Markov model (HMM) that integrates multiple sources of information to infer CNV calls for genotyped samples. The HMM is a statistical technique that assumes that the distribution of an observed intensities data point depends on an unobserved (hidden) copy number state at each locus, where the elements of the hidden states follow a Markov process.

PennCNV software incorporates different information (LRR and BAF) for each SNPs into the HMM, in order to detect CNVs and to differentiate copy number neutral (LOH) regions from normal state regions. Both the LRR and BAF values can be displayed given an appropriate clustering file with canonical cluster positions for each SNP (Figure B.3). The distance among neighbouring SNPs determines the probability of having a copy number state change. Each SNP has two alleles referred to the A and B alleles, thus the term "population frequency of B allele" is used to differentiate it from the BAF term that measures the allelic intensity ratio. Six hidden states are identified as reported in Table B.3 (Wang et al., 2007).

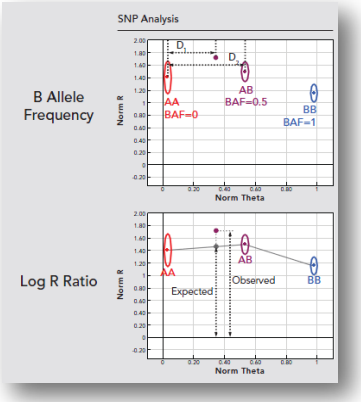


Figure B.3 Graphic representation of log R ratio (LRR) and the B allele frequency (BAF) estimation from GenomeStudio

Table B.3 Hidden states and their corresponding copy number and description as modelled in PennCNV software.

HIDDEN STATE	TOTAL COPY NUMBER	DESCRIPTION FOR AUTOSOMES	CNV GENOTYPES
1	0	homozygous deletion	Null
2	1	heterozygous deletion	A, B
3	2	normal state	AA, AB, BB
4	2	copy neutral with LOH	AA, BB
5	3	heterozygous duplication	AAA, AAB, ABB, BBB
6	4	homozygous duplication	AAAA, AAAB, AABB, ABBB, BBBB

A flowchart outlining the procedure for CNV calling from genotyping data in PennCNV software is represented in Figure B.4.

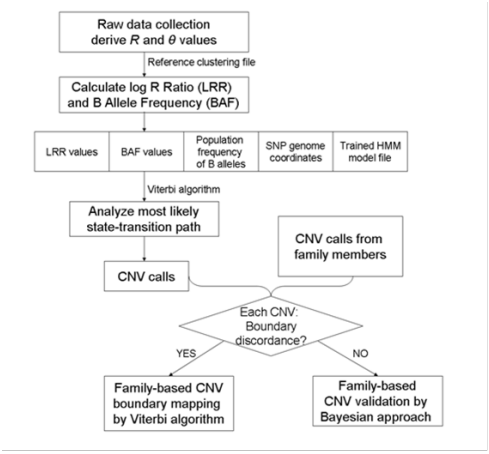


Figure B.4 Flowchart that represents CNV calling from genotyping data by PennCNV software (When genotype data are available for family members, the pedigree information can be incorporated to model CNV events more accurately)

B.3.2 Copy Number Module (CNAM) of Golden Helix SNP software and variation Suite 7.6.4 (SVS7)

The Golden Helix software[10], and in particular it's CNV package, offers the possibility to process raw intensity data, to identify regions of copy number variation, and to visualize copy number data .

The CNAM algorithm delineates CNV boundaries even at a single probe level, with controllable sensitivity and false discovery rate.

The procedure for CNV calling from genotyping data in SVS7 is summarized as following:

- Process raw data and generate log ratios. The LRR, also called "Log2 ratio", is the commonly used measurement to determine copy number status. For the LRR determination, a reference panel is required to determine the "normal" or baseline intensity expected at each marker.

[10] (Golden Helix, Bozeman, MT, www.goldenhelix.com)

- Quality assurance. This is the most important step in copy number analysis to reduce the false positive and not replicable association findings. This software offers several types of quality filters:
  - ✓ the derivative log ratio spread (DLRS): DLRS is a measurement of point to point consistency or noisiness in LRR data. This value is correlated with low quality SNP call rates and over/under abundance of identified copy number segments. Samples with higher values of DLRS tend to have poor signal-to-noise properties and accurate CNV detection is often difficult for these samples.
  - ✓ Genomic waves detection in log ratio data: Genomic waves are phenomena occurring when the LRR data appear to have a long-range wave pattern after plotted in genomic space. Waviness seems to be correlated with the GC content of the probes themselves in addition to the GC content of the region around the probes. The approach used in S7S removes samples with extreme wave factors.
  - ✓ Principal component analysis (PCA): this procedure is needed to detect the presence of batch effects and other technical artefacts for the LRR data correction before the CNV detection.
- Detect of CNVs. S7S provides two segmentation algorithms to CVN detection. The univariate method, which is suitable for detecting rare and/or large CNVs, considers only one sample at a time; contrariwise, the multivariate method uses all samples simultaneously and is ideal for detecting small, common CNVs. The objective in this step is to determine regions in the genome where a given sample's mean LRR value differs from the reference. These regions are referred to CNVs. A mean LRR around zero indicates that a sample has the same number of copies as the reference. A LRR segment mean above zero typically means that there is a copy number gain, and a LRR segment mean below zero means that there is a copy number loss.

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3

IDENTIFICATION AND VALIDATION OF COPY NUMBER VARIANTS IN ITALIAN BROWN SWISS DAIRY CATTLE USING ILLUMINA BOVINE SNP50 BEADCHIP

Submitted: ***Animal genetics***

Short comunication

IDENTIFICATION AND VALIDATION OF COPY NUMBER VARIANTS IN ITALIAN BROWN SWISS DAIRY CATTLE USING ILLUMINA BOVINE SNP50 BEADCHIP

A. Bagnato*§, **M. G. Strillacci***, L. Pellegrino*, F. Schiavini*, E. Frigo*, A. Rossoni^, L. Fontanesi§, M.A. Dolezal†

* Department of Health, Animal Science and Food Safety (VESPA), University of Milan, Via Celoria 10, 20133, Milan, Italy
†Institut für Populationsgenetik Veterinärmedizinische, University Wien, Josef Baumann Gasse 1, 1210 Wien, Austria
§Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Viale Fanin 46, 40127 Bologna, Italy
^ Associazione Nazionale Allevatori Razza Bruna, Loc. Ferlina 204, 37012 Bussolengo (VR), Italy

3.1 Abstract

The aim of this study was to obtain a copy number variation (CNV) genome map in 651 bulls of the Italian Brown Swiss breed using the Illumina Bovine SNP50 BeadChip data. Hidden Markov Model (HMM) of PennCNV and SVS7 software (Golden Helix) were used for the identification of the CNVs and Copy Number Variation Regions (CNVRs). A total of 5,099 and 1,289 CNVs were identified using PennCNV and SVS7 software, respectively. These were grouped at the population level into 1,101 (220 losses, 774 gains, 107 complex) and 277 (185 losses, 56 gains and 36 complex) CNVRs, covering 682 Mb (27.14%) and 33.7 Mb (1.35%) of the autosome, respectively. Ten of the selected CNVRs were experimentally validated with qPCR and the proportions of confirmed positive samples for each region varied from 50% to 100%. The GO and pathway analyses identified genes (false discovery rate corrected) in the CNVRs related to biological processes, cellular component, molecular function and metabolic pathways.

Although there is variability in the CNVRs detection across methods, platforms, this study allowed the identification CNVRs in Italian Brown Swiss, overlapping those already detected in other breeds and finding additional ones.

The understanding of the genetic variation in livestock species, such as cattle, is crucial to associate genomic regions to the traits of interest. Copy Number Variations (CNVs) are classes of polymorphic DNA regions including deletions, duplications and insertions of DNA fragments from at least 0.5 kb to several Mb, that are copy number variable when compared with a reference genome (Jiang et al. 2013). The CNVs are important sources of genetic diversity providing structural genomic information comparable to single nucleotide polymorphism (SNP) data; they influence gene expression, phenotypic variation, environmental adaptability and disease susceptibility (Wang et al. 2012).

The development of SNP arrays allowed the identification of CNVs by high-throughput genotyping on different types of cattle breeds. CNV loci were identified in several indicine and taurine breeds, and CNV maps of the bovine genome, using SNP, Next Generation Sequencing (NGS), CGH arrays, were reported (Matukumalli et al. 2009; Bae et al. 2010; Fadista et al. 2010; Hou et al. 2012; Bickhart et al., 2012). In cattle, Meyers et al. (2010) identified the association between CNV in a deletion state in SLC4A2 gene and the osteoporosis in Red Angus cows. Additionally, it has been reported that a Copy Number Variation Region (CNVR) located on BTA18 is associated with the index of total merit and protein production, fat production and herd life in Holstein cattle (Seroussi et al. 2010).

Several CNV detection algorithms based on SNP data array are available. Winchester et al. (2009), Pinto et al. (2011) and Tsuang et al. (2010) recommended the use of a minimum of two algorithms for the identification of CNVs in order to reduce the false discovery rates. The aim of this study was to obtain a consensus CNV genome map in

the Italian Brown Swiss cattle based on the Illumina Bovine SNP50 BeadChip and two SNP based CNV calling algorithms.

The Italian Brown Cattle Breeders Association (A.N.A.R.B.) provided commercial semen samples for 1,342 bulls (born between 1951 and 2005). Genomic DNA was extracted from semen using the ZR Genomic DNA™ Tissue MiniPrep (Zymo, Irvine, CA, U.S.A.). Sample DNA was quantified using NanoQuant Infinite®m200 (Tecan, Männedorf, Switzerland) and diluted to 50 ng/μl as required to apply the Illumina Infinium protocol. DNA samples were genotyped using Illumina Bovine SNP50 BeadChip (Illumina Inc., San Diego, USA) interrogating 54,001 polymorphic SNPs with an average probe spacing of 51.5 kb and a median spacing of 37.3 kb. In this study, the UMD3.1 assembly was used as the reference genome.

All SNPs were clustered and genotyped using the Illumina BeadStudio software V.2.0 (Illumina Inc.). Samples that showed a call rate below 98% were excluded for the CNVs detection. The signal intensity data of Log R Ratio (LRR) and B allele frequency (BAF) were exported from Illumina BeadStudio software. The overall distribution of derivative log ratio spread (DLRS) values was used in the SVS7 software to identify and filter outlier samples as described by Pinto et al. (2011).

Principal component analysis (PCA) for LRR was performed using the SVS7 software to detect the presence of batch effects and correct the signal intensity values accordingly.

A total of 691 bulls were discarded from the dataset after the application of the DLRS filter and the wave factor corrections, reducing the number of bull sample to be analysed to 651.

Individual-based CNV calling was performed by PennCNV (http://www.openbioinformatics.org/penncnv/) for all autosomes, using the default parameters of the Hidden Markov Model (HMM) To reduce the false discover rate in CNVs calling we used high quality samples with a standard deviation (SD) of LRR <0.30 and with default set of BAF drift as 0.01. Samples whose absolute wave factor exceeded a threshold of 0.05, as suggested by Diskin et al. (2008), were

excluded from the analysis. Genomic waves were calculated as GC content of the 1 Mb genomic region surrounding each marker (500 kb each side). In addition, we deleted the CNVs which overlapped at least 10% of telomere length (the first and last 500 kb of each autosome were considered representing the telomeres).

A total of 5,099 CNV events were detected using PennCNV, that were located in all 29 autosomes with a mean size of 350 kb (±165.259) ranging from 40.4 kb to 4.46 Mb (median = 230 kb). The predicted status for the CNVs was: for homozygous deletion, no. 97 (2%); heterozygous deletion, no. 2,086 (41%); for, heterozygous duplication, no. 2,915 (57%); homozygous duplication, no. 1.

The CNV identification was also carried out by Copy Number Analysis Module (CNAM) of SVS7 software (Golden Helix Inc.), with a univariate analysis. The criteria considered for the analysis were: univariate outlier removal, a maximum of 10 per 10,000 markers, with a minimum of 1 marker per segment, and 2,000 permutations per segment pair p-value cut-off of 0.005. A total of 1,289 CNVs calls have been identified by SVS7 in all the 29 autosomes, which encompassed 762 (59%) losses and 527 (41%) gains. The length of the CNVs ranged from 11.3 kb to 1.4 Mb with median and average values equal to 45 kb and 88.9 kb, respectively. Table 1 showed the descriptive statistics of the identified CNV length using PennCNV and SVS7 software.

CNVRs were defined as in Redon et al. (2006) with the BedTools software (Quinlan & Hall, 2010) for each of the two software.

A total of 1,101 CNVRs were mapped with the PennCNV. Among these 220 were in homozygous or heterozygous deletion state, 774 were in homozygous or heterozygous duplication state and 107 represented complex regions. The total length of the sequence covered by the CNVRs was 682 Mb, which corresponded to the 27.14% of the bovine autosomal genome in the Brown Swiss breed. The percentage by chromosome of sequence covered by CNVRs ranged from 16.59 (BTA 12) to 50.14% (BTA 19).

The CNVs calls identified with SVS7 were summarized at population level according to Redon's approach, resulting into 277 (185 losses, 56 gains and 36 complex) CNVRs. The total length of the sequence covered by the CNVRs was 33.71 Mb (1.35%) of the bovine autosomes. The percentage by chromosome of sequence covered by CNVRs ranged from 0.12% (BTA 10) to 3.5% (BTA 12). Differences in relative abundance of CNVRs may be due to the use of different algorithms for their detection.

A consensus was then performed between the two software using the approach suggested by Wain et al. (2009) using the BedTools software. A total of 150 consensus regions were generated with a total length of 17.1 Mb (0.68 % of the autosomes), as shown in Table S1. The comparison between the CNVRs detected in this study by Wain's approach and in literature was reported in Figure 1, confirming both the existence of high variability in CNVRs detection across platforms, methods, cattle breeds and sub-species and the overlapping of the regions detected in this study with other CNVRs dataset cattle studies.

Quantitative PCR (qPCR) experiments were performed to validate the CNVRs among those identified. Eleven CNVRs were selected for the validation; three of those were in common between PennCNV and SVS7 detection, six and two of those were randomly chosen among the CNVRs identified with PennCNV and SVS7 software, respectively. Table S2 reports the primer list for the eleven regions that were selected for the validation. Ten CNVRs (91%) were confirmed by qPCR experiments. Additionally, the proportions of confirmed positive CNV in each sample varied from 50% to 100% in each of the confirmed CNVRs; however, the average of false negative rate was equal to 25%. Jiang et al. (2013) reported similar values rates in Holstein breed.

The full Ensembl v76 gene set for the autosomal chromosomes was downloaded (http://www.ensembl.org/biomart/martview/76d1cab099658c68bde77f7daf55117e). A gene ontology (GO) and pathways analyses using the DAVID Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/) was performed (using the high

classification stringency option and the FDR correction) to identify molecular function, biological processes, cellular component and pathway for the genes included in the CNVRs consensus (Tables S3, S4). Among the identified genes, in Table 2 we highlighted those showing differential expression of association with quantitative traits in cattle in literature.

However, some of the CNVRs detected in this study are located in gene-poor regions.

Modern studies have highlighted the genome-wide distribution of CNVs in regions covering noncoding sequences, thus affecting the regulation of distant target genes. Further studied are envisaged to clarify this suggested impact of CNVs in noncoding regions (Liu *et al.*, 2013). In this study, the first on this breed and on a such a large number of individuals, we detected CNVs in the Italian Brown Swiss cattle population based on whole genome SNP genotyping data, using two algorithms for mapping the CNVs, with the aim to reduce the high error rate commonly recognised in copy number discovery.

The results will enrich the bovine CNV map in the cattle genome providing new information for association studies with traits of economic and healthy interest.

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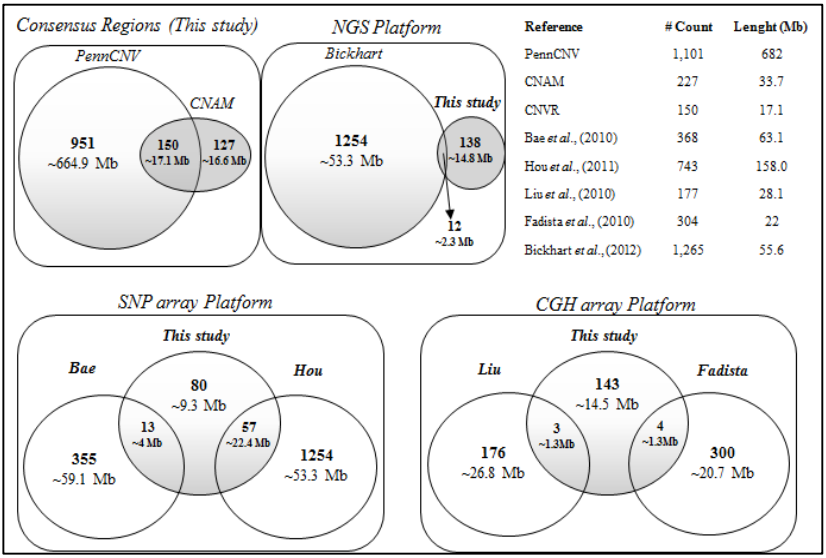
Table I Descriptive statistics for CNVs identified with PennCNV software (0 = homozygous deletion, 1 heterozygous deletion, 3 heterozygous duplication, and 4 homozygous duplication) and SVS7 softwares.

Copy	Number of	Mean	Median	Total Length	Min	Max
PennCNV						
0	97	311345	245646	30200500	46665	1053143
1	2086	159066	134534	331711379	40374	1688267
3	2915	488559	385138	1423739019	41449	4457756
4	1	511301	511301	511301	511301	511301
SVS7						
Loss	762	94830	57612	72260727	11315	1440751
Gain	527	80324	37591	42330968	20342	770044

Table 2 Genes mapping in CNVRs with differential expression or association with cattle quatitative traits reported in literarture.

Bta	CNVRs Consensus		Genes in Consensus				References
3	7957960	7983149	7928113	7944607	ENSBTAG00000021842	*FCGR2B*	Lewandowska-Sabat et al., 2013
5	116895329	117247824	117151549	117233112	ENSBTAG00000008063	*PPARA*	White et al., 2011
12	30418611	30646042	30519852	30558210	ENSBTAG00000009340	*KATNAL1*	Zang et al., 2014
12	13179696	13204137	13183734	13266310	ENSBTAG00000034785	*DNAJC15*	Zang et al., 2011
14	3885798	4017201	3870893	4065010	ENSBTAG00000009578	*PTK2*	Wang et al., 2013
14	9300228	9345140	9262251	9508938	ENSBTAG00000007823	*TG*	Fortes et al., 2009 Anton et al., 2008
19	42976859	43170256	43056660	43132624	ENSBTAG00000021523	*STAT3*	Ozawa et al., 2011
19	42976859	43170256	42960226	42996671	ENSBTAG00000010125	*STAT5B*	He et al., 2011
19	42976859	43170256	43033597	43054075	ENSBTAG00000009496	*STAT5A*	Khatib et al., 2008 X et al., 2012
20	2880532	3189118	3111198	3123860	ENSBTAG00000015316	*NPM1*	Huang et al., 2010
22	59951940	60243916	60016985	60024586	ENSBTAG00000019707	*GATA2*	Bai et al., 2011
25	609241	983759	724446	775899	ENSBTAG00000019745	*LMF1*	Gang et al., 2011
26	25828973	25982293	25856475	25865594	ENSBTAG00000017710	*ECHS1*	Li et al., 2014

Figure 1 Comparisons between the 150 CNVRs (consensus between the two softwares) identified in this study and other existing cattle CNVRs datasets.



Reference	# Count	Lenght (Mb)
PennCNV	1,101	682
CNAM	227	33.7
CNVR	150	17.1
Bae *et al.*, (2010)	368	63.1
Hou *et al.*, (2011)	743	158.0
Liu *et al.*, (2010)	177	28.1
Fadista *et al.*, (2010)	304	22
Bickhart *et al.*, (2012)	1,265	55.6

Supporting information

Table S1 List of the copy number variant region (CNVRs) identified using PennCNV and SVS7 softwares and the consensus CNVRs (PennCNV/SVS7) generated according to Wain et al. (2009).

pennCNV (CNVRs)			lenght	pennCNV (CNVRs)			lenght
chr1	1359951	1603944	243993	chr11	67498111	68298497	800386
chr1	1625471	2013659	388188	chr11	73934314	74197538	263224
chr1	4727118	5010974	283856	chr11	75295616	76060924	765308
chr1	5319965	5741816	421851	chr11	77235847	79314962	2079115
chr1	12889396	13019983	130587	chr11	80450475	80839258	388783
chr1	17021138	17160556	139418	chr11	82833655	82959206	125551
chr1	18170722	18980052	809330	chr11	87124625	89371911	2247286
chr1	19829143	20192420	363277	chr11	89552045	89680768	128723
chr1	27357510	27761036	403526	chr11	90738123	90911719	173596
chr1	28034525	28216247	181722	chr11	91961667	92500678	539011
chr1	41315418	41465449	150031	chr11	92963716	93050541	86825
chr1	43317853	43861492	543639	chr11	93546324	93612322	65998
chr1	46321775	46648008	326233	chr11	94640611	94837245	196634
chr1	47389301	49230292	1840991	chr11	95150490	99180813	4030323
chr1	52191701	52316632	124931	chr11	99497380	99738023	240643
chr1	56505444	56901848	396404	chr11	99774304	104856815	5082511
chr1	60753573	61329180	575607	chr11	105593624	106825407	1231783
chr1	66188527	66630647	442120	chr12	2058297	2273370	215073
chr1	68066718	69250962	1184244	chr12	2814820	3705325	890505
chr1	71229187	71623173	393986	chr12	9079069	9165632	86563
chr1	72573950	73640200	1066250	chr12	13140896	13273888	132992
chr1	80875014	80974985	99971	chr12	15726574	16098884	372310
chr1	81018906	81160609	141703	chr12	18472407	18584913	112506
chr1	82059960	82468782	408822	chr12	20079121	20402284	323163
chr1	83030921	83672872	641951	chr12	20510073	20800774	290701
chr1	88086502	88262684	176182	chr12	21073393	21441958	368565
chr1	91031267	91358383	327116	chr12	22664255	22863845	199590
chr1	92958471	93279488	321017	chr12	25141005	25255436	114431
chr1	95793312	95910972	117660	chr12	27006686	27133552	126866
chr1	97276664	97484342	207678	chr12	29520358	29778424	258066
chr1	101103098	101366448	263350	chr12	30099199	31555734	1456535
chr1	101403755	101664632	260877	chr12	31639399	31848902	209503
chr1	101773849	102056710	282861	chr12	34480373	34635671	155298
chr1	103728420	103926075	197655	chr12	35189028	35703502	514474
chr1	104842361	105264358	421997	chr12	41384223	41479027	94804
chr1	106228836	106295981	67145	chr12	45002070	45573176	571106
chr1	106512965	106824629	311664	chr12	47300401	47528422	228021
chr1	107094743	107172243	77500	chr12	48758328	49421215	662887
chr1	108154057	108782509	628452	chr12	50125707	50324576	198869
chr1	109649036	109869888	220852	chr12	52546816	52663318	116502
chr1	113631332	113780811	149479	chr12	55097796	55674695	576899
chr1	114945541	115185215	239674	chr12	55867003	56204081	337078
chr1	115389278	115456741	67463	chr12	56367819	56695055	327236
chr1	117619160	118226740	607580	chr12	57077252	57211328	134076
chr1	118532290	118882592	350302	chr12	57931622	58461348	529726
chr1	120915343	121003901	88558	chr12	63969950	64152261	182311
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chr1	146587678	146632014	44336
chr1	155835816	155955828	120012
chr2	5757355	5802738	45383
chr2	14525350	14565726	40376
chr2	27489458	27724930	235472
chr2	28215944	28246133	30189
chr2	32108568	32154806	46238
chr2	39976359	39999947	23588
chr2	57373897	57416172	42275
chr2	58772041	59143632	371591
chr2	83029887	83050544	20657
chr2	110311653	110353189	41536
chr14	80082712	80543545	460833
chr15	47780178	47819771	39593
chr15	76438547	76466667	28120
chr15	80369812	81139855	770043
chr16	4158997	4233985	74988
chr16	8317477	8582055	264578
chr16	9171622	9267151	95529
chr16	9670453	9729773	59320
chr16	15154212	15362423	208211
chr16	22272329	22302686	30357
chr16	29441057	29636822	195765
chr16	36768083	36817218	49135
chr16	41131268	41194530	63262
chr16	49355913	49455109	99196
chr16	68811897	68889225	77328
chr16	70906202	71125864	219662
chr17	8170089	8297151	127062
chr17	20275502	20484740	209238

chr2	124137395	124204421	67026	chr17	27459029	27491589	32560
chr3	7957960	7983149	25189	chr17	32762909	32964041	201132
chr3	33514564	33741850	227286	chr17	39963957	40071626	107669
chr3	39097420	39118317	20897	chr17	42425636	42661925	236289
chr3	40977107	41079360	102253	chr17	55713369	55764236	50867
chr3	68402961	68432145	29184	chr18	3080400	3114628	34228
chr3	81379707	81411041	31334	chr18	35971459	36107915	136456
chr3	91910014	92190368	280354	chr18	42659289	42826428	167139
chr3	100468099	100493684	25585	chr18	50388296	50465387	77091
chr3	105715727	105739637	23910	chr18	51571629	51592949	21320
chr3	115888900	115937988	49088	chr18	53132012	53195763	63751
chr3	116781408	116801749	20341	chr18	60978019	61054591	76572
chr4	6248795	6337362	88567	chr18	61095214	61156737	61523
chr4	10401625	10450547	48922	chr18	61438125	61920892	482767
chr4	11472235	11500911	28676	chr18	63119361	63167945	48584
chr4	17902915	17978432	75517	chr19	6709868	6768232	58364
chr4	22093546	22180214	86668	chr19	11863651	11970132	106481
chr4	24087424	24150445	63021	chr19	34836416	34905583	69167
chr4	28384911	28413603	28692	chr19	35585081	35619269	34188
chr4	41444423	41657868	213445	chr19	38519698	38546855	27157
chr4	66781385	66830563	49178	chr19	42352691	42423488	70797
chr4	69158293	69220543	62250	chr19	42976859	43170256	193397
chr4	73699663	73791282	91619	chr19	46655940	46723662	67722
chr4	78440044	78523846	83802	chr19	50336021	50395622	59601
chr4	81497187	81554820	57633	chr19	51767413	51842198	74785
chr4	86811243	86948025	136782	chr19	52175916	52264019	88103
chr4	89850655	89921990	71335	chr19	54306610	54446207	139597
chr4	99574406	99691481	117075	chr19	55379112	55527962	148850
chr4	106980782	107007048	26266	chr19	56072306	56202223	129917
chr4	108168742	108198485	29743	chr19	56754737	56837932	83195
chr4	108867683	108904498	36815	chr20	1741145	1792368	51223
chr4	111990062	112164314	174252	chr20	2880532	3189118	308586
chr4	118608842	118655986	47144	chr20	6360647	6385223	24576
chr5	30684691	30838906	154215	chr20	21018903	21048672	29769
chr5	46279541	46306149	26608	chr20	33773531	33817557	44026
chr5	58847022	58966295	119273	chr20	34241862	34264853	22991
chr5	114543256	114698428	155172	chr20	34953795	34981347	27552
chr5	116895329	118329917	1434588	chr20	41239866	41289921	50055
chr5	119729902	119949553	219651	chr20	45052283	45369517	317234
chr6	5025746	5086136	60390	chr20	60902173	60928704	26531
chr6	9736332	9981135	244803	chr20	64185456	64376028	190572
chr6	12648459	12703601	55142	chr21	54186710	54208626	21916
chr6	22613578	22672648	59070	chr21	70089833	71210609	1120776
chr6	40107367	40208497	101130	chr22	12869969	12948282	78313
chr6	50981312	51007189	25877	chr22	16219978	16407075	187097
chr6	53428838	53449439	20601	chr22	19409002	19588936	179934
chr6	68291351	68332579	41228	chr22	21431682	21455286	23604
chr6	81551479	81604925	53446	chr22	25141851	25321072	179221
chr6	90966250	90989420	23170	chr22	26527854	26604789	76935
chr6	100620998	100709082	88084	chr22	26865100	26924506	59406
chr6	104493834	104587477	93643	chr22	31649896	31675722	25826
chr6	117013231	117148273	135042	chr22	36527685	36548339	20654
chr7	1293067	1353317	60250	chr22	39545402	39657636	112234
chr7	2597655	2680354	82699	chr22	44430993	44595995	165002
chr7	22524899	22681472	156573	chr22	47510478	47537080	26602
chr7	42788788	43132401	343613	chr22	48858472	49131324	272852
chr7	43709405	43808593	99188	chr22	54028803	54183730	154927
chr7	76886696	77011685	124989	chr22	57098389	57111693	13304

chr7	78081511	78307528	226017
chr7	87158251	87359924	201673
chr8	9133270	9156221	22951
chr8	15417359	15441189	23830
chr8	15665796	15773921	108125
chr8	20018829	20067861	49032
chr8	29068846	29100768	31922
chr8	33356720	33747904	391184
chr8	34898163	34920926	22763
chr8	43628838	43779600	150762
chr8	73715997	73829090	113093
chr8	87038169	87141059	102890
chr8	94115663	94973599	857936
chr8	105683974	105695288	11314
chr9	4239500	4439872	200372
chr9	68973776	69002105	28329
chr9	102258435	102271805	13370
chr10	5437359	5540505	103146
chr10	39823420	39846476	23056
chr11	14979948	15029477	49529
chr11	16425876	16514661	88785
chr11	33642979	33682867	39888
chr11	46657176	46701073	43897
chr11	72555948	72598008	42060
chr11	84899274	84947879	48605
chr11	88028793	88377200	348407
chr11	93445185	93587894	142709
chr11	101750113	101802657	52544
chr11	105699664	105778702	79038
chr12	13179696	13204137	24441
chr12	20129895	20402284	272389
chr12	30418611	30646042	227431
chr12	41384223	41479027	94804
chr12	43601825	43638160	36335
chr12	50385487	50451289	65802
chr12	55867003	55931940	64937
chr12	57931622	58461348	529726
chr12	59437039	59609816	172777
chr12	67538730	67564989	26259
chr12	70649671	72090421	1440750
chr12	78212571	78235491	22920
chr12	82159124	82199690	40566
chr12	82450106	82661747	211641
chr13	5594384	5623697	29313
chr13	58043371	58070117	26746
chr13	65965727	66336246	370519
chr13	70496054	70523797	27743
chr13	80026050	80144645	118595
chr14	2721633	2803998	82365
chr14	3765019	4017201	252182
chr14	6778397	6850767	72370
chr14	8064004	8113083	49079
chr14	8499902	8551460	51558
chr14	9300228	9345140	44912
chr14	20119611	20157384	37773
chr14	30449596	30595032	145436
chr14	50955416	50996515	41099
chr14	51285167	51430094	144927
chr22	59951940	60243916	291976
chr22	60435042	60508872	73830
chr23	25250595	25339818	89223
chr23	28503248	28563533	60285
chr24	1027534	1137518	109984
chr24	3321961	3342966	21005
chr24	21071943	21129533	57590
chr24	24499452	24582206	82754
chr24	28060569	28083770	23201
chr24	28175885	28196203	20318
chr24	38640377	38694114	53737
chr24	39320770	39365195	44425
chr24	53328928	53434181	105253
chr25	609241	983759	374518
chr25	7952738	7992272	39534
chr25	12190414	12217941	27527
chr25	16017661	16048464	30803
chr25	18206998	18372002	165004
chr25	19082329	19145490	63161
chr25	22238007	22320002	81995
chr25	24125205	24228117	102912
chr25	37988321	38142895	154574
chr25	39286957	39424763	137806
chr25	39544407	39570754	26347
chr25	39785037	39844749	59712
chr26	5258082	5288263	30181
chr26	5472360	5504271	31911
chr26	12136498	12236803	100305
chr26	19686897	19942669	255772
chr26	21902497	21955137	52640
chr26	25828973	25982293	153320
chr26	28303383	28398156	94773
chr26	39655739	39681664	25925
chr26	48693316	48713332	20016
chr26	49027625	49090826	63201
chr27	4544917	4773381	228464
chr27	6922514	7188361	265847
chr27	8730441	8827679	97238
chr27	9096031	9191858	95827
chr27	12282518	12330184	47666
chr27	18036224	18164172	127948
chr27	33813284	33845584	32300
chr27	38025744	38233675	207931
chr27	43237090	43260976	23886
chr28	2313753	2638563	324810
chr28	6334557	6547497	212940
chr28	12717523	12973750	256227
chr28	13713042	13894573	181531
chr28	21982457	22148051	165594
chr28	26994978	27072121	77143
chr28	37514643	37624697	110054
chr28	38026506	38074472	47966
chr28	44030986	44056044	25058
chr29	7401774	7480356	78582
chr29	19618823	19701179	82356
chr29	21930571	21987120	56549
chr29	27184360	27465875	281515
chr29	28192104	28248785	56681

chr14	53415847	53436763	20916	chr29	33329702	33353664	23962
chr14	54023420	54123146	99726	chr29	35136093	35169599	33506
chr14	75571250	76043148	471898	chr29	41212959	41264801	51842
chr14	76217573	76269650	52077	chr29	48178151	48252404	74253
chr14	79178022	79322701	144679				

CNVRS Consensus (pennCNV/SVS7)			lenght	CNVRS Consensus (pennCNV/SVS7)			lenght
chr1	91066621	91358383	291762	chr15	76438547	76466667	28120
chr1	144134237	144190645	56408	chr16	4158997	4233985	74988
chr1	146587678	146632014	44336	chr16	8486467	8582055	95588
chr1	155835816	155955828	120012	chr16	29441057	29636822	195765
chr2	14525350	14565726	40376	chr16	49355913	49455109	99196
chr2	27489458	27724930	235472	chr16	70906202	71125864	219662
chr2	32108568	32154806	46238	chr17	27459029	27491589	32560
chr2	39976359	39999947	23588	chr17	32762909	32964041	201132
chr2	57373897	57416172	42275	chr17	39963957	40071626	107669
chr2	83029887	83050544	20657	chr17	42425636	42661925	236289
chr2	110311653	110353189	41536	chr17	55713369	55764236	50867
chr2	124137395	124204421	67026	chr18	35971459	36040190	68731
chr3	7957960	7983149	25189	chr18	42659289	42826428	167139
chr3	33514564	33741850	227286	chr18	51571629	51592949	21320
chr3	39097420	39118317	20897	chr18	53132012	53195763	63751
chr3	40977107	41079360	102253	chr18	61095214	61156737	61523
chr3	81379707	81411041	31334	chr18	61438125	61597742	159617
chr3	91910014	92190368	280354	chr18	63119361	63167945	48584
chr3	100468099	100493684	25585	chr19	11863651	11970132	106481
chr3	115888900	115937988	49088	chr19	35585081	35619269	34188
chr3	116781408	116801749	20341	chr19	34836416	34905583	69167
chr4	6248795	6337362	88567	chr19	42393606	42423488	29882
chr4	41556810	41657868	101058	chr19	42976859	43170256	193397
chr4	66781385	66830563	49178	chr19	46655940	46723662	67722
chr4	69158293	69220543	62250	chr19	50336021	50395622	59601
chr4	73699663	73791282	91619	chr19	52175916	52264019	88103
chr4	99574406	99635737	61331	chr19	51767413	51842198	74785
chr4	106980782	107007048	26266	chr19	56754737	56837932	83195
chr4	108867683	108904498	36815	chr19	54306610	54446207	139597
chr4	111990062	112164314	174252	chr19	55379112	55527962	148850
chr4	118608842	118655986	47144	chr19	56072306	56202223	129917
chr5	46279541	46306149	26608	chr20	1741145	1792368	51223
chr5	114543256	114698428	155172	chr20	2880532	3189118	308586
chr5	116895329	117247824	352495	chr20	6360647	6385223	24576
chr5	119729902	119949553	219651	chr20	41239866	41289921	50055
chr5	117738204	118329917	591713	chr20	60902173	60928704	26531
chr6	9914189	9981135	66946	chr21	70089833	71136925	1047092
chr6	12648459	12703601	55142	chr22	12869969	12948282	78313
chr6	81551479	81604925	53446	chr22	16219978	16342830	122852
chr6	100620998	100709082	88084	chr22	19409002	19588936	179934
chr6	104493834	104587477	93643	chr22	39545402	39657636	112234
chr6	117013231	117148273	135042	chr22	47510478	47537080	26602
chr7	1293067	1353317	60250	chr22	57098389	57111693	13304
chr7	2597655	2680354	82699	chr22	60435042	60508872	73830
chr7	22524899	22681472	156573	chr22	59951940	60243916	291976
chr7	42788788	43132401	343613	chr23	25250595	25339818	89223
chr8	33668705	33747904	79199	chr24	1027534	1094942	67408
chr8	34898163	34920926	22763	chr24	3321961	3342966	21005
chr8	94115663	94386951	271288	chr24	24499452	24582206	82754
chr8	94579362	94973599	394237	chr24	28175885	28196203	20318
chr8	105683974	105695288	11314	chr24	39320770	39365195	44425
chr9	4239500	4439872	200372	chr24	53328928	53434181	105253
chr10	5437359	5540505	103146	chr25	609241	983759	374518
chr11	46657176	46701073	43897	chr25	37988321	38142895	154574
chr11	88028793	88377200	348407	chr25	39544407	39570754	26347
chr11	93546324	93587894	41570	chr25	39785037	39844749	59712
chr11	101750113	101802657	52544	chr25	39286957	39424763	137806
chr11	105699664	105778702	79038	chr26	5258082	5288263	30181
chr12	13179696	13204137	24441	chr26	5472360	5504271	31911

chr12	20129895	20402284	272389	chr26	25828973	25982293	153320
chr12	30418611	30646042	227431	chr26	48693316	48713332	20016
chr12	41384223	41479027	94804	chr26	49027625	49090826	63201
chr12	55867003	55931940	64937	chr27	4544917	4773381	228464
chr12	57931622	58461348	529726	chr27	6922514	6955584	33070
chr13	80026050	80144645	118595	chr27	8781446	8827679	46233
chr14	2721633	2803998	82365	chr27	38109791	38233675	123884
chr14	3885798	4017201	131403	chr27	43237090	43260976	23886
chr14	8064004	8113083	49079	chr28	6334557	6547497	212940
chr14	8499902	8551460	51558	chr28	13713042	13894573	181531
chr14	9300228	9345140	44912	chr28	26994978	27072121	77143
chr14	20119611	20157384	37773	chr29	19618823	19701179	82356
chr14	53415847	53436763	20916	chr29	33329702	33353664	23962
chr14	54023420	54123146	99726	chr29	35136093	35169599	33506
chr14	79178022	79322701	144679	chr29	41212959	41264801	51842
chr14	80475984	80543545	67561	chr29	48178151	48252404	74253

Table S2 Summary of the results of the qPCR analysis of the eleven CNVRs selected after the consensus analysis in the Italian Brown Swiss breed.

BTA	PRIMER (FORWARD)	PRIMER (REVERSE)	PROBE	START*	END*	LENGHT	PENN CNV STATE	CNAM CNV STATE	NUMBER OF SAMPLES	CONFIRMED SAMPLES	CONFIRMED RATE	VALIDATED	GENE (Btau4.6.1)
2	TGCATGCACACAGGAATGTTAC	TGCCCCTAAGAAGGAGTCGTT	ACTCTGTTCAGCCCTTC	63870167	64073748	203581	GAIN		11	8	0,73	YES	MGAT5
3	GACTAATGGCAAGAGCCGTGTA	AGGCAGGAACAGAAGGAGGAA	TGAGCATGTCACTTTAA	7957198	7966700	9502	GAIN	GAIN	3	2	0,67	YES	FCGR2B
4	GCCCGGCGGACACTAAG	CCAGCATTATGTCCTTCATCAACA	TCAGGAAGCTGTGGCCA	6228016	6450000	221984	GAIN		11	11	1,00	YES	-
4	TCCTGCCCAGATACCATATCCTT	CGAGGCAAGCTCTACAGGAAA	TGGCATTCAAATCAC	73686740	73785101	98361	LOSS	LOSS	10	9	0,90	YES	ZNF804B
5	GGAGATAGGATAGAAAGAAATGGAGAAC	ATGGGAGTGATGGAAAATTGAAG	CACTCTTAAATTCCC	58965609	59140571	174962	LOSS		10	7	0,70	YES	LOC787945
12	GGACAGTCACCTCAGGATGCA	TTGCCACAAGTGAGGCTTCTC	AACGGTCACCTAAGAGACA	58071208	58427000	355792	LOSS	LOSS	11	1	0,09	NO	-
12	GCCTGGTGTTGTCATGATGAA	CCCGTACACTGACACCAAAGTG	TTTTGCGCTTGAAGCAG	67534765	67579929	45164		LOSS	11	10	0,91	YES	-
13	TGCGAAATTCTGGAAGAGGAA	GGGTGCCTGGTGCAATTC	CCTGAGGACATGAAGTT	53931895	53983934	52039	GAIN		5	3	0,60	YES	SIRPB1
19	CAGTGAGCCAAAGCCAATCC	AATCCAACTTGCCGGCTAGTATT	CCTCCACAGGAATC	2585940	2607218	21278	LOSS		12	9	0,75	YES	-
28	ACATTCAGGCTGCCATTTTGT	GAGGCGGGATGTCACAGAAA	TCCAATATCGTCAACCATT	2553716	2635632	81916		LOSS	12	6	0,50	YES	OR5AS1
29	CACGGGCGCACCACTT	CCCCCGATGAATGGCTATC	AGCTCCCTGCTCGAC	5414600	5444000	29400	GAIN		10	9	0,90	YES	-
REF_BTF3	GCTGAGACAAAGCAACTGACAGA	TCGGCACCAAGCTGGTTTA	TGCTCCCCAGCATC										

The BTF3 gene was selected as a reference location for all the qPCR experiments (Bae et al. 2010). Primers for the selected target regions and for the reference gene were designed with the Primer Express® Software v3.0.1 (Life Technologies™, Milano, Italy) using the MGB quantification parameters. All the qPCR experiments were run in quadruple using the qPCR protocol described by TaqMan® Copy Number Assays kit (Life Tecnologies™) on 7500 Fast Real-time PCR System instrument (Applied Biosystems, Life Technologies™). The samples for each qPCR experiment were randomly selected with or without CNVs for each CNVR. The analysis of the crossing thresholds (Ct) for each samples tested was carried out using CopyCaller™ software (Applied Biosystems). The validated CNVRs positions were converted from Bos_taurus_UMD3.1 to Btau_4.6.1 assembly using the Batch Coordinate Conversion option in UCSC database (https://genome.ucsc.edu/) in order to identify potential candidate CNV genes for complex traits.

Table S3 Annotation of copy number variant regions (CNVRs).

CNVRS_pennCNV			CNVRS_SVS7		CNVRS_Consensus		CNVRs State	Gene included in CNVRs_Cconsensus			
chr1	141903958	145280015	144134237	144190645	144134237	144190645	gain/loss	144176745	144180011	ENSBTAG00000030814	TFF2
chr1	155654021	156710174	155835816	155955828	155835816	155955828	gain/loss	155833805	156185921	ENSBTAG00000030581	TBC1D5
chr2	14496313	14565726	14525350	14565726	14525350	14565726	loss	14502890	14623643	ENSBTAG00000044009	PPP1R1C
chr2	26781358	28215944	27489458	27724930	27489458	27724930	gain/loss	27629813	27629887	ENSBTAG00000044462	bta-mir-2353
								27407917	27758923	ENSBTAG00000044179	CERS6
chr2	39976359	40021512	39976359	39999947	39976359	39999947	loss	39999717	40017015	ENSBTAG00000003650	NR4A2
chr2	109740844	110393192	110311653	110353189	110311653	110353189	gain/loss	110251546	110405363	ENSBTAG00000010030	EPHA4
chr3	7866803	8021336	7957960	7983149	7957960	7983149	gain	7928113	7944607	ENSBTAG00000021842	FCGR2B
chr3	33276539	34344799	33514564	33741850	33514564	33741850	gain/loss	33702816	33702894	ENSBTAG00000044953	bta-mir-2413
								33607139	33621030	ENSBTAG00000000283	CSF1
								33513768	33556281	ENSBTAG00000018893	AHCYL1
chr3	39097420	39143931	39097420	39118317	39097420	39118317	loss	39113552	39114954	ENSBTAG00000015180	none
chr3	91703200	93847303	91910014	92190368	91910014	92190368	gain/loss	91901853	91911965	ENSBTAG00000013241	BSND
								91919532	91925379	ENSBTAG00000046583	TMEM61
								91994928	91995058	ENSBTAG00000042369	SNORA8
								92098624	92100051	ENSBTAG00000040313	PARS2
								92023811	92054136	ENSBTAG00000017145	C1orf177
								92059023	92083335	ENSBTAG00000017132	TTC22
								92106927	92132082	ENSBTAG00000015931	TTC4
								91981619	92014282	ENSBTAG00000004688	DHCR24
								92136670	92190804	ENSBTAG00000030623	none
								92136706	92165969	ENSBTAG00000044141	HEATR8
chr3	100442675	100982336	100468099	100493684	100468099	100493684	gain/loss	100472063	100495789	ENSBTAG00000013322	POMGNT1
								100472499	100483275	ENSBTAG00000024144	LURAP1
chr3	115630359	118715700	115888900	115937988	115888900	115937988	gain/loss	115843770	116226449	ENSBTAG00000016504	none
			116781408	116801749							
chr4	66583105	66978951	66781385	66830563	66781385	66830563	gain	66785266	66851245	ENSBTAG00000016223	SCRN1
chr4	73699663	73791282	73699663	73791282	73699663	73791282	loss	73326980	73897041	ENSBTAG00000046430	ZNF804B
chr4	99542142	99635737	99574406	99691481	99574406	99635737	loss	99475015	99580189	ENSBTAG00000013953	CALD1
								99591042	99690829	ENSBTAG00000013976	AGBL3
chr4	106885171	107060437	106980782	107007048	106980782	107007048	gain/loss	106989816	106993917	ENSBTAG00000015510	none
								106996940	107013515	ENSBTAG00000024219	TRPV6
			111990062	112164314	111990062	112164314	gain/loss	112048065	112048217	ENSBTAG00000047873	none

chr4	117170573	120412745	118608842	118655986				112126680	112126805	ENSBTAG00000045871	none
chr5	114543256	114859696	114543256	114698428	114543256	114698428	gain/loss	114576427	114588012	ENSBTAG00000002413	MCAT
								114596006	114608640	ENSBTAG00000018073	TSPO
								114610664	114627819	ENSBTAG00000001708	TTLL12
								114644629	114767260	ENSBTAG00000011275	SCUBE1
chr5	115010779	117247824	116895329	118329917	116895329	117247824	gain/loss	117119385	117119458	ENSBTAG00000029772	bta-let-7a-3
								117119640	117119712	ENSBTAG00000045309	bta-mir-2443
								117120188	117120270	ENSBTAG00000036417	bta-mir-3596
								116959716	116966318	ENSBTAG00000009532	WNT7B
								117240033	117248391	ENSBTAG00000008065	CDPF1
								116756235	116897163	ENSBTAG00000009351	ATXN10
								117151549	117233112	ENSBTAG00000008063	PPARA
			116895329	118329917	117738204	118329917	gain/loss	117743264	117758670	ENSBTAG00000005595	TRMU
								117975282	118012354	ENSBTAG00000021803	GRAMD4
								118023267	118036020	ENSBTAG00000046654	CERK
								117677522	117738845	ENSBTAG00000007102	GTSE1
								117764821	117853214	ENSBTAG00000008036	CELSR1
								118086468	118343833	ENSBTAG00000012291	TBC1D22A
chr5	117738204	120783915	119729902	119949553	119729902	119949553	gain/loss	119821435	119829363	ENSBTAG00000019574	MAPK12
								119771356	119791795	ENSBTAG00000000647	SELO
								119814028	119819426	ENSBTAG00000011000	HDAC10
								119832293	119837101	ENSBTAG00000030182	MAPK11
								119840726	119854120	ENSBTAG00000014966	PLXNB2
								119874457	119885052	ENSBTAG00000024756	DENND6B
								119791795	119813398	ENSBTAG00000000650	TUBGCP6
								119926286	120029002	ENSBTAG00000018660	PPP6R2
chr6	81467492	81604925	81551479	81604925	81551479	81604925	gain/loss	81511554	81653990	ENSBTAG00000024826	TECRL
chr7	1127439	2008771	1293067	1353317	1293067	1353317	loss	1317088	1334619	ENSBTAG00000015602	C7H5orf45
								1334566	1346051	ENSBTAG00000015591	SQSTM1
								1273635	1313897	ENSBTAG00000015611	TBC1D9B
chr7	2565484	6669157	2597655	2680354	2597655	2680354	gain/loss	2586101	2598115	ENSBTAG00000001604	none
								2600732	2618482	ENSBTAG00000040028	MGC166429
chr7	21462645	23074262	22524899	22681472	22524899	22681472	gain/loss	22533096	22537819	ENSBTAG00000004521	LSM7
								22569683	22574912	ENSBTAG00000016477	C19orf35
								22577243	22580777	ENSBTAG00000018522	OAZ1
								22519686	22532977	ENSBTAG00000004524	SPPL2B
								22560008	22561849	ENSBTAG00000016478	LINGO3
								22629692	22680307	ENSBTAG00000009996	DOT1L

chr7	42788788	43479777	42788788	43132401	42788788	43132401	gain/loss	42787986	42788915	ENSBTAG00000007557	OR2AK2
								42811931	42813032	ENSBTAG00000047016	none
								42833645	42834607	ENSBTAG00000046417	none
								42868129	42869064	ENSBTAG00000027241	none
								42890345	42891283	ENSBTAG00000045733	none
								42913832	42914770	ENSBTAG00000046474	none
								42947455	42948392	ENSBTAG00000046042	none
								43044539	43045551	ENSBTAG00000040033	OR2AJ1
								43101693	43102628	ENSBTAG00000030725	none
								43119732	43120670	ENSBTAG00000047180	none
chr8	94115663	94386951	94115663	94973599	94115663	94386951	loss	94230962	94231065	ENSBTAG00000042843	U6
								94205191	94210057	ENSBTAG00000015608	CYLC2
chr11	46307696	47430509	46657176	46701073	46657176	46701073	gain/loss	46699166	46706152	ENSBTAG00000019665	IL1RN
chr11	87124625	89371911	88028793	88377200	88028793	88377200	gain/loss	88012967	88104077	ENSBTAG00000002329	ASAP2
chr11	93546324	93612322	93445185	93587894	93546324	93587894	gain/loss	93563425	93564411	ENSBTAG00000038726	none
								93584334	93585269	ENSBTAG00000037542	none
chr11	99774304	104856815	101750113	101802657	101750113	101802657	gain/loss	101728372	101793685	ENSBTAG00000020791	RAPGEF1
chr11	105593624	106825407	105699664	105778702	105699664	105778702	gain/loss	105698114	105702610	ENSBTAG00000030246	ENTPD8
								105702496	105711512	ENSBTAG00000012121	NOXA1
								105728961	105770612	ENSBTAG00000023788	EXD3
chr12	13140896	13273888	13179696	13204137	13179696	13204137	gain/loss	13183734	13266310	ENSBTAG00000034785	DNAJC15
chr12	30099199	31555734	30418611	30646042	30418611	30646042	gain	30587084	30587189	ENSBTAG00000045239	SNORA70
								30519852	30558210	ENSBTAG00000009340	KATNAL1
chr12	57931622	58461348	57931622	58461348	57931622	58461348	loss	58187519	58187639	ENSBTAG00000045992	none
chr13	79866776	82559505	80026050	80144645	80026050	80144645	gain/loss	80015601	80114072	ENSBTAG00000018270	NFATC2
chr14	1435005	3664511	2721633	2803998	2721633	2803998	gain/loss	2755206	2762197	ENSBTAG00000000158	LY6K
								2770551	2775678	ENSBTAG00000037824	none
								2715416	2742638	ENSBTAG00000004595	GML
								2801383	2803020	ENSBTAG00000034498	LY6D
chr 14	3885798	6371334	3765019	4017201	3885798	4017201	gain/loss	3870893	4065010	ENSBTAG00000009578	PTK2
chr 14	6850767	8264685	8064004	8113083	8064004	8113083	gain/loss	8080292	8080361	ENSBTAG00000029987	bta-mir-30d
								8084721	8084808	ENSBTAG00000029972	bta-mir-30b
chr 14	8385937	10549180	9300228	9345140	9300228	9345140	gain/loss	9334778	9371281	ENSBTAG00000007828	SLA
								9262251	9508938	ENSBTAG00000007823	TG
chr 14	53415847	54164119	54023420	54123146	54023420	54123146	loss	53901591	54429251	ENSBTAG00000038281	CSMD3
chr 14	79178022	79486476	79178022	79322701	79178022	79322701	gain/loss	79296713	79298474	ENSBTAG00000002851	none
chr16	3987821	4610955	4158997	4233985	4158997	4233985	gain/loss	4221210	4242621	ENSBTAG00000010432	EIF2D
								4144349	4218744	ENSBTAG00000010427	RASSF5

chr16	29441057	29661958	29441057	29636822	29441057	29636822	gain/loss	29552152	29561665	ENSBTAG00000033322	SRP9
								29624572	29655286	ENSBTAG00000002854	TMEM63A
								29585792	29624037	ENSBTAG00000000140	EPHX1
								29238992	29442791	ENSBTAG00000016185	ENAH
chr16	49355913	49491331	49355913	49455109	49355913	49455109	loss	49429155	49429874	ENSBTAG00000046062	none
								49447984	49564506	ENSBTAG00000021919	NAV1
chr16	69911268	71125864	70906202	71125864	70906202	71125864	gain/loss	71019535	71024141	ENSBTAG00000004790	UBE2T
								71077717	71077907	ENSBTAG00000033994	U2
								70925149	70928253	ENSBTAG00000047073	none
								70932238	71016271	ENSBTAG00000004789	LGR6
								71062132	71137463	ENSBTAG00000011772	PPP1R12B
								70902206	70917245	ENSBTAG00000003016	PTPN7
chr17	32731007	32964041	32762909	32964041	32762909	32964041	gain/loss	32712712	32889849	ENSBTAG00000003345	FAT4
chr17	55713369	55941040	55713369	55764236	55713369	55764236	loss	55707870	55719927	ENSBTAG00000004175	HPD
								55727012	55747669	ENSBTAG00000004172	SETD1B
								55759606	55773011	ENSBTAG00000032534	RHOF
chr18	35971459	36040190	35971459	36107915	35971459	36040190	gain/loss	36008030	36029234	ENSBTAG00000007488	ZFP90
chr18	42638878	42826428	42659289	42826428	42659289	42826428	gain/loss	42749252	42750804	ENSBTAG00000003856	none
chr18	51321651	52024379	51571629	51592949	51571629	51592949	gain/loss	51520760	51578983	ENSBTAG00000011723	GRIK5
								51587793	51603479	ENSBTAG00000018635	ATP1A3
chr18	53132012	53224638	53132012	53195763	53132012	53195763	loss	53154421	53158137	ENSBTAG00000013702	ZNF296
								53129172	53153714	ENSBTAG00000013697	CLASRP
								53160659	53167519	ENSBTAG00000010668	GEMIN7
								53169569	53207223	ENSBTAG00000018834	PPP1R37
chr18	61095214	61597742	61438125	61920892	61095214	61156737	gain/loss	61145844	61145922	ENSBTAG00000036392	bta-mir-371
								61091348	61143063	ENSBTAG00000038149	NLRP12
chr18	63029071	64901743	63119361	63167945	63119361	63167945	gain/loss	63119873	63124888	ENSBTAG00000045989	CDC42EP5
								63146729	63154412	ENSBTAG00000019547	none
chr19	11049355	12032389	11863651	11970132	11863651	11970132	gain/loss	11865527	11891936	ENSBTAG00000009968	TBX4
								11943185	11951411	ENSBTAG00000014278	TBX2
chr19	34371541	36710214	35585081	35619269	35585081	35619269	gain/loss	35557245	35646258	ENSBTAG00000010534	M-RIP
			34836416	34905583	34836416	34905583	gain/loss	34832961	34860869	ENSBTAG00000003705	FAM83G
								34878438	34899068	ENSBTAG00000014858	PRPSAP2
								34817325	34872403	ENSBTAG00000003700	SLC5A10
chr19	42393606	43170256	42352691	42423488	42393606	42423488	gain/loss	42413413	42418215	ENSBTAG00000047165	KRT9
chr19	46396064	46770465	46655940	46723662	42976859	43170256	gain/loss	43033597	43054075	ENSBTAG00000009496	STAT5A
								43148013	43162165	ENSBTAG00000039684	PTRF
								43056660	43132624	ENSBTAG00000021523	STAT3

								42960226	42996671	ENSBTAG00000010125	STAT5B
chr19	46396064	46770465	46655940	46723662	46655940	46723662	gain/loss	46650344	46775847	ENSBTAG00000012564	KANSL1
chr19	50087148	50567992	50336021	50395622	50336021	50395622	gain/loss	50388667	50536976	ENSBTAG00000015414	TBCD
			52175916	52264019	52175916	52264019	gain/loss	52189629	52189720	ENSBTAG00000029775	bta-mir-338
								52181458	52196943	ENSBTAG00000019049	AATK
								52198617	52263294	ENSBTAG00000019044	BAIAP2
chr19	51148913	52911677	51767413	51842198				51768184	51769908	ENSBTAG00000000354	PDE6G
								51781191	51829647	ENSBTAG00000019105	NPLOC4
								51833821	51842965	ENSBTAG00000019104	C17orf70
								51771254	51775213	ENSBTAG00000040573	TSPAN10
chr19	53038373	59742080	56754737	56837932	56754737	56837932	gain/loss	56754112	56818424	ENSBTAG00000004736	GRB2
			54306610	54446207	54306610	54446207	gain/loss	54343819	54343903	ENSBTAG00000047060	none
								54404340	54441590	ENSBTAG00000000675	PGS1
								54312243	54405245	ENSBTAG00000000920	DNAH17
			55379112	55527962	55379112	55527962	gain/loss	55419632	55419819	ENSBTAG00000044443	SCARNA16
			56072306	56202223	56072306	56202223	gain/loss	56072684	56078553	ENSBTAG00000013792	UBALD2
								56171932	56175103	ENSBTAG00000007916	FOXJ1
								56112050	56167541	ENSBTAG00000016240	RNF157
								56190846	56208380	ENSBTAG00000007910	EXOC7
chr20	1627053	2585844	1741145	1792368	1741145	1792368	gain/loss	1452376	1893741	ENSBTAG00000014612	DOCK2
chr20	2880532	5096097	2880532	3189118	2880532	3189118	gain	2985749	2986486	ENSBTAG00000034824	none
								3004314	3005537	ENSBTAG00000022684	none
								3064510	3066676	ENSBTAG00000010003	TLX3
								3111198	3123860	ENSBTAG00000015316	NPM1
								2680574	3054892	ENSBTAG00000024801	RANBP17
								3132655	3195021	ENSBTAG00000000128	FGF18
chr20	6360647	6416157	6360647	6385223	6360647	6385223	loss	6360600	6365489	ENSBTAG00000013873	MSX2
chr21	70089833	71136925	70089833	71210609	70089833	71136925	gain/loss	70113045	70118400	ENSBTAG00000026886	MP68
								70114200	70114755	ENSBTAG00000046186	none
								70133009	70134255	ENSBTAG00000046401	RD3L
								70717761	70727303	ENSBTAG00000006673	TMEM179
								70870027	70874415	ENSBTAG00000017622	SIVA1
								70903809	70905041	ENSBTAG00000005038	ZBTB42
								70999273	71004734	ENSBTAG00000015160	PLD4
								71011580	71016820	ENSBTAG00000046828	none
								71041900	71047128	ENSBTAG00000010370	C14orf79
								71052184	71052852	ENSBTAG00000031242	CDCA4
								71077800	71078924	ENSBTAG00000005647	GPR132

								70123917	70233306	ENSBTAG00000020402	TDRD9
								70269910	70292666	ENSBTAG00000017194	ASPG
								70313782	70351845	ENSBTAG00000021904	KIF26A
								70707408	70715785	ENSBTAG00000022775	C14orf180
								70827525	70839732	ENSBTAG00000007187	INF2
								70845971	70861972	ENSBTAG00000017616	ADSSL1
								70878138	70895537	ENSBTAG00000017636	AKT1
								70919570	70983506	ENSBTAG00000004802	CEP170B
chr22	16219978	16342830	16219978	16407075	16219978	16342830	gain	16310518	16348300	ENSBTAG00000016622	TCAIM
								16247929	16308333	ENSBTAG00000044167	TOPAZ1
chr22	19409002	19588936	19409002	19588936	19409002	19588936	gain/loss	18740484	19647747	ENSBTAG00000013047	GRM7
chr22	39545402	39702951	39545402	39657636	39545402	39657636	loss	39175038	40360572	ENSBTAG00000021911	PTPRG
chr22	54440045	61040701	60435042	60508872	60435042	60508872	gain/loss	60443564	60493810	ENSBTAG00000018248	MGLL
								60502719	60507367	ENSBTAG00000018238	ABTB1
								60507746	60556978	ENSBTAG00000018031	PODXL2
			59951940	60243916	59951940	60243916	gain/loss	60016985	60024586	ENSBTAG00000019707	GATA2
								60039910	60040605	ENSBTAG00000006179	DNAJB8
								59952594	59965985	ENSBTAG00000005191	RPN1
								60179708	60211026	ENSBTAG00000020998	RUVBL1
								60217808	60229341	ENSBTAG00000004937	SEC61A1
								60063340	60172924	ENSBTAG00000030962	EEFSEC
chr24	541784	1094942	1027534	1137518	1027534	1094942	gain/loss	1018253	1099817	ENSBTAG00000000656	NFATC1
chr24	39320770	39365195	39320770	39365195	39320770	39365195	gain/loss	39312037	39404644	ENSBTAG00000019251	EPB41L3
chr25	472458	5156189	609241	983759	609241	983759	gain/loss	613234	615345	ENSBTAG00000020198	METRN
								618418	619923	ENSBTAG00000002467	FAM173A
								624818	627214	ENSBTAG00000002481	HAGHL
								627456	633977	ENSBTAG00000002484	NARFL
								649253	652835	ENSBTAG00000000177	MSLN
								665058	668257	ENSBTAG00000000179	RPUSD1
								676665	677047	ENSBTAG00000033526	GNG13
								787493	790981	ENSBTAG00000020737	SOX8
								857167	858273	ENSBTAG00000039974	SSTR5
								865752	867111	ENSBTAG00000033481	C1QTNF8
								871131	877122	ENSBTAG00000012601	TEKT4
								620322	623768	ENSBTAG00000002470	CCDC78
								668529	676616	ENSBTAG00000019743	CHTF18
								724446	775899	ENSBTAG00000019745	LMF1
								959758	984950	ENSBTAG00000026461	CACNA1H

								653105	660655	ENSBTAG00000033580	MSLNL
chr25	36980815	38698430	37988321	38142895	37988321	38142895	gain	38041960	38053172	ENSBTAG00000045896	NPTX2
chr25	38856905	39921068	39286957	39424763	39544407	39570754	gain/loss	39565711	39589412	ENSBTAG00000012049	WIPI2
			39785037	39844749	39785037	39844749	gain/loss	39840013	39840088	ENSBTAG00000047050	bta-mir-2890
								39761774	39816244	ENSBTAG00000019310	FOXK1
			39286957	39424763	39286957	39424763	gain/loss	39292721	39302192	ENSBTAG00000003191	FSCN1
								39308651	39309366	ENSBTAG00000047781	none
								39343633	39347044	ENSBTAG00000026199	ACTB
								39359290	39376730	ENSBTAG00000010264	FBXL18
chr26	5258082	5526925	5472360	5504271	5258082	5288263	loss	5017714	5578654	ENSBTAG00000045905	PCDH15
					5472360	5504271	loss	5017714	5578654	ENSBTAG00000045905	PCDH15
chr26	25501890	26124236	25828973	25982293	25828973	25982293	gain/loss	25856475	25865594	ENSBTAG00000017710	ECHS1
								25872737	25875735	ENSBTAG00000005715	FUOM
								25881752	25885322	ENSBTAG00000013717	PRAP1
								25928928	25930145	ENSBTAG00000046499	none
								25938181	25941636	ENSBTAG00000012416	ZNF511
								25828813	25855778	ENSBTAG00000018321	PAOX
								25893499	25904421	ENSBTAG00000000791	CALY
								25960809	25972557	ENSBTAG00000023832	ADAM8
								25944197	25958640	ENSBTAG00000006395	TUBGCP2
chr27	4357162	5000552	4544917	4773381	4544917	4773381	gain	4677302	4677407	ENSBTAG00000043496	U6
								4766514	4783646	ENSBTAG00000007473	XKR5
								4313369	4554747	ENSBTAG00000011032	MCPH1
								4679600	4727246	ENSBTAG00000004922	AGPAT5
chr28	6334557	6547497	6334557	6547497	6334557	6547497	gain/loss	6492389	6559855	ENSBTAG00000004515	KCNK1
					26994978	27072121	gain/loss	26985668	27080093	ENSBTAG00000021177	ADAMTS14
chr29	35051920	36669359	35136093	35169599	35136093	35169599	gain	35154689	35575203	ENSBTAG00000010032	NTM
chr29	45817015	50999092	48178151	48252404	48178151	48252404	gain/loss	48167168	48194210	ENSBTAG00000006071	CTTN
								48217044	48378574	ENSBTAG00000003171	SHANK2

Table S4 Go and pathways analyses performed using DAVID on line database with high classification stringency option and the FDR correction (sheet 1: gene clustered_DAVID; sheet 2: genes not clustered_DAVID).

sheet 1: gene clustered

Category	ID	Term	P-Value	FDR
Annotation Cluster 1		**Enrichment Score: 2,73**		
GOTERM_BP_FAT	GO:0043434	response to peptide hormone stimulus	1,22E+12	1.9E-1
	GO:0032870	cellular response to hormone stimulus	1,40E+12	2.1E-1
	GO:0009719	response to endogenous stimulus	2,67E+11	4.1E-1
	GO:0009725	response to hormone stimulus	1,46E-03	2.2E0
KEGG_PATHWAY	bta05221	Acute myeloid leukemia	3,29E-03	3.5E0
	bta05220	Chronic myeloid leukemia	5,08E-02	4.3E1
	bta04630	Jak-STAT signaling pathway	6,72E-02	5.3E1
	AKT1, GRB2, STAT5A, STAT5B, NR4A2, STAT3			
Annotation Cluster 2		**Enrichment Score: 2,68**		
GOTERM_BP_FAT	GO:0060397	JAK-STAT cascade involved in growth hormone signaling pathway	2,57E+12	3.9E-1
	GO:0060396	growth hormone receptor signaling pathway	8,45E+11	1.3E0
	GO:0060416	response to growth hormone stimulus	8,45E+11	1.3E0
	GO:0007259	JAK-STAT cascade	3,69E-03	5.5E0
	GO:0040014	regulation of multicellular organism growth	5,23E-03	7.7E0
	GO:0019221	cytokine-mediated signaling pathway	2,25E-02	2.9E1
	STAT5A, CSF1, STAT5B, STAT3			
Annotation Cluster 3		**Enrichment Score: 1,69**		
GOTERM_BP_FAT	GO:0045137	development of primary sexual characteristics	6,92E-03	1.0E1
	GO:0003006	reproductive developmental process	1,22E-02	1.7E1
	GO:0007548	sex differentiation	1,45E-02	2.0E1
	GO:0046661	male sex differentiation	2,08E-02	2.7E1
	GO:0008406	gonad development	4,87E-02	5.3E1
	GO:0048608	reproductive structure development	6,08E-02	6.2E1
	FOXJ1, STAT5A, CSF1, STAT5B, DHCR24			
Annotation Cluster 4		**Enrichment Score: 1,60**		
GOTERM_BP_FAT	GO:0051056	regulation of small GTPase mediated signal transduction	4,08E-03	6.0E0
	GO:0030695	GTPase regulator activity	4,38E-02	4.4E1
	GO:0005083	small GTPase regulator activity	4,52E-02	4.5E1
	GO:0060589	nucleoside-triphosphatase regulator activity	4,92E-02	4.7E1
	CSF1, TBC1D5, ASAP2, MGC166429, RAPGEF1, TBC1D22A, TBC1D9B			
Annotation Cluster 5		**Enrichment Score: 1,45**		
GOTERM_BP_FAT	GO:0002763	positive regulation of myeloid leukocyte differentiation	2,32E-03	3.5E0
	GO:0040014	regulation of multicellular organism growth	5,23E-03	7.7E0
	GO:0045639	positive regulation of myeloid cell differentiation	8,35E-03	1.2E1
	GO:0040018	positive regulation of multicellular organism growth	8,35E-03	1.2E1
	GO:0002761	regulation of myeloid leukocyte differentiation	1,33E-02	1.8E1
	GO:0045637	regulation of myeloid cell differentiation	3,56E-02	4.2E1
	GO:0070665	positive regulation of leukocyte proliferation	4,20E-02	4.8E1
	GO:0032946	positive regulation of mononuclear cell proliferation	4,20E-02	4.8E1
	GO:0045927	positive regulation of growth	4,42E-02	5.0E1
	GO:0045597	positive regulation of cell differentiation	5,02E-02	5.4E1
	GO:0032944	regulation of mononuclear cell proliferation	5,58E-02	5.8E1
	GO:0070663	regulation of leukocyte proliferation	5,58E-02	5.8E1
	GO:0051094	positive regulation of developmental process	8,86E-02	7.6E1
	GO:0048872	homeostasis of number of cells	1,02E-01	8.1E1

	GO:0030155	regulation of cell adhesion	1,17E-01	8.5E1
	GO:0008284	positive regulation of cell proliferation	1,71E-01	9.4E1
	GO:0051240	positive regulation of multicellular organismal process	2,53E-01	9.9E1
	METRN, STAT5A, CSF1, STAT5B, STAT3			
Annotation Cluster 6		**Enrichment Score: 1,40**		
	GO:0032318	regulation of Ras GTPase activity	1,38E-02	1.9E1
	GO:0043087	regulation of GTPase activity	2,08E-02	2.7E1
	GO:0008047	enzyme activator activity	3,23E-02	3.4E1
	GO:0032313	regulation of Rab GTPase activity	3,56E-02	4.2E1
GOTERM_BP_FAT	GO:0032483	regulation of Rab protein signal transduction	3,56E-02	4.2E1
	GO:0005097	Rab GTPase activator activity	3,67E-02	3.8E1
	GO:0005083	small GTPase regulator activity	4,52E-02	4.5E1
	GO:0005096	GTPase activator activity	5,53E-02	5.2E1
	GO:0005099	Ras GTPase activator activity	6,16E-02	5.6E1
	GO:0051336	regulation of hydrolase activity	1,42E-01	9.0E1
	TBC1D5, ASAP2, MGC166429, TBC1D22A, TBC1D9B, NOXA1			
Annotation Cluster 7		**Enrichment Score: 1,11**		
	GO:0006468	protein amino acid phosphorylation	5,68E-02	5.9E1
GOTERM_BP_FAT	GO:0006796	phosphate metabolic process	7,71E-02	7.1E1
	GO:0006793	phosphorus metabolic process	7,71E-02	7.1E1
	GO:0016310	phosphorylation	1,12E-01	8.4E1
	PTPN7, AKT1, EPHA4, PTK2, MAPK12, PTPRG, STAT5A, STAT5B, MAPK11, PDE6G, AATK			
Annotation Cluster 8		**Enrichment Score: 1,031**		
	GO:0008344	adult locomotory behavior	2,25E-02	2.9E1
GOTERM_BP_FAT	GO:0030534	adult behavior	6,84E-02	6.6E1
	GO:0007610	behavior	2,04E-01	9.7E1
	GO:0007626	locomotory behavior	2,39E-01	9.8E1
	EPHA4, ATP1A3, NR4A2, STAT3			
Annotation Cluster 9		**Enrichment Score: 1,03**		
	GO:0017076	purine nucleotide binding	6,80E-02	5.9E1
	GO:0001883	purine nucleoside binding	7,18E-02	6.1E1
	GO:0001882	nucleoside binding	7,46E-02	6.3E1
	GO:0032555	purine ribonucleotide binding	8,30E-02	6.7E1
GOTERM_MF_FAT	GO:0032553	ribonucleotide binding	8,30E-02	6.7E1
	GO:0000166	nucleotide binding	9,38E-02	7.2E1
	GO:0030554	adenyl nucleotide binding	1,17E-01	8.0E1
	GO:0005524	ATP binding	1,38E-01	8.5E1
	GO:0032559	adenyl ribonucleotide binding	1,45E-01	8.6E1
	ACTB, PGS1, ADSSL1, SETD1B, TDRD9, ATP1A3, MAPK11, PDE6G, TPK1, AKT1, EPHA4, NAV1, MAPK12, PARS2, ENTPD8, RUVBL1, EEFSEC, RHOF, UBE2T, DHCR24, AATK			
Annotation Cluster 10		**Enrichment Score: 0,97**		
	GO:0032989	cellular component morphogenesis	2,99E-02	3.7E1
	GO:0000904	cell morphogenesis involved in differentiation	3,39E-02	4.1E1
	GO:0000902	cell morphogenesis	7,64E-02	7.0E1
	GO:0007409	axonogenesis	9,60E-02	7.9E1
	GO:0030182	neuron differentiation	1,03E-01	8.1E1
	GO:0048812	neuron projection morphogenesis	1,05E-01	8.1E1
GOTERM_BP_FAT	GO:0048667	cell morphogenesis involved in neuron differentiation	1,17E-01	8.5E1
	GO:0048858	cell projection morphogenesis	1,23E-01	8.6E1
	GO:0032990	cell part morphogenesis	1,42E-01	9.0E1
	GO:0031175	neuron projection development	1,48E-01	9.1E1
	GO:0048666	neuron development	2,39E-01	9.8E1
	GO:0006928	cell motion	4,03E-01	1.0E2
	ACTB, EPHA4, PTK2, NR4A2, NFATC1, STAT3			
Annotation Cluster 11		**Enrichment Score: 0,83**		
	GO:0006417	regulation of translation	8,74E-02	7.5E1
GOTERM_BP_FAT	GO:0010608	posttranscriptional regulation of gene expression	1,85E-01	9.6E1
	GO:0032268	regulation of cellular protein metabolic process	2,00E-01	9.7E1

		AKT1, CSF1, EEFSEC, SRP9		
Annotation Cluster 12		**Enrichment Score: 0,81**		
	GO:0005856	cytoskeleton	3,83E-02	3.5E1
GOTERM_CC_FAT	GO:0043228	non-membrane-bounded organelle	3,12E-01	9.8E1
	GO:0043232	intracellular non-membrane-bounded organelle	3,12E-01	9.8E1
		AKT1, ACTB, FGF18, CYLC2, PTK2, TUBGCP6, EXOC7, CALD1, NPM1, TEKT4, TUBGCP2, RHOF		
Annotation Cluster 13		**Enrichment Score: 0,76**		
	GO:0009165	nucleotide biosynthetic process	1,65E-01	9.4E1
GOTERM_BP_FAT	GO:0034654	nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process	1,81E-01	9.5E1
	GO:0034404	nucleobase, nucleoside and nucleotide biosynthetic process	1,81E-01	9.5E1
		ADSSL1, ENTPD8, ATP1A3, PRPSAP2		
Annotation Cluster 14		**Enrichment Score: 0,70**		
	bta04664	Fc epsilon RI signaling pathway	4,92E-02	4.2E1
KEGG_PATHWAY	bta04914	Progesterone-mediated oocyte maturation	2,34E-01	9.5E1
	bta04620	Toll-like receptor signaling pathway	2,89E-01	9.8E1
GOTERM_MF_FAT	GO:0004674	protein serine/threonine kinase activity	4,78E-01	1.0E2
		AKT1, MAPK12, MAPK11, AATK, GRB2		
Annotation Cluster 15		**Enrichment Score: 0,66**		
	GO:0043066	negative regulation of apoptosis	1,56E-01	9.2E1
	GO:0043069	negative regulation of programmed cell death	1,60E-01	9.3E1
GOTERM_BP_FAT	GO:0060548	negative regulation of cell death	1,60E-01	9.3E1
	GO:0042981	regulation of apoptosis	2,96E-01	1.0E2
	GO:0043067	regulation of programmed cell death	3,03E-01	1.0E2
	GO:0010941	regulation of cell death	3,05E-01	1.0E2
		MSX2, SIVA1, STAT5A, STAT5B, NR4A2		
Annotation Cluster 16		**Enrichment Score: 0,62**		
	GO:0019216	regulation of lipid metabolic process	4,87E-02	5.3E1
	GO:0010628	positive regulation of gene expression	1,41E-01	9.0E1
	GO:0045944	positive regulation of transcription from RNA polymerase II promoter	1,58E-01	9.3E1
	GO:0051254	positive regulation of RNA metabolic process	2,17E-01	9.8E1
	GO:0045893	positive regulation of transcription, DNA-dependent	2,17E-01	9.8E1
GOTERM_BP_FAT	GO:0045941	positive regulation of transcription	2,99E-01	1.0E2
	GO:0045935	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	3,61E-01	1.0E2
	GO:0051173	positive regulation of nitrogen compound metabolic process	3,79E-01	1.0E2
	GO:0010557	positive regulation of macromolecule biosynthetic process	4,04E-01	1.0E2
	GO:0031328	positive regulation of cellular biosynthetic process	4,32E-01	1.0E2
	GO:0009891	positive regulation of biosynthetic process	4,39E-01	1.0E2
		PPARA, STAT5A, CSF1, STAT5B, NR4A2		
Annotation Cluster 17		**Enrichment Score: 0,49**		
GOTERM_MF_FAT	GO:0003700	transcription factor activity	1,24E-01	8.2E1
	GO:0030528	transcription regulator activity	3,35E-01	9.9E1
	GO:0006355	regulation of transcription, DNA-dependent	3,91E-01	1.0E2
GOTERM_BP_FAT	GO:0051252	regulation of RNA metabolic process	4,11E-01	1.0E2
	GO:0045449	regulation of transcription	5,34E-01	1.0E2
		MSX2, PPARA, TRMU, ZFP90, STAT5A, TBX4, STAT5B, NARFL, HDAC10, NR4A2, STAT3, NFATC1		
Annotation Cluster 18		**Enrichment Score: 0,30**		
	GO:0031981	nuclear lumen	4,50E-01	1.0E2
GOTERM_CC_FAT	GO:0070013	intracellular organelle lumen	5,03E-01	1.0E2
	GO:0043233	organelle lumen	5,04E-01	1.0E2
	GO:0031974	membrane-enclosed lumen	5,41E-01	1.0E2
		ACTB, FGF18, NPM1, HDAC10, ECHS1, GEMIN7		
Annotation Cluster 19		**Enrichment Score: 0,25**		
GOTERM_BP_FAT	GO:0010558	negative regulation of macromolecule biosynthetic	5,21E-01	1.0E2

		process		
	GO:0031327	negative regulation of cellular biosynthetic process	5,30E-01	1.0E2
	GO:0009890	negative regulation of biosynthetic process	5,47E-01	1.0E2
	GO:0010605	negative regulation of macromolecule metabolic process	6,40E-01	1.0E2
		PPARA, HDAC10, SRP9		
Annotation Cluster 20		**Enrichment Score: 0,25**		
	GO:0046872	metal ion binding	5,35E-01	1.0E2
GOTERM_MF_FAT	GO:0043169	cation binding	5,61E-01	1.0E2
	GO:0043167	ion binding	5,82E-01	1.0E2
PPARA, NPLOC4, ADSSL1, TRMU, STAT5A, ZNF296, SCUBE1, STAT5B, ASAP2, NR4A2, CELSR1, KCNK1, STAT3, PRPSAP2, POMGNT1, SQSTM1, ZFP90, ENTPD8, HAGHL, ADAM8, SLC5A10, TBC1D9B, HPD				
Annotation Cluster 21		**Enrichment Score: 0,24**		
	GO:0032561	guanyl ribonucleotide binding	5,08E-01	1.0E2
GOTERM_MF_FAT	GO:0019001	guanyl nucleotide binding	5,15E-01	1.0E2
	GO:0005525	GTP binding	7,44E-01	1.0E2
		ADSSL1, EEFSEC, PDE6G, RHOF		
Annotation Cluster 22		**Enrichment Score: 0,19**		
	GO:0006886	intracellular protein transport	5,61E-01	1.0E2
	GO:0034613	cellular protein localization	5,98E-01	1.0E2
	GO:0070727	cellular macromolecule localization	6,00E-01	1.0E2
GOTERM_BP_FAT	GO:0008104	protein localization	6,04E-01	1.0E2
	GO:0015031	protein transport	7,33E-01	1.0E2
	GO:0045184	establishment of protein localization	7,35E-01	1.0E2
	GO:0046907	intracellular transport	7,43E-01	1.0E2
		RANBP17, LMF1, SEC61A1, SRP9, DHCR24		
Annotation Cluster 23		**Enrichment Score: 0,14**		
	GO:0005216	ion channel activity	7,11E-01	1.0E2
GOTERM_MF_FAT	GO:0022838	substrate specific channel activity	7,17E-01	1.0E2
	GO:0015267	channel activity	7,24E-01	1.0E2
	GO:0022803	passive transmembrane transporter activity	7,24E-01	1.0E2
		GRIK5, CACNA1H, KCNK1		

sheet 2: genes not clustered

Category	Term		Genes	FDR	P-Value
	GO:0009719	response to endogenous stimulus	AKT1, GRB2, STAT5A, STAT5B, NR4A2, STAT3	2,70E-04	2,67E+11
	GO:0010033	response to organic substance	MSX2, AKT1, GRB2, STAT5A, STAT5B, NR4A2, EPHX1, STAT3	1,90E-03	1,92E-03
	GO:0051056	regulation of small GTPase mediated signal transduction	CSF1, TBC1D5, ASAP2, MGC166429, RAPGEF1, TBC1D22A, TBC1D9B	4,10E-03	4,08E-03
	GO:0040014	regulation of multicellular organism growth	STAT5A, CSF1, STAT5B, STAT3	5,20E-03	5,23E-03
	GO:0046578	regulation of Ras protein signal transduction	CSF1, TBC1D5, ASAP2, MGC166429, TBC1D22A, TBC1D9B	6,30E-03	6,34E-03
	GO:0045137	development of primary sexual characteristics	FOXJ1, STAT5A, STAT5B, DHCR24	6,90E-03	6,92E-03
	GO:0007167	enzyme linked receptor protein signaling pathway	MSX2, EPHA4, GRB2, STAT5A, STAT5B, STAT3	9,20E-03	9,23E-03
	GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	EPHA4, GRB2, STAT5A, STAT5B, STAT3	1,10E-02	1,06E-02
	GO:0003006	reproductive developmental process	FOXJ1, STAT5A, CSF1, STAT5B, DHCR24	1,20E-02	1,22E-02
	GO:0007243	protein kinase cascade	GRB2, STAT5A, STAT5B, PDE6G, STAT3	1,40E-02	1,40E-02
	GO:0007548	sex differentiation	FOXJ1, STAT5A, STAT5B, DHCR24	1,50E-02	1,45E-02
	GO:0000226	microtubule cytoskeleton organization	PTK2, TUBGCP6, TEKT4, TUBGCP2	1,80E-02	1,83E-02
	GO:0007010	cytoskeleton organization	ACTB, PTK2, TUBGCP6, TEKT4, TUBGCP2, RHOF	2,00E-02	1,97E-02
	GO:0046661	male sex differentiation	STAT5A, STAT5B, DHCR24	2,10E-02	2,08E-02
	GO:0030030	cell projection organization	EPHA4, PTK2, BAIAP2, NR4A2, TEKT4	2,20E-02	2,17E-02
	GO:0032989	cellular component morphogenesis	ACTB, EPHA4, PTK2, NR4A2, NFATC1	3,00E-02	2,99E-02
	GO:0000904	cell morphogenesis involved in differentiation	EPHA4, PTK2, NR4A2, NFATC1	3,40E-02	3,39E-02
GOTERM_BP_FAT	GO:0045596	negative regulation of cell differentiation	PPARA, PTK2, STAT5A, STAT5B	3,50E-02	3,50E-02
	GO:0040008	regulation of growth	PTK2, STAT5A, CSF1, STAT5B, STAT3	3,70E-02	3,72E-02
	GO:0006575	cellular amino acid derivative metabolic process	PAOX, STAT5A, STAT5B, NR4A2	4,40E-02	4,35E-02
	GO:0008406	gonad development	FOXJ1, STAT5A, STAT5B	4,90E-02	4,87E-02
	GO:0019216	regulation of lipid metabolic process	PPARA, STAT5A, STAT5B	4,90E-02	4,87E-02
	GO:0045597	positive regulation of cell differentiation	METRN, STAT5A, CSF1, STAT5B	5,00E-02	5,02E-02
	GO:0006468	protein amino acid phosphorylation	AKT1, EPHA4, PTK2, MAPK12, STAT5A, STAT5B, MAPK11, PDE6G, AATK	5,70E-02	5,68E-02
	GO:0048608	reproductive structure development	FOXJ1, STAT5A, STAT5B	6,10E-02	6,08E-02
	GO:0044271	nitrogen compound biosynthetic process	TPK1, ADSSL1, ENTPD8, ATP1A3, NR4A2, PRPSAP2	7,60E-02	7,64E-02
	GO:0000902	cell morphogenesis	EPHA4, PTK2, NR4A2, NFATC1	7,60E-02	7,64E-02
	GO:0008544	epidermis development	AKT1, PPARA, DHCR24	7,60E-02	7,64E-02
	GO:0006793	phosphorus metabolic process	PTPN7, AKT1, EPHA4, PTK2, MAPK12, PTPRG, STAT5A, STAT5B, MAPK11, PDE6G, AATK	7,70E-02	7,71E-02
	GO:0006796	phosphate metabolic process	PTPN7, AKT1, EPHA4, PTK2, MAPK12, PTPRG, STAT5A, STAT5B, MAPK11, PDE6G, AATK	7,70E-02	7,71E-02
	GO:0006357	regulation of transcription from RNA polymerase II promoter	PPARA, STAT5A, STAT5B, HDAC10, NR4A2, STAT3	8,10E-02	8,06E-02
	GO:0007398	ectoderm development	AKT1, PPARA, DHCR24	8,50E-02	8,46E-02
	GO:0006790	sulfur metabolic process	TPK1, STAT5A, STAT5B	8,50E-02	8,46E-02

GO:0006417	regulation of translation	AKT1, EEFSEC, SRP9	8,70E-02	8,74E-02
GO:0051094	positive regulation of developmental process	METRN, STAT5A, CSF1, STAT5B	8,90E-02	8,86E-02
GO:0006631	fatty acid metabolic process	PPARA, STAT5A, STAT5B, ECHS1	9,00E-02	9,04E-02
GO:0030182	neuron differentiation	EPHA4, PTK2, NR4A2, STAT3	1,00E-01	1,03E-01
GO:0016310	phosphorylation	AKT1, EPHA4, PTK2, MAPK12, STAT5A, STAT5B, MAPK11, PDE6G, AATK	1,10E-01	1,12E-01
GO:0007017	microtubule-based process	PTK2, TUBGCP6, TEKT4, TUBGCP2	1,30E-01	1,28E-01
GO:0010628	positive regulation of gene expression	PPARA, STAT5A, CSF1, STAT5B, NR4A2	1,40E-01	1,41E-01
GO:0010604	positive regulation of macromolecule metabolic process	AKT1, PPARA, STAT5A, CSF1, STAT5B, NR4A2	1,60E-01	1,56E-01
GO:0008284	positive regulation of cell proliferation	FGF18, STAT5A, CSF1, STAT5B	1,70E-01	1,71E-01
GO:0010608	posttranscriptional regulation of gene expression	AKT1, EEFSEC, SRP9	1,80E-01	1,85E-01
GO:0032268	regulation of cellular protein metabolic process	AKT1, CSF1, EEFSEC, SRP9	2,00E-01	2,00E-01
GO:0007610	behavior	EPHA4, ATP1A3, NR4A2, STAT3	2,00E-01	2,04E-01
GO:0032940	secretion by cell	EXOC7, LMF1, SCRN1	2,20E-01	2,19E-01
GO:0009100	glycoprotein metabolic process	POMGNT1, RPN1, DHCR24	2,30E-01	2,29E-01
GO:0042127	regulation of cell proliferation	MSX2, FGF18, STAT5A, CSF1, STAT5B	2,50E-01	2,55E-01
GO:0046903	secretion	EXOC7, LMF1, SCRN1	3,10E-01	3,05E-01
GO:0009967	positive regulation of signal transduction	FGF18, CSF1, PDE6G	3,20E-01	3,19E-01
GO:0010647	positive regulation of cell communication	FGF18, CSF1, PDE6G	3,40E-01	3,40E-01
GO:0006355	regulation of transcription, DNA-dependent	MSX2, PPARA, ZFP90, STAT5A, TBX4, STAT5B, HDAC10, NR4A2, STAT3, NFATC1	3,90E-01	3,91E-01
GO:0048609	reproductive process in a multicellular organism	CYLC2, STAT5A, STAT5B	4,00E-01	4,00E-01
GO:0032504	multicellular organism reproduction	CYLC2, STAT5A, STAT5B	4,00E-01	4,00E-01
GO:0043085	positive regulation of catalytic activity	CSF1, NR4A2, PDE6G	4,10E-01	4,07E-01
GO:0051252	regulation of RNA metabolic process	MSX2, PPARA, ZFP90, STAT5A, TBX4, STAT5B, HDAC10, NR4A2, STAT3, NFATC1	4,10E-01	4,11E-01
GO:0042592	homeostatic process	STAT5A, CSF1, STAT5B, NARFL, STAT3	4,20E-01	4,20E-01
GO:0044093	positive regulation of molecular function	CSF1, NR4A2, PDE6G	4,90E-01	4,86E-01
GO:0006350	transcription	PPARA, STAT5A, STAT5B, NR4A2, STAT3, NFATC1	4,90E-01	4,89E-01
GO:0007242	intracellular signaling cascade	GRB2, STAT5A, STAT5B, PDE6G, RHOF, STAT3	5,00E-01	5,00E-01
GO:0006811	ion transport	ATP1A3, GRIK5, CACNA1H, KCNK1, SLC5A10, NFATC1	5,20E-01	5,23E-01
GO:0045449	regulation of transcription	MSX2, PPARA, TRMU, ZFP90, STAT5A, TBX4, STAT5B, NARFL, HDAC10, NR4A2, STAT3, NFATC1	5,30E-01	5,34E-01
GO:0015672	monovalent inorganic cation transport	ATP1A3, KCNK1, SLC5A10	5,80E-01	5,82E-01
GO:0008104	protein localization	RANBP17, LMF1, SEC61A1, SRP9, DHCR24	6,00E-01	6,04E-01
GO:0006812	cation transport	ATP1A3, KCNK1, SLC5A10, NFATC1	6,50E-01	6,54E-01
GO:0016192	vesicle-mediated transport	CALY, EXOC7, SCRN1	6,90E-01	6,91E-01
GO:0050877	neurological system process	PTK2, ATP1A3, PDE6G	7,00E-01	6,99E-01
GO:0007166	cell surface receptor linked signal transduction	CALY, GRB2, STAT5A, STAT5B, ATP1A3, GPR132, CELSR1, STAT3, MSX2, EPHA4, SSTR5, WNT7B, GRM7	7,20E-01	7,21E-01
GO:0015031	protein transport	RANBP17, LMF1, SEC61A1, SRP9	7,30E-01	7,33E-01

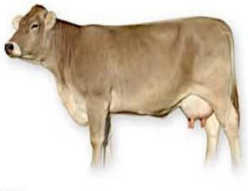
	GO:0045184	establishment of protein localization	RANBP17, LMF1, SEC61A1, SRP9	7,30E-01	7,35E-01
	GO:0030001	metal ion transport	KCNK1, SLC5A10, NFATC1	7,50E-01	7,51E-01
	GO:0009057	macromolecule catabolic process	AKT1, UBE2T, DHCR24	8,20E-01	8,15E-01
	GO:0055085	transmembrane transport	CACNA1H, SLC5A10, SEC61A1	8,80E-01	8,84E-01
	GO:0006508	proteolysis	ADAM8, UBE2T, DHCR24	9,80E-01	9,82E-01
	GO:0007186	G-protein coupled receptor protein signaling pathway	SSTR5, CALY, GRM7, GPR132, CELSR1	1,00E+00	9,97E-01
	GO:0031252	cell leading edge	AKT1, CTTN, PTK2, BAIAP2	6,60E-03	6,59E-03
	GO:0005938	cell cortex	ACTB, CTTN, EXOC7, CALD1	2,10E-02	2,13E-02
	GO:0030027	lamellipodium	AKT1, CTTN, PTK2	2,40E-02	2,39E-02
	GO:0042995	cell projection	AKT1, CTTN, PTK2, BAIAP2, FSCN1, TEKT4	3,30E-02	3,27E-02
	GO:0005856	cytoskeleton	AKT1, ACTB, CYLC2, PTK2, TUBGCP6, EXOC7, CALD1, TEKT4, TUBGCP2, RHOF	3,80E-02	3,83E-02
	GO:0044448	cell cortex part	ACTB, EXOC7, CALD1	5,70E-02	5,66E-02
	GO:0005819	spindle	AKT1, TUBGCP6, TUBGCP2	8,80E-02	8,84E-02
	GO:0015630	microtubule cytoskeleton	AKT1, TUBGCP6, EXOC7, TEKT4, TUBGCP2	1,00E-01	9,98E-02
	GO:0044430	cytoskeletal part	AKT1, CYLC2, TUBGCP6, EXOC7, CALD1, TEKT4, TUBGCP2	1,00E-01	1,03E-01
	GO:0005815	microtubule organizing center	TUBGCP6, EXOC7, TUBGCP2	1,50E-01	1,54E-01
	GO:0048471	perinuclear region of cytoplasm	CYLC2, ATXN10, CSF1	2,10E-01	2,06E-01
	GO:0043228	non-membrane-bounded organelle	AKT1, ACTB, FGF18, CYLC2, PTK2, TUBGCP6, EXOC7, CALD1, NPM1, TEKT4, TUBGCP2, RHOF	3,10E-01	3,12E-01
	GO:0043232	intracellular non-membrane-bounded organelle	AKT1, ACTB, FGF18, CYLC2, PTK2, TUBGCP6, EXOC7, CALD1, NPM1, TEKT4, TUBGCP2, RHOF	3,10E-01	3,12E-01
GOTERM_CC_FAT	GO:0005654	nucleoplasm	ACTB, NPM1, HDAC10, GEMIN7	3,70E-01	3,66E-01
	GO:0031981	nuclear lumen	ACTB, FGF18, NPM1, HDAC10, GEMIN7	4,50E-01	4,50E-01
	GO:0012505	endomembrane system	POMGNT1, GRB2, SCRN1, SEC61A1	4,60E-01	4,62E-01
	GO:0005886	plasma membrane	AKT1, PTK2, CALY, CALD1, FSCN1, ENTPD8, GRIK5, CELSR1, RHOF, NTM, STAT3	4,90E-01	4,89E-01
	GO:0044451	nucleoplasm part	ACTB, HDAC10, GEMIN7	5,50E-01	5,49E-01
	GO:0000267	cell fraction	ACTB, CALD1, ENTPD8	5,70E-01	5,73E-01
	GO:0005783	endoplasmic reticulum	PGS1, LMF1, RPN1, SEC61A1	7,00E-01	6,98E-01
	GO:0005739	mitochondrion	PGS1, TRMU, TSPO, AGPAT5, ECHS1, MP68	7,10E-01	7,14E-01
	GO:0031090	organelle membrane	POMGNT1, GRB2, SCRN1, SEC61A1	8,20E-01	8,22E-01
	GO:0005576	extracellular region	TG, FGF18, WNT7B, METRN, FOXJ1, IL1RN	8,60E-01	8,61E-01
	GO:0031224	intrinsic to membrane	TSPO, CALY, CSF1, LMF1, SPPL2B, GRIK5, ATP1A3, GPR132, KCNK1, EPHA4, SSTR5, POMGNT1, TSPAN10, TECRL, GRM7, ENTPD8, RPN1, CACNA1H, SLC5A10, SEC61A1, NTM, AATK	8,80E-01	8,85E-01
	GO:0016021	integral to membrane	TSPO, CALY, CSF1, LMF1, SPPL2B, ATP1A3, GRIK5, GPR132, KCNK1, EPHA4, SSTR5, POMGNT1, TSPAN10, TECRL, GRM7, ENTPD8, RPN1, CACNA1H, SLC5A10, SEC61A1, AATK	9,00E-01	8,98E-01
	GO:0044459	plasma membrane part	PTK2, CALY, GRIK5, RHOF	9,40E-01	9,37E-01
GOTERM_MF_FAT	GO:0008047	enzyme activator activity	NOXA1, TBC1D5, ASAP2, TBC1D22A, TBC1D9B	3,20E-02	3,23E-02
	GO:0030695	GTPase regulator activity	TBC1D5, ASAP2, MGC166429, RAPGEF1, TBC1D22A, TBC1D9B	4,40E-02	4,38E-02

GO:0005083	small GTPase regulator activity	TBC1D5, ASAP2, MGC166429, TBC1D22A, TBC1D9B	4,50E-02	4,52E-02
GO:0060589	nucleoside-triphosphatase regulator activity	TBC1D5, ASAP2, MGC166429, RAPGEF1, TBC1D22A, TBC1D9B	4,90E-02	4,92E-02
GO:0019904	protein domain specific binding	PTK2, GRB2, SQSTM1, BAIAP2	6,30E-02	6,27E-02
GO:0017076	purine nucleotide binding	ACTB, PGS1, ADSSL1, TDRD9, ATP1A3, MAPK11, PDE6G, TPK1, AKT1, EPHA4, MAPK12, PARS2, ENTPD8, RUVBL1, EEFSEC, RHOF, UBE2T, DHCR24, AATK	6,80E-02	6,80E-02
GO:0001883	purine nucleoside binding	ACTB, PGS1, TDRD9, ATP1A3, MAPK11, PDE6G, TPK1, AKT1, EPHA4, MAPK12, PARS2, ENTPD8, RUVBL1, UBE2T, DHCR24, AATK	7,20E-02	7,18E-02
GO:0001882	nucleoside binding	ACTB, PGS1, TDRD9, ATP1A3, MAPK11, PDE6G, TPK1, AKT1, EPHA4, MAPK12, PARS2, ENTPD8, RUVBL1, UBE2T, DHCR24, AATK	7,50E-02	7,46E-02
GO:0032553	ribonucleotide binding	ACTB, PGS1, ADSSL1, TDRD9, ATP1A3, MAPK11, PDE6G, TPK1, AKT1, EPHA4, MAPK12, PARS2, ENTPD8, RUVBL1, EEFSEC, RHOF, UBE2T, AATK	8,30E-02	8,30E-02
GO:0032555	purine ribonucleotide binding	ACTB, PGS1, ADSSL1, TDRD9, ATP1A3, MAPK11, PDE6G, TPK1, AKT1, EPHA4, MAPK12, PARS2, ENTPD8, RUVBL1, EEFSEC, RHOF, UBE2T, AATK	8,30E-02	8,30E-02
GO:0000166	nucleotide binding	ACTB, PGS1, ADSSL1, SETD1B, TDRD9, ATP1A3, MAPK11, PDE6G, TPK1, AKT1, EPHA4, NAV1, MAPK12, PARS2, ENTPD8, RUVBL1, EEFSEC, RHOF, UBE2T, DHCR24, AATK	9,40E-02	9,38E-02
GO:0030554	adenyl nucleotide binding	ACTB, PGS1, TDRD9, ATP1A3, MAPK11, TPK1, AKT1, EPHA4, MAPK12, PARS2, ENTPD8, RUVBL1, UBE2T, DHCR24, AATK	1,20E-01	1,17E-01
GO:0003700	transcription factor activity	MSX2, PPARA, STAT5A, TBX4, STAT5B, NR4A2, STAT3, NFATC1	1,20E-01	1,24E-01
GO:0005524	ATP binding	ACTB, PGS1, TDRD9, ATP1A3, MAPK11, TPK1, AKT1, EPHA4, MAPK12, PARS2, ENTPD8, RUVBL1, UBE2T, AATK	1,40E-01	1,38E-01
GO:0032559	adenyl ribonucleotide binding	ACTB, PGS1, TDRD9, ATP1A3, MAPK11, TPK1, AKT1, EPHA4, MAPK12, PARS2, ENTPD8, RUVBL1, UBE2T, AATK	1,40E-01	1,45E-01
GO:0008083	growth factor activity	FGF18, FOXJ1, CSF1	2,10E-01	2,07E-01
GO:0016563	transcription activator activity	PPARA, NR4A2, STAT3	2,60E-01	2,65E-01
GO:0030528	transcription regulator activity	MSX2, PPARA, STAT5A, TBX4, STAT5B, HDAC10, NR4A2, STAT3, NFATC1	3,30E-01	3,35E-01
GO:0005509	calcium ion binding	STAT5A, SCUBE1, ENTPD8, STAT5B, CELSR1, STAT3, TBC1D9B	3,60E-01	3,57E-01
GO:0016879	ligase activity, forming carbon-nitrogen bonds	ADSSL1, UBE2T, TTLL12	3,90E-01	3,89E-01
GO:0019899	enzyme binding	SQSTM1, HDAC10, STAT3	4,70E-01	4,65E-01
GO:0004674	protein serine/threonine kinase activity	AKT1, MAPK12, MAPK11, AATK	4,80E-01	4,78E-01
GO:0032561	guanyl ribonucleotide binding	ADSSL1, EEFSEC, PDE6G, RHOF	5,10E-01	5,08E-01
GO:0019001	guanyl nucleotide binding	ADSSL1, EEFSEC, PDE6G, RHOF	5,10E-01	5,15E-01
GO:0004672	protein kinase activity	AKT1, EPHA4, MAPK12, MAPK11, AATK	5,50E-01	5,49E-01
GO:0043565	sequence-specific DNA binding	MSX2, PPARA, NR4A2, NFATC1	5,60E-01	5,62E-01
GO:0046983	protein dimerization activity	CSF1, NR4A2, STAT3	6,00E-01	6,02E-01
GO:0008092	cytoskeletal protein binding	BAIAP2, CALD1, FSCN1	6,00E-01	6,02E-01
GO:0003723	RNA binding	NPM1, RPUSD1, EEFSEC, SRP9	6,20E-01	6,18E-01
GO:0003677	DNA binding	MSX2, PPARA, TRMU, STAT5A, TBX4, STAT5B, NR4A2, SOX8, STAT3, NFATC1	6,30E-01	6,25E-01

	GO:0042802	identical protein binding	ACTB, PTPRG, CSF1	6,70E-01	6,72E-01
	GO:0005525	GTP binding	ADSSL1, EEFSEC, RHOF	7,40E-01	7,44E-01
	GO:0008270	zinc ion binding	PPARA, NPLOC4, TRMU, SQSTM1, ZFP90, ZNF296, NR4A2, ASAP2, HAGHL, ADAM8	9,00E-01	8,99E-01
	GO:0046914	transition metal ion binding	PPARA, NPLOC4, TRMU, POMGNT1, SQSTM1, ZFP90, ZNF296, NR4A2, ASAP2, HAGHL, ADAM8, HPD	9,30E-01	9,30E-01
KEGG_PATHWAY	bta04370	VEGF signaling pathway	AKT1, PTK2, MAPK12, MAPK11, NFATC2, NFATC1	1,30E-03	1,30E-03
	bta04660	T cell receptor signaling pathway	AKT1, MAPK12, GRB2, MAPK11, NFATC2, NFATC1	6,40E-03	6,37E-03
	bta04012	ErbB signaling pathway	AKT1, PTK2, GRB2, STAT5A, STAT5B	1,10E-02	1,14E-02
	bta05200	Pathways in cancer	AKT1, FGF18, WNT7B, PTK2, RASSF5, GRB2, STAT5A, STAT5B, STAT3	2,00E-02	1,97E-02
	bta04010	MAPK signaling pathway	PTPN7, AKT1, FGF18, MAPK12, GRB2, CACNA1H, MAPK11, NFATC2	2,50E-02	2,49E-02
	bta04670	Leukocyte transendothelial migration	ACTB, PTK2, RASSF5, MAPK12, MAPK11	3,70E-02	3,73E-02
	bta04360	Axon guidance	EPHA4, PTK2, PLXNB2, NFATC2, NFATC1	3,90E-02	3,94E-02
	bta04662	B cell receptor signaling pathway	AKT1, GRB2, NFATC2, NFATC1	4,30E-02	4,27E-02
	bta04062	Chemokine signaling pathway	AKT1, PTK2, GRB2, STAT5B, GNG13, STAT3	4,30E-02	4,32E-02
	bta04722	Neurotrophin signaling pathway	AKT1, MAPK12, GRB2, MAPK11, RAPGEF1	4,40E-02	4,39E-02
	bta04664	Fc epsilon RI signaling pathway	AKT1, MAPK12, GRB2, MAPK11	4,90E-02	4,92E-02
	bta05220	Chronic myeloid leukemia	AKT1, GRB2, STAT5A, STAT5B	5,10E-02	5,08E-02
	bta05223	Non-small cell lung cancer	AKT1, RASSF5, GRB2	1,10E-01	1,10E-01
	bta04510	Focal adhesion	AKT1, ACTB, PTK2, GRB2, RAPGEF1	1,50E-01	1,50E-01
	bta04920	Adipocytokine signaling pathway	AKT1, PPARA, STAT3	1,60E-01	1,59E-01
	bta04530	Tight junction	AKT1, ACTB, EPB41L3, CTTN	1,60E-01	1,60E-01
	bta04810	Regulation of actin cytoskeleton	ACTB, FGF18, ENAH, PTK2, BAIAP2	1,70E-01	1,67E-01
	bta05211	Renal cell carcinoma	AKT1, GRB2, RAPGEF1	1,70E-01	1,68E-01
	bta04910	Insulin signaling pathway	AKT1, EXOC7, GRB2, RAPGEF1	1,70E-01	1,68E-01
	bta04310	Wnt signaling pathway	WNT7B, RUVBL1, NFATC2, NFATC1	2,20E-01	2,17E-01
	bta04914	Progesterone-mediated oocyte maturation	AKT1, MAPK12, MAPK11	2,30E-01	2,34E-01
	bta04912	GnRH signaling pathway	MAPK12, GRB2, MAPK11	2,70E-01	2,68E-01
	bta04620	Toll-like receptor signaling pathway	AKT1, MAPK12, MAPK11	2,90E-01	2,89E-01
	bta04650	Natural killer cell mediated cytotoxicity	GRB2, NFATC2, NFATC1	3,30E-01	3,35E-01
	bta00230	Purine metabolism	ADSSL1, ENTPD8, PDE6G	5,10E-01	5,08E-01
	bta04080	Neuroactive ligand-receptor interaction	SSTR5, TSPO, GRM7, GRIK5	5,10E-01	5,14E-01

4

GENERAL DISCUSSION



The aim of the animal genetic improvement in livestock production is to change genetic frequency of genes related to traits of economic interest in order to maximize their phenotypic amount and thus the revenue for the farmer.
In the last decades, selection objectives aimed at optimization of qualitative-quantitative aspects of milk production and functional characterists (e.g. longevity, fertility, mastitis resistance).
In particular, the marker-assisted selection (MAS) approach involves selecting individuals based on their genotype at specific loci. This approach is known to be particularly beneficial when the traits of interest are difficult or expensive to measure (Boichard et al., 2006).
Genomic selection (GS), defined by Meuwissen (2007) as MAS on a genome-wide scale, is a new and important tool for the genetic improvement of livestock that allows to estimate direct genomic values (DGV) of candidates using dense marker maps without need to record the phenotypic performances of the animals (Meuwissen et al., 2001).
The QTL involved in susceptibility/resistance to infectious diseases and in the productive traits variations, are characterized by genetic heterogeneity and multifactorial inheritance, involving gene polymorphisms from different alternative pathways. With the accessibility of the single nucleotide polymorphism (SNP) array, the linkage analysis and the genome-wide association study (GWAS) has been frequently used to study the genetic component of complex trait, and using the data from the same SNP array, the Ccpy number variations (CNVs) can be identified.
In the studies included in this PhD thesis, using a selective DNA pooling approach, the first QTL mapping were performed for CLA, VA, D9D and for SCS, in Italian Brown Swiss and in Valdostana Red Pied cattle populations, resulting in the identification of various

genomic regions associated with the traits. These genomic regions will help to understand which potential candidate genes may be responsible for the genetic variation in milk fat composition and in mastitis resistance/susceptibility.
In addition, the thesis include a genome scan study performed in bulls of Italian Brown Swiss breed, for the identification of CNVs, providing a rich source of additional genetic variation in this breed.
The studies included in this PhD thesis integrate the knowledge available about QTL association with fatty acids and resistance to mastitis, and their possible use in MAS and in GS. In addition, the CNVs identified in this study, will enrich the bovine CNV map in the cattle genome, providing new information for association studies with traits of economic and healthy interest.

The results were shared with the respective Breeders Associations allowing the results of these studies to be applied in ongoing selection process in the populations.

The information delivered to the scientific community allow a step forward in the advancement of the state of the art and constitute a solid base for additional scientific research in structural variantion studies in cattle (CNV) and association analysis for health and productive traits (SCC and FA).

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5

INTERNATIONAL AND NATIONAL CONFERENCES

Abstracts of poster presentations

5.I.I. Abstract number: P-065

QUANTITATIVE TRAIT LOCI MAPPING FOR MILK FATTY ACIDS IN ITALIAN BROWN SWISS DAIRY CATTLE BREED

Alessandro Bagnato[1], Erika Frigo[1], Fabiola Canavesi[1], Fausta Schiavini[1], Morris Soller[2], Ehud Lipkin[2], Ruth Tal-Stein[2], Yechezkel Kashi[3], Eyal Shimoni[3], Yael Ungar[3], **Maria Giuseppina Strillacci**[1]

[1]Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Università di Milano, Italy; [2]Department of Genetics. The Hebrew University of Jerusalem, Israel; [3]Faculty of Biotechnology & Food Engineering, Technion-Israel, Institute of Technology, Technion City, 32000 Haifa, Israel.

The detection of genomic regions affecting complex traits has leaded the interest in using large panels of single nucleotide polymorphisms (SNPs) to identify quantitative trait loci (QTL). Selective DNA pooling strategy is a method to reduce costs in genomic studies by genotyping pooled DNA samples from selected individuals at each of the two phenotypic extremes. The identification of genomic regions responsible for genetic variation in milk fat composition may help to understand the biological pathways involved in fatty acid synthesis. In this study, a selective DNA pooling approach in Italian Brown Swiss cattle was applied to identify QTLs for Δ9-Desaturase (D9D), conjugated linoleic and vaccenic (CLA and VA) acids. A total of 120 daughters for each of the five selected families (60 animals with higher residual values and 60 animal with lower residual values) were pooled and genotyped using Illumina Bovine SNP50 BeadChip. In this study, the generation of B-allele frequency was performed

automatically using the self-normalization algorithm of Illumina BeadStudio software. Statistical analysis was performed with respect to SNPs for which the sires were heterozygous. Using the R software a procedure has been implemented in order to perform a single marker sire test. A multiple testing correction was applied using the proportion of false positives (PFP) among all positive test results. Association tests were carried out in order to identify genes with an important role in pathways for milk fat and fatty acids metabolism. Several chromosome regions were significantly associated with the traits studied, being some of these regions harboring genes known to be involved in fat synthesis as reported in literature.

5.I.2. Abstract number: P-048

SOME OF THE MAIN RESULTS OF QUANTOMICS EU PROJECT: CNV DETECTION AND GWA ANALYSIS IN THE ITALIAN BROWN SWISS DAIRY CATTLE.

Laura Pellegrino*, Marlies A. Dolezal*, Christian Maltecca#, Dinesh Velayutham*, **Maria Giuseppina Strillacci***, Erika Frigo*, Karin Schlangen*, Antonia B. Samoré*, Fausta Schiavini*, Enrico Santus°, Chris Warkup^, Alessandro Bagnato *

*Dept. VESPA, Università degli Studi di Milano, Milan, Italy. #NC State University, Raleigh, NC, USA; °ANARB, Verona, Italy; ^Biosciences KTN, Edinburgh, UK.

Amongst Quantomics results here we present identified QTL regions for mastitis resistance and a medium resolution map of CNVRs obtained in the Italian Brown Swiss. A total number of 1,489 bulls were genotyped on Illumina's BovineSNP50 BeadChip on UMD3.1 autosomes and a subset of 1,342 bulls were used for CNV detection. Among these, 192 bulls were genotyped on Illumina's HD chip (777k) and utilized for GWA analysis jointly with the remaining bulls which genotypes were imputed to Illumina's HD chip interrogating 735,238 loci. PennCNV and SVS7 software were used

for CNVs detection for a total of 46,728 loci. We corrected for GC score and wave factor and employed PCA for SVS7 to correct for technical background noise to reduce false positive calls. PennCNV and SVS7 CNVs results were summarized into 1,101 (220 losses, 774 gains, 107 complex), and 277 (185 losses, 56 gains and 36 complex) CNVRs on 651 bulls, respectively. The consensus between the CNV scans was obtained using the Redon et al. (2006), and Wain et al. (2009) approaches, covering 146 Mb (5.88%) and 17.1 Mb (0.68%), respectively. CNVRs were annotated with the bovine Ensembl gene set v69 and tested for enrichment of GO terms using DAVID database. Consensus CNVRs were enriched for protein-coding genes. GO analysis identified genes in the CNVRs related to cytoplasm, intercellular part, cellular processes, cytoplasmic part, and intracellular organelles. For the GWA analysis, after data filtering, a total of 35,566 SNPs were retained for with MAF >0.02, call rate >0.90 at SNP and bull level. Stratification in the population was corrected for PCA. Success of correction for stratification was empirically assessed based on Q-Q plots of expected versus observed P-values. We employed single SNP regression and multiple SNP regression in sliding windows of three to five SNPs. Significance was declared employing a false discovery rate approach. Several QTL regions were found across the genome. The most interesting regions were located on BTA1, BTA4, BTA7, BTA13, BTA16, BTA20, BTA21 and BTA27. Acknowledgement. This study was funded by EC-FP7/2007-2013, agreement n°222664, "Quantomics".

5.2. 64th ANNUAL MEETING OF THE EUROPEAN FEDERATION OF ANIMAL SCIENCE (EAAP). NANTES 26-30 August 2013

5.2.1. Abstract number: 17437

A MEDIUM RESOLUTION SNP ARRAY BASED CNV SCAN IN ITALIAN BROWN SWISS DAIRY CATTLE

L. Pellegrino[1], M.A. Dolezal[1], C. Maltecca[2], D. Velayutham[1], **M.G. Strillacci**[1], E. Frigo[1], K. Schlangen[1], A.B. Samoré[1], F. Schiavini[1], E. Santus[3], C. Warkup[4], A. Bagnato[1]

[1]University of Milan, Via Celoria 10, 20133 Milano, Italy; [2]North Carolina State University, Box 7621, NC 27695-7621, Raleigh, USA; [3]ANARB, Loc. Ferlina, 37012, Bussolengo (VR), Italy; [4]Biosciences KTN, Easter Bush, Midlothian, EH25 9RG, Roslin, United Kingdom.

Recent reports indicate copy number variations (CNVs) to be functionally significant. This study presents a medium resolution map of CNV regions (CNVRs) in the Italian Brown Swiss dairy cattle, from - to this day - the largest CNV genome scan in any cattle breed. We genotyped 1,489 bulls and after quality filtering on males we called CNVs with PennCNV and with "Copy Number Analysis Module" (CNAM) of SVS7 software (Goldenhelix) for a total of 46,728 loci anchored on the UMD3.1 assembly on 983 and 561 bulls respectively. We corrected for sequence composition flanking each SNP and employed principal component analysis for CNAM to correct for technical background noise to reduce false positive calls. PennCNV and SVS7 identified a total of 4,501 and 1,289 CNVs segregating in 983 and 559 bulls respectively. These were summarized at the population level into 483 (401 losses, 61 gains, 21 complex) and 277 (185 losses, 56 gains and 36 complex) CNVRs, covering

114 Mb (4.59%) and 33.7 Mb (1.35%) of the autosome, respectively. We then obtained the consensus between the two CNV scans using the approaches suggested by Redon et al. (2006), union set, and by Wain et al. (2009), intersection, covering 37.7 Mb (1.51%.) and 13.4 Mb (0.54%), respectively. CNVRs were annotated with the bovine Ensembl gene set v69 and tested for enrichment of GO terms using DAVID database. Consensus CNVRs are enriched for protein-coding genes and genes involved in MHC class II protein complex and VEGF signalling pathway. Acknowledgement: This study funded by EC-FP7, agreement n°222664, "Quantomics".

5.2.2. Abstract number: 17542

MAPPING QTL FOR FATTY ACIDS IN ITALIAN BROWN SWISS BREED USING A SELECTIVE DNA POOLING

M.G. Strillacci[1], E. Frigo[1], F. Canavesi[1], F. Schiavini[1], M. Soller[2], E. Lipkin[2], R. Tal-Stein[2], Y. Kashi[3], E. Shimoni[3], Y. Ungar[3], A. Bagnato[1]

[1]Facoltà di Medicina Veterinaria, Università di Milano, Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e Sicurezza Alimentare, Via Celoria 10, Milano, 20133, Italy; [2]The Hebrew University of Jerusalem, Department of Genetics, Jerusalem, 91904 , Israel; [3]Faculty of Biotechnology & Food Engineering, Technion-Israel, Institute of Technology, Technion City , 32000 Haifa, Israel.

A selective DNA pooling approach in a daughter design was applied to perform a GWA in Italian Brown Swiss cattle, to identify QTLs for Δ9desaturase (D9D), conjugated linoleic and vaccenic (CLA and VA) acids. A total of 120 daughters for each of five selected families (60 animals with higher residual values and 60 with lower residual values) were pooled. DNA samples, extracted from sire's semen and milk pools were genotyped using Illumina Bovine SNP50 BeadChip. Statistical analysis was performed with respect to SNPs for which

sires were heterozygous. Using the R software a procedure was implemented to perform a single and multiple marker sire test. A multiple testing correction was applied using the proportion of false positives among all positive test results. Association tests were carried out to identify genes with an important role in pathways for milk fat and fatty acids metabolism. Above all, BTA 19 showed a highly significant association with CLA, VA and D9D. A large number of regions were significantly associated with the studied traits. Some of these regions harboring genes known to be involved in fat synthesis as reported in literature. The feasibility and the effectiveness of a selective DNA pooling approach using Bovine SNP50 BeadChip for the identification of QTLs was underlined in this study. Acknowledgement: This study was part of QuaLAT project financially supported by Regione Lombardia.

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5.3.1. Abstract number: P540

GENOME WIDE ASSOCIATION ANALYSIS ON IMPUTED HIGH-DENSITY SNP GENOTYPES IN THE ITALIAN AND SWISS BROWN SWISS DAIRY CATTLE POPULATION FOR MILK SOMATIC CELL COUNT

Marlies A. Dolezal (*Università degli Studi di Milano*), Birgit Gredler (*Qualitas AG*), Attilio Rossoni (*ANARB - Associazione Nazionale Allevatori di Razza Bruna*), Franz R. (Seefried *Qualitas AG*), Fausta Schiavini (*Università degli Studi di Milano*), **Maria Strillacci** (*Università degli Studi di Milano*), Hossein Jorjani (*Interbull Centre*), Enrico Santus (*ANARB - Associazione Nazionale Allevatori di Razza Bruna*), Alessandro Bagnato (*Università degli Studi di Milano*)

Mastitis is one of the most costly diseases in dairy cattle and a huge concern to animal welfare. Milk Somatic Cell Count (MSCC) is an indirect measure widely used for years to select individuals to reduce mastitis susceptibility in dairy cattle. The purpose of this study was to identify regions underlying phenotypic variation for mastitis resistance in the Brown Swiss dairy cattle population. We report on a whole genome association study on a total of 2,979 mainly Italian-, Swiss- and US-Brown Swiss bulls imputed from Illumina's Bovine 50k v1 and v2 SNP chip with FImpute to Illumina's 777k chip for 628,415 SNPs with MAF > 0.5% anchored on the UMD3.1 autosome. Association testing with MSCC-EBVs for 2,834 bulls with EBV reliability greater than 0.3 provided by Interbull, was performed for 604,568 SNPs with MAF > 2% employing EMMAX as implemented in SVS7.7.8. Stratification was controlled by fitting a genomic relationship matrix, calculated as suggested by VanRaden based on all genome wide SNPs in the model. Success of stratification correction was empirically assessed via quantile-quantile plots. Significance was declared employing a false discovery rate approach. Several QTL regions were found across the genome. The most interesting regions were located on BTA6, BTA10, BTA13 and BTA19. We thank Braunvieh Schweiz and ANARB for providing genotypes and Genotype pool Germany-Austria, Beltsville Agricultural Research Centre and LowInputBreeds, FP7 – project KBBE 222 632 for providing genotypes used for imputation. This study was supported by the FP7 project QUANTOMICS contract n. 222664-2.

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