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TITOLO TESI

IDENTIFICATION OF CNV AND QTL FOR PRODUCTIVE AND FUNCTIONAL TRAITS IN DAIRY CATTLE USING DENSE SNP CHIPS

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GENERAL INTRODUCTION

Genomic Selection in livestock populations

The relatively recent application of genomic selection in cattle (Meuwissen et al 2001, Goddard & Hayes, 2009) is based on the association of genomic variants to phenotypic variation. The identification and knowledge of genomic structural variation associated to phenotypic variability can be used in populations to select individuals and improve the accuracy and efficiency of selection schemes.

The recent advances in sequencing and SNP genotyping technologies, together to reduction in costs, delivered in livestock populations a large amount of genomic data. Projects like the 1000K Bull genome project (Hayes et al. 2013) focussed on the identification of SNP structural variation from genome wide sequence data, disclosing million of mutations in the bovine genome. The possibility to use genotyping by sequencing technologies, as in the 1000k Bull genome project, is driving the methods to improve the accuracy of genomic information, used as inputs in genomic evaluation, at maximum possible: i.e. at individual sequence level. This is the major goal in the genomic evaluation research field, as the accuracy of genomic selection is a key factor in the success of many breeding companies among livestock species.

Nevertheless other source of structural variants may play a role in improving accuracy of genomic selection. Among those the Copy Number Variants (CNV). The disclosure of other structural variants and their possible association with complex trait variation (Redon et al 2006) encouraged the mapping of CNV in cattle populations and the subsequent validation of these structural variants as markers for genomic selection.

Recent studies (Clop et al., 2012) have mapped CNV in several livestock populations indicating them as an additional source of genetic variation that can be possibly used in selection of individuals.

If it is true that genomic selection process is trying to improve its accuracy using SNP genotyping by sequencing and CNV as markers, selection in livestock is also looking towards novel traits.

Selection in the past decades was directed towards improvement of quantity and quality of production. Nowadays functional traits are more and more a priority in selection schemes. Additionally nutritional properties of food, as content of specific fatty acids, are of possible interest for breeding purposes (Stoop et al. 2008)

Identification of genomic region harbouring genes involved in resistance to specific diseases (e.g. mastitis in dairy cattle) or in fatty acid content in milk has been the goal of several research project in the recent past .

Genome Wide Association Studies (GWAS) have been developed in the last decades to identify chromosomal regions linked to genes involved in functional traits and in nutritional properties of food.

Copy Number Variants

The term Copy number variants (CNVs) appeared in a review by Feuk et al. (2006) describing DNA segments, originally bigger than 1 kb, that differ in copy number among individuals. As compared to a reference sample they can be classified as deletions, insertions and duplications (Lee and Scherer, 2010).

CNVs are a structural variation much less abundant than Single Nucleotide Polymorphisms in the genome. However they can affect the DNA sequence up to several megabases making the inter individual variability based on CNVs much higher, as highlighted by Redon et al. (2006) who published the first comprehensive CNV maps in human. Clop et al. (2012) published a review on CNV mapped in the domestic animals including mammals and avian species. Studies on CNV have advanced from simple mapping studies to population genetics of structural variants and in assessing the influence of CNVs on phenotypic variation.

Several studies in humans have shown the possible impact of CNV on susceptibility to certain complex disease and disorders as Crohn’s disease, susceptibility to HIV/AIDS and Kawasaki disease, autism, bipolar disorder, schizophrenia, age-related macular degeneration and cancer (see Estivil and Armengol http://www.nature.com/ejhg/journal/v18/n8/full/ejhg201035a.html - bib4, 2007, Wain et al., 2009; Lee and Scherer, 2010; Almal and Padh, 2011)

As reported in a review by Clop et al, (2012) in livestock coat color is, in several species, partly determined by CNVs.

Array comparative genomic hybridisation (aCGH), has been the first technology available to perform whole genome scans for CNVs in a single experiment. With the decrease in costs of SNP genotyping arrays, these are today’s the main standard for CNV detection. Nonetheless with ever decreasing costs and ever higher output, high throughput sequencing techniques are becoming a major player in the structural variation detection, including CNVs.

SNP array based studies usually compare to the reference population used in the array design or a large reference dataset specific to the population under study. Several algorithms has been developed to map CNV from information delivered by SNP array as Log R Ratio and B Allele Frequency. Among those PennCNV, CNAM (Golden Helix), CNVnator are the most and widely used in CNV mapping studies.

GWAS for Health and Nutritional Traits in Dairy Cattle

Mastitis is one of the major costly disease in dairy cattle causing a loss of about 200 Euro per case of infection. Selection for mastitis resistance is undergoing in cattle populations for over a decade using the Milk Somatic Cell Count (SCC) as indicator of mastitis susceptibility. A large number of studies have mapped Quantitative Trait Loci related to SCC using microsatellite and SNP markers.

More recently interest for bioactive compounds in food have raised the attention of researcher to investigate the genetic bases of nutritional components in milk, as fatty acids content (Stoop et al. 2008). Among the fatty acids, Conjugated Linoleic Acid has been extensively studied in livestock as nutritional component in meat and milk. CLA nutritional properties and their predicted effect on human health has been in fact widely studied.

In livestock a portal grouping a wide range of data bases[1] reporting QTL, candidate gene, association data from GWAS and copy number variations mapped on genomes is available at www.animalgenome.org.

Among the various databases, the Cattle QTLdb, regularly updated, reports about 9,180 QTL for 472 different traits[2]. Among those traits those related to mastitis resistance and to fatty acid content.

QTL for mastitis resistance

The phenotypes widely used to study the resistance to mastitis are the milk somatic cell count (SCC) and its log transformation, somatic cell score (SCS).The reason resides in the possibility to record the measure during functional recording and for the positive and strong genetic correlation with clinical mastitis occurrence that range from .50 to more than .90 according to studies and populations (Rupp and Boichard 2003, Samoré et al. 2008).

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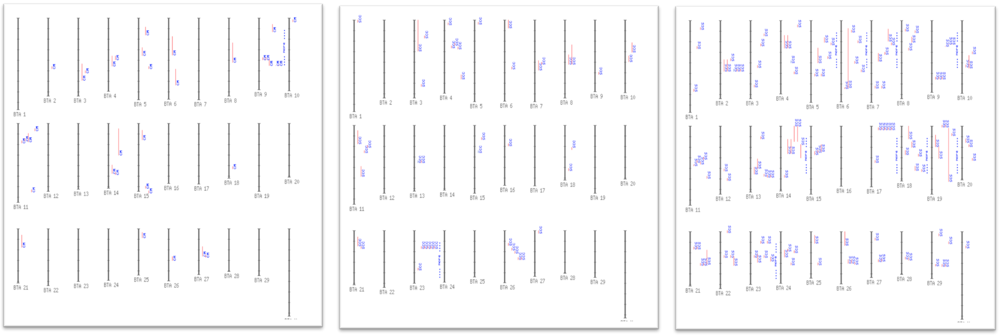
[1] Examples of Livestock animal QTL databases: AnimalQTLdb (http://www.animalgenome.org); Bovine QTL Viewer (http://genomes.sapac.edu.au/bovineqtl/); cgQTL database: QTL for milk production traits in cattle identified from expression experiments (http://cowry.agri.huji.ac.il/QTLMAP/qtlmap.htm).

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

Several authors mapped the QTLs for mastitis resistance (SCC and SCS) and regions have been reported on almost all bovine chromosomes (www.animalgenome.org/QTLdb/)(Figure 1).



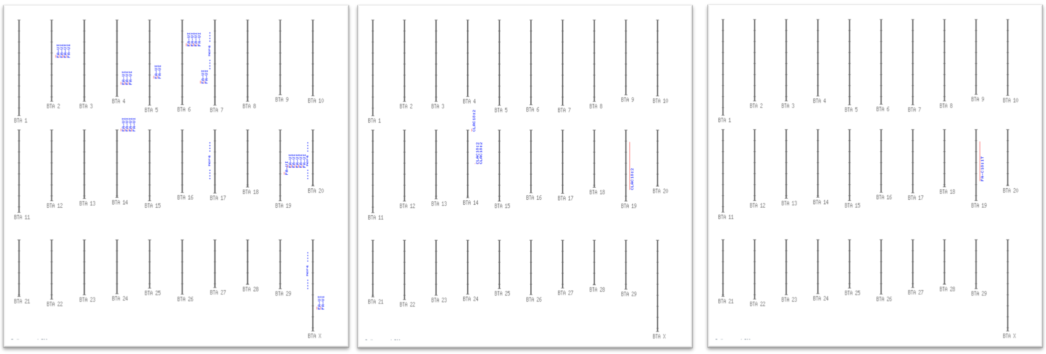
Clinical mastitis (CM) Somatic Cell Count (SCC) Somatic Cell Score (SCS)

Figure 1. Graphical representation of QTL on all bovine chromosomes associated to clinical mastitis (CM), somatic cell count (SCC) and somatic cell score (SCS).

QTL for Fatty Acid Component

Among the bioactive components in milk, the conjugated linoleic acids (CLA) is one of the most studied polyunsaturated fatty acids for its effect on human health. The 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 literature results of the studies mapping QTL for CLA, VA and D9D are graphically summarized in Figure 2 (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index)



Milk conjugated linoleic acid percentage Milk fatty acid unsaturated index Milk trans-vaccenic acid percentage

Figure 2. Graphical representation of QTL associated with milk conjugated linoleic acid percentage, milk fatty acid unsaturated index and milk trans-vaccenic acid percentage on all bovine chromosomes.

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AIM

The aim of the thesis is to identify CNV structural variants as possible markers for genomic selection and to identify QTL regions for Fatty Acid Content in the Italian Brown Swiss population. Additionally the identification of QTL for mastitis resistance in the Valdostana Red Pied cattle is a study that can be used to validate the QTL mapped in the Brown Swiss Population and to improve the selection accuracy in a native population.

The mapping of CNV in the Brown Swiss population has been done using the Illumina Bovine SNP50 BeadChip, the most used in sire genotyping. The possibility to use the data from a medium density SNP array is here tested and CNV regions identified has been validated by qPCR. This study is exposed in Chapter 1 and was part of the EU funded project Quantomics “From sequence to consequence – tools for the exploitation of livestock genome”.

The mapping of fatty acid content in the Italian Brown Swiss cattle population was the second objective of this thesis and was developed given the particular interest of the Brown Swiss Association (ANARB) for nutritional properties of milk. ANARB has been in fact one of the partners in 2 projects funded by Regione Lombardia: QuaLAT aimed to the identification of QTL for fatty acid contents in the Brown Swiss and Israel Holstein cattle populations; LattOMEGA aimed at identifying the basis for the implementation of selection for fatty acid contents in the Italian Brown Swiss and Italian Holstein selection schemes. The results are in Chapter 2.

Chapter 3 shows the results relative to the objective to map QTL for mastitis resistance in the Valdostana Red Pied cattle population. The Valdostana Red Pied cattle breed is selected for double purpose, meat and milk. The expectation is that a part of the QTL mapped in this population overlaps those mapped in the other populations. Additionally it is expected that proprietary QTLs for mastitis resistance of the Valdostana Red Pied are disclosed. This study was part of the EU funded project Quantomics.

In the final discussion some results relative to the CNV mapping in the Valdostana Red Pied are also reported with an overview to association of these markers with traits under selection.

CHAPTER 1

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

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1.1 ABSTRACT

The determination of copy number variation (CNV) is very important for the evaluation of genomic traits in several species because they are a major source for the genetic variation, influencing gene expression, phenotypic variation, adaptation and the development of diseases. The aim of this study was to obtain a CNV genome map using the Illumina Bovine SNP50 BeadChip data of 651 bulls of the Italian Brown Swiss breed. PennCNV and SVS7 (Golden Helix) software were used for the detection of the CNVs and Copy Number Variation Regions (CNVRs).

A total of 5,099 and 1,289 CNVs were identified with 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) CNVR. Ten of the selected CNVR were experimentally validated with a qPCR experiment. The GO and pathway analyses were conducted and they identified genes (false discovery rate corrected) in the CNVR related to biological processes, cellular component, molecular function and metabolic pathways. Among those, we found the *FCGR2B, PPARα, KATNAL1, DNAJC15, PTK2, TG, STAT family, NPM1, GATA2, LMF1, ECHS1* genes, already known in literature because of their association with various traits in cattle.

Although there is variability in the CNVRs detection across methods and platforms, this study allowed the identification of CNVRs in Italian Brown Swiss, overlapping those already detected in other breeds and finding additional ones, thus producing new knowledge for association studies with traits of interest in cattle.

Keywords: CNV, Italian Brown Swiss breed, Illumina Bovine SNP50 BeadChip, qPCR

1.2 INTRODUCTION

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 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 and provide structural genomic information comparable to single nucleotide polymorphism (SNP) data; they influence gene expression, phenotypic variation, environmental adaptability and disease susceptibility (Wang *et al.*, 2009).

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 SNPs, Next Generation Sequencing (NGS) and 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 livestock, recent studies underlined the effects of the CNVs in intron 1 of the *SOX5* gene causing the pea-comb phenotype in chickens (Wright *et al.*, 2009), in the *STX17* gene responsible for premature hair graying and susceptibility to melanoma in horses (Rosengren *et al.*, 2008). Also, the CNVs in the *ASIP* gene are responsible in the leading of different coat colours in goats (Fontanesi *et al.*, 2009). In cattle, Meyers *et al.* in 2010 identified the association between CNVs in a deletion state in the *SLC4A2* gene and 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 array are available (Xu *et al.*, 2013). 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 as the algorithms differ in performance and impact in CNV calling (Xu *et al*., 2013).

The Italian Brown Swiss breed represents the Italian strain of the Swiss Brown Alpine Breed, originally native of central Switzerland. The typical rusticity of the breed, together with its good production attitude, have leaded its spread all over many European and American countries, with the differentiation of different genetic groups in relation to various environmental conditions.

The milk of the Italian Brown Swiss breed has a good cheese-making attitude due to the low frequency of the allele A of the K-casein, in respect to other breeds (http://www.anarb.it/).

In order to support the attitude of the Italian Brown Swiss breed milk to be processed for cheese making, the Associazione Nazionale Allevatori Razza Bruna Italiana (ANARB) sponsored the “disolabruna®” registered mark used for the commercialization of typical cheeses made with only milk coming from Brown Swiss breed cows (http://www.disolabruna.it/).

Nowadays in literature, there is not a whole-genome CNV map for the Italian Brown Swiss in a large population dataset. 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.

1.3 MATERIALS AND METHODS

1.3.1 Sampling and genotyping

ANARB provided commercial semen samples for 1,342 bulls. Genomic DNA was extracted from semen using the ZR Genomic DNA TM 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.

1.3.2 Editing data

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 CNV detection. The signal intensity data of Log R Ratio (LRR) and B allele frequency (BAF) were exported from the Illumina BeadStudio software and the overall distribution of derivative log ratio spread (DLRS) values was used in the SVS7 software (Golden Helix Inc.) 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. Samples with extreme wave factors were excluded from the analysis through the SVS7 software wave correction algorithm. This because waviness is hypothesized to be correlated with the GC content of the probes in addition to the GC content of the region around the probes (Diskin *et al*., 2008).

1.3.3 CNVs detection

Two softwares were chosen for the detection of CNVs: PennCNV (http://www.openbioinformatics.org/penncnv/) and Copy Number Analysis Module (CNAM) of SVS7 software. The use of two software based on different algorithms has the final aim to reduce the false discovery calls resulting from the limitations of the identification of CNVs based on the Illumina Bovine SNP50 BeadChip.

1.3.4 PennCNV detection

PennCNV is the freely available most commonly utilized software for CNV calling in bovine studies; it considers multiple sources of information, among those the LRR and BAF at each SNP. Also, the software reports data quality control measurements for each CNV dataset.

Individual-based CNV calling was performed by PennCNV for all autosomes, using the default parameters of the Hidden Markov Model (HMM). The HMM is a statistical technique that assumes that the distribution of an observed intensity data point depending on an unobserved (hidden) copy number state at each locus, where the elements of the hidden states follow a Markov process (Wang., *et al*., 2007). To reduce the false discovery 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. 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).

1.3.5 SVS7 detection

SVS7 software has a user-friendly graphical interface, efficient pipelines for analysis and workflow, optimized computational speed as well as a technical support. The univariate analysis was used for the CNVs identification. The univariate method segments each sample independently, resulting efficient to find individual variations. 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.

1.3.6 CNVRs definition

CNVRs were defined as in Redon *et al*. (2006) with the BedTools software (Quinlan *et al*., 2010) within software. In addition, consensus regions were created among those identified within the two software, using the Wain *et al.* (2009)'s approach, which identified only CNVRs that fully overlapped each other.

1.3.7 CNVRs validation by quantitative PCR

Quantitative PCR (qPCR) experiments were performed to validate the CNVRs among those identified. The *BTF3* gene was selected as a reference location for all 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™) using the minor groove binder (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 CNVR positions were converted from Bos_taurus_UMD3.1 to Btau_4.6.1 assembly using the Batch Coordinate Conversion option in the UCSC database (https://genome.ucsc.edu/) in order to identify potential candidate CNV genes for complex traits.

1.3.8 CNVR annotation

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/) were performed (using the high classification stringency option and the false discovery rate (FDR) correction) to identify molecular functions, biological processes, cellular components and pathways for the genes included in the consensus CNVRs.

1.4 RESULTS AND DISCUSSION

The application of stringently quality filters above described reduced the number of bull samples to be analysed to 651.

1.4.1 CNVs and CNVRs detection

Table 1 shows the descriptive statistics of the identified CNVs length using PennCNV and SVS7 softwares. Using PennCNV, a total of 5,099 CNVs were detected, 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 highest number of CNVs was detected on BTA7 (8.4%). In detail, the homozygous deletion, heterozygous deletion and the heterozygous duplication CNVs with the highest frequency were observed on BTA5 (12.4%), BTA7 (13.4%), BTA2 (7.9%), respectively. Only one homozygous duplication CNV was identified on BTA25.

A total of 1,289 CNVs were identified by SVS7 in all the 29 autosomes. 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. The highest frequency of gain (23.9%) and loss (21%) CNVs were detected on BTA28, which also showed the highest number of CNVs in total (22.2%).

The discrepancies among the number of CNVs detected from the two software packages is ascribed to the lack of identification of shorter CNVs of the SVS7 univariate approach (here used) (http://doc.goldenhelix.com/).

A graphical representation of CNVs obtained by PennCNV and SVS7 software for each chromosome was visualized by HD-CNV software (http://daleylab.org/lab/?page_id=125) and reported in Figure 1. The graph files allow the visualization of the regions where CNVs were observed across samples with a strong amount of overlap.

A total of 1,101 CNVRs were mapped with PennCNV software (Table 2). The total length of the sequence covered by the CNVRs was 682 Mb, which corresponded to 27.14% of the bovine autosomal genome in the Brown Swiss breed. The percentage of sequence covered by CNVRs by chromosome ranged from 16.59 (BTA 12) to 50.14 (BTA 19).

The CNVs identified with SVS7 software were summarized at the population level according to Redon *et al*. (2006)'s approach, resulting into 277 CNVRs (Table 2). 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). The highest frequency of CNVRs were identified on BTAs 8 and 4 for PennCNV and SVS7 software, respectively. The consensus performed between the two software generated 150 consensus CNVRs with a total length of 17.1 Mb (0.68 % of the autosomes), as shown in Supplementary File 1.

Table 3 shows the comparison between the CNVRs detected and those reported in literature, confirming both the existence of high variability in CNVRs detection across platforms, methods, population size, cattle breeds and species. It is evident that only a small proportion of CNVRs in our study overlapped with those in other studies, probably because only the 150 consensus CNVR were considered in the comparison, to enhance the power of CNVs detection in this study.

The highest overlapping coverage (38%) was found with the study of Hou *et al.* (2011), in which CNVs detection was performed using BovineSNP50 assay including animals from taurine dairy and beef breeds, breeds of predominantly indicine back-ground, Taurine × Indicine composite and African groups. The previous mentioned dataset included 24 Brown Swiss individuals in which 22 CNVRs were identified on 13 BTAs. Only one CNVR on BTA9 from Hou *et al.* (2011) (4305338 -4386831 Mbp) resulted in common with the region identified in our study (4050528-4476378 Mbp).

The comparison between CNVRs here identified with PennCNV software and those detected in the study of Hou *et al.* (2011) in Brown Swiss cattle, using the same software, provided five common CNVRs on BTAs 2, 9, 12, 14, 18. The Table 4 shows a list of the QTL included in these regions (http://www.animalgenome.org/QTLdb/doc/genomeversion#UMD_3.1). Since some QTL covered very large chromosome regions, we considered only those with a small interval of confidence.

1.4.2 CNVRs validation by quantitative PCR

Eleven CNVRs were selected for the validation; three of which were in common between PennCNV and SVS7 software, six and two of which were randomly chosen among the CNVRs identified with the two software, respectively.

Supplementary File 2 reports the primer list for the eleven regions. Ten CNVRs (91%) were confirmed by qPCR experiments. Additionally, the proportions of confirmed positive CNVs 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 the Holstein breed.

1.4.3 CNVRs annotation

Supplementary File 3 shows the 252 Ensembl annotated genes, which correspond to 218 gene symbols in the consensus CNVRs. Supplementary File 4 reports the GO and the pathways analyses for 158 genes among those included in Supplementary File 3. The KEGG pathway analysis revealed that these genes are mainly represented in the pathway of immune system. Among the identified genes, in Table 5 we highlighted those showing differential expression of association with various traits in cattle reported in literature.

In detail, Lewandowska-Sabat *et al.* (2013) in their *in vitro* study, highlighted the role of the *TREM1 (triggering receptor expressed on myeloid cells 1)* signaling pathway in which the *FCGR2B (Fc fragment of IgG, low affinity IIb, receptor (CD32))* gene is included. The *TREM1*, in synergy with the *TLR2 (Toll-like receptor 2)* pathway, are involved in phagocytosis and production of proinflammatory cytokines, determining optimal host defense during bovine mastitis.

The *PPARα* gene, encoding for the *peroxisome proliferator-activated receptor-α,* maps in the CNVRs found in this study on BTA5. The role of this gene in the fatty acid metabolism is widely described in literature (Bionaz *et al.,* 2013)

The CNVRs detected on BTA12 in our study enclose the *KATNAL1 (Katanin p60 subunit A-like 1)* and *DNAJC15 (DnaJ (Hsp40) homolog, subfamily C, member 15)* genes. Zhang *et al.* (2014) characterized the *KATNAL1* gene and found the promoter polymorphism associated with semen traits in Chinese bulls. Moreover, SNPs in the *DNAJC15* gene were found to be associated with bovine blastocyst rate by Zhang *et al.* (2011).

The polymorphism in the *PTK2 (Protein tyrosine kinase 2)* gene, annotated in one of the CNVR on BTA14 was found to be associated with milk production traits in Chinese Holstein (Wang *et al.*, 2013). On the same BTA, the gene encoding for the thyroglobulin *(TG)* was associated with fat distribution, carcass and meat traits in beef cattle (Bennet *et al.*, 2013). Fernàndez *et al*. (2014) also found the association of SNPs in the *TG* gene with age of puberty in bulls.

The *STAT3 (signal transducer and activator of transcription 3 (acute-phase response factor))*, the *STAT5B (signal transducer and activator of transcription 5B)* and the *STAT5A (signal transducer and activator of transcription 5A)* genes are annotated in the CNVR on BTA19 detected in our study. The main bovine *STAT*s family members *STAT3* and *STAT5* are involved in *prolactin receptor (PRLR)* signaling by *JAK/STAT* pathway (*Janus kinase (JAK) and Signal Transducer and Activator of Transcription*) that activates the expression of milk protein genes (Zhang *et al.,* 2010).

The *NPM1* gene (*nucleophosmin 1*) is located in the CNVR found on BTA20. This gene encoding a multifunctional nucleolar phosphoprotein that plays a crucial role in the control of various aspects of cell growth and homeostasis, is a candidate gene for growth traits in cattle (Huang *et al.*, 2010)

The *GATA2* (*GATA binding protein 2*) gene, included in the CNVR on BTA22, is involved in the regulation of trophoblast-specific gene transcription in bovine trophoblast CT-1 cells, as described by Bai *et al.* (2011).

The *LMF1* (*lipase maturation factor 1*) gene maps in the CNVR found on BTA25. The mutations of this gene are involved in glyceridemia and hypertriglyceridemia in human and animals, playing an important role in the lipase maturation (Ren *et al.*, 2011).

The *ECHS1* (*enoyl coenzyme A hydratase, short chain, 1*) gene, included in the CNVR on BTA26, was associated with the conjugated linoleic and vaccenic acids in milk in a quantitative trait loci mapping study of Strillacci *et al*. (2014) in the Italian Brown Swiss cattle breed.

1.5 CONCLUSIONS

In this study, the first on this breed 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 software packages (PennCNV and SVS7), with the aim to reduce the high error rate commonly recognised in copy number discovery. CNVRs identified by PennCNV software overlapped in part with the SVS7 data, which emphasised the diversities and the shared features of the two detection methods.

The GO and pathway analyses here conducted identified genes that have shown differential expression of association with production traits, carcass and meat traits, reproduction traits and growth traits in cattle. The results enrich the bovine CNVs map providing new information for association studies with economic and health-related traits of interest.

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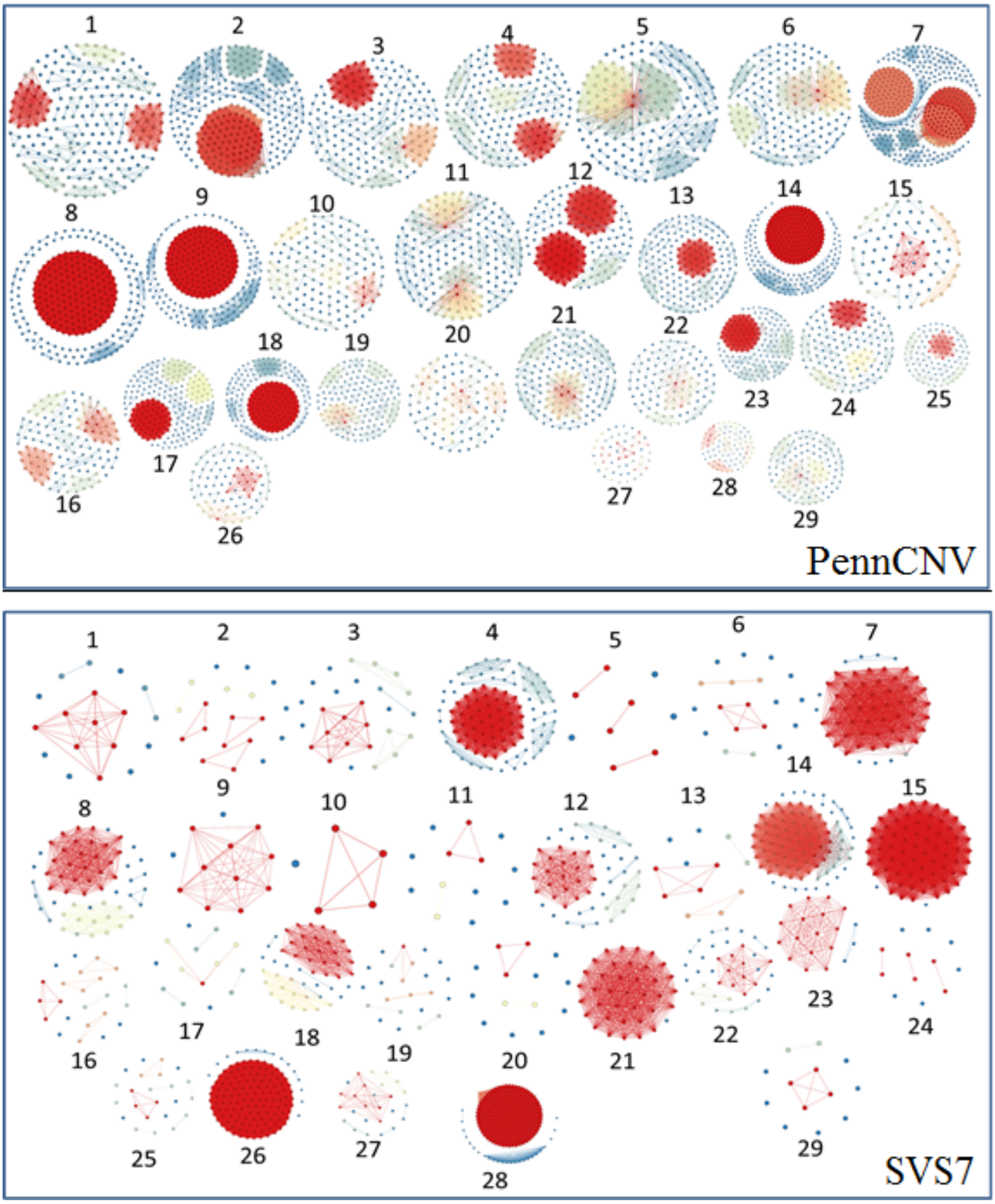
Figure1. Graphical representation of CNVs visualized by HD-CNV software.



Detailed legend. Each graph represents CNVs identified by PennCNV and SVS7 software for each of the 29 bovine autosomes. The size of the graphical representation of each chromosome depends on their total length. Each note represents a CNV, and edges are added between nodes that share 40% (default) overlap. The darker the red spots the more overlapping of CNVs across samples. The blue spots identify the unique events.

Table 1. Descriptive statistics for CNVs identified with PennCNV and SVS7 software.

Copy number	Number of events	Mean	Median	Total Length	Min Length	Max Length
*PennCNV**						
0	97	311345.2	245646.2	30200500	46665	1053143
1	2086	159066.4	134534.5	331711379	40374	1688267
3	2915	488559.5	385138.7	1423739019	41449	4457756
4	1	511301.5	511301.5	511301	511301	511301
Total	5,099					
*SVS7***						
Loss	762	94830.3	57612.5	72260727	11315	1440751
Gain	527	80324.2	37591.4	42330968	20342	770044
Total	1,289					

*0 = homozygous deletion, 1 heterozygous deletion, 3 heterozygous duplication, and 4 homozygous duplication

**loss= homozygous or heterozygous deletion; gain= homozygous or heterozygous duplication

Table 2. Descriptive statistics for CNVRs identified with PennCNV and SVS7 softwares.

CNVRs	Number of events	Mean	Median	Total Length	Min Length	Max Length
*PennCNV**						
Loss	220	210454.3	148427.5	46299963	40754	977685
Gain	774	596255.2	403827.3	461501583	45465	3873856
Complex**	107	1625208.1	1068260.7	173897266	179707	6703707
Total	1,101	210454.4	148427.5	46299963	40754	977685
*SVS7**						
Loss	185	116378.6	61523.6	21530044	11314	1440750
Gain	56	115358.1	83498.5	6460049	20341	460833
Complex**	36	158863.1	127525.5	5719073	21916	770043
Total	277					

*loss= homozygous or heterozygous deletion; gain= homozygous or heterozygous duplication

**complex= CNVRs defined both as deletion and duplication across samples

Table 3. Comparison between results of this study and results from literature.

Results from different studies						**Overlapped CNVRs of this study**		
Reference	Methods of detection	Total CNVRs	N° samples	Breeds	Lenght (Mb)	Count	Percentage overlap	Total length (Mb)
Bae *et al*. (2010)	SNP-based Studies (54k)	368	265	1	63.1	13	8,7%	4.1
Hou *et al*. (2011)	SNP-based Studies (54K)	682	521	21	158.0	57	38,0%	22.4
Jian *et al*. (2013)	SNP-based Studies (HD)	367	96	1	42.7	15	10,0%	0.92
Liu *et al*. (2010)	CGH-based Studies	177	90	17	28.1	3	2,0%	1.3
Fadista *et al*. (2010)	CGH-based Studies	304	20	4	22.0	4	2,7%	1.3
Bickhart *et al*. (2012)	Resequencing-based Studies	1,265	5	3	55.6	12	8,0%	2.3
This study	*SNP-based Studies (54k)*	*150*			*17.1*			

Table 4. Common CNVRs in the Brown Swiss breed between Hou *et al*. (2011) and this study.

Hou *et al.* (2011) (UMD3.1 assembly) PennCNV				**This study (UMD3.1 assembly) PennCNV**				**QTL**		
bta	start CNVR	end CNVR	lenght CNVR	bta	start CNVR	end CNVR	lenght CNVR	start QTL	end QTL	trait (ID)
2	8788219	9113368	325150	2	8788219	9040720	252501	9003563	9867063	trans-15-C18:1 fatty acid content (20510)
9	4305338	4386831	81494	9	4050528	4476378	425850	2148415	9159784	Interval from first to last insemination (5006)
12	31368562	31679957	311396	12	30099199	31555734	1456535	30751141	30912583	Longissimus muscle area (11733)
14	17378950	17457836	78887	14	17322658	17457836	135178	9884020	19204282	Calving ease (maternal) (10958)
								12169925	20562022	Longissimus muscle area (4550)
								16752147	18440442	Shear force (20791)
18	57565406	57659303	93898	18	56364657	58090087	1725430	55181080	60030732	Stillbirth (direct) (15198), (15199)
								55181080	60030732	Birth index (15200)
								55181080	60030732	Calving ease (direct) (15201) (15202)
								55181080	60030732	Calf size (direct) (15203), (15204)
								55777394	55928978	Marbling score (10014)
								55860765	63144054	Bilateral convergent strabismus with exophthalmus (10051)
								56701305	56852890	Dystocia (direct) (11363)

Table 5. List of genes located in identified Brown Swiss CNVRs reported in literature.

Consensus CNVRs			**Genes in Consensus CNVRs**				**References**
Bta	Start	End	Start	End	Ensembl code	Gene symbol	
3	7957960	7983149	7928113	7944607	ENSBTAG00000021842	*FCGR2B*	Lewandowska-Sabat *et al*. (2013)
5	116895329	117247824	117151549	117233112	ENSBTAG00000008063	*PPARα*	Bionaz *et al*. (2013)
12	30418611	30646042	30519852	30558210	ENSBTAG00000009340	*KATNAL1*	Zhang *et al*. (2014)
12	13179696	13204137	13183734	13266310	ENSBTAG00000034785	*DNAJC15*	Zhang *et al*. (2011)
14	3885798	4017201	3870893	4065010	ENSBTAG00000009578	*PTK2*	Wang *et al*. (2013)
14	9300228	9345140	9262251	9508938	ENSBTAG00000007823	*TG*	Fernàndez *et al*. (2014) Bennet *et al*. (2013)
19	42976859	43170256	43056660	43132624	ENSBTAG00000021523	*STAT3*	Zhang *et al*. (2010)
19	42976859	43170256	42960226	42996671	ENSBTAG00000010125	*STAT5B*	
19	42976859	43170256	43033597	43054075	ENSBTAG00000009496	*STAT5A*	
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*	Ren *et al*. (2011)
26	25828973	25982293	25856475	25865594	ENSBTAG00000017710	*ECHS1*	Strillacci *et al*. (2014)

Supplementary Files

Supplementary File 1: List of the copy number variant regions (CNVRs) identified using PennCNV and SVS7 software and the consensus CNVRs (PennCNV/SVS7) generated according to Wain *et al.* (2009).

	pennCNV (CNVRs)		lenght
chr1	1359951	1603944	243993
chr1	1625471	2013659	388188
chr1	4727118	5010974	283856
chr1	5319965	5741816	421851
chr1	12889396	13019983	130587
chr1	17021138	17160556	139418
chr1	18170722	18980052	809330
chr1	19829143	20192420	363277
chr1	27357510	27761036	403526
chr1	28034525	28216247	181722
chr1	41315418	41465449	150031
chr1	43317853	43861492	543639
chr1	46321775	46648008	326233
chr1	47389301	49230292	1840991
chr1	52191701	52316632	124931
chr1	56505444	56901848	396404
chr1	60753573	61329180	575607
chr1	66188527	66630647	442120
chr1	68066718	69250962	1184244
chr1	71229187	71623173	393986
chr1	72573950	73640200	1066250
chr1	80875014	80974985	99971
chr1	81018906	81160609	141703
chr1	82059960	82468782	408822
chr1	83030921	83672872	641951
chr1	88086502	88262684	176182
chr1	91031267	91358383	327116
chr1	92958471	93279488	321017
chr1	95793312	95910972	117660
chr1	97276664	97484342	207678
chr1	101103098	101366448	263350
chr1	101403755	101664632	260877
chr1	101773849	102056710	282861
chr1	103728420	103926075	197655
chr1	104842361	105264358	421997
chr1	106228836	106295981	67145
chr1	106512965	106824629	311664
chr1	107094743	107172243	77500
chr1	108154057	108782509	628452
chr1	109649036	109869888	220852
chr1	113631332	113780811	149479

	pennCNV (CNVRs)		lenght
chr12	2058297	2273370	215073
chr12	2814820	3705325	890505
chr12	9079069	9165632	86563
chr12	13140896	13273888	132992
chr12	15726574	16098884	372310
chr12	18472407	18584913	112506
chr12	20079121	20402284	323163
chr12	20510073	20800774	290701
chr12	21073393	21441958	368565
chr12	22664255	22863845	199590
chr12	25141005	25255436	114431
chr12	27006686	27133552	126866
chr12	29520358	29778424	258066
chr12	30099199	31555734	1456535
chr12	31639399	31848902	209503
chr12	34480373	34635671	155298
chr12	35189028	35703502	514474
chr12	41384223	41479027	94804
chr12	45002070	45573176	571106
chr12	47300401	47528422	228021
chr12	48758328	49421215	662887
chr12	50125707	50324576	198869
chr12	52546816	52663318	116502
chr12	55097796	55674695	576899
chr12	55867003	56204081	337078
chr12	56367819	56695055	327236
chr12	57077252	57211328	134076
chr12	57931622	58461348	529726
chr12	63969950	64152261	182311
chr12	64384089	64761912	377823
chr12	66068259	66877440	809181
chr12	69969069	70092297	123228
chr12	77109094	77347204	238110
chr12	78099072	78212571	113499
chr12	78276736	78750520	473784
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chr11	15788936	15919622	130686
chr11	20755386	20960589	205203
chr11	22558808	23085190	526382
chr11	24354910	26162970	1808060
chr11	27989531	28207767	218236
chr11	28992259	29367145	374886
chr11	30651364	31130270	478906
chr11	31173613	31259588	85975
chr11	36713471	37261133	547662
chr11	38733905	39104584	370679
chr11	46307696	47430509	1122813
chr11	51963535	52677772	714237
chr11	54193489	54246625	53136
chr11	60128321	60572203	443882
chr11	63686208	64313748	627540
chr11	67498111	68298497	800386
chr11	73934314	74197538	263224
chr11	75295616	76060924	765308
chr11	77235847	79314962	2079115
chr11	80450475	80839258	388783
chr11	82833655	82959206	125551
chr11	87124625	89371911	2247286
chr11	89552045	89680768	128723
chr11	90738123	90911719	173596
chr11	91961667	92500678	539011
chr11	92963716	93050541	86825
chr11	93546324	93612322	65998
chr11	94640611	94837245	196634
chr11	95150490	99180813	4030323
chr11	99497380	99738023	240643
chr11	99774304	104856815	5082511
chr11	105593624	106825407	1231783

chr29	42620218	45023665	2403447
chr29	45817015	50999092	5182077

SVS7 (CNVRs)			lenght
chr1	4626587	4648383	21796
chr1	52748123	52772080	23957
chr1	64501909	64525174	23265
chr1	91066621	91435586	368965
chr1	123825385	123921894	96509
chr1	144134237	144190645	56408
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
chr2	124137395	124204421	67026
chr3	7957960	7983149	25189
chr3	33514564	33741850	227286
chr3	39097420	39118317	20897
chr3	40977107	41079360	102253
chr3	68402961	68432145	29184
chr3	81379707	81411041	31334
chr3	91910014	92190368	280354
chr3	100468099	100493684	25585
chr3	105715727	105739637	23910
chr3	115888900	115937988	49088
chr3	116781408	116801749	20341
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chr4	10401625	10450547	48922
chr4	11472235	11500911	28676
chr4	17902915	17978432	75517
chr4	22093546	22180214	86668
chr4	24087424	24150445	63021
chr4	28384911	28413603	28692
chr4	41444423	41657868	213445
chr4	66781385	66830563	49178
chr4	69158293	69220543	62250
chr4	73699663	73791282	91619
chr4	78440044	78523846	83802
chr4	81497187	81554820	57633
chr4	86811243	86948025	136782
chr4	89850655	89921990	71335
chr4	99574406	99691481	117075
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SVS7 (CNVRs)			lenght
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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
chr17	27459029	27491589	32560
chr17	32762909	32964041	201132
chr17	39963957	40071626	107669
chr17	42425636	42661925	236289
chr17	55713369	55764236	50867
chr18	3080400	3114628	34228
chr18	35971459	36107915	136456
chr18	42659289	42826428	167139
chr18	50388296	50465387	77091
chr18	51571629	51592949	21320
chr18	53132012	53195763	63751
chr18	60978019	61054591	76572
chr18	61095214	61156737	61523
chr18	61438125	61920892	482767
chr18	63119361	63167945	48584
chr19	6709868	6768232	58364
chr19	11863651	11970132	106481
chr19	34836416	34905583	69167
chr19	35585081	35619269	34188
chr19	38519698	38546855	27157
chr19	42352691	42423488	70797
chr19	42976859	43170256	193397
chr19	46655940	46723662	67722
chr19	50336021	50395622	59601
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chr19	52175916	52264019	88103
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chr19	55379112	55527962	148850
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chr20	1741145	1792368	51223

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chr5	46279541	46306149	26608
chr5	58847022	58966295	119273
chr5	114543256	114698428	155172
chr5	116895329	118329917	1434588
chr5	119729902	119949553	219651
chr6	5025746	5086136	60390
chr6	9736332	9981135	244803
chr6	12648459	12703601	55142
chr6	22613578	22672648	59070
chr6	40107367	40208497	101130
chr6	50981312	51007189	25877
chr6	53428838	53449439	20601
chr6	68291351	68332579	41228
chr6	81551479	81604925	53446
chr6	90966250	90989420	23170
chr6	100620998	100709082	88084
chr6	104493834	104587477	93643
chr6	117013231	117148273	135042
chr7	1293067	1353317	60250
chr7	2597655	2680354	82699
chr7	22524899	22681472	156573
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chr7	43709405	43808593	99188
chr7	76886696	77011685	124989
chr7	78081511	78307528	226017
chr7	87158251	87359924	201673
chr8	9133270	9156221	22951
chr8	15417359	15441189	23830
chr8	15665796	15773921	108125
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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
chr20	2880532	3189118	308586
chr20	6360647	6385223	24576
chr20	21018903	21048672	29769
chr20	33773531	33817557	44026
chr20	34241862	34264853	22991
chr20	34953795	34981347	27552
chr20	41239866	41289921	50055
chr20	45052283	45369517	317234
chr20	60902173	60928704	26531
chr20	64185456	64376028	190572
chr21	54186710	54208626	21916
chr21	70089833	71210609	1120776
chr22	12869969	12948282	78313
chr22	16219978	16407075	187097
chr22	19409002	19588936	179934
chr22	21431682	21455286	23604
chr22	25141851	25321072	179221
chr22	26527854	26604789	76935
chr22	26865100	26924506	59406
chr22	31649896	31675722	25826
chr22	36527685	36548339	20654
chr22	39545402	39657636	112234
chr22	44430993	44595995	165002
chr22	47510478	47537080	26602
chr22	48858472	49131324	272852
chr22	54028803	54183730	154927
chr22	57098389	57111693	13304
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
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chr25	22238007	22320002	81995
chr25	24125205	24228117	102912
chr25	37988321	38142895	154574

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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
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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
chr14	53415847	53436763	20916
chr14	54023420	54123146	99726
chr14	75571250	76043148	471898
chr14	76217573	76269650	52077
chr14	79178022	79322701	144679
chr14	80082712	80543545	460833
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
chr29	33329702	33353664	23962
chr29	35136093	35169599	33506
chr29	41212959	41264801	51842
chr29	48178151	48252404	74253

	CNVRs Consensus (pennCNV/SVS7)		lenght
chr1	91066621	91358383	291762
chr1	144134237	144190645	56408
chr1	146587678	146632014	44336
chr1	155835816	155955828	120012
chr2	14525350	14565726	40376
chr2	27489458	27724930	235472
chr2	32108568	32154806	46238
chr2	39976359	39999947	23588
chr2	57373897	57416172	42275
chr2	83029887	83050544	20657
chr2	110311653	110353189	41536
chr2	124137395	124204421	67026
chr3	7957960	7983149	25189
chr3	33514564	33741850	227286
chr3	39097420	39118317	20897
chr3	40977107	41079360	102253
chr3	81379707	81411041	31334
chr3	91910014	92190368	280354
chr3	100468099	100493684	25585
chr3	115888900	115937988	49088
chr3	116781408	116801749	20341
chr4	6248795	6337362	88567
chr4	41556810	41657868	101058
chr4	66781385	66830563	49178
chr4	69158293	69220543	62250
chr4	73699663	73791282	91619
chr4	99574406	99635737	61331
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chr4	108867683	108904498	36815
chr4	111990062	112164314	174252
chr4	118608842	118655986	47144
chr5	46279541	46306149	26608
chr5	114543256	114698428	155172
chr5	116895329	117247824	352495
chr5	119729902	119949553	219651
chr5	117738204	118329917	591713
chr6	9914189	9981135	66946
chr6	12648459	12703601	55142
chr6	81551479	81604925	53446
chr6	100620998	100709082	88084
chr6	104493834	104587477	93643
chr6	117013231	117148273	135042
chr7	1293067	1353317	60250
chr7	2597655	2680354	82699
chr7	22524899	22681472	156573
chr7	42788788	43132401	343613
chr8	33668705	33747904	79199

	CNVRs Consensus (pennCNV/SVS7)		lenght
chr15	76438547	76466667	28120
chr16	4158997	4233985	74988
chr16	8486467	8582055	95588
chr16	29441057	29636822	195765
chr16	49355913	49455109	99196
chr16	70906202	71125864	219662
chr17	27459029	27491589	32560
chr17	32762909	32964041	201132
chr17	39963957	40071626	107669
chr17	42425636	42661925	236289
chr17	55713369	55764236	50867
chr18	35971459	36040190	68731
chr18	42659289	42826428	167139
chr18	51571629	51592949	21320
chr18	53132012	53195763	63751
chr18	61095214	61156737	61523
chr18	61438125	61597742	159617
chr18	63119361	63167945	48584
chr19	11863651	11970132	106481
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chr19	52175916	52264019	88103
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chr19	56754737	56837932	83195
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chr19	56072306	56202223	129917
chr20	1741145	1792368	51223
chr20	2880532	3189118	308586
chr20	6360647	6385223	24576
chr20	41239866	41289921	50055
chr20	60902173	60928704	26531
chr21	70089833	71136925	1047092
chr22	12869969	12948282	78313
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chr22	47510478	47537080	26602
chr22	57098389	57111693	13304
chr22	60435042	60508872	73830
chr22	59951940	60243916	291976
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chr24	1027534	1094942	67408

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chr8	94115663	94386951	271288
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chr9	4239500	4439872	200372
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chr13	80026050	80144645	118595
chr14	2721633	2803998	82365
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chr14	8064004	8113083	49079
chr14	8499902	8551460	51558
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chr14	20119611	20157384	37773
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chr14	54023420	54123146	99726
chr14	79178022	79322701	144679
chr14	80475984	80543545	67561

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chr24	39320770	39365195	44425
chr24	53328928	53434181	105253
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chr25	39785037	39844749	59712
chr25	39286957	39424763	137806
chr26	5258082	5288263	30181
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chr26	25828973	25982293	153320
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chr26	49027625	49090826	63201
chr27	4544917	4773381	228464
chr27	6922514	6955584	33070
chr27	8781446	8827679	46233
chr27	38109791	38233675	123884
chr27	43237090	43260976	23886
chr28	6334557	6547497	212940
chr28	13713042	13894573	181531
chr28	26994978	27072121	77143
chr29	19618823	19701179	82356
chr29	33329702	33353664	23962
chr29	35136093	35169599	33506
chr29	41212959	41264801	51842
chr29	48178151	48252404	74253

Supplementary File 2: Summary of the results of the qPCR analysis of the eleven CNVRs selected after the consensus analysis **in the Italian Brown Swiss breed.**

CHR	PRIMER (FORWARD)	PRIMER (REVERSE)	PROBE
2	TGCATGCACACAGGAATGTTAC	TGCCCCTAAGAAGGAGTCGTT	ACTCTGTTCAGCCCTTC
3	GACTAATGGCAAGAGCCGTGTA	AGGCAGGAACAGAAGGAGGAA	TGAGCATGTCACTTTAA
4	GCCCGGCGGACACTAAG	CCAGCATTATGTCCTTCATCAACA	TCAGGAAGCTGTGGCCA
4	TCCTGCCCAGATACCATATCCTT	CGAGGCAAGCTCTACAGGAAA	TGGCATTCAAATCAC
5	GGAGATAGGATAGAAAGAAATGGAGAAC	ATGGGAGTGATGGAAAATTGAAG	CACTCTTAAATTCCC
12	GGACAGTCACCTCAGGATGCA	TTGCCACAAGTGAGGCTTCTC	AACGGTCACCTAAGAGACA
12	GCCTGGTGTTGTCATGATGAA	CCCGTACACTGACACCAAAGTG	TTTTGCGCTTGAAGCAG
13	TGCGAAATTCTGGAAGAGGAA	GGGTGCCTGGTGCAATTC	CCTGAGGACATGAAGTT
19	CAGTGAGCCAAAGCCAATCC	AATCCAACTTGCCGGCTAGTATT	CCTCCACAGGAATC
28	ACATTCAGGCTGCCATTTTGT	GAGGCGGGATGTCACAGAAA	TCCAATATCGTCAACCATT
29	CACGGGCGCACCACTT	CCCCCGATGAATGGCTATC	AGCTCCCTGCTCGAC
REF_BTF3	GCTGAGACAAAGCAACTGACAGA	TCGGCACCAAGCTGGTTTA	TGCTCCCCAGCATC

*The UMD3.1 assembly was used to indicated the position of the CNVRs

CHR	**START***	**END***	**LENGHT**	**PENN CNV STATE**	**CNAM CNV STATE**	**NUMBER OF SAMPLES**	**CONFIRMED SAMPLES**	**CONFIRMED RATE**	**VALIDATED**	**GENE (Btau4.6.1)**
2	63870167	64073748	203581	GAIN		11	8	0,73	YES	MGAT5
3	7957198	7966700	9502	GAIN	GAIN	3	2	0,67	YES	FCGR2B
4	6228016	6450000	221984	GAIN		11	11	1,00	YES	-
4	73686740	73785101	98361	LOSS	LOSS	10	9	0,90	YES	ZNF804B
5	58965609	59140571	174962	LOSS		10	7	0,70	YES	LOC787945
12	58071208	58427000	355792	LOSS	LOSS	11	1	0,09	NO	-
12	67534765	67579929	45164		LOSS	11	10	0,91	YES	-
13	53931895	53983934	52039	GAIN		5	3	0,60	YES	SIRPB1
19	2585940	2607218	21278	LOSS		12	9	0,75	YES	-
28	2553716	2635632	81916		LOSS	12	6	0,50	YES	OR5AS1
29	5414600	5444000	29400	GAIN		10	9	0,90	YES	-
REF_BTF3										

Supplementary File 3: Annotation of copy number variant regions (CNVRs).

CNVRs_pennCNV			CNVRs_SVS7		CNVRs_Consensus		CNVRs State	Genes included in Consensus CNVRs			
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
18	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

Supplementary File 4: 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).

Clustered_DAVID

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	6.08E-02	6.2E1

		development		
	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**			
GOTERM_BP_FAT	GO:0032318	regulation of Ras GTPase activity	1.38E-02	1.9E1
GOTERM_BP_FAT	GO:0043087	regulation of GTPase activity	2.08E-02	2.7E1
GOTERM_MF_FAT	GO:0008047	enzyme activator activity	3.23E-02	3.4E1
GOTERM_BP_FAT	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
GOTERM_MF_FAT	GO:0005097	Rab GTPase activator activity	3.67E-02	3.8E1
GOTERM_MF_FAT	GO:0005083	small GTPase regulator activity	4.52E-02	4.5E1
GOTERM_MF_FAT	GO:0005096	GTPase activator activity	5.53E-02	5.2E1
GOTERM_MF_FAT	GO:0005099	Ras GTPase activator activity	6.16E-02	5.6E1
GOTERM_BP_FAT	GO:0051336	regulation of hydrolase activity	1.42E-01	9.0E1
TBC1D5, ASAP2, MGC166429, TBC1D22A, TBC1D9B, NOXA1				
Annotation Cluster 7	**Enrichment Score: 1.11**			
GOTERM_BP_FAT	GO:0006468	protein amino acid phosphorylation	5.68E-02	5.9E1
GOTERM_BP_FAT	GO:0006796	phosphate metabolic process	7.71E-02	7.1E1
GOTERM_BP_FAT	GO:0006793	phosphorus metabolic process	7.71E-02	7.1E1
GOTERM_BP_FAT	GO:0016310	phosphorylation	1.12E-01	8.4E1
PTPN7, AKT1, EPHA4, PTK2, MAPK12, PTPRG, STAT5A, STAT5B, MAPK11, PDE6G, AATK				
Annotation Cluster 8	**Enrichment Score: 1.03**			
GOTERM_BP_FAT	GO:0008344	adult locomotory behavior	2.25E-02	2.9E1
GOTERM_BP_FAT	GO:0030534	adult behavior	6.84E-02	6.6E1
GOTERM_BP_FAT	GO:0007610	behavior	2.04E-01	9.7E1
GOTERM_BP_FAT	GO:0007626	locomotory behavior	2.39E-01	9.8E1
EPHA4, ATP1A3, NR4A2, STAT3				
Annotation Cluster 9	**Enrichment Score: 1.03**			
GOTERM_MF_FAT	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
	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**			
GOTERM_BP_FAT	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
	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**			
GOTERM_BP_FAT	GO:0006417	regulation of translation	8.74E-02	7.5E1
	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**			
GOTERM_CC_FAT	GO:0005856	cytoskeleton	3.83E-02	3.5E1
	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**			
GOTERM_BP_FAT	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
GOTERM_BP_FAT	GO:0034404	nucleobase, nucleoside and nucleotide biosynthetic process	1.81E-01	9.5E1
	ADSSL1, ENTPD8, ATP1A3, PRPSAP2			
Annotation Cluster 14	**Enrichment Score: 0.70**			
KEGG_PATHWAY	bta04664	Fc epsilon RI signaling pathway	4.92E-02	4.2E1
KEGG_PATHWAY	bta04914	Progesterone-mediated oocyte maturation	2.34E-01	9.5E1
KEGG_PATHWAY	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**			
GOTERM_BP_FAT	GO:0043066	negative regulation of apoptosis	1.56E-01	9.2E1
	GO:0043069	negative regulation of programmed cell death	1.60E-01	9.3E1
	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**			
GOTERM_BP_FAT	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	2.17E-01	9.8E1

		transcription, DNA-dependent		
	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
GOTERM_BP_FAT	GO:0006355	regulation of transcription, DNA-dependent	3.91E-01	1.0E2
	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**			
GOTERM_CC_FAT	GO:0031981	nuclear lumen	4.50E-01	1.0E2
GOTERM_CC_FAT	GO:0070013	intracellular organelle lumen	5.03E-01	1.0E2
GOTERM_CC_FAT	GO:0043233	organelle lumen	5.04E-01	1.0E2
GOTERM_CC_FAT	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 process	5.21E-01	1.0E2
	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**			
GOTERM_MF_FAT	GO:0046872	metal ion binding	5.35E-01	1.0E2
	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**			
GOTERM_MF_FAT	GO:0032561	guanyl ribonucleotide binding	5.08E-01	1.0E2
GOTERM_MF_FAT	GO:0019001	guanyl nucleotide binding	5.15E-01	1.0E2
GOTERM_MF_FAT	GO:0005525	GTP binding	7.44E-01	1.0E2
ADSSL1, EEFSEC, PDE6G, RHOF				
Annotation Cluster 22	**Enrichment Score: 0.19**			
GOTERM_BP_FAT	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
	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**			
GOTERM_MF_FAT	GO:0005216	ion channel activity	7.11E-01	1.0E2
	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				

Category	Term		Genes	FDR	P-Value
GOTERM_ BP_FAT	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	EPHA4, PTK2, NR4A2, NFATC1	3.40E-02	3.39E-02

	differentiation			
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,	4.10E-01	4.11E-01

			NFATC1		
	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
GOTERM_CC_FAT	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
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,	7.50E-02	7.46E-02

		DHCR24, AATK		
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

CHAPTER 2

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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2.1. 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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2.2. 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.

2.3. MATERIALS AND METHODS

2.3.1. 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.

2.3.2. 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).

2.3.3. 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:

$$y_{ijklmnp} = \mu + P_i + AG_j + YM_k + S_l + DIM_m + PR_n + a_p + e_{ijklmnp}$$

where:

$y_{ijklmnp}$ is the value of VA, CLA or D9D determined for each daughter milk sample;

μ is the factor common to all observations;

P_i (i=1,…4) is the fixed effect of the class of parity;

AG_j (j=1,…4) is the fixed effect of class age at calving;

YM_k (k=1, ….16) is the fixed effect of the interaction between year and month of calving;

S_l (l=1,…4) is the fixed effect of the season of calving;

DIM_m (m=1,…15) is the fixed effect of the class of days in milk;

PR_n (n=92) is the fixed effect of province;

a_p is the random additive genetic effect of the animal p (0, As2a);

$e_{ijklmnp}$ is a random residual (0, Is2e).

Parity was classified into 4 classes for first, second, third and later parities. Age at calving was classified into 4 classes (class 1: 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 (1=spring; 2=summer; 3=autumn; 4=winter). Days in milk were grouped in 30-days classes (15 classes).

2.3.4. 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.

2.3.5. 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.

2.3.6. 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:

$$Z_{ij}=Dtest_{ij}/SD(Dnull_{ij})$$

where $Dtest_{ij} = [(H1 + H2) - (L1 + L2)]/2$ is the difference in sire allele frequencies between the high and low daughters pools of the i^{th} sire with respect to the j^{th} marker, averaged over the two subpools of the same tail.

$Dnull_{ij} = [(H1 - H2) + (L1 - L2)]/2$ is the difference in allele frequencies between the 2 subpools of the same tail of the i^{th} sire with respect to the j^{th} marker, averaged over the high and low pools.

$Dtest_{ij}$ and $Dnull_{ij}$ were calculated only for markers for which the sire was heterozygous. Because $Dnull_{ij}$ 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 $Dnull_{ij}$ values obtained across all markers [SD($Dnull_{ij}$)] is an empirical estimate of the standard error of $Dtest_{ij}$ under the null hypothesis.

Under the null hypothesis, Z_{ij} values distribute as a standardized normal variable and P-values for the individual sire-marker combinations were obtained accordingly. The test statistic (TS_j) for the j^{th} marker was then calculated by summing the Z^2_{ij} across all heterozygous sires:

$$TS_j=\sum(Z^2_{ij})$$

Under the null hypothesis, TS_j 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.

2.4. 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.

2.4.1. 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.

2.4.2. 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 (*ACOX1*) 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.

2.4.3. 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.

2.5. 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**
D^9D					
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	*DGK1*
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^9D					
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^9D					
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^9D					
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).

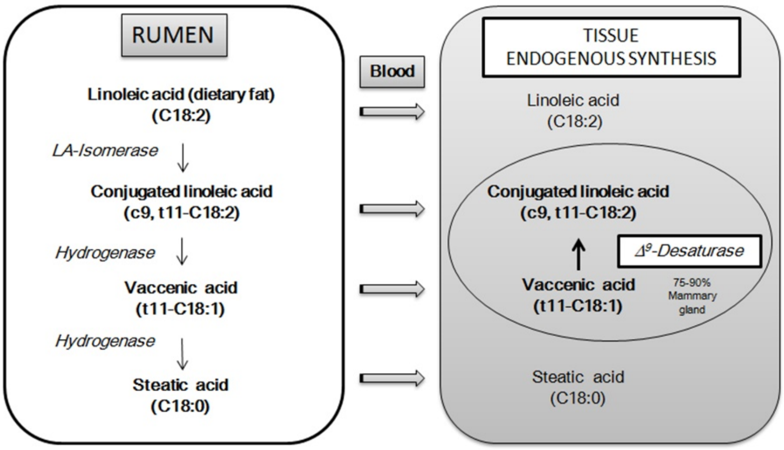


Figure 2. Quantile-quantile plots of the observed distribution of the p-value at marker level for CLA, VA and D9D

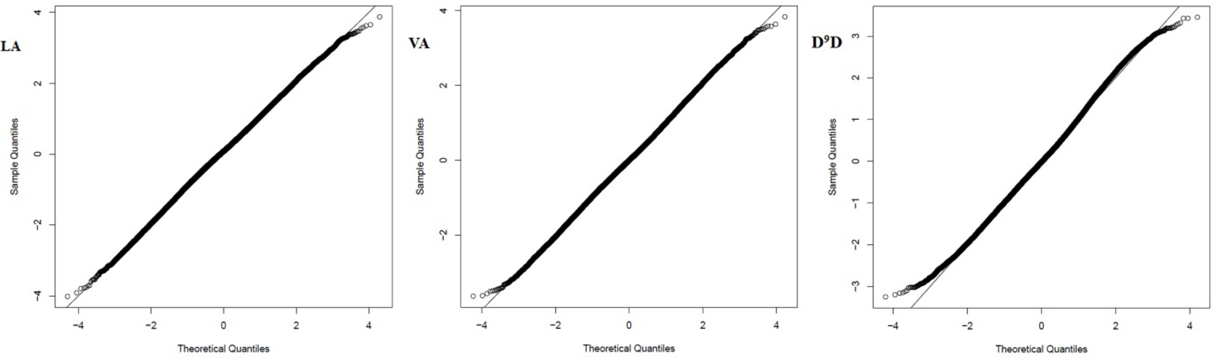


Figure 3. Quantile-quantile plots of SNPs at marker level for CLA, VA and D9D comparing the association statistics expected under the null hypothesis of no association.

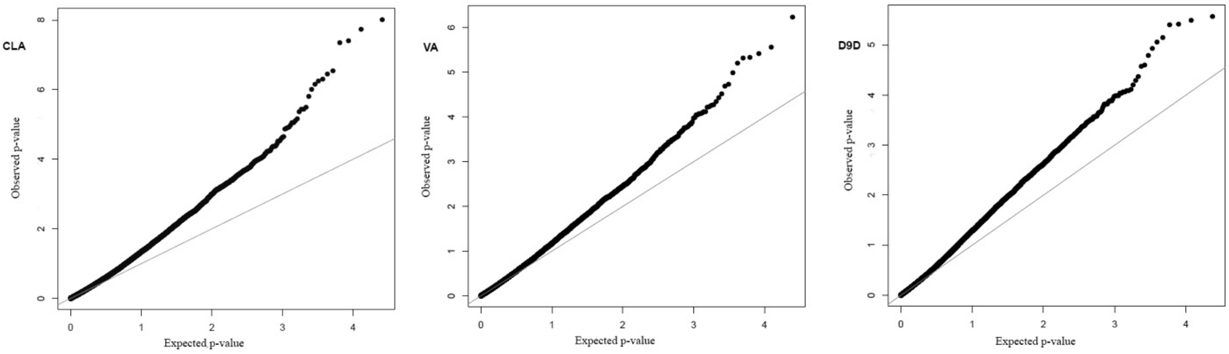


Figure 4. The location of SNPs associated with CLA VA and D9D shown as a Manhattan plot. Odd-numbered chromosomes are shown in orange; even-numbered chromosomes are shown in black. The horizontal blue and dashed line represent the 5% proportion of false positives (PFP) threshold; the horizontal red and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

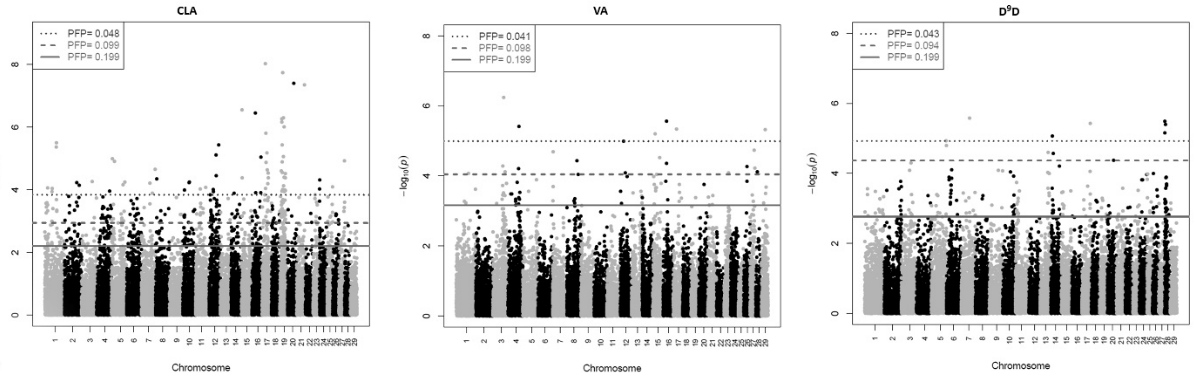


Figure 5. Association regions for CLA in all chromosomes

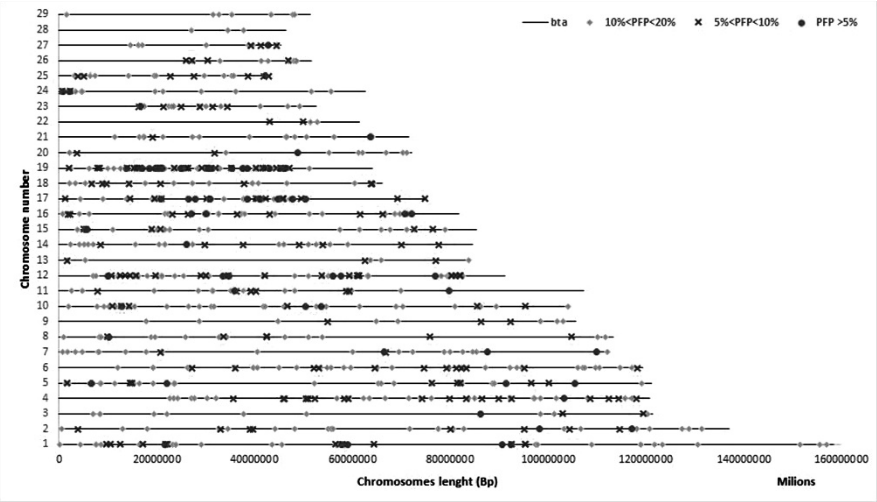


Figure 6. Association regions for VA in all chromosomes

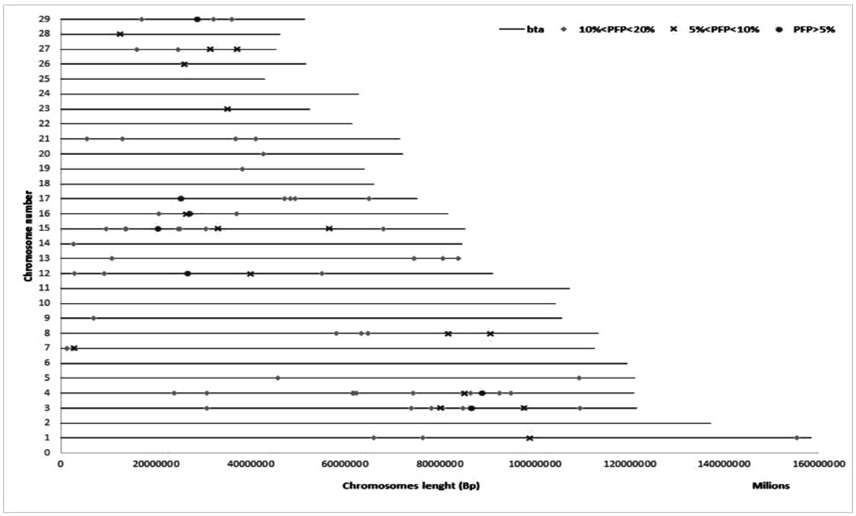


Figure 7. Association regions for D9D in all chromosomes

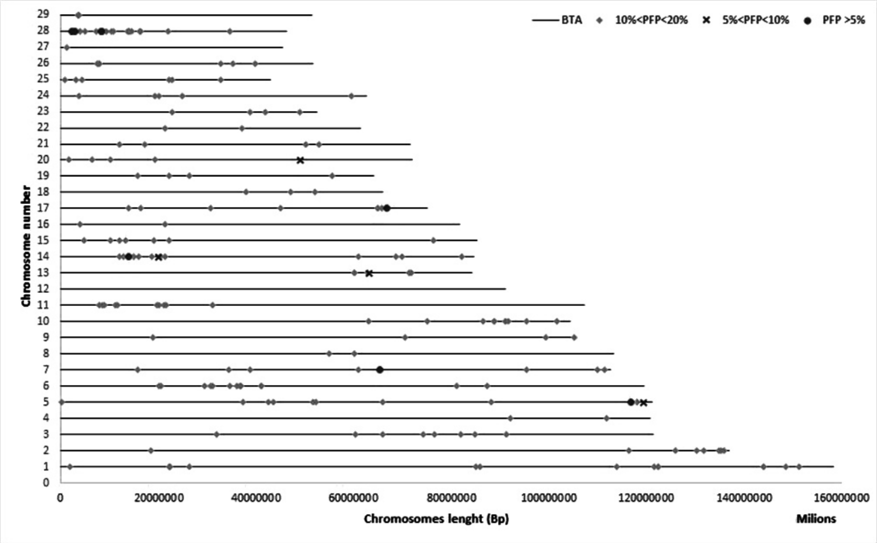
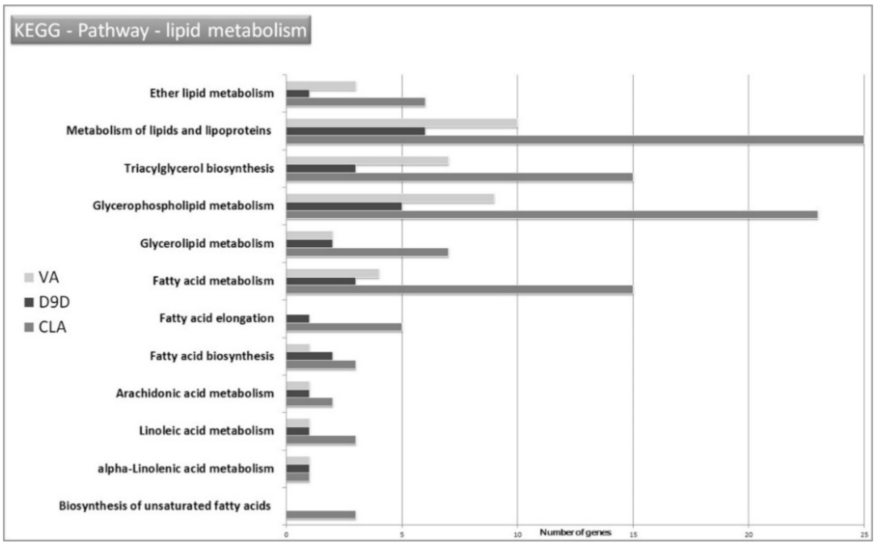


Figure 8. Metabolic lipid pathways in which genes are involved according to KEGG database



CHAPTER 3

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

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3.1. 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.

3.2. 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.

3.3. 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 1 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.

3.4. 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.

3.5. 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 c2 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

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

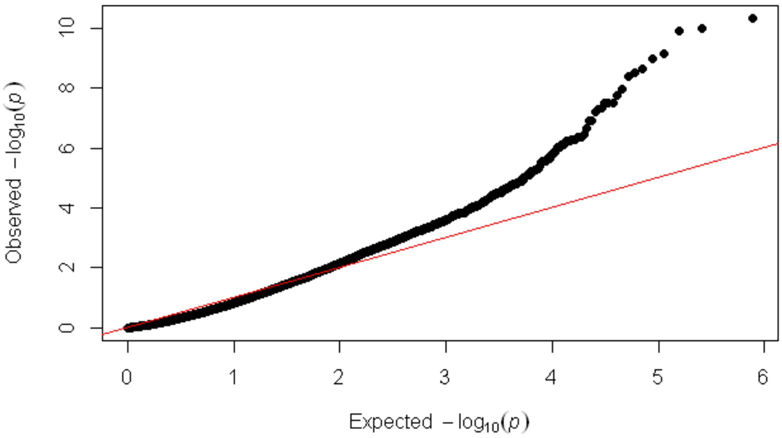
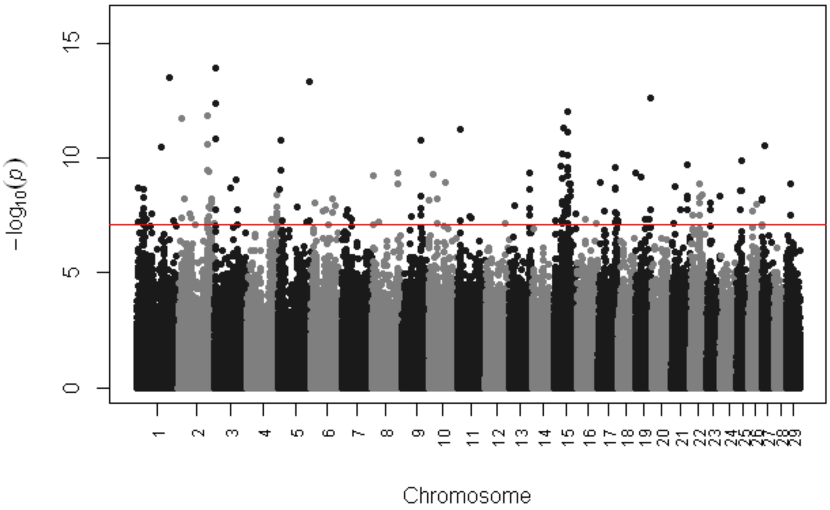


Figure 2. Manhattan plot of genome-wide associations for SCS in Valdostana Red Pied breed. The red line represents the Bonferroni correction threshold.



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-59263	*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	49477988-

									mastitis	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 1708	clinical mastitis SCC	8909340-26179970 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 synthetase short-chain family member 3)	GOTERM_CC_FAT	mitochondrion
	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	GOTERM_BP_FAT	protein folding

containing TCP1, subunit 7 (eta))	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 1 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	GOTERM_BP_FAT	phosphorus metabolic, phosphate metabolic, dephosphorylation
	GOTERM_MF_FAT	phosphatase activity

related protein 3)		
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	11140	Body weight (birth)	Chr22:12171103-35097286
		11142	Body weight (weaning)	Chr22:12171103-35097286
		11149	Body weight (yearling)	Chr22:32628727-43319438

	A1	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

GENERAL CONCLUSION

The mapping of CNV in the Brown population identified several CNV Regions that may be considered as possible source of variation in genomic selection for this cattle population. The mapping has been here performed using the Illumina Bovine SNP50 BeadChip array, a medium density one. The limit in the detection of CNVs with this array may be related to the density of the array. In fact the algorithms here used uses Log R Ratio and B allele frequency of the SNP included in the array. The SNPs probe spacing in this tool average 51.5 Kb thus leaving room for misidentification of CNV. The HD SNP Chip is more than 10 times denser with 777K SNP considered. Nevertheless the validation here performed with the qPCR, showed true CNV among those identified. The comparison with results obtained with HD SNP chip in the same population may provide insight on the capability of the tool to identify CNVs. Additionally comparison with other populations may deliver a meta-analysis to identify CNV conserved across populations. During the development on this thesis a CNV mapping in the Valdostana Red Pied has been produced (results not shown). The comparison among the two population is particularly interesting because they derive from a common environment, the Alps, but they undergo in the recent past to a different selection. Common CNV regions among the two breeds may disclosed interesting genomic regions.

In genomic selection the imputation is a technique to improve SNP genotype density of individuals. This is particularly used to impute low to high density genotypes of individuals in genomic selections. As CNV may be used as additional markers in calculating prediction equations (and in subsequent genomic selection of individuals), it can be envisaged to develop an imputation process for CNV as done for SNP genotypes. This would be particularly important whenever females will enter genomic evaluation process, because is unlikely that they will be genotyped with HD SNP chip or using genotyping by sequencing.

The QTL identified in this thesis for fatty acid components in the Italian Brown Swiss are of particular interest as is the first study on this population. Given the majority of milk is processed for cheese production, the selection for milk quality in the Italian Brown Swiss is undergoing for decades: k-casein variant B is desired and weighted positively in the official selection index. The Brown Association investigated for some year the possibility to improve the selection for quality including in their index true protein content and fatty acid composition. The results here obtained (i.e. heritability estimate and presence of QTL) indicates clearly the possibility to include in the selection objectives the fatty acid composition. The possibility that the Breeders Association will include in the selection index these objectives is nevertheless linked to the market that such a products will have. The enhanced nutritional value is not, at present market conditions, recognised as an additional economic value by the consumers. Some niche market for milk with added nutritional value is active in France where a farmers cooperative is successfully marketing the “Blue-Blanc-Coeur” milk, a product rich with Omega-3 fatty acid.

Finally QTLs mapped for mastitis resistance provide a step forward in the gene mapping research perfused in the Italian dairy cattle populations. The study is here done in a small population using a selective DNA pooling design. This approach allows to overcome the limitation from the small size of the population defining two extreme groups of individuals and identifying the markers with alternative allele frequencies associated with the considered phenotype. This is the first attempt to map QTL with SNP chips genotypes for a health trait is a small native population. This is particularly relevant as the vast majority of research in cattle populations is done in cosmopolitan and large cattle breeds. The possibility to enlarge GWAS studies in small populations is a key challenge to disclosed alleles and genomic variants that may be lost in highly selected breeds.