



Data in Brief

Gene expression profile of *Musculus longissimus dorsi* in bulls of a Charolais × Holstein F₂-cross with divergent intramuscular fat content



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ABSTRACT

Transcriptomes of *Musculus longissimus dorsi* (MLD) were compared between bulls from a F₂-cross derived from Charolais and Holstein Friesian. Two groups of 10 bulls were selected which differed significantly in intramuscular fat (IMF) deposition despite standardized husbandry and feeding conditions and identical sires in both groups. Consequently, genetic factors underlying the different capability of IMF deposition should be identified. A total of 32 differentially expressed genes (DEGs) were found of which 11 were up-regulated and 21 were down-regulated in the high IMF group. Ingenuity Pathway Analysis (IPA) identified a gene network comprising DEGs with functions in carbohydrate metabolism, lipid metabolism and molecular transport. The data from this study were deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE75347. We provide here a dataset which is of potential value to dissect molecular pathways influencing differences in IMF deposition in crossbred cattle with standardized genetic background.

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Specification	
Organism/cell line/tissue	<i>Bos taurus/Musculus longissimus dorsi</i>
Sex	Male
Sequencer or array type	GeneChip® Bovine Genome Array (Affymetrix)
Data format	Raw CEL files, Normalized data: PLIER signal intensity
Experimental factors	High vs. low intramuscular fat content in <i>M. longissimus</i>
Experimental features	Microarray gene expression profiling to identify differentially regulated genes in MLD of bulls with divergent intramuscular fat content
Consent	Publicly available from NCBI GEO
Sample source location	Dummerstorf, Germany

1. Direct link to deposited data

The deposited data can be found at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75347>

2. Experimental design, materials and methods

2.1. Experimental design

Intramuscular fat content in bovine skeletal muscle is a major quality trait of beef and depends largely on nutritional and other environmental

factors. Beside this, genetic factors like breed, sex and age determine the individual capability for IMF deposition [1,2]. An experimental F₂-population was generated by crossing the beef breed Charolais with the dairy breed Holstein Friesian to dissect the genetic background of productive traits in cattle [3]. The F₂-offspring is characterized by large variation in phenotypic traits and well-defined genetic structure. A partial phenotypic characterization of the bulls from this population was performed by Pfuhl et al. [4] and Hoehne et al. [5]. In this study, we selected two groups of 10 bulls according to low ($1.9 \pm 0.6\%$) and high ($7.0 \pm 0.6\%$) IMF content in MLD. The same sires were represented in both groups in identical number [6]. Moreover, husbandry and feeding conditions were identical for all animals. In the current analysis, we profiled mRNA expression in MLD of these bulls by microarray analysis. The aim of this analysis was the identification of differentially expressed genes (DEGs) related to the different genetic capacity to deposit fat in MLD.

2.2. Animals

Details on feeding and husbandry conditions are given by Kuehn et al. [3] and Pfuhl et al. [4]. Briefly, bulls were kept tethered and were fed a silage-based diet usual in intensive cattle fattening. All animals were reared, kept and slaughtered according to German Law of Animal Protection and the experimental protocol was approved by the Animal Care Committee of the State Mecklenburg-Western Pomerania, Germany (State Office for Agriculture, Food Safety and Fishery;

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Table 1

List of differentially expressed genes (DEGs) in bulls with high IMF (7.0%) and low IMF (1.9%), p value ≤ 0.05 , threshold of FC ≥ 1.3 .

11 up-regulated genes in high IMF group	21 down-regulated genes in high IMF group
ACLY, AGRN, CIDEC, CILP, FABP4, ICMT, INSIG1, MGST1, MRAS, SCD, THRSP	ART3, ATXN7L1, BACH2, C8orf22, CDC40, CETN3, CIR, FRAT2, GNAS, GPD2, HIST1H1E, IL4R, MGC13057, PROX1, RAD50, SESN1, SLC43A2, SMARCAD1, TAP2, TBXAS1, TRA@

LALLF M-V/TSD/7221.3-2.1-010/03). The bulls were slaughtered at an age of 18 months resulting in cold carcass weights of 408.1 ± 8.1 kg (low IMF) and 389.7 ± 8.1 kg (high IMF).

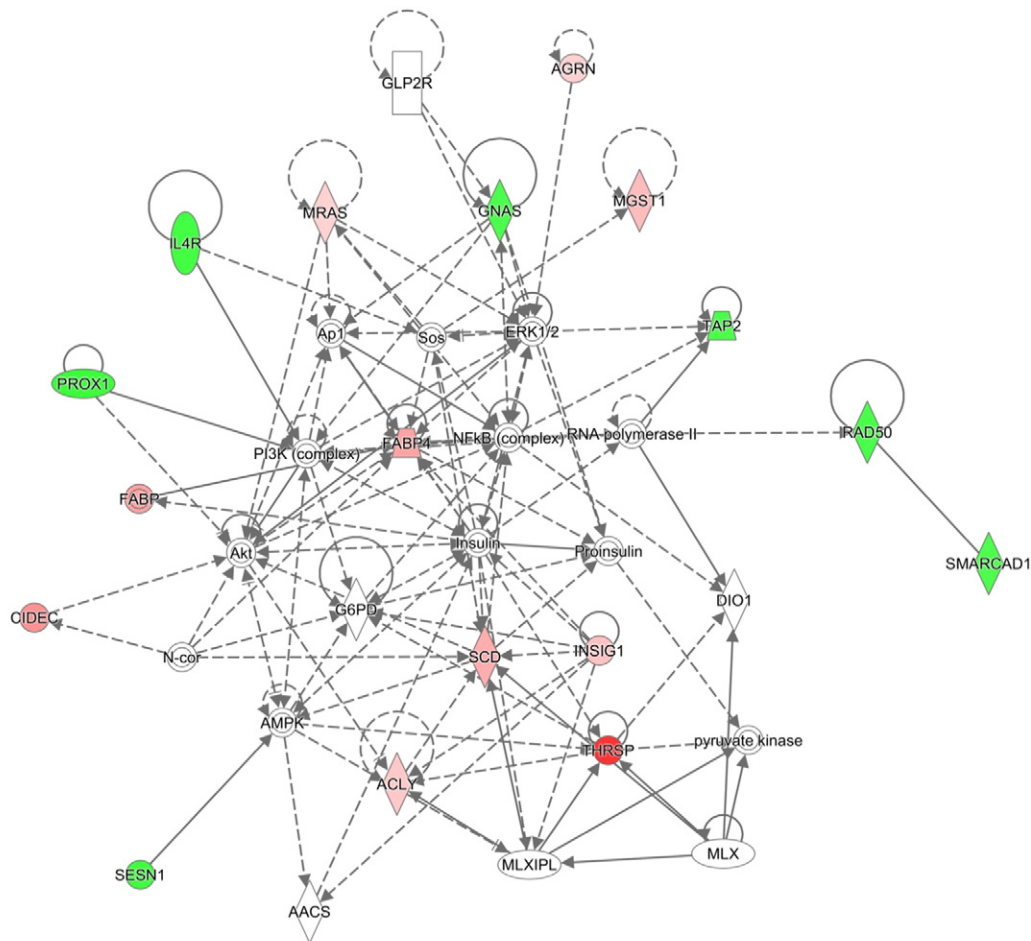
2.3. RNA isolation

Samples of MLD were obtained immediately after slaughter and shock frozen in liquid nitrogen. Total RNA was isolated from the samples with TRI Reagent (Sigma, Taufkirchen, Germany) and extracted with phenol-chloroform according to the manufacturers' instructions. RNA preparations were treated with DNase (Qiagen, Hilden, Germany) to remove genomic DNA and purified with an RNeasy kit (Qiagen). To assess RNA integrity, 1 μ l of RNA was loaded onto 1% agarose gel containing ethidium bromide. To rule out DNA contamination, PCR was performed on all RNA

samples using primers for the bovine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

2.4. Bovine microarray and data analysis

For each sample, 500 ng of total RNA was reverse-transcribed to cDNA and then transcribed to cRNA according to the protocols supplied by the manufacturer. Labeling of cRNA was done using an Affymetrix One-Cycle Synthesis and Labeling kit to prepare antisense biotinylated RNA targets (Affymetrix, High Wycombe, U.K.). The GeneChip® Bovine Genome Array (Affymetrix) was used in this experiment. Labeled RNA (10 μ g) was hybridized to the arrays at 42 °C for 16 h, washed and stained in a Fluidics Station 400, and scanned on a G3000 GeneArray Scanner (Affymetrix). Data were analyzed with Affymetrix GCOS 1.1.1 software using global scaling to a target signal of 500. Data were processed with MAS 5.0 to generate cell intensity files (CEL; present or absent). Quantitative expression levels of the present transcripts were estimated using the PLIER (Probe Logarithmic Intensity Error) algorithm for normalization in Expression Console software (Affymetrix). Statistical analysis was performed by JMP GENOMICS 6 (SAS Institute, Cary, USA) which includes a logarithmic scaling of expression values and Student's t-test. Only transcripts with "present" calls in at least 7 out of 10 animals per group were included in further analyses. Expression differences were considered significant when



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Fig. 1. Top-network identified by Ingenuity Pathway Analysis using DEGs from *M. longissimus dorsi* of bulls with high and low IMF; "Carbohydrate Metabolism, Lipid Metabolism, Molecular Transport" (red: up-regulated in high IMF group, green: down-regulated in high IMF group).

$p \leq 0.05$ and fold-changes (FC) ≥ 1.3 in transcripts between both experimental groups.

2.5. Ingenuity Pathway Analysis

Functional annotation was performed with Ingenuity Pathway Analysis (IPA, www.ingenuity.com) using predefined pathways and functional categories of the Ingenuity Knowledge Base. Fisher's exact test and Benjamini–Hochberg correction were applied to identify significantly enriched DEGs as members of pathways and functional categories. Relevant gene regulatory networks were identified using the Ingenuity Knowledge Base.

3. Results

We identified 32 unique protein coding genes with significantly different expression between both groups. Of them, 11 were up-regulated in bulls of the high IMF group whereas 21 were higher expressed in bulls of the low IMF group (Table 1). Increased expression of several genes identified as up-regulated in this study has been related to IMF in different cattle breeds in previous studies [7,8]. In contrast, data on genes down-regulated in the high IMF group are scarce in cattle.

Ingenuity Pathway Analysis was performed with all DEGs that are listed in Table 1. The identified top-network of genes unified the functional terms “Carbohydrate Metabolism, Lipid Metabolism, Molecular Transport” (Fig. 1). Ten out of 11 DEGs up-regulated in bulls with high IMF were included in this network. The network furthermore contains several members of general signaling pathways (e.g. MAPK, ERK 1 and 2, and AMPK) indicating a complex regulation of IMF deposition.

4. Summary

In this study, we have generated a dataset from transcriptome profiling of MLD in crossbred cattle with defined genetic background

differing in IMF content of the muscle. Of the 32 unique identified DEGs, 11 were up-regulated in muscle with high IMF content and assigned to pathways of lipid and carbohydrate metabolism. The DEGs presented here confirm in part earlier results but provide also a number of genes which have not been related to IMF deposition so far. This can serve as a basis for further investigations on lipid deposition in skeletal muscle of cattle.

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