Transcriptome profiling of *Musculus longissimus dorsi* in two cattle breeds with different intramuscular fat deposition

Elke Albrecht [a], Katrin Komolka [a], Siriluck Ponsuksili [b], Takafumi Gotoh [c], Klaus Wimmers [b], Steffen Maak [a,*]

[a] *Institute for Muscle Biology and Growth, Leibniz Institute for Farm Animal Biology, D-18197 Dummerstorf, Germany*
[b] *Institute for Genome Biology, Leibniz Institute for Farm Animal Biology, D-18197 Dummerstorf, Germany*
[c] *Kuju Agricultural Research Center, Kyushu University, 878-0201 Kuju-cho, Oita, Japan*

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ABSTRACT

Intramuscular fat (IMF) deposition is a physiological process in cattle and is highly variable among breeds suggesting a large influence of genetic factors besides environmental factors. In order to elucidate molecular pathways underlying the genetic variation in this trait we compared transcriptomes of *Musculus longissimus dorsi* (MLD) in steers of Japanese Black and Holstein Friesian cattle breeds fed a high energy diet typically applied in Japan to achieve maximum IMF content. We identified a total of 569 differentially expressed genes (DEGs) with the majority (433) up-regulated in Japanese Black cattle. This breed is characterized by an extreme capacity for IMF deposition. Subsequent Ingenuity Pathway Analysis (IPA) revealed a gene network linking parameters of cell morphology and maintenance with lipid metabolism. The data from this study were deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE75348. We provide here a dataset which is of potential value to dissect molecular pathways influencing differences in fat deposition under high-energy nutrition.

Specification	
Organism/cell line/tissue	*Bos taurus/Musculus longissimus dorsi*
Sex	Male (castrated)
Sequencer or array type	GeneChip® Bovine Genome Array (Affymetrix)
Data format	Raw CEL files, normalized data: PLIER signal intensity
Experimental factors	Japanese Black vs. Holstein Friesian
Experimental features	Microarray gene expression profiling to identify differentially regulated genes in MLD of Japanese Black steers compared to Holstein Friesian steers
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=GSE75348

* Corresponding author.
  E-mail address: maak@fbn-dummerstorf.de (S. Maak).

2. Experimental design, materials and methods

2.1. Experimental design

Intramuscular fat (IMF) – also known as marbling – comprises adipose tissue within skeletal muscle and is considered a key factor for meat quality, especially in the *M. longissimus dorsi* (MLD) [1]. The IMF content in bovine skeletal muscle is influenced by various genetic and environmental factors like breed, sex, age, and nutrition [1,2]. Japanese Black (JB) is a cattle breed with an exceptional capability for IMF deposition under high-energy feeding conditions in Japan [3]. In a previous investigation of Wang et al., expression profiles of MLD in Japanese Black were compared with the dairy breed Holstein Friesian (HS) under standard feeding conditions and revealed 24 differentially expressed genes (DEGs) [4]. These DEGs were mainly assigned to functional categories "lipid and energy metabolism" and "skeletal muscle contraction". In order to elucidate breed differences under high-energy feeding conditions an experiment was conducted to compare the maximum capacity for IMF deposition in Japanese Black and Holstein cattle [5]. The feeding regimen was described elsewhere [5] and the average IMF content in MLD was 34.3 ± 4.2% in Japanese Black and 20.4 ± 5.5% in Holstein at slaughter with 26 months of age. In the current analysis,

Table 1
DEGs with highest fold-changes (FC) in *M. longissimus* of Japanese Black and Holstein steers.

Up-regulated in Japanese Black			Down-regulated in Japanese Black		
Locus	FC	p-value	Locus	FC	p-value
WFDC3	8.54	0.017	SCN3B	−6.56	0.012
LRRN1	4.17	0.043	IFITM1	−4.06	0.020
LOC152573	3.82	0.013	DNAJB5	−3.27	0.006
GHR	3.48	0.005	S100A1	−3.25	0.008
CIB2	3.28	0.012	SGK	−3.24	0.048
KIAA1363	3.19	0.018	PLEK	−3.02	0.019
EIF4A2	3.14	0.043	BCL6	−2.98	0.043
FLJ14054	2.97	0.023	CNOT6	−2.79	0.006
RHOBTB3	2.94	0.042	FN5	−2.68	0.019
RAB26	2.91	0.002	SLK	−2.62	0.006
MBNL2	2.82	0.003	S100A2	−2.50	0.012
NKIRAS1	2.74	0.004	FRAS1	−2.50	0.003
ART3	2.60	0.044	MYL3	−2.46	0.045
RSC1A1	2.57	0.001	CYP1A1	−2.44	0.014
CRLF3	2.53	0.041	TSAP6	−2.39	0.027
SMOC2	2.46	0.007	RRAD	−2.36	0.001
KIAA1522	2.34	0.022	DSCR1	−2.12	0.048
RPP14	2.33	0.010	ANP32E	−2.11	0.004
MTUS1	2.31	0.001	VLDLR	−2.10	0.043
PYGO1	2.27	0.028	TFPI2	−2.07	0.029

we conducted microarray gene expression profiling on RNA isolated from MLD of Japanese Black and Holstein steers at slaughter to identify DEGs related to the differences in the capacity of IMF deposition.

2.2. Animals

Details on feeding regimen and husbandry conditions are given in [5]. Briefly, steers of both breeds were fed a diet with increasing concentrate percentage (36.8–86.4%) from 10 to 18 months of age. During the final fattening period (18–26 months) the concentrate share was 86.4–84.2%. Each 3 age matched steers of both breeds were investigated in this study (body weight at slaughter JB: 625 ± 30 kg and HS: 827 ± 45 kg). The animals were cared for and slaughtered according to Guidelines for Animal Experiments in the Faculty of Agriculture of Kyushu University and to laws of the Japanese Government (Law No. 105, Notification No. 6).

2.3. RNA isolation

Total RNA was isolated from MLD samples stored in RNAlater (Applied Biosystems, Tokyo, Japan) with TRI Reagent (Sigma, Taufkirchen, Germany) and extracted with phenol-chloroform as described by the manufacturer. Samples were treated with DNase and purified with an RNeasy kit (Qiagen, Hilden, Germany). To assess RNA integrity, 1 μl 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.
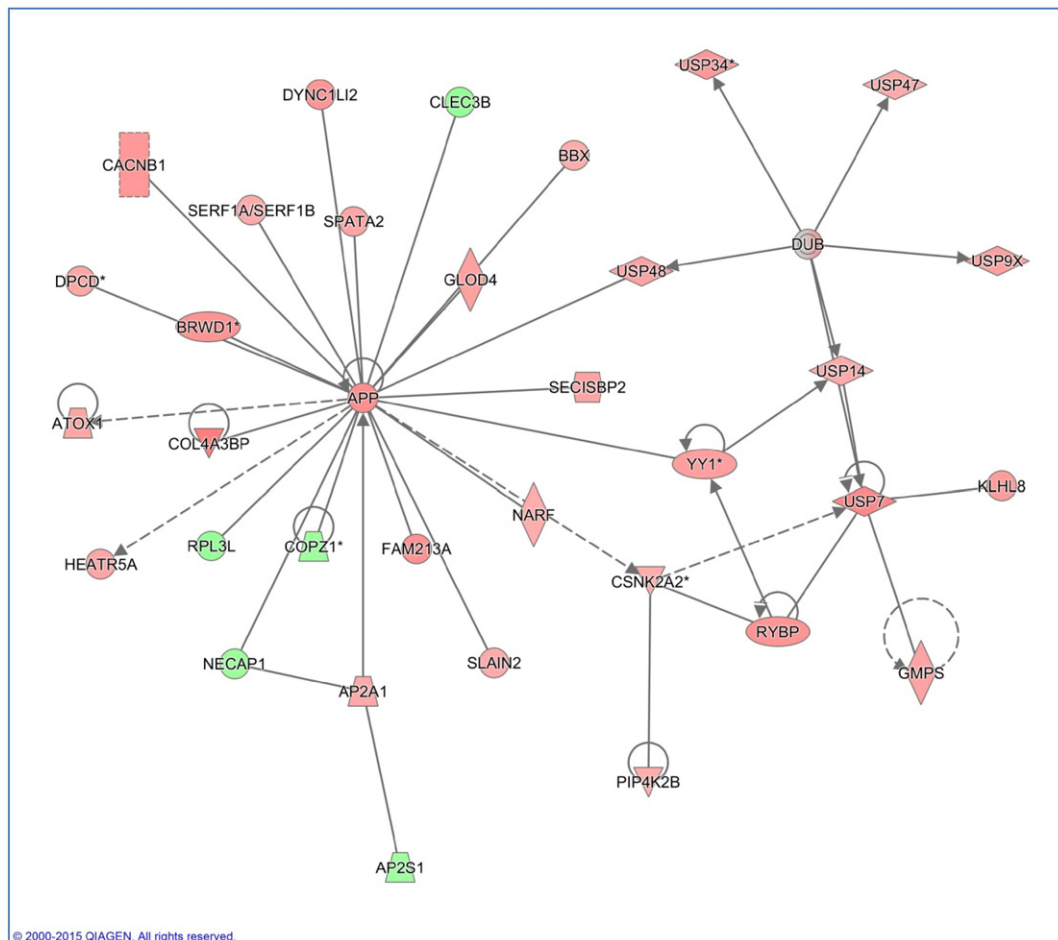
Fig. 1. Top-network identified by Ingenuity Pathway Analysis using DEGs from MLD of Japanes Black and Holstein steers: "Cell Morphology, Cellular Function and Maintenance, Lipid Metabolism"(red: up-regulated in Japanese Black, green: down-regulated in Japanese Black)

2.4. Bovine microarray and data analysis

The GeneChip® Bovine Genome Array (Affymetrix, High Wycombe, U.K.) was used in this experiment. 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. 10 μg labeled cRNA was then hybridized at 42 °C for 16 h to the arrays, washed and stained in a Fluidics Station 400, and scanned on a G3000 GeneChip 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 test. Only transcripts with "present" calls in all six samples were included in further analyses. Expression differences were considered significant when $p \leq 0.05$. A cut-off value of 1.3 was set for fold-changes (FC) of transcripts between both breeds.

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

A total of 569 unique coding genes were found to be differentially expressed in MLD of Japanese Black compared to Holstein steers applying the criteria $p \leq 0.05$ and FC ≥ 1.3. The majority of DEGs was up-regulated in MLD of Japanese Black (433) whereas 136 were up-regulated in Holstein (Supplemental Table S1). Forty DEGs with highest fold-changes between both breeds are given in Table 1. In contrast to a previous study of Wang et al. in the same breeds [4], no genes with obvious function in lipid metabolism were found to be differentially regulated in our analysis.

Ingenuity Pathway Analysis was performed with all DEGs meeting the cut-off criteria. The most significant network comprised associated functions "cell morphology, cellular function and maintenance, lipid metabolism" (Fig. 1). DEGs involved in this network are also marked in Supplementary Table 1.

4. Summary

We describe here a dataset of transcriptome profiling of MLD in Japanese Black and Holstein steers fed a diet to achieve maximum intramuscular fat deposition. A total of 569 DEGs was identified as associated with different intramuscular fat deposition. Pathway analyses linked genes involved in cell morphology and cell maintenance with lipid metabolism and provides a sound basis for further investigations.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.12.014.

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