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The comprehensive liver transcriptome of two cattle breeds with different intramuscular fat content

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ABSTRACT

Intramuscular fat (IMF) content is an important determinant factor of meat quality in cattle. There is significant difference in IMF content between Jinnan and Simmental cattle. Here, to identify candidate genes and networks associated with IMF deposition, we deeply explored the transcriptome architecture of liver in these two cattle breeds. We sequenced the liver transcriptome of five Jinnan and three Simmental cattle, yielding about 413.9 million sequencing reads. 124 differentially expressed genes (DEGs) were detected, of which 53 were up-regulated and 71 were down-regulated in Jinnan cattle. 1282 potentially novel genes were also identified. Gene ontology analysis revealed these DEGs (including *CYP21A2*, *PC*, *ACACB*, *APOA1*, and *FADS2*) were significantly enriched in lipid biosynthetic process, regulation of cholesterol esterification, reverse cholesterol transport, and regulation of lipoprotein lipase activity. Genes involved in pyruvate metabolism pathway were also significantly overrepresented. Moreover, we identified an interaction network which related to lipid metabolism, which might be contributed to the IMF deposition in cattle. We concluded that the DEGs involved in the regulation of lipid metabolism could play an important role in IMF deposition. Overall, we proposed a new panel of candidate genes and interaction networks that can be associated with IMF deposition and used as biomarkers in cattle breeding.

1. Introduction

Beef cattle are one of the most important protein sources of human food. Between different breeds of cattle, there are significant differences in growth rate, muscle mass, and meat quality. The Jinnan cattle are one of the five major varieties of yellow cattle in China, which are famous for their good quality with tender, distinct marble-textured meat [8]. On the contrast, the Simmental cattle, which are originated from Switzerland, are the best dairy-meat or dual purpose cattle breed in the world for intensive production [22], they have markedly high growth rate and lean content, which result in lower intramuscular fat (IMF) content and tenderness than Jinnan cattle. IMF content and muscle fiber characteristics are the main determinants of meat quality, including sensory traits (tenderness, juiciness, and flavor) and nutritional values [12,23]. IMF content is positively correlated to the sensory quality traits of beef [9,24]. The difference in IMF content between Simmental and Jinnan cattle makes them as great materials for comparative genomics studies to understand the molecular mechanism of intramuscular fatty acid deposition in cattle.

The liver, together with adipose tissue and skeletal muscle, is one of the most important organs to regulate lipid metabolism and other metabolic processes in mammals [7,18]. It is the central organ for uptake, oxidation, and metabolic conversion of non-esterified fatty acids. Moreover, it has the enzymatic capacity for *de novo* lipogenesis, cytoplasmic triacylglycerol stores, the synthesis of fatty acids from glucose and other non-lipid precursors [18]. With the development of high-throughput sequencing technologies, comparative analysis of liver transcriptome has been performed to

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explore potential candidate genes which affect IMF composition in pigs [20] and squab [28]. The transcriptome profiling of *longissimus dorsi* in Japanese Black and Holstein Friesian cattle breeds revealed most of differentially expressed genes were up-regulated in Japanese Black cattle, which is characterized by an extreme capacity for IMF deposition. A gene network related to cell morphology and lipid metabolism was also identified [2]. However, as we know, no comparative transcriptome studies in liver have been taken in different cattle breeds with distinct IMF levels.

In the present study, RNA-Seq approach was used to explore the liver transcriptome of Simmental and Jinnan cattle. The main goal of this study was to identify differentially expressed genes and interaction networks, which might be contributed to intramuscular fatty acid composition in cattle. In addition, the candidate genes we identified might be useful for the molecular breeding of beef cattle.

2. Material and methods

2.1. Animals and sample collection

All animal experiments were finished according to the animal procedures established by the Ministry of Agriculture of China, and were approved by the Institutional Animal Care and Use Committee at Shanxi Agricultural University. To eliminate sex differences in liver transcriptome, only male cattle were selected in this study. The liver tissues of three Simmental and five Jinnan cattle were harvested within 30 min after slaughter at the age of 1.5 years. All samples were quickly stored in liquid nitrogen until use.

2.2. RNA extraction and quantification

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. RNA concentration was measured using Qubit RNA Assay Kit in Qubit 2.0 Flurometer (Life Technologies, CA, USA), and RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

2.3. Transcriptome library preparation and sequencing

1 μg RNA per sample was used for library construction. Sequencing libraries were generated using NEBNext UltraTM RNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads and fragmented using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer(5X). First and second strand cDNA synthesis was synthesized. After adenylation of 3′ ends of DNA fragments, NEBNext Adaptors with hairpin loop structure were ligated to prepare for hybridization, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then PCR was performed and purified, library quality was assessed on the Agilent Bioanalyzer 2100 system. The clustering of the index-coded samples was performed using TruSeq PE Cluster Kit v4-cBot-HS (Illumina, USA) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq 3000 platform and 150 bp paired-end reads were generated.

2.4. Sequencing data analysis

After obtained the raw data, we removed reads containing adapter, reads containing ploy-N and low quality reads to get clean data through in-house perl scripts. At the same time, Q30, GC-content, and sequence duplication level of the clean data were calculated. The clean data with high quality were used for the downstream analyses. The clean reads were mapped to the reference genome of *Bos taurus* (version UMD 3.1.1) using Tophat2 software (version 2.1.0) [16]. Only reads with a perfect match or one mismatch were further analyzed and annotated based on the reference genome.

2.5. Gene expression quantification and differential expression analysis

Gene expression levels were estimated by fragments per kilobase of transcript per million fragments mapped reads (FPKM) method using cufflinks (version 1.3.0) [26]. Differential expression genes were identified between Simmental and Jinnan cattle using the DESeq R package (1.10.1) based on the negative binomial distribution [3]. The P values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate [4]. Genes with a FDR value < 0.05 and $|\log2\text{-fold change}| > 2$ were assigned as differentially expressed.

2.6. Functional enrichment analysis of differentially expressed genes

GO and KEGG pathway enrichment analyses of the DEGs were implemented by the Database for Annotation, Visualization and Integrated Discovery (DAVID) website [11]. Bovine Ensembl gene IDs of DEGs were submitted to the database for enrichment analysis of the significantly overrepresented GO biological process terms and KEGG pathway categories.

2.7. Protein-protein interaction

We construct protein-protein interaction networks of the DEGs to identify the extent of gene connectivity using Ingenuity Pathways Analysis (IPA) software (Qiagen, USA), which based on evidences in the literature. A cutoff of 35 molecules per network and 25 networks per analysis were selected. The network score is calculated with the right-tailed Fisher's Exact Test according to the hypergeometric distribution. The score is the −log of Fisher's Exact test P value, with the higher the score corresponding to a lower the probability of finding the observed number of network eligible molecules in a given network by random chance.

3. Results

3.1. Transcriptome profile of liver tissues in Simmental and Jinnan cattle

The liver transcriptome of Simmental ($n = 3$) and Jinnan ($n = 5$) cattle were sequenced using Illumina Hiseq 3000 platform. After removing reads containing ploy-N or adaptors and low quality reads, a total of 413.9 million 150-bp pair-end clean reads were obtained, the total read length was 61.33 gigabases (Gb), representing about 23-fold of the cattle genome size. At least 45.7 million clean raw reads (6.78 Gb) were obtained for each library. The Q30 scores of clean bases were more than 93% for all these eight samples, implying the high quality of our sequencing data. We next aligned the clean reads onto the *Bos taurus* reference genome (version UMD 3.1.1) [29], about 80.27–85.48% of reads were mapped (Table 1), of which 63.4–70.3% assigned to annotated exons; 10.49–13.08% located in introns; and the remaining 18.4–23.5% were fell into intergenic regions. The mapped reads were evenly distributed into the positive and negative strands of the genome. Moreover, 71.5–76.3% of reads were uniquely mapped to the bovine reference genome (Table 1).