Inheritance patterns of leukocyte gene expression under heat stress in F₁ hybrid cattle and their parents

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ABSTRACT

Crossbreeding capitalizes on heterosis effects and results in increased performance of crossbred animals. Dominance hypothesis and overdominance hypothesis are 2 common models proposed to explain heterosis. Differential gene expression between parents and hybrids is hypothesized to be responsible for heterosis. This study aimed to investigate the heat tolerance and inheritance patterns of leukocyte transcriptomics in F₁ hybrid cattle (Angus males × Droughtmaster females) and their parents Red Angus (AN) and Droughtmaster (DR) under heat stress. According to the respiratory rate and heat tolerance coefficient index, DR was better adapted to heat stress than AN. The physiological responses to heat stress of F₁ hybrids were similar to AN. We identified 802 differentially expressed genes in leukocytes between AN and DR under heat stress using mRNA sequencing. Compared with AN, upregulated genes in DR were enriched in biological processes of response to stress, external and chemical stimulus, and cytokine, cell surface receptor signaling pathway, and cardiovascular system development. In contrast, upregulated genes in AN were enriched in B cell activation and regulation of B cell activation. Gene expression levels can be inherited additively or nonadditively and are classified into additive (35%), dominance (44%), and overdominance and underdominance (18%) modes in F₁ hybrids and their parents. Inheritance patterns of gene expression showed that 97% (249/255) of the dominant genes were classified as paternal AN dominant in hybrids. The paternal imprinted *PEG10* gene and its regulatory transcription factor *MYC* showed an AN dominant expression pattern. The *MYC* interacted with most AN dominant genes. These transcriptomic analyses revealed that DR and AN had specific cellular

and humoral immunity and cardiovascular systems development function under heat stress. Inheritance pattern analyses from gene expression partly explained phenotypic differences between parents and F₁ hybrids. The paternal imprinted *PEG10* gene interaction with transcription factor *MYC* may contribute to explaining paternal dominant gene expression in hybrids.

Key words: crossbreeding, inheritance pattern, heterosis, heat tolerance

INTRODUCTION

Crossbreeding is a common practice for pigs and poultry. In cattle, the use of crosses or composite breeds contributes to the beef and dairy industry. Crossbreeding capitalizes on heterosis effects and complementarity between breeds and results in increased performance of crossbred animals compared with purebred animals (Wei and van der Werf, 1994). Two common models proposed to explain heterosis are the dominance (or complementation) hypothesis and the overdominance hypothesis. However, our knowledge of the genetic mechanisms of heterosis lags behind its wide application.

Over the past decade, differential gene expression in parents and hybrids has been hypothesized to be responsible for heterosis (Song and Messing, 2003; Hubner et al., 2005). Studies of humans and mice showed that much variation in gene expression levels is heritable (Cheung et al., 2003; Cui et al., 2006; Pickrell et al., 2010). The expression levels of genes in a genetically segregating population can be treated as quantitative traits for genetic analysis (Schadt et al., 2003). Like most traits, gene expression levels can be inherited additively or nonadditively (Gibson et al., 2004). Inheritance for differentially expressed genes (DEG) is classified into 5 modes. A hybrid can accumulate transcript levels equal to the mid-parent (additivity) or the high or low parent (high or low parent dominance); above the high parent (overdominance); or below the

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low parent (underdominance) (Swanson-Wagner et al., 2006; McManus et al., 2010). Additive alleles affect the phenotype in heterozygous individuals and are readily “visible” to selection (McManus et al., 2010). Therefore, investigating the inheritance patterns of gene expression is essential for understanding the genetic basis of complex phenotypes and animal crossbreeding.

In bovines, indicine (*Bos indicus*) breeds are generally better adapted to heat stress than taurine (*Bos taurus*) breeds (Beatty et al., 2006). These cattle differ in their resistance to thermal stress, ticks, and disease (Hansen, 2004; Jonsson et al., 2014). In beef cattle systems, indicine cattle are used extensively for crossbreeding with taurine. Several breeds are based on Brahman (*B. indicus*) crossbred foundations including Santa Gertrudis, Beefmaster, Brangus, Brahmousin, Charbray, and Droughtmaster (DR; Koger, 1980). In Southwest China, European cattle breeds such as Angus and Simmental are used to crossbreed with indigenous cattle to improve meat production. However, high ambient summer temperatures causing heat stress are likely to become major threats to the sustainability of beef cattle production systems. To adapt to warm climates, an alternative scheme to crossbreeding is using the zebu genotype to confer thermal tolerance in European breeds. Droughtmaster cattle are a tropical breed of beef cattle developed in North Queensland of Australia. This breed was produced by crossing Brahman and British breed cattle (*B. taurus*) and selecting for meat quality, parasite resistance, heat tolerance, and ability to adapt to the environment. Thus, crossbreeding between Angus and Droughtmaster was investigated in this study.

Peripheral blood is an accessible and informative source of transcriptional information for many metabolic changes, immune response function, and heat stress studies (Mehla et al., 2014). Gene expression profiling of leukocytes provides an opportunity to clarify the response of the animal to heat stress on a global level (Mehla et al., 2014; Srikanth et al., 2017). Thus, this study aimed to investigate thermal tolerance and leukocyte transcriptomic differences between Red Angus (AN) and DR purebreds and inheritance of gene expression levels of their F₁ hybrids (AN males × DR females, AD).

MATERIALS AND METHODS

Animals

The F₁ hybrids were produced by crossing AN males to DR females. Female (about 10 mo old) F₁ hybrids (AD, n = 12), purebred AN (n = 12), and DR (n =

11) were used in this study. All animals were managed under an open housing system and fed per standard management practices followed at the breeding farm (Yunyang, Chongqing, China). Feed and water were offered ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Southwest University (Chongqing, China).

Record of Temperature-Humidity Index

Ambient temperature and humidity were recorded daily at 0800, 1400, and 2000 h from 1.5 m above the floor during the experimental period. The summer experimental period was August 11–16, 2016, and the autumn experimental period was November 9–13, 2016. Temperature-humidity index (THI) was calculated as $THI = 0.72 (T_{db} + T_{wb}) + 40.6$, where T_{db} was dry bulb temperature and T_{wb} was wet bulb temperature (Maust et al., 1972).

Physiological Phenotype

The physiological responses of cattle to heat stress including increased respiratory rate and rectal temperature have been well described (Beatty et al., 2006). Respiratory rates (diaphragm movements per minute), rectal temperature (°C), and heat tolerance coefficient (HTC) were used to evaluate the performance of cattle under heat stress in previous studies (Beatty et al., 2006; Liu et al., 2011; Otto et al., 2019). Respiratory rates and rectal temperature were measured at 0800, 1400, and 2000 h. Individual heat tolerance coefficient was calculated as $HTC = 100 - 10 (ART - 38.3)$, where ART was average rectal temperature, 38.3 was physiological bovine body temperature in degrees Celsius, 10 was a correction factor to convert deviations in body temperature to a unit basis, and 100 was perfect efficiency in maintaining temperature at 38.3°C (Rhead, 1944). An index of HTC was calculated for each animal to assess heat adaptability. High HTC value indicated better heat resistance.

Blood RNA Sampling

At the end of the summer experimental period, blood was collected from tail veins of healthy cattle and anticoagulated with EDTA. Blood samples (5 mL) were centrifuged at $3,000 \times g$ for 15 min at 4°C and white coat (leukocytes) was transferred to a new 2-mL cryopreservation tube to which 1 mL of RNAiso Plus (Takara, Dalian, China) was added before snap freezing in liquid nitrogen for transport. Storage was at −80°C until RNA isolation.

mRNA-Seq and DEG Analysis

Total RNA was extracted with RNAiso Plus (Takara) according to the manufacturer's protocols. Quantity and purity of RNA were detected using a Nanodrop 2000C (Thermo Fisher Scientific, Wilmington, DE) and Qubit Fluorometer (Invitrogen, Carlsbad, CA). The RNA was stored at -80°C until further analyses. A total 18 RNA samples from 6 individuals of AD, AN, and DR were selected for mRNA-seq. All mRNA-Seq libraries were sequenced on an Illumina Novaseq platform (San Diego, CA) following the manufacturer's protocol. Quantity and purity of RNA and RNA-Seq libraries were determined by Beijing Novogene Bioinformatics Technology (Beijing, China). Raw data were submitted to the NCBI Gene Expression Omnibus (GEO) under accession number GSE151817.

Clean reads that passed quality control were mapped to the *B. taurus* genome ARS-UCD1.2 from Ensembl 97 using HISAT2 v2.0.5 (Kim et al., 2019). Read numbers mapped to each gene were counted by featureCounts v1.5.0 (-p3). Fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM) of each gene were calculated based on the length of the gene and read counts mapped to this gene. Read count data were sent to iDEP90, an integrated web application for differential expression and pathway analysis of RNA-Seq data (Ge et al., 2018). The DESeq2 algorithm was applied to filter the DEG (Love et al., 2014), after fold-change analysis and false discovery rate (FDR) analysis under the following criteria: (1) $|\log_2\text{foldchange}| \geq 1$; and (2) $\text{FDR} \leq 0.05$. Volcano and pathway plots were drawn by iDEP90.

Functional Enrichment Analysis

Gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted on DEG to investigate their biological processes and functions using iDEP90 and DAVID 6.8 (<https://david.ncifcrf.gov/>). The GO terms and KEGG were set for significance threshold at $\text{FDR} \leq 0.05$.

Inheritance Classifications

The expression data of hybrids and parents were $\log_2$ -transformed, enabling directions and fold changes to be viewed directly. A threshold of 1.25-fold FPKM change was set to determine differences in expression level (McManus et al., 2010). These DEG were classified as having additive, dominant, underdominant, or overdominant inheritance, based on the magnitude of the difference between total expression in the hybrid

and each parental species. Genes for which expression in AD hybrids were lower than in AN and greater than in DR (or vice versa) were classified as additive. Genes for which expression in AD hybrids were similar to one of the parents were classified as dominant. Genes for which expression in AD hybrids was either greater than or less than both AN and DR were considered mis-expressed and classified, respectively, as overdominant and underdominant (Figure 3B; McManus et al., 2010). Graphs for inheritance of gene expression were produced with GraphPad Prism 7.0 (La Jolla, CA). Heatmap and enriched GO terms of additivity, AN dominance, and underdominance genes were determined using iDEP90. The network of *PEG10*, *MYC*, *IGF2BP3*, and other AN dominance genes was generated by STRING (<https://string-db.org/>; Szklarczyk et al., 2019).

Validation by Reverse-Transcription Quantitative PCR

Gene expression levels were validated using reverse-transcription quantitative PCR (RT-qPCR). Each of 6 RNA samples of AN, DR, and AD were treated with DNase and first-strand cDNA was generated using a PrimeScript RT Reagent Kit with gDNA Eraser (Takara). The RT-qPCR reactions (SuperReal PreMix Plus, SYBR Green I, Tiangen, China) were performed on a Bio-Rad CFX-96 system (Bio-Rad, Hercules, CA) using conditions of 95°C for 3 min followed by 40 cycles with 10 s at 95°C , 30 s at the specific annealing temperature, and a standard melting curve analysis to validate specificity of PCR products (Zhang et al., 2019). All RT-qPCR reactions were performed in triplicate. One Angus cattle was used as the control sample. β -Actin and *HPRT* genes were used as reference genes. Thus, mRNA relative fold-change was analyzed with the CFX-96 system's relative quantification software based on the $2^{-\Delta\Delta C_q}$ method (Bio-Rad).

Statistical Analysis

Physiological phenotype and RT-qPCR data were expressed as mean $\pm$ standard deviation. For all statistical analyses, $P < 0.05$ was considered significant. One-way ANOVA and Tukey's multiple comparisons tests were conducted for all statistical significance analyses using GraphPad Prism 7 software.

RESULTS

Heat Tolerance of F_1 Hybrids Was Similar to Angus

During the summer experimental period, THI were 77 at 0800 h and 82 at 2000 h from d 3 to 6 (Figure

1A). This result indicated that the cattle were under middle heat stress status. Average respiratory rate was 45.15 ± 5.01 and average rectal temperature was $39.12 \pm 0.15^\circ\text{C}$ for AN, 42.24 ± 4.07 and $39.19 \pm 0.12^\circ\text{C}$ for AD, and 40.88 ± 2.56 and $38.92 \pm 0.12^\circ\text{C}$ for DR. Average respiratory rate was significantly higher for AN than DR ($P = 0.043$) with no significant difference between AD compared with AN or DR (Figure 1B). The HTC index was significantly higher for DR (93.82 ± 1.20) than AN (91.82 ± 1.51) and AD (91.13 ± 1.28 ; Figure 1C, $P = 0.001$). Average respiratory rate was significantly increased for afternoon compared with morning for the AN and AD groups during the summer experimental period with no significant change in the DR group (Figure 1D). Average rectal temperature was increased about 0.2°C for afternoon compared with morning for the AN and AD groups during the summer experimental period with almost no change in the DR group (Figure 1E). During the autumn experimental period, THI was 48 to 60 (Figure 1A) and average respiratory rate for AN was significantly higher for DR (Figure 1B, 26.95 ± 2.34 vs. 24.26 ± 2.74 , $P = 0.030$). These results indicated that DR was better adapted than AN to heat stress. Physiological responses to heat stress of AD were similar to AN.

Gene Expression Differences Between AN and DR

Gene expression was quantified by paired-end mRNA-seq from each of 6 leukocyte samples of AN, DR, and AD (Supplemental Table S1; <https://doi.org/10.3168/jds.2020-18410>). We obtained an average of 53.90 million reads from AN, 50.45 million from DR, and 49.11 million from AD libraries. Clean reads uniquely mapped to bovine reference genome *Bos_taurus* ARS-UCD1.2 were 48.09 million (89.2%) for the AN library, 42.24 million (84.1%) for DR, and 44.13 million (89.7%) for AD (Supplemental Table S1). Expression levels of transcripts were normalized as FPKM.

Using the integrated web application iDEP90, 13,423 genes passed a filter of average read counts more than 20 and 0.5 counts per million in at least 4 samples. We identified 802 significant DEG between AN and DR with 541 upregulated (higher expression in DR) and 261 downregulated (higher expression in AN) genes using a threshold of $\text{FDR} \leq 0.05$ and fold change ≥ 2 (Figure 2A). These significant DEG were useful for studying the molecular mechanism of differences between AN and DR cattle under heat stress. The upregulated genes had significant enrichment in inflammatory response, response to external stimulus, response to cytokine,

Figure 1. Farm temperature-humidity index and respiratory rates of cattle during the summer and autumn experimental period. (A) Farm temperature-humidity index at 0800, 1400, and 2000 h during the summer and autumn period. (B) Average respiratory rates (diaphragm movements per min) of different breeds at the summer and autumn period. (C) Heat tolerance coefficient of different breeds at the summer and autumn period. (D) Respiratory rates (diaphragm movements per min) of cattle at 0800, 1400, and 2000 h during the summer period. (E) Rectal temperature of cattle at 0800, 1400, and 2000 h during the summer period. ΔT = rectal temperature at 1400 h – rectal temperature at 0800 h.

response to stress, cell surface receptor signaling pathway, cellular response to cytokine stimulus, immune response, and defense response, constructed from a biological processes GO term network (Figure 2B, $FDR < 7.52 \times 10^{-11}$). Downregulated genes were involved in B cell activation, immunoglobulin production, and regulation of B cell activation (Figure 2B, $FDR < 0.007$).

All 802 DEG were used in GO functional and KEGG pathway analyses with DAVID 6.8. The enrichment of biological processes GO terms was similar using DAVID 6.8 and iDEP90. To evaluate the relative importance of each GO term in the biological process categories, we calculated the percent enriched genes for each GO term out of the total higher-expression DEG for AN or DR. For example, 6 genes with higher expression were enriched in inflammatory response in AN and 36 genes in DR. Thus, the relative importance of the immune response GO term was 2.30% (6/261) for AN and 6.65% (36/541) for DR (Figure 2C). Similar relative importance was found for response to stress, response to stimulus, response to external stimulus, defense response, immune system process, and immune response GO terms in AN and DR. The DR had specific enrichment genes in inflammatory response, cytokine production, cytokine secretion, regulation of cytokine secretion, chemotaxis, and cardiovascular system development (Figure 2C, $FDR < 2.02 \times 10^{-4}$). In contrast, AN had genes enriched in B cell activation and regulation of B cell activation (Figure 2C, $FDR = 0.001$). Pathway analysis showed these DEG were significantly enriched in cytokine-cytokine receptor interaction (Figure 2D, $FDR = 0.001$). These results indicated that different breeds had different genes that contributed to response to stress and stimulus and cardiovascular system function. Under heat stress, DR had higher cellular immunity and AN had higher humoral immunity gene expression.

Inheritance Patterns of Transcript Levels in F_1 Hybrid Cattle

We found 557 DEG between AD and DR with 107 with higher expression in AD and 450 with higher expression in DR (Figure 3A). No genes were significantly different between AD and AN. A total of 988 genes were significantly different between AN, AD, and DR (Figure 3A). A total of 371 DEG between AD and DR also differentially expressed between AN and DR (Figure 3A). Using the average FPKM ≥ 1 for all 3 genotypes as the cutoff, 580 high-confidence genes passed a filter for inheritance analysis. Regardless of statistical significance, any pair of genotypes with expression that differed less than 1.25-fold FPKM change was considered to have similar expression. Hypotheti-

cal gene expression patterns were classified as having conserved, additive, dominant, underdominant, or overdominant inheritance (Figure 3B). Of the remaining 580 genes, 255 (44.0%) were dominance genes with 249 showing AN dominance expression and 6 showing DR dominance, 203 (35.0%) were classified as additive, and 122 (21.0%) were misexpressed in AD and classified as 103 underdominant and 19 overdominant (Figure 3C).

The heatmap of additive, AN dominant, and underdominant inheritance genes is in Figure 3D. Additive genes were enriched in immune response and B cell activation. The AN dominant genes were enriched in regulation of response to stimulus, response to external stimulus, and response to LPS. Response to stress and defense response genes mostly had AN dominant and underdominant patterns (Figure 3D). Expression patterns validated by RT-qPCR included the AN dominant genes *LIPA*, *S100A8*, *S100A9*, *S100A12*, *IL12B*, and *CD14*, the additive gene *BOLA-DOB*, and the conserved gene *CLEC7A* (Figure 3E). Most genes involved with cardiovascular system were significantly different between AN and DR and had AN dominance (Figure 3F). These results suggested that response to stimulus, response to stress, and cardiovascular system genes were inherited as AN dominant in AD.

Paternal Imprinted Gene *PEG10* Showed AN Dominant Expression in F_1 Hybrids

To investigate the association between imprinted genes and inheritance patterns, we checked the expression patterns of 22 imprinted genes in hybrids and their parents. A total of 13 imprinted genes were expressed in leukocytes (Figure 4A-B). Only paternal imprinted gene paternally expressed gene 10 (*PEG10*) was significantly different between AN and DR and showed AN dominant pattern (Figure 4B, $P = 0.008$). The *PEG10* regulatory network member genes *MYC* and *IGF2BP3* also showed AN dominant expression patterns (Figure 4B, $P < 0.05$). The AN dominant genes involved with cellular response to chemical stimulus, cellular response to stimulus, response to stress, and response to stimulus significantly interacted with *MYC* (Figure 4C). These results suggested that the expression patterns of paternal imprinted gene *PEG10* and *MYC* were associated with paternal AN dominant gene expression.

DISCUSSION

In this study, we found that DR was better adapted to heat stress than AN in Southwest China. However, crossbreeding males AN with females DR did not improve physiological responses to heat stress in their F_1 hybrids. Dominance inheritance pattern analyses using

Figure 3. Inheritance patterns of gene expression in F_1 hybrids of Angus (AN) and Droughtmaster (DR). (A) Venn diagrams of differentially expressed genes between DR versus AN and DR versus F_1 hybrids (AD) based on upregulated and downregulated genes. (B) Hypothetical gene expression patterns in AN (red), DR (green), and AD (yellow) illustrating conserved, additive, dominant, underdominant, and overdominant modes of inheritance. (C) Scatterplot comparing differences in expression level between F_1 hybrids and parental species (DR, x-axis; AN, y-axis) for differentially expressed genes. (D) Heatmap and enrichment gene ontology terms of additive, AN dominant, and underdominant modes. (E) Gene expression levels in AN, DR, and AD were detected using reverse-transcription quantitative (RT-q) PCR. (F) Expression patterns of genes involved in cardiovascular system development. FPKM = fragments per kilobase of transcript sequence per million base pairs sequenced. Data were expressed as mean $\pm$ SD.

Figure 4. The paternal imprinted gene *PEG10* and transcription factor *MYC* showed Angus (AN) dominant expression in F₁ hybrids (AD). (A) Expression patterns of 12 imprinted genes showed no significant differences between 3 groups. (B) Expression patterns of the paternal imprinted gene *PEG10* and its regulatory network member genes. (C) Network of *PEG10*, *MYC*, *IGF2BP3*, and other AN dominance genes. Genes involved in cellular response to chemical stimulus, cellular response to stimulus, response to stress, and response to stimulus are shown in different colors. DR = Droughtmaster; FPKM = fragments per kilobase of transcript sequence per million base pairs sequenced. Data were expressed as mean $\pm$ SD.

gene expression partly explain phenotypic differences between parents and F₁ hybrids.

Transcriptomic variation among cattle breeds may provide mechanistic knowledge about differences in phenotypes including appearance and physiological, behavioral, and production traits. For example, differential gene expression analysis of leukocytes using mRNA-seq showed high similarity within taurine breeds Holstein and Jersey, and a much greater difference between taurine breeds and the indicine breed Cholistani. Enriched genes with differential expression between Holstein and Cholistani were involved in the immune response and inflammatory response of animals (Huang et al., 2012). Whole-blood transcriptome analysis showed that gene expression patterns in Northern Finncattle are more similar to Yakutian cattle (Pokharel et al., 2019). A transcriptomic study of peripheral leukocytes from tick-infested *B. taurus* and *B. indicus* showed significant differences between the 2 cattle breeds in genes in biological processes including metabolic and immune response processes (Piper et al., 2009). In our study, DR was better adapted to heat stress than AN; DR had significantly lower respiratory rate and higher HTC during summer in Southwest China. The blood transcriptome revealed that under heat stress, DR had higher cellular immunity and AN had humoral immunity gene expression. These results were consistent with previous studies showing that immune response and inflammatory response genes are important in adaptability to heat stress (Huang et al., 2012; Mehla et al., 2014). The biological process GO terms for the cardiovascular system were enriched in DEG between DR and AN and showing AN dominance (Figure 3F). This difference in gene expression was consistent with differences in phenotypes with *B. indicus* having less pronounced heart rates changes than *B. taurus* during heat stress (Beatty et al., 2006). In total, leukocyte transcriptomes revealed that the immune and cardiovascular systems may contribute to resistance to heat stress in DR cattle.

An important finding of this study was that inheritance pattern analyses of the transcriptome can partly explain heat tolerance phenotype differences between F₁ hybrids and their parents. Consistent with the similar physiological responses to heat stress between AD and AN, inheritance patterns of transcript levels showed that 97% (249/255) of high-confidence dominant genes were AN dominant in AD (Figure 3C). A previous study showed that high-parent dominance DEG are important in heterosis in *Drosophila melanogaster* and contribute specifically to BW heterosis (Wu et al., 2016). In chickens, heterosis of abdominal fat percentage was 69.28 to 154.71% in reciprocal crosses and high-parent dominance accounted for approximately 50%

of nonadditive genes in reciprocal crosses (Mai et al., 2019). A threshold of gene expression 1.25-fold change was used to classify inheritance categories in our study and previous studies (McManus et al., 2010; Gu et al., 2019). Although different threshold criteria may lead to more stringent or more lenient filter results, this would not have a substantial effect on our statistical analyses of AN dominance ($432/459 = 94.12\%$ for 1.5-fold vs. $249/255 = 97.65\%$ for 1.25-fold). Parental dominant genes seem to be important for heterosis or phenotype differences between F₁ hybrids and their parents.

Another important finding of this study was that the paternal imprinted gene *PEG10* may contribute to explaining paternal dominant gene expression in hybrids. The intrinsic regulatory mechanisms of gene expression classified as dominant are still unknown. Changes in *cis*-acting elements (e.g., promoters and enhancers) and *trans*-acting factors (e.g., transcription factors and chromatin modifiers) or both likely to contribute to variation in gene expression (Signor and Nuzhdin, 2018). A recent study in chickens found that *trans*-acting regulation of gene expression may be largely responsible for differential gene expression between parental lines and between the crosses and parental lines, thus contributing to heterosis effects in the F₁ hybrids (Zhuo et al., 2019). Genomic imprinting is an epigenetic mechanism that results in monoallelic expression of genes depending on parent-of-origin-specific allele silencing (Khatib et al., 2007). The *PEG10* gene is paternally imprinted (Khatib et al., 2007) and the transcription factors E2F1 (Wang et al., 2018), c-MYC (Li et al., 2006), and transforming growth factor β 1 (TGF- β 1; Shinohara et al., 2017) are reported to participate in *PEG10* expression regulation (Xie et al., 2018). The H19-*PEG10*/*IGF2BP3* axis promotes gastric cancer progression in patients with high lymph node ratios (Ishii et al., 2017). In our study, AN cattle were used as the paternal breed for the hybrids. The *PEG10*, *MYC*, and *IGF2BP3* genes were expressed as paternal dominant in hybrids and other paternal dominant genes significantly interacted with *MYC* (Figure 4B-C). One limitation of our study is that it lacked reciprocal cross samples to verify the paternal expression patterns of *PEG10* and *MYC* genes. Thus, the role of paternal imprinted gene *PEG10* in paternal dominant gene expression should be studied further.

In summary, transcriptomic analysis showed that response to stress, immune function, and blood circulation capacity were different between DR and AN. The response to stress and cardiovascular system genes showing AN dominant expression patterns may partly explain the similar heat tolerance phenotype between AD and AN. The paternal imprinted gene *PEG10* and its regulatory transcription factor *MYC* had an

AN dominant expression pattern. The *MYC* interacted with most AN dominant genes. These results suggested that imprinted genes *PEG10* and *MYC* may contribute to explaining paternal dominant gene expression in hybrids.

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