Whole-genome scanning for the heat-resistance-associated genes in the Droughtmaster breed (*Bos taurus*)

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Abstract

The Droughtmaster is a tropical breed of beef cattle developed in North Queensland that exhibits a combination of heat resistance and parasitic resistance from long-term artificial selection. Therefore, we used next-generation sequencing technology to screen the chromosomal regions and genes related to heat-resistance in cattle to provide data for improving cattle breeding. A total of 15,569,067 variants including 14,249,316 SNPs were obtained from two mixed pools by genome-wide resequencing. According to the results of the selective sweep analysis of the Droughtmaster pool compared to the nonheat resistant breeds pool, 81 candidate genes under selection in Droughtmaster were identified by combining χ^2_{HP} and F_{ST} analyses with a threshold standard of the top 1%, including SLC7A11, GYPC, and GYPC. In addition, 40 GO terms and 44 pathways were annotated from newly identified candidate genes. These signaling pathways were involved in environmental information processing, organismal systems, and metabolism. A majority of these genes have not been implicated in the previous studies of heat resistance. This study explored the genomic changes that result from long-term artificial selection, our findings help to explain the molecular mechanism of heat resistance in cattle.

Keywords Droughtmaster · Whole-genome scanning · Heat resistance · Cattle

Introduction

Heat stress is one of the most challenging and dangerous environmental factors affecting commercial domestic animals, such as poultry (Nawab et al. 2018; El-Kassas et al. 2018), pigs and cattle (Davis et al. 2017). Consequently,

heavy economic losses occur often in animal husbandry (Belhadj Slimen et al. 2016).

Many studies have attempted to characterize heat stress resistance in domestic animals, including pigs (Johnson et al. 2016; Liu et al. 2016), dairy (Kmiha et al. 2017; Gantner et al. 2017), and chickens (e.g., Chen et al. 2017). Notably, genetic markers and functional genes have been identified, including heat shock protein (HSP) genes (e.g., Cedraz et al. 2017), PtoEXPA12 (e.g., Zhang et al. 2018), NAC domain-containing protein 19 (NAC019), and respiratory supercomplex factor 2 (RCF2) (Guan et al. 2014). Thus, effective genetic variants and genes were used to breed heat-resistant dairy cows using genetic breeding systems (Davis et al. 2017; Ortega et al. 2016).

The Droughtmaster is a tropical breed of beef cattle developed in North Queensland by crossing the Brahman and British breeds, principally the Beef Shorthorn, during the early 1900s. The Droughtmaster breed was conceived in response to the need for a breed of cattle that had good tick resistance and would be able to use the environment in the most efficient manner, resulting in increased weight gain and fertility (Stephens et al. 2000). Therefore, this breed exhibits a combination of heat resistance and parasitic resistance

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via long-term artificial selection. Using the Droughtmaster breed as a model, we used next-generation sequencing technology to screen the chromosomal region and genes related to heat resistance in cattle. Our goal was to determine the molecular mechanism of heat resistance and to provide data to further accelerate adaptive breeding of cattle with environmental adaptability.

Materials and methods

All procedures were observed by the administration of animal affairs and approved by the Ethics Committee at Southwest University, and all procedures were in accordance with the agreement of Ethics Committee at Southwest University.

We compared two groups of commercial cattle with distinct physiological characteristics. The Droughtmaster cattle comprised the heat-pasture resistance group (HPR), which contained 20 individuals. The control group (CHR, nonheat resistant breeds) also contained 20 individuals, including 8 Angus, 6 Simmental, and 6 Sherlock cattle. Genomic DNA was extracted from the venous blood of each cow using the QIAamp DNA Mini Kit (QIAGEN, 51304, Hilden, Germany). DNA from each group member was pooled in equimolar quantities (1.5 µg/sample) to construct two mixed sequencing libraries, and sequencing was performed on an Illumina HiSeqX Ten PE150™ platform.

We filtered out adapters and low-quality raw paired reads as described as Lai et al. (2016). BWA software version 14 (Comparison parameters: -k 32-M) was utilized to map the high-quality clean reads to the *Bos taurus* reference genome (UMD_3.1.1, GCF_000003055.6). SNPs were identified from each pool using SAMtools 1.4 (Li et al. 2009), and ANNOVAR was used for the classification of variants (Wang et al. 2010).

Two independent approaches of selective sweep analysis were performed: pairwise fixation index (F_{ST}) and heterozygosity scores (H_p). For the H_p analysis, according to the number of alleles at each SNP locus in a population, the most frequent allele in a particular window is defined as the major allele (Maj) and the least frequent is defined as the minor allele (Min). The formula for the heterozygous rate of genes within populations was calculated as follows: $H_p = \frac{2 \times \sum n_{MAX} \times \sum n_{MIN}}{(\sum n_{MAX} + \sum n_{MIN})^2}$. The H_p values were homogenized using the formula $Z(H_p) = \frac{H_p - \mu_{H_p}}{\sigma_{H_p}}$. For F_{ST} analysis, we calculated the genetic divergence between populations with the formula $F_{ST} = \frac{\pi_{Between} - \pi_{Within}}{\pi_{Between}}$, where π_{Within} is the average number of differentially paired bases of reads in a population, and $\pi_{Between}$ is the average number of differentially paired bases of reads between populations. A 50 kb sliding window approach with a 25 kb step size was applied to quantify these

parameters using in-house PERL scripts. All related graphs were drawn by R-scripts. Candidate genes within the sweep regions were extracted for further analysis.

GO enrichment was analyzed using the Gene Ontology database (<http://www.geneontology.org/>), and Pathway-based analysis (KEGG) was performed using the public pathway-related database (<http://www.genome.jp/kegg/pathway.html>). Detail information of bioinformatics analysis operation as Support material I.

Results and discussion

After sequencing the two DNA libraries, 84.6 (SRS2374593) and 83.3 GB (SRS2374594) raw data were generated with $32.5 \times$ and $32.0 \times$ depth of the two pools, respectively. After removing the low-quality reads, the sequences were mapped to the cattle genome, with mapping rates of 99.16% and 99.28% (Supplemental Material II). A total of 15,569,067 variants including 14,249,316 SNPs and 1,319,751 indels were identified from the two mixed pools (Supplemental Material III).

We used a window size of 50 kb and steps of 25 kb to screen whole genomic regions with H_p and F_{ST} analysis (Fig. 1). F_{ST} analysis reflects the degree of differentiation between groups. A total of 604 chromosome windows were identified using F_{ST} [$zF_{ST} > 3.6878$ (top 1%)] and contained 1087 genes. In addition, 596 candidate windows were found by zH_p [zH_p (HPR) < -2.6841 (top 1%)] and contained 940 genes. Then, we combined H_p and F_{ST} values to map the intersection of these areas as potentially selected areas and found 81 selective candidate genes.

We classified these candidate genes based on functional classification and annotation of signaling pathways. A total of 40 GO terms were obtained from 3 main GO Ontologies (Fig. 2a, Supplemental Material IV). According to the KEGG analysis results (Fig. 2b, Supplemental Material V), 44 pathways were annotated, and the top 20 significant annotated pathways are shown in Fig. 2c. Most of the pathways were clustered into the categories organismal systems, environmental information processing, and metabolism. The classification of the candidate genes indicated the complex physiological and genetic changes that have occurred under long-term artificial selection.

We also identified genes such as RAR related orphan receptor B (RORB) and guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-5 (LOC104968964), which both belong to environmental adaptation relative pathways (Circadian entrainment and Circadian rhythm), but do not appear related to heat resistance.

Genes identified in this study could help further our understanding of the genetic and physiological developmental changes. For example, cytoplasmic-like dynein light chain

Fig. 1 Genome-wide scan for identifying chromosome regions with heat-resistance genes in the Droughtmaster breed. Sliding window analysis (50 kb window with 25 kb step increment) using a top 1% cutoff

1 (LOC101902172), which was annotated as vasopressin-regulated water reabsorption, could be a novel candidate gene to explain the molecular mechanism of heat resistance in the Droughtmaster breed. The main functions of this protein are to improve the permeability of the distal convoluted tubules and collecting tubes to water, promote the absorption of water, and regulate hormones for urine concentration and dilution. Therefore, an increase in vasopressin can promote renal tubules and enhance the reabsorption of water by the collecting tube. In addition, the gene cholinergic receptor muscarinic 3 (CHRM3) is related to salivary secretion and was shown to be involved in neurological diseases (Chee and Cumming 2018), but it is also involved in blood pressure regulation (Cowley 2018). Some studies have revealed a negative correlation between blood pressure and the exposed temperature of animals (Madaniyazi et al. 2016; Xu et al. 2018). Another identified gene, FOXG1 (forkhead box G1), is associated with nervous system development (Bulstrode et al. 2017; Papandreou et al. 2016) and dyskinesia (Kimira et al. 2017)

and belongs to the FoxO-signaling pathway, which is closely related to energy metabolism (Pan et al. 2017).

Energy metabolism in mammals is affected by many environmental factors such as temperature and light (Zhu and Gao 2017). In the current study, several metabolic genes were found including two glycan biosynthesis and metabolism-related genes, HS6ST3 (heparan sulfate 6-*O*-sulfotransferase 3) and GALNTL6 (polypeptide *N*-acetylgalactosaminyltransferase such as 6). These genes are involved in glycosaminoglycan biosynthesis—heparan sulfate/heparin and mucin type *O*-glycan biosynthesis, respectively.

High-temperature conditions may affect the digestive enzyme activity of an animal's intestine, the intestinal structure and the residence time of the diet in the intestine, which affects the digestion and absorption functions of the animal; taken together, this can result in a decrease in nutrient digestibility and metabolic energy (Diao et al. 2017). Therefore, heat-tolerant traits not only accelerate adaptive physiological changes but also improve the evolution of selective genes.

Fig. 2 Selective gene distribution of GO and KEGG categories of the Droughtmaster breed. **a** GO functional annotations are summarized in three main categories: biological process, cellular component and

molecular function. **b** Number of candidate genes and distribution of pathways. **c** Top 20 significantly enriched pathways of candidate selected genes

Our study used genome-wide differential scanning to identify heat resistance traits in cattle using H_p and F_{ST} analysis. We identified 81 candidate genes including SLC7A11, GYPC, and GYPC. However, because we were limited by costs and experimental design, further verification of the newly identified genes is necessary.

Conclusions

In this study, a total of 14,249,316 SNPs were obtained and used to perform selective sweep analysis of the Droughtmaster cattle breed in comparison with nonheat-resistant breeds using next-generation sequencing technology. Eighty-one candidate genes under selection in the Droughtmaster breed were identified and allocated into 40 GO terms and 44 related signaling pathways, and most of the signaling pathways were involved with environmental information processing and organismal systems. Our results provide insight into the selected genes related to heat-resistance in cattle.

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Author contributions AJ and JZ, GXE conceived and designed the experiments. CBY, BJX, DJH, and LFG performed the lab work. GXE and JZ analyzed the data and wrote the paper.

Funding The sequencing data generated in this study has been uploaded to the NCBI SRA database with the accession number (SRS2374593 and SRS2374594).

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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