RESEARCH ARTICLE

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Body Size Traits and Association with the Genetic Polymorphism of Melatonin Receptor 1A (*MTNR1A*) Gene in Shuxuan Cattle

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ABSTRACT

Background: Shuxuan cattle is one newly cultivated dual-purpose breed in Sichuan province, China, by crossing the indigenous Xuanhan cattle with external breeds. Body sizes have been widely regarded as the important breed characteristics in cattle. The current study was aimed to evaluate the body sizes and its molecular inherite marker for futhure herd improvement.

Methods: In the present study, we first investigated a total of 11 body size traits and their overall distribution and pairwise relationships of 169 healthy Shuxuan cattle. Subsequently, the SNP c.A455G of melatonin receptor 1A (*MTNR1A*) gene were genotyped among 169 Shuxuan cattle and subjected to association analysis with body size traits.

Result: We revealed that adult females have the mean withers height of 113.5 cm, body length of 131.8 cm, chest girth of 157.5 cm and hip height of 104.8 cm, respectively. Among all these traits, the highest correlation was observed in withers height and hip height with spearman's coefficient of 0.85. We found that Shuxuan cattle had an average heterozygosity of 0.5008 and polymorphism information content of 0.3747. Accordingly to the very stringent criterion of Bonferroni multiple adjustments, only chest width was associated with different genotypes of this candidate SNP ($P<0.05$). The results provide an overall profile of the morphological and growth characteristics in Shuxuan cattle.

Key words: Association analysis, Body size traits, *MTNR1A* gene, Shuxuan cattle.

INTRODUCTION

Body size traits have the moderate to high heritability in dairy and beef cattle and therefore have been involved in the genetic selection programs. In China, all Chinese cattle breeds can be divided into three groups with corresponding to northern, central and southern regions and their body size declines continuously along a north-to-south axis. The Shuxuan cattle are dual-purpose breed in Sichuan Province of Southwest China and had been successfully bred by crossing the indigenous breed of Xuanhan cattle with both Simmental and Holstein semen during the past 30 years. However, the overall profiles of body size traits and genetically associated genes have not been reported in Xuanhan cattle.

During the past decade, many candidate genes and the related single nucleotide polymorphisms (SNPs) have been proposed to affect or be associated with various body size traits in cattle. In the Chinese Qinchuan cattle, SNPs of the melatonin receptor 1A (*MTNR1A*) gene were found to be significantly associated with both chest depth and chest girth (Yang *et al.* 2015). Other candidate genes, such as LYR motif containing 1 (*LYRM1*), sine oculis homeobox homolog 4 (*SIX4*), signal transducer and activator of transcription 3 (*STAT3*), have also been individually reported (Wu *et al.* 2018). More recently, a large-scale meta-analysis of genome-wide association studies suggested that a large number of variants of small effect comprehensively affect the stature in cattle (Bouwman *et al.* 2018). Therefore, it was concluded that body sizes are the complex quantitative traits in cattle.

Melatonin is an indole hormone secreted by the pineal gland and plays an important role in regulating circadian rhythm and seasonal reproductive activities in animals. Melatonin is also a key regulator of both glucose metabolism and energy balance (Owino *et al.* 2019). In mammals the melatonin receptors are classified into MT1 (MTNR1A) and MT2 (MTNR1B) subtypes and MTNR1A has a unique capacity for high-affinity binding to melatonin (Owino *et al.* 2018). Three synonymous and one non- synonymous SNPs were reported in the *MTNR1A* gene and associated with body size traits in Chinese Qinchuan cattle (Yang *et al.* 2015). In the present study, we first analyzed the overall profiles for 11 body size traits in Shuxuan cattle and then investigated the association with polymorphism of the *MTNR1A* gene.

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MATERIALS AND METHODS

Ethics statement

Collection of biological samples and experimental procedures involved in this study were approved by the Institutional Animal Care and Use Committee in Sichuan Animal Science Academy, China.

Animals and body size traits

The experiment was carried out from 2019-05 to 2020-4 at the Sichuan Animal Science Academy. A total of 169 healthy female Shuxuan cattle with more than 12 months of age were involved in the present study. According to pedigree records, the genetic relationships within three generations were avoided for all animals. They were subjected to a collection of jugular venous blood samples and measurements of body size traits. We totally measured and collected 11 body size traits, including the withers height (WH, from the highest point of shoulder blade to the ground), body length (BL, from shoulder joint to tuber ischiadicum), head length (HL, from poll to nose), forehead width (FW, maximum distance between the temples), chest width (HW, height immediately posterior to the front leg), chest depth (CD, width immediately posterior to the front leg), chest girth (CG, body circumference immediately posterior to the front leg), shin circumference (SC, circumference of left metacarpus at its narrowest), rump width (RW, distance between tuber ischiadicum), hip width (PW, distance between the lateral surfaces of the tuber coxae) and hip height (HH, from hip to the ground). All body size traits were measured when the animal was standing naturally.

Genotyping of *MTNR1A* gene

The candidate SNP (c.A455G with GenBank accession number of rs208053155) was selected from the former publication (Yang *et al.* 2015) and subjected to genotyping in the present study. Genomic DNA was extracted using AxyPrep Genomic DNA Miniprep Kit (Axygen Bioscience, USA) and then stored at -20°C for later analysis. This candidate SNP was genotyped using a custom-by-design SNPscan^{TM} Kit (Genesky Biotechnologies Inc., Shanghai, China). Briefly, 100-200 ng genomic DNA was used for producing 20 μL ligation mixture containing 1X Ligase buffer, 1X probe and 0.5 μL ligase. The ligation reaction was performed on a ABI 2720 Thermal Cycler (Applied Biosystems, USA) under the following program: 2 min at 98°C, 4 cycles of (94°C 1 min, 58°C 4 hr), 94°C 2 min, hold at 4°C and immediately stopped by adding 20μl of 2xStop Buffer. Multiplex fluorescence PCR reactions were performed for each ligation product. Each PCR reaction was prepared in a 20 μL mixture containing 1xPCR Master Mix, 1 μL Primer Mix SetA or SetB and 1 μL ligation product. PCR products were separated and detected by capillary electrophoresis on ABI3730XL sequencer (Applied Biosystems, USA).

Data analysis

We first analyzed the overall distribution and pairwise relationships among the 11 body size traits using the R package of Performance Analytics, in which the spearman correlation coefficient was derived. After both the genotype and allele frequencies of SNP were calculated, the Hardy-Weinberg equilibrium (HWE) was measured through the chi-square test (χ^2 test). The polymorphism information content (PIC) and heterozygosity (H_e) were calculated. The associations between genotypes and body size traits were investigated under the linear mixed model (LMM) as:

$$Y_{ijk} = \mu + G_i + Age_j + \varepsilon_{ijk}$$

Where

Y_{ijk} is measurement values of body size traits, μ is overall of mean for each trait, G_i is the effect of genotypes (AA, AG and GG); Age_j is the fixed effect of animal age (having three levels of 12 ~ 16 months, 17 ~ 23 months and > 24 months); ε_{ijk} is random residual effects. The model fitting and statistical tests were conducted using R package of lmerTest (Kuznetsova *et al.* 2017). The Bonferroni method was used for adjustment for multiple comparisons.

RESULTS AND DISCUSSION

The overall distribution and pairwise correlations among the 11 body size traits were shown in Fig 1. Beside the SC, all other traits showed the normal distribution more or less. Among all these traits, the highest correlation was observed in WH and HH with spearman's coefficient of 0.85, which was followed by BL and CD (0.61). In contrast, the FW and RW had the lowest correlation with spearman's coefficient of 0.14. We further analyzed 133 adult animals that are more than 24 months of age (Table 1). The mean values of WH, BL, CG and HL were 113.5 cm, 131.8 cm, 157.5 cm and 104.8 cm, respectively.

The SNP c.A455G of *MTNR1A* gene had been successfully genotyped among all the animals, by which a total of 35 AA, 93 AG and 41 GG genotypes were determined. The computed allele frequencies were 48.22% of A and 51.78% of G, respectively. The χ^2 suitability test indicated that SNP c.A455G was under in Hardy-Weinberg equilibrium ($\chi^2 = 1.466$ and $P\text{-value} = 0.2259$). Accordingly, we calculated that Shuxuan cattle had an anverage heterozygosity of 0.5008 and polymorphism information content of 0.3747. The associations between genotypes of SNP c.A455G and body size traits were shown in Table 2. Accordingly to the very stringent criterion of Bonferroni multiple adjustment, only the individuals of GG genotype had the higher HW (39.40±1.33 cm) than that of AA genotype with pre-adjusted P-value<0.05 (36.00±2.92 cm).

In dairy cattle, body size traits and the related conformation scores have been widely included into the genetic selection programs. Genome-wide association study revealed that tens of SNPs are significantly associated with most of body conformation scores in Chinese Holstein. Also in beef cattle, body size traits have important economic implications because they are directly associated with live weight and carcass characteristics. In the present study, we first analyzed a total of 11 body size traits of Shuxuan cattle in Sichuan and found that this breed has a moderate stature in comparison with other representative breeds of Chinese cattle, such as Qinchuan cattle (Liu *et al.* 2014). However, body size traits of Shuxuan cattle are smaller than most of European breeds. This could be explained by its breeding history because Shuxuan cattle had been bred by crossing the indigenous breed in Sichuan with external breeds. Further more, we investigated pairwise correlations among the 11 traits in Shuxuan cattle and provided the basic overview about the body growth in this breed.

It has been widely acknowledged that melatonin plays important roles in regulating energy metabolism. Many genetic variants in *MTNR1A* gene have also been reported to be associated with litter size, egg production traits, body

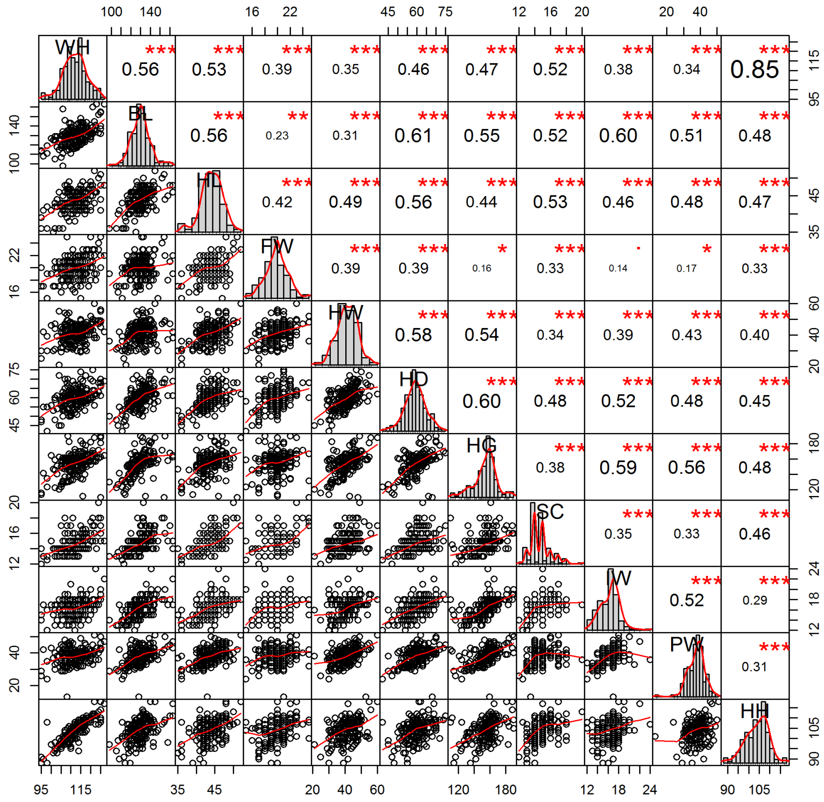

Fig 1: Correlation matrix for the body size traits. The overall distribution of traits are individually demonstrated as histogram plots on the diagonal. The spearman correlation coefficient and statistical tests ("***"<0.001, "**"<0.01, "*"<0.05 and "."<0.1) are on the upper triangular. The bivariate scatterplots are placed on the lower triangular.

Table 1: Summaries of the 11 body size traits in Shuxuan cattle.

Traits (cm)	Summaries of measurements				
	1st Quartile	Median	Mean	3rd Quartile	Variation coefficient (95% confidence interval)
Withers height	110.0	113.5	113.5	117.0	4.89 (4.37-5.58)
Body length	126.0	130.0	131.8	136.0	6.80 (6.08-7.76)
Head length	42.00	44.00	44.41	46.00	5.72 (5.11-6.52)
Forehead width	19.00	20.00	20.12	21.00	8.69 (7.76-9.91)
Chest width	38.0	43.0	42.2	46.0	7.21 (6.14-8.36)
Chest depth	58.00	60.00	60.77	64.00	7.87 (7.03-8.98)
Chest girth	150.0	159.0	157.5	163.0	8.20 (7.32-9.35)
Shin circumference	14.00	15.00	14.89	15.00	8.20 (7.33-9.36)
Rump width	16.00	17.00	16.79	18.00	9.97 (8.91-11.38)
Hip width	36.00	39.00	39.03	42.00	12.53 (11.19-14.32)
Hip height	101.0	105.0	104.8	108.0	4.67 (4.18-5.33)

Note: Only 133 adult animals that are more than 24 months of age were included.

Table 2: Association between genotypes and body measurement traits

Traits (cm)	Genotypes (Mean±Standard Errors)			*P*-values
	AA (N=35)	AG (N=93)	GG (N=41)	
Withers height	111.00±3.02	110.06±1.14	109.43±1.32	0.4953
Body length	125.32± 5.53	126.57±1.90	124.81±2.20	0.5733
Head length	41.94±1.43	42.34±0.55	42.58±0.63	0.5960
Forehead width	19.40±0.62	19.76±0.35	19.51±0.40	0.5090
Chest width	36.00±2.92	38.23±1.52	39.40±1.33	0.0370
Chest depth	55.72±3.00	57.64±1.01	57.22±1.17	0.1710
Chest girth	144.14±8.03	146.27±2.58	147.01±2.98	0.6043
Shin circumference	14.50±0.78	14.87±0.23	14.75±0.27	0.2897
Rump width	16.17±0.95	15.87±0.33	15.53±0.38	0.2416
Hip width	36.08±2.11	35.83±0.97	35.21±1.12	0.7076
Hip height	102.35±1.78	102.12±1.05	102.36±1.21	0.9600

size traits and meat quality traits (Yang *et al.* 2015; Alsiddig *et al.* 2017; He *et al.* 2019). Yang *et al.* (2015) identified three synonymous and one nonsynonymous mutations in *MTNR1A* gene and proposed that these four SNPs were associated with body size traits in Qinchuan cattle (Yang *et al.* 2015). In the present study, we genotyped a candidate SNP c.A455G of *MTNR1A* gene in Shuxuan cattle and found a high genetic diversity with respect to both an anverage heterozygosity and polymorphism information content. However, we only detected a significant association between this SNP of c.A455G and HW in Shuxuan cattle. This result would indicate inter-breed heterogeneity for genetically controlling the body size traits.

In conclusion, we first investigated abundant of body size traits in Shuxuan cattle and then investigated their association with the genetic polymorphism of *MTNR1A* gene. The results would help us better understanding the morphological and growth characteristics for this breed.

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