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Polymorphisms of the DHCR24 Gene are Associated with Carcass and Fat Deposition in Chinese Simmental Steers

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PZ and AI conceived the idea, carried out the experiments and data analysis, and drafted the manuscript. GZ and LJ helped with sample collection and data analysis. FX helped in the bioinformatic analysis. JP and ZZ revised the manuscript.

Key words
mi-RNA, DHCR24, PCR-RFLP, Carcass, Fat deposition

ABSTRACT

3-β-hydroxysteroid-Δ-24-reductase (DHCR24) gene regulates the abdominal fat of broilers and it is hypothesized that it can also play a role in the fat metabolism of cattle. A mutation in the 3' untranslated region of the DHCR24 gene was identified that led to the differential expression of DHCR24 in cattle. In this study, we detected mutation at g.32435 from G > A in 3'UTR of DHCR24 gene in a Chinese Simmental steer population. In mutated individuals at g. 32435 G > A of DHCR24 the kidney fat weight of GG was higher than that of GA and AA individuals. Steers of the GG and AA genotype having high backfat thickness ($p < 0.05$) and a higher carcass fat coverage rate ($p < 0.05$). Furthermore, GG genotype individuals had higher marble grade than GA genotype individuals ($p < 0.05$). It is predicted through bioinformatics analysis that bta-miR-12059 can bind to 3'UTR of the DHCR24 gene. Our results indicate that single nucleotide polymorphisms might be used as a molecular marker for marker-assisted selection in beef cattle breeding.

INTRODUCTION

3-β-hydroxysteroid-Δ-24-reductase (DHCR24), also known as seladin-1, was first identified by studying the effect of the *DIMINUTO* gene on the growth of *Arabidopsis* cells (Takahashi *et al*., 1995). In previous studies, the *DHCR24* gene was thought to be involved in steroid synthesis, albeit through further extrapolation by researchers, it was found that DHCR24 was mainly involved in the conversion of lanosterol into cholesterol and played an important role in the stability of cholesterol and can act as anti-apoptosis (Kandutsch and Russell, 1960; Johnston and Bloch, 1957). Recent studies showed that the *DHCR24* gene could provide a neuroprotective effect (Martiskainen *et al*., 2017; Hernández-Jiménez *et al*., 2016). Greeve *et al*. (2000) found that the *DHCR24* gene can inhibit the activation of CASOASE3 in nerve cells and proved that DHCR24 could protect nerves. Interestingly, Giannini *et al*. (2008) pointed out that IGF-1 stimulation and hypoglycemia can inhibit the expression of the *DHCR24* gene in neurons. It shows that DHCR24 was involved in diabetic neuropathy. Further, the results of the mouse liver proteomics and bioinformatics analysis by Kim *et al*. (2013) showed that the mRNA and protein levels of the *DHCR24* gene in the liver of diabetic mice were 10 times higher than those of normal mice. Recently, Zhang *et al*. (2018) showed that the expression of *DHCR24* in ovaries of estrus and non-estrus pigs was significantly higher than that of estrus pigs which indicated that the expression of the *DHCR24* gene was significantly associated with the development of ovaries. Other studies have shown that DHCR24 is associated with Alzheimer's disease, cardiovascular disease, and prostate cancer (Lämsä *et al*., 2007; Wu *et al*., 2013; Hendriksen *et al*., 2006).

Presently, only a few studies on single nucleotide polymorphism (SNP) of the *DHCR24* gene in Chinese Simmental cattle are available with major focus on reproductive and growth traits. Mu *et al*., (2019) reported that *DHCR24* gene expression in broiler abdominal fat was significantly higher in ovariectomized than non-

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ovariectomized broilers. They further reported that the *DHCR24* gene was also involved in fat deposition. On the contrary, Cochran *et al.* (2013) reported that the *DHCR24* gene was not involved in the reproductive traits of Holstein cows. The present study aimed at find the polymorphisms of the DHCR24 gene and their associated with carcass and fat deposition in Chinese Simmental steers.

MATERIALS AND METHODS

Animals and sampling

A total of 445 Chinese Simmental steers were randomly selected from 15 cattle farms from the Wulagai administrative district of Inner Mongolia in China. Steers were slaughtered at 28 months of age at the Inner Mongolia Baolongshan cattle farm (Tongliao, China). Carcass composition traits such as meat quality traits as well as fatty acid composition traits were measured at the Chinese Academy of Agricultural Sciences meat laboratory as described previously by Fang *et al.* (2017). Fatty acid content was extracted from longissimus muscle and stated as grams per 100g fresh tissue. Blood samples (10 mL per animal) were collected from the jugular vein with anticoagulant (acid citrate dextrose, ACD) and stored at -80°C until used for performing further analysis. All animal experiments in this study were strictly abided by the ordinance for the care and use of laboratory animals of the Jilin University Animal Care and Use Committee (permit number: SYXK (Ji) pzpx 20181227083).

DHCR24 variant detection and genotyping by sequencing

DNA of leukocytes from whole blood samples was extracted using a TIANamp Blood DNA Kit (Tiangen, Beijing, China) and following the manufacturer's protocol. The purity and concentration of the genomic DNA were determined using a NanoDropND-2000 ultraviolet spectrophotometer (Thermo Fisher Scientific Inc., USA) and the quality was verified by agarose gel electrophoresis. The PCR primers for 3'UTR polymorphism were 5'- GCATCTCCCCAATTCATGGT -3' and 5'-AACTGTCCCACTCTATCCTG -3'. The primers were designed using Primer Premier 6.0 (Premier Bio soft International, Canada) and synthesized by GENEWIZ, Inc. (Suzhou, China). The 3'UTR sequence of *DHCR24* gene was amplified by PCR in a 25 µL reaction volume including 12.5 µL of 2× Taq PCR Master Mix (Tiangen, Beijing), 0.5 µL of forward and reverse primers (10 µM), 1.0 µL of template DNA, and 10.5 µL of nuclease-free water. The PCR conditions were as follows: 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 60 °C for the 30s, and 72 °C for 30 s; and a final 5 min extension at 72 °C. The PCR products of the 3'UTR sequence and mRNA of the *DHCR24* gene were run on 1% agarose gel and visualized under UV light (Tanon, China). The remaining PCR product was sent to Sangon Biotech (Shanghai, China) for sequencing. Sequencing data were analyzed by Chromas (Technelysium, Australia) to determine the location of SNP and genotypes.

miRNA binding site prediction for DHCR24

miRNAs that bind to the 3'UTR sequence of *DHCR24* gene were predicted by miRNA binding site prediction site (http://www.mirbase.org/search.shtml), and to analyze the effect of SNPs on miRNA that may bind to 3'UTR of *DHCR24* gene.

Statistical analysis

The genotype frequency and allelic frequency of each SNP were calculated according to genotyping results. The polymorphism information component (PIC) was determined through Botstein's methods (Botstein *et al.*, 1980). The Hardy–Weinberg equilibrium of the polymorphisms was tested with the chi-squared (x^2) test. Variance effective population size Ne is defined as the size of the ideal population with the same variance. Associations between *DHCR24* gene polymorphisms and economic traits of beef cattle were analyzed by two-way analysis of variance (ANOVA) using SPSS 13.0 for Windows. The fixed model was Equation 1.

$$Y_{ijk} = u + ysi + mj + eijk \quad \ldots.(1)$$

Where; Y_{ijk} is the observed value of the kth individual from the Simmental breed of genotype j in the i^{th}-year season, u is the lowest square mean of the observed values, *ysi* is the effective value of the i^{th}-year season, *mj* is the effective value of genotype j, and *eijk* is the random residual effect corresponding to the observed value. For SNPs that correlated with the carcass and fat-related traits, the haplotype test and linkage disequilibrium (LD) were performed and measured by D' and r^2 with the Haplo-View software (Daly Lab at the Broad Institute Cambridge, USA, ver. 3.32) (Barrett *et al.*, 2005).

RESULTS

SNP loci in DHCR24 *gene*

We had screened SNP in the key functional domain of the bovine *DHCR24* gene in the Chinese Simmental cattle population. Based on the sequencing results and sequencing chromatographs of PCR products, one SNP, g.32435 G > A, was identified in 3'UTR of the *DHCR24* gene in a population of Chinese Simmental steers (Fig. 1). We used restriction endonuclease *Ava I* to digest the PCR products of the *DHCR24* gene and detected by gel electrophoresis (Fig. 2). Subsequent single sample PCR product sequencing results were consistent with digestion results.

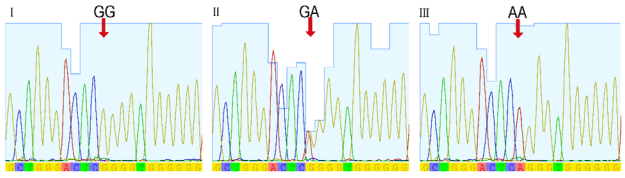

Fig. 1. Molostide sequencing of PCR product of *MDHCR24* gene GG (I), GA (II); AA (III) genotype.

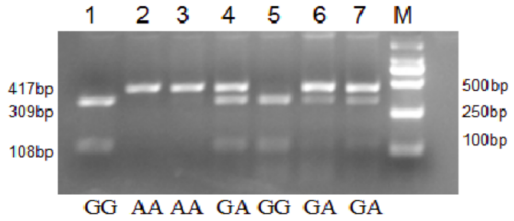

Fig. 2. PAGE map of *DHCR24* gene 3'UTR digestion results. Lanes 1-7 show different Chinese Simmental cattle populations in which two bands are GG genotype, three bands are GA genotype and one band is AA genotype.

Genetic diversity of SNP in the DHCR24 *gene*

Values of genotypic frequencies, allelic frequencies, PIC, Ne (variance effective size), Ho (gene homozygosity), and He (gene heterozygosity) were calculated to illustrate the genetic diversity of *DHCR24* in Chinese Simmental steers (Table I). The percentage of Ho in SNP was lower than that of He. The PIC value of g. 32435 G > A, 0.416, indicated an intermediate polymorphism frequency of SNP. The g. 32435 G > A fit the Hardy–Weinberg equilibrium in this population ($p > 0.05$).

DHCR24 *polymorphisms correlation with carcass and fat deposition*

The associations between variants and traits are presented in Table II. These analyses revealed that the SNP in the 3'-terminal sequence of the *DHCR24* gene was significantly associated with carcass and meat quality trail. At g. 32435 G > A of *DHCR24*, a higher carcass weight ($p<0.05$) and deeper fat color ($p<0.05$) were observed in an individual with the GG genotype as compared to the GA genotype. The kidney fat weight of GG individuals was higher than that of GA and AA individuals. Steers of the GG and AA genotype had higher liver weight ($p<0.05$), thicker back fat ($p<0.05$), and a higher carcass fat coverage rate ($p<0.05$). Furthermore, GG genotype individuals have higher marble grade than GA genotype individuals ($p<0.05$). There was no significant correlation between the mutation site and the ocular muscle area.

miRNA binding site prediction

The prediction results showed that bta-miR-12059 might recognize (Fig. 3) possible binding sites for bta-miR-12059 and *DHCR24* gene 3'UTR sequences 3'*UTR* sequence of *DHCR24* gene containing g. 32435 G > A site. If the base of g. 32435 G > A is A, bta-miR-12059 could recognize the sequence containing the site the bta-miR-12059 is combined with *DHCR24*, it would reduce the translation rate of mRNA and affect the performance of body traits (Fig. 3). However, if the base of the SNP site is G, bta-miR-12059 does not recognize the sequence containing the site.

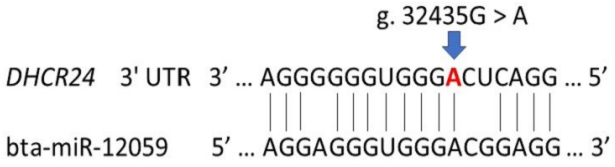

Fig. 3. Indicating binding site for the *DHCR24* for bta-miR-12059.

Table I. Genetic polymorphism analysis of *DHCR24* gene g. 32435 G > A site.

Site	Gene frequency		Genotype frequency			H	Ne	χ^2	PIC
	G	A	GG	GA	AA				
g.32435G > A	0.706	0.294	0.492(191/445)	0.553(246/445)	0.018(8/445)	0.4154	1.7107	48.05	0.416

$\chi^2_{0.05}$(df=2) = 5.99, $\chi^2_{0.01}$(df= 2)= 4.61, $\chi^2>\chi^2_{0.05}$ mean $p>0.05$.

Table II. Correlation analysis between *DHCR24* gene g. 32435 G > A locus and 445 Chinese Simmental cattle traits.

Genotype	Traits							
	Carcass weight /kg	Liver weight/kg	Kidney fat /kg	Back fat /cm	Fat cover-age /%	Eye muscle area /cm²	Marbling rating	Fat color
GG	269.72 ± 34.25^{a}	6.48 ± 0.69^{a}	6.19 ± 2.063^{a}	1.29 ± 0.46^{a}	1.98 ± 0.28^{a}	23.61 ± 2.32	5.16 ± 0.74^{b}	84.08 ± 12.81^{a}
GA	246.43 ± 38.43^{b}	5.47 ± 1.16^{b}	3.54 ± 2.74^{b}	0.72± 0.63^{b}	1.69 ± 0.34^{b}	23.51 ± 2.56	5.55 ± 0.64^{a}	75.70 ± 11.64^{b}
AA	272.12 ± 51.42^{ab}	6.41 ± 0.83^{a}	5.92 ± 2.94^{b}	1.18± 0.54^{a}	1.97 ± 0.37^{a}	23.61 ± 2.70	5.40 ± 0.89^{ab}	77.60 ± 6.35^{ab}

Lowercase letters in the same column indicate a significance difference (p <0.05), lower case letters indicate the same difference was not significant (p> 0.05).

DISCUSSION

According to the results of SNP microarray screening, there was no significant correlation between the *DHCR24* gene and reproductive traits in 550 Holstein cattle (Cochran *et al*., 2013). This result may suggest that the main function of the *DHCR24* gene is not related with cattle reproduction. At present, the main research results showed that *DHCR24* can regulate carcinogenesis and oxidative stress and play a protective role in the nerve (Crameri *et al*., 2006; Cecchi *et al*., 2008; Kuehnle *et al*., 2008). According to Mu *et al*. (2019) the *DHCR24* gene is involved in the fat deposition of broilers. However, the gene expression profile of Kim *et al*. (2013) on human subcutaneous and visceral fat stem cells showed that the expression of DHCR24 in visceral fat was significantly higher than that in sebum. Hitherto, the single nucleotide polymorphism analysis of the *DHCR24* gene in Chinese Simmental cattle has not appeared. The correlation between the *DHCR24* gene with carcass traits and fat deposition traits has never been studied before. Our results indicated that the SNP site (g. 32435 G > A) was detected in 3 'UTR of the *DHCR24* gene in 445 Chinese Simmental cattle. The GA genotype frequency of this locus was the highest (0.553), followed by GG (0.429). The results showed that the SNP site (G. 32435 g > A) was detected in 3 'UTR of the *DHCR24* gene in 445 Chinese Simmental cattle. The GA genotype frequency of this locus was the highest (0.553), followed by GG (0.429). The results of correlation analysis between SNP and traits showed that there was a significant correlation between g. 32435g > a locus to the carcass and fat deposition traits ($p < 0.05$): individuals with GG genotype had higher body weight, liver weight, kidney fat weight, backfat thickness, marbling grade, and fat color grade ($p < 0.05$); individuals with GA genotype had lower liver weight, kidney fat weight, backfat thickness and fat coverage ($p < 0.05$) than those with other two genotypes. We also detected the SNP at the 3'UTR at the bovine *DHCR24* locus. Among them, the mutation of the g. 32435 G > A showed significant association with traits related to fat deposition in 445 Chinese Simmental steers. The site of g. 32435 G > A occurred in 3' untranslated regions and did not alter the amino acid sequence of DHCR24. However, mutations in the non-coding region may also influence the expression and function of a gene by aberrant splicing (Komar, 2007). This also indirectly indicates that bovine DNA is highly conserved. We use miRNA binding site prediction software predicted miRNA binding site, when g. 32435 G > A site is nucleotide A, bta-miR-12059 may recognize 3'UTR of the *DHCR24* gene. Based on the prediction results, it can be inferred that bta-miR-12059 can regulate the expression of the *DHCR24* gene, thus affecting cholesterol production and finally causing the difference of phenotype. Albeit, how *DHCR24* genes are involved in the regulation of body fat deposition in cattle and whether bta-miR-12059 can regulate the expression of *DHCR24* gene still need to be explored.

CONCLUSION

In summary, the single nucleotide polymorphisms of DHCR24 showed significant correlations with fat-related traits in Chinese Simmental steers. This suggests that DHCR24 is involved in fat deposition at different stages of growth and fattening of cattle, e.g. intramuscular fat, back fat, and peri-renal fat deposition. Bioinformatics analysis predicts that bta-miR-12059 can bind to the *DHCR24* gene, so it can regulate the expression of the *DHCR24* gene in bovine fat through bta-miR-12059. This mutation might be molecular markers for beef breeding selection.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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