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Research paper

Identification of a 13 bp indel polymorphism in the 3′-UTR of *DGAT2* gene associated with backfat thickness and lean percentage in pigs

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ABSTRACT

DGAT2 (acyl-CoA: diacylglycerol acyltransferase, EC2.3.1.20) is a member of acyl-CoA: monoacylglycerol acyltransferase (MGAT) family, which catalyzes one fatty acyl-CoA and diacylglycerol (DG) molecule to form triacylglycerols (TG) and is the final and rate-limiting step in the reaction of TG synthesis pathways. We previously showed that, during pig development, the fold change of *DGAT2* mRNA in backfat tissue is much higher than that of *DGAT1*, implying that DGAT2 is more important in regulating porcine fat deposition. In this study, a 13 bp indel polymorphism located at 905 bp downstream from the stop codon (TGA) of porcine *DGAT2* was found and two alleles of A (with 13 bp insertion) and B (no insertion) were designated. Allele A is dominant in all pig populations investigated. The backfat thickness of individuals with genotype AA is significantly lower than those with genotype AB ($p < 0.01$), and the lean percentage of individuals with genotype AA is significantly higher than those with genotype AB ($p < 0.05$) in Junmu No. 1 white pig population. The secondary structure of 3′-UTR without the 13 bp insertion is slightly less stable than with the 13 bp insertion type. *In vitro* assay indicates that, after differentiation, the luciferase activity was significantly higher for pGL3-B compared to pGL3-A vector ($p < 0.001$). Moreover, the *DGAT2* mRNA expression in the backfat tissue of pigs with genotype BB was significantly higher than AB in commercial DLY pigs ($p < 0.05$). These results suggest that the 13 bp indel polymorphism in the 3′-UTR of porcine *DGAT2* most likely affects fat deposition by altering its expression in pigs.

1. Introduction

In animals, triacylglycerols are mainly synthesized and deposited in the liver, intestines and adipose tissue and serve mainly as a store of energy. The final and rate-limiting step of triacylglycerol synthesis is catalyzed by the enzyme acyl-CoA:diacylglycerol acyltransferase (DGAT, EC2.3.1.20). Up to date, two isoforms of the enzyme DGATs, i.e. DGAT1 and DGAT2, were identified in mammals. *DGAT2*-deficient mice are lipopenic and die soon after birth, suggesting that DGAT2 plays a predominant role in mammalian triglyceride synthesis and is required for survival (Stone et al., 2004; Liu et al., 2012). DGAT2 is found both in endoplasmic reticulum and mitochondria-associated membranes (Stone et al., 2009). Murine DGAT2 catalyzes triacylglycerol synthesis and promotes lipid droplet formation independent of its localization to the endoplasmic reticulum (McFie et al., 2011). Studies indicated that, DGAT2 is primarily responsible for incorporating endogenously synthesized fatty acids into triacylglycerol, by using nascent diacylglycerol and *de novo* synthesized fatty acids as substrates, largely determines the rate of *de novo* synthesis of triglyceride (Qi et al., 2012; Wurie et al., 2012).

Given that DGAT2 plays an essential role in mammalian triglyceride synthesis, investigations on associations of *DGAT2* gene polymorphism with lipid traits were reported. In human, 15 DNA variants in the genomic region of *DGAT2*, including four non-synonymous exchanges were identified (Friedel et al., 2007) and studies indicate that individuals carrying two or one copies of the minor T allele of SNP rs1944438 has a smaller decrease in liver fat compared with subjects homozygous for the C allele (Kantartzis et al., 2009), and the rs3060 and rs101899116 polymorphisms are associated with a smaller reduction in liver fat content in response to niacin (Hu et al., 2012).

The intramuscular fat content (IMF) is a very important trait affected mainly by fat deposition, and is a major determinant factor of meat quality traits like flavor, shear force and drip loss. Appropriate amount of triacylglycerols improves the meat quality traits of livestock and,

Abbreviations: DGAT2, acyl-CoA: diacylglycerol acyltransferase; MGAT, acyl-CoA: monoacylglycerol acyltransferase; DG, diacylglycerol; TG, triacylglycerols; 3′-UTR, 3′-untranslated region; IMF, intramuscular fat content.

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therefore, is more acceptable by consumers. In cattle, the expression of *DGAT2* exhibited significant positive correlations with IMF content in the *longissimus dorsi* muscle of Korean cattle steers (Jeong et al., 2012), and polymorphisms located either outside exons or silent mutation of *DGAT2* were detected (Winter et al., 2003). In cow, feeding saturated long-chain fatty acids led to sustained up-regulation of *DGAT2* (Schmitt et al., 2011) and progesterone supplementation alters the time of *DGAT2* expression pattern in endometrium (Forde et al., 2009).

The pig is an important agricultural animal as well as a biomedical model for human disease. The capacity of fat deposition differs greatly between western lean-type pig breeds and Chinese indigenous pigs. Our previous study indicated that, during pig development, the fold change of *DGAT2* mRNA in backfat tissue is much higher than that of *DGAT1* (Hu et al., 2010), implying DGAT2 likely plays a more important role in porcine fat deposition. Porcine *DGAT2* is highly expressed in the liver and a single nucleotide polymorphism in its 3′-UTR region is associated with backfat thickness between the 6th and 7th ribs (Yin et al., 2012). Up to date, the mechanism that *DGAT2* expression affects fat deposition in pigs remains largely unknown. In this study, a 13 bp indel polymorphism in the 3′-UTR of porcine *DGAT2* associated with backfat thickness and lean percentage was identified and its mechanism on *DGAT2* expression was analyzed.

2. Materials and methods

2.1. Animals and sample collection

Three western pig populations of Duroc (190), Landrace (96) and Yorkshire (277) were randomly sampled from large pig populations which were reared under similar conditions in the Pig Breeding Center of Shandong Academy of Agricultural Sciences (Jinan, Shandong, PRC). Thirty-seven Laiwu pigs and 95 Lulai Black pigs were sampled randomly from the Laiwu Pig Preservation Center (Laiwu, Shandong, PRC), 57 Dapulian pigs were sampled randomly from the Dapulian Pig Preservation Center (Jining, Shandong, PRC). One hundred and forty-one castrated Junmu No. 1 white pigs, with similar birth date, were sampled from the breeding farm in the Agronomy Ministry of Jilin University. A small piece of ear tissue from each individual was obtained and stored at −20 °C in 70% ethanol for DNA isolation. One hundred and twenty-five commercial crossbred pigs of Duroc sires and Landrace × Large White dams (DLY) were slaughtered from Xingyue Food Company (Taian, Shandong, PRC) and backfat tissue was sampled for DNA and RNA isolation.

2.2. Production trait measurement

Production traits of 141 castrated Junmu No. 1 white pigs that were reared under equalized environmental and feeding conditions were measured on farm. The lion muscle thickness, backfat thickness and lean ratio traits were measured with Piglog 105 machine (Carometec A/S, Denmark) on pigs with the body weight reaching 100 kg. The animal management and sample collection protocol was approved by the ethics committee of Jilin University in accordance with the guidelines on animal care and use.

2.3. Isolation of DNA and total RNA

Genomic DNA was isolated from ear and backfat tissues using classical phenol-chloroform method and stored at −20 °C. Total RNA was isolated from backfat tissues of Laiwu pigs and commercial crossbred pigs with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and stored at −80 °C. The quality and concentration of nucleic acids were verified by 1% gel electrophoresis and BioPhotometer plus UV spectrophotometer (Eppendorf, Hamburg, Germany), respectively.

2.4. PCR amplification

For the determination of possible poly(A) site of porcine *DGAT2* transcripts, PCR was performed by mixing 4 μl of 5 × PrimeSTAR buffer (Mg^{2+} plus), 1.6 μl of dNTPs (2.5 mM for each), 0.5 μl of SS-f (forward) and 0.5 μl of one of the three reverse primers (SS-r1, SS-r2 and SS-r3, 10 μM) (Table 1), 0.2 μl of PrimeSTAR HS DNA polymerase (2.5 U/μl, TaKaRa, Dalian, PRC) and 0.5 μl of genomic DNA (50–100 ng) in a 25 μl volume, and run on a Mastercycler gradient (Eppendorf, Hamburg, Germany) according to the following program: 95 °C for 3 min, 35 cycles of 95 °C for 30 s, annealing at Tm °C (Table 1) for 30 s, and 72 °C for 1 min and final extension at 72 °C for 5 min. For the amplification of 722 bp and ~120 bp fragments, the PCR amplification conditions were similar except that different annealing temperatures were used (Table 1).

2.5. cDNA synthesis and RT-PCR amplification.

First-stranded cDNA synthesis was performed according to the following procedure: First, 1 μg of total RNA, 1 μl of oligo$(dT)_{18}$ primer (5 μM, TaKaRa, Dalian, PRC) and 11 μl nuclease free H_2O were mixed and incubated at 65 °C for 10 min and placed immediately on ice; then 0.5 μl of RNase inhibitor (20 U/μl, Fermentas, Glen Burnie, USA), 2 μl of dNTPs (10 mM, TaKaRa, Dalian, PRC), 4 μl of Transcriptor RT Reaction Buffer and 0.5 μl of Transcriptor Reverse Transcriptase (20 U/μl, Roche, Penzberg, Germany) were added and incubated at 55 °C for 30 min, then 85 °C for 5 min. The resultant cDNA was stored at −20 °C. With 1 μl of cDNA as template, RT-PCR was carried out according to conditions used for PCR with primers SS-f (forward) and one of the three reverse primers (SS-r1, SS-r2 and SS-r3) (Table 1).

2.6. Sequencing

The amplified fragments of PCR and RT-PCR were purified and ligated into pJET1.2/blunt cloning vector (Fermentas, Glen Burnie, USA) and transformed into the competent *Escherichia coli* DH5α cells. At least four positive clones for each fragment were sequenced with ABI 3730 sequencer (Applied Biosystems, CA, USA).

2.7. Genotyping

The genotyping of 13 bp indel polymorphism in the 3′-UTR region of porcine *DGAT2* was performed by loading 1.5 μl of the ~120 bp amplified fragment and running 15% polyacrylamide gel (arc:bis = 29:1) at 200 V for 12 h and resolved by silver staining according to reference (Liu et al., 2011).

Table 1
Primers used for searching polymorphic loci, RT-PCR, vector construction and quantitative RT-PCR analysis of porcine *DGAT2* gene.

Primer	Sequence (5′-3′)	Tm(°C)	size(bp)
3UTR-f	AGTGTGGATGTGGAGAAGGG	58.9	772
3UTR-r	AGCGGAAAGCCTAGAGTCCC		
D3R2-F	AGTGTGGATGTGGAGAAGGG	59	~120
D3R2-R	ACAAATACACGAGCCTGAGG		
SS-f	GAGGTCCTGGAGGTGAACTGA		
SS-r1	TGGGAGTCTGTGATTTTATTTATC	60	2464
SS-r2	GGACTCTCGTTTTCATTTATTCAT	59	1120
SS-r3	GCCTTTTCCAACATTTATTTCTG	59	1195
Dgat23tur-f	GCtctagaGAGGTCCTGGAGGTGAACTGA	68.4	1203
Dgat23tur-r	GCtctagaGCCTTTTCCAACATTTATTTCTG		
Dgat2f	5′-TACTTCACTTGGCTGGCGT-3′	60	241
Dgat2r	5′-GGAACTTCTTGCTCACTTCG-3′		
GAPDHf	5′-ACTCACTCTTCTACCTTTGATGATGCT-3′	60	100
GAPDHr	5′-TGTTGCTGTAGCCAAATTCA-3′		

Lower case indicates restriction digestion site.