African Journal of Biotechnology Vol. 10(27), pp. 5263-5267, 15 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1534
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

A genetic polymorphism and its genetic effects on goat *myogenin* gene in intron 1

Zheng-Zhu Liu, Yuan-Fang Gong*, Chuan-Sheng Zhang, Zhi-Xin Fu and Wen-Xiang Zhang

College of Animal Science and Technology, Hebei Normal University of Science and Technology, Changli 066600, China.

Accepted 9 March, 2011

Single nucleotide polymorphisms (SNPs) of the *myogenin* (*MyoG*) gene were tested using primer induced restriction fragment length polymorphism assay-polymerase chain reaction (PIRA-PCR) from Bore goat and its upgrading offspring to Tangshan diary goat (including F1, F2 and F3). The effects of the *myogenin* gene on the birth weight, 1-month body weight and the weaning weight were also analyzed. On the basis of the DNA sequence of the goat *myogenin* gene (FJ607135), primers were designed to amplify *myogenin* gene. The result showed that one polymorphism (transition of g.*558C>T*) was found in intron 1 of goat *myogenin* gene, in which two alleles (*A* and *B*) and three genotypes (*AA*, *AB* and *BB*) were examined. The distributions of three genotypes were basically identical in four goat populations, and allele *A* was the dominant gene. The effect of the *myogenin* genotypes on the birth weight, 1-month body weight and the weaning weight were all not significant (P > 0.05) due to the small number of *BB* gaots; however, the values of *AA* genotype goats and *AB* genotype goats were obviously higher than those of *BB* genotype goats for three growth traits, in the order of *AA* > *AB* > *BB*. These results suggest that the *myogenin* genotype has some effects on partial growth traits of goat, and selecting the individuals with *A* allele could be favorable to the birth weight, 1-month body weight and the weaning weight.

Key words: goats, *myogenin* gene, primer induced restriction fragment length polymorphism assay-polymerase chain reaction (PIRA-PCR), genetic polymorphisms, genetic effects.

INTRODUCTION

The meat production capacity of animals is related to the number of myofibers in their muscles. Muscle fiber formation occurs only during embryonic development when it is under the control of the basic helix-loop-helix (bHLH) family consisting of *myogenin*, *MyoD1*, *myf-5* and *myf-6* (Olson, 1990; Weintraub et al., 1991; Te Pas et al., 1999). The *myogenin* has a central position with the bHLH family because its expression abrogates myoblast proliferation potential and regulates the differentiation of single nucleated myobasts into multinucleated myofibers (Wright et al., 1989; Weintraub et al., 1991). The *myogenin* gene contains three exons and two introns. It is well known that some genes can affect myofiber numbers. Disruption of the *myogenin* gene also affects myofiber numbers (Hasty et al., 1993; Nabeshima et al., 1993). For many years, the meat production for high yield and good quality in the same level has been pursued by the livestock producers and achieving the ideal goal is quite uneasy. However, it offers the possibility of achieving this ideal goal because of the discovery of the bHLH family and the further research on their physiological functions especially on identification and expression of the *myogenin* gene. At present, many studies have been mainly carried out on *myogenin* gene in pigs. The previous ones not only physically mapped *myogenin* gene to the porcine chromosome 9q2.1-q2.6 (Ernst et al., 1998), but also for analysis of the structure and genetic variation (Ernst et al., 1993; Soumillion et al., 1997), the phylogeny analysis of *myogenin* gen in

*Corresponding author. E-mail: gongyuanfang 1992@ yahoo. com.cn.

Abbreviations: PIRA-PCR, Primer induced restriction fragment length polymorphism assay-polymerase chain reaction; **RFLP,** restriction fragment length polymorphism; **bHLH,** basic helix-loop-helix.

AGCCAGGG*GTAAGTGGCC[A/G]C[C/T]CCACCCGCCTGCCCCGGGAGGGGGCACAGGAGGCATCTG*

GACAGCCTCAGGGGACCCATCGTGGGCTCAGTCAGATGGCTGGAGCAGGAGCCAGGCAGGGTCTT

Figure 1. Nucleotide sequence of the *myogenin* gene fragment (bases 538-662 of accession No. FJ607135). The intron 1 is in italics and coding sequence is in normal type. Primers are shown shadowed, and the mismatch site (556 A/G) and the mutation site (g. *558 C>T*) are in square brackets. The *Mbi*I recognition sequence is underlined.

different breeds (Zhu et al., 2010) and the analysis of the correlation between different *myogenin* genotypes, and some economic traits including growth traits were completed (Te Pas et al., 1999; Anton et al., 2006; Xue and Zhou, 2006; Zhao et al., 2005). Up till now, little is known about this gene in goat and only the partial sequences of the gene were reported by Gao et al. (2009). Bore goat is one of the famous meat type of goat breed, which grows faster and has heavier body weight and higher meat production (Campher et al., 1998). It was introduced into many countries to improve the meat production of the native goats and a very good hybridization effects had been obtained (Gong et al., 2002; Goonewardene et al., 1998; Waldron et al., 1995). The objective of this study is to analyze the DNA polymorphism of the caprine *myogenin* gene and to investigate the relationships between *myogenin* genotypes and the partial growth traits in goat.

MATERIALS AND METHODS

Population samples, DNA extraction and data collection

A total of two hundred and nine samples were investigated, including 48 Bore goats and 161 upgrading offspring of Bore goat and Tangshan Diary goat (including 20 F1, 48 F2 and 93 F3) from Qinhuangdao Bore goat Breeding Farm. Genomic DNA was extracted from the blood using the phenol extraction method. In this study, the partial growth traits including the birth weight, 1-month body weight and the weaning weight were recorded. The three traits were recorded within one day after birth, within the 30th day and within the 90th day, respectively.

Primer induced restriction fragment length polymorphism assay-polymerase chain reaction (PIRA-PCR) design and restriction fragment length polymorphism (RFLP)

In order to identify the polymorphism of goat *myogenin* gene, four sequenced individuals were aligned by Clustalw program as implemented in BioEdit software (Version 7.0.5.2). The transition of g.*558C >T* (according to FJ607135) in intron 1 of *myogenin* gene was defined, aligned and checked for sequencing results. But a restriction enzyme site was not detected in the sequence with mutition site. A pair of polymerase chain reaction (PCR) primers (Forward: 5'-AGCCAGGGGTAAGTGGCCGC-3', and reverse: 5'-AAGACCCTGCCTGGCTCCTGCT-3') was designed to detect a single-base substitution at nucleotide number 558 using a www-based computer program (PIRA-PCR, http://cedar.genetics.soton.ac.uk/public_html/primer2.html) (Ke et al., 2001). The first primer contains a nucleotide G that is mismatched with its target DNA to artificially create a restriction site of Mbi I (5'-CCG|CTC-3', Fermentas) (Figure 1). The base substitution within the restriction site would result in presence/absence of the restriction site in the amplified product to generate a polymorphism.

PCR amplification was carried out in a PTC-100TM PCR instrument (MJ Research, Inc., Massachusetts, USA) with a total reaction volume of 50 µl. The reaction volume contained 100 ng genomic DNA, 400 pmol/l for each forward and reverse primer, 5.0 µl 10× PCR buffer (with Mg^{2+}), 4.0 µl of 2.5 mM dNTP, and 2.5 U Taq DNA polymerase from TaKaRa BIOTECH Co., Ltd (Dalian, China). The PCR protocols was as follows: denaturation at 94℃ for 4 min, followed by 35 amplification cycles comprising of denaturation at 94℃ for 30 s, annealing at 57℃ for 30 s and extension at 72℃ for 1 min, followed by an extended elongation at 72℃ for 10 min. PCR products were detected on 2% agarose gel including 0.5 µg/ml of ethidium bromide, photographed under UV light and sequenced by Shanghai Sangon Biological Engineering Technology, Biological and Technology and Service Co., Ltd. (Shanghai, China).

The RFLP was carried out according to the variation site (g.*558C>T*), Mbi I recognizing 5'-CCG|CTC-3' and cutting at position 556 (position 19 according to the 125bp PCR product). The digestion solution with a total volume of 20 µl containing 10 µl of PCR products, 0.2 µl (10 U/µl) of Mbi I, 2.0 µl of 10×Buffer, and 7.8 µl distilled water, was incubated at 37℃ for 3 h in the Programmable Thermal Controller. Genotypes were detected by running digested products on 3% agarose gel including 0.5 µg/ml of ethidium bromide.

Statistical analysis

The genotype frequencies from the examined goats were calculated. An analysis of the genotypic effects of the *myogenin* gene was carried out using the general linear model (GLM) procedure of the Statistical Analysis System (SAS) 8.2 (version).

The fixed model was:

$$Y_{ijklmno} = \mu + p_i + s_j + y_k + g_l + tc_m + tx_n + e_{ijklmno}$$

Where, $Y_{ijklmno}$ is the observed value of individuals, μ is the least square means of the observed values, p_i is the effective value of the population *i*, s_j is the effective value of the sex *j*, y_k is the effective value of the *k*th birth season, g_l is the effective value

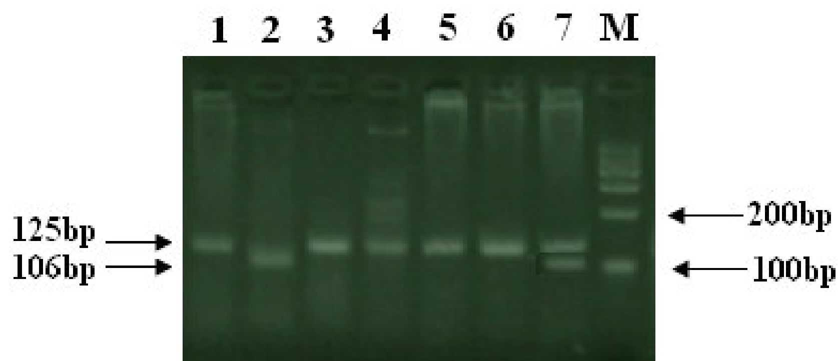

Figure 2. Examples of the PIRA-PCR-RFLP products of the *myogenin* gene intron 1 after digestion with *Mbi*I and separation by electrophoresis through 3% agarose gel. Lanes 1, 3 to 6: *AA* homozygotes; Lane 2: *BB* homozygotes; Lane 7: *AB* heterozygote; M: 100 bp DNA marker.

of the genotype *l*, tc_m is the effective value of the *m*th birth order, tx_n is the effective value of the *n*th embryo type and $e_{ijklmno}$ is the random residual effect corresponding to the observed value.

RESULTS AND DISCUSSION

PIRA-PCR-RFLP analysis

One polymorphism was found in the region of *myogenin* gene intron 1, which resulted in three genotypes, and designated as *AA*, *AB* and *BB*. Homozygote *AA* was defined when base C exists at position 558 forming CCGCCC which is not recognized by Mbi I and forming 125 bp. Homozygote *BB* was defined when base T exists at the 558 position forming CCGCTC recognized by Mbi I forming 106 and 19 bp, and heterozygote *AB* was defined when allele T and C both exist at the same position of homologous chromosome, forming 125, 106 and19 bp, whereas the fragment (19bp) was too small to be visualized on 3% agarose gel (Figure 2). The sequence comparison of homozygote *AA*, *BB* and heterozygote *AB* is shown in Figure 3.

Genotypes distribution

The genotypes frequencies and allele frequencies of g.*558C>T* in four goat populations are shown in Table 1. The distributions of three genotypes were basically identical in four goat populations, and allele *A* was the dominant gene.

Effects of the different genotypes

The genotypic effects of the *myogenin* gene are summarized in Table 2. Significant differences (P < 0.05 or P < 0.01) were not found among the different *myogenin* genotypes for the three growth traits. The least square means and standard errors concerning the birth weight, 1-month body weigh and the weaning weight are presented in Table 3. Although there were no significant differences (P > 0.05) for the three growth traits between the goats of *AA* vs. *AB* genotype and those of the *BB* genotype, the values of *AA* genotype goats and *AB* genotypes goats were both obviously higher than those of *BB* genotype goats, in the order of *AA* > *AB* > *BB*. With regard to three growth traits, allele *A* had high positive additive effect, in which its additive value was 0.05, 0.63 and 1.57 kg, respectively.

The RFLP of all the experimental samples was carried out and three genotypes (*AA*, *AB* and *BB*) were detected. The distributions of three genotypes were basically identical in four goat populations, and allele *A* was the dominant gene. Therefore, it may be suggested that the resemblances between the Boer goats and their upgrading offspring to Tangshan Diary goat (F1, F2 and F3) with regard to the three growth traits are related to the genotypes of the *myogenin* gene, which result from the long-term upgrading and breeding.

Recently, a number of studies have proved that the *myogenin* gene fulfilled a key function in muscle differentiation by controlling the onset of myoblast fusion and the establishment of myofibers (Te Pas et al., 1999; Ernst et al., 1993). The different *myogenin* genotypes are related to the variation in the number of muscle fibers and the growth rate, which led to a variation in the muscle mass (Te pas et al., 1999; Soumillion et al., 1997). For pigs, Te pas et al. (1999), Xue et al. (2007) and Anton et al. (2006) found that significant differences exist among the *myogenin* genotypes for the partial growth traits, such as birth weight, growth rate, lean weight and so on. For chickens, significant differences (P < 0.05) or extremity significant differences (P < 0.01) were examined among *myogenin* genotypes on the partial carcass traits and the partial meat traits by Wang et al. (2007). However, whether

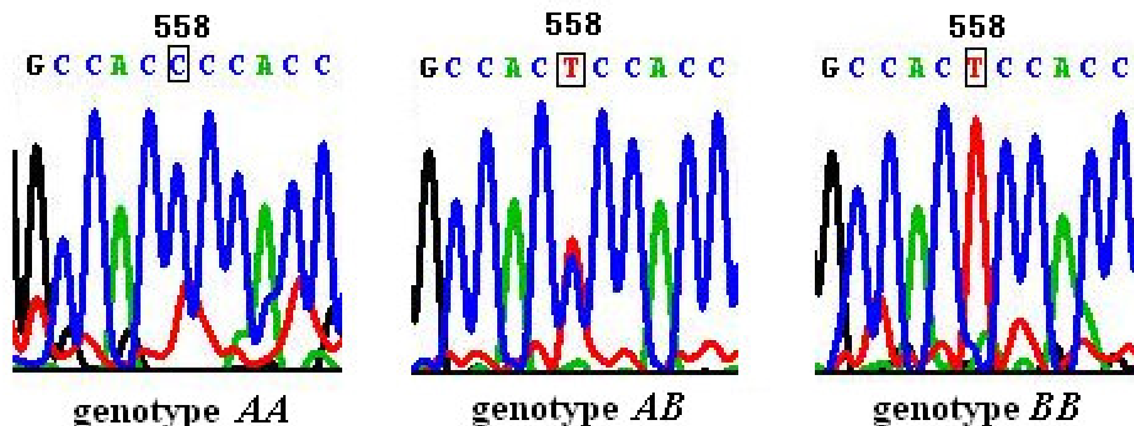

Figure 3. The sequence comparison of the homozygotes *AA*, *BB* and heterozygote *AB* of the *myogenin* gene intron 1 revealed a C>T transition.

Table 1. The genotypes frequencies and allele frequencies of *myogenin* gene intron 1 (g. *558C>T*) in four goat populations.

Goat population	Population size	Genotype frequency			Allele frequency	
		AA	*AB*	*BB*	*A*	*B*
Bore goat	48	0.50 (24)	0.48 (23)	0.02 (1)	0.74	0.26
F1	20	1.00 (20)	0.00 (0)	0.00 (0)	1.00	0.00
F2	48	0.51 (25)	0.43 (21)	0.04 (2)	0.74	0.26
F3	93	0.52 (50)	0.44 (40)	0.04 (3)	0.75	0.25

Table 2. Effect of the source of variation on the measured growth traits.

Source	*df*	F value		
		Birth weight	1-month body weight	Weaning weight
Population	4	6.53**	4.97**	3.55*
Sex	1	23.31**	2.59	1.63
Season	3	2.63	1.63	2.79*
Birth order	6	5.57**	3.75**	2.49*
Embryo type	3	24.35**	32.38**	13.52**
Genotype	2	0.10	1.66	1.66

Values with * differ at P < 0.05; values with ** differ at P < 0.01.

Table 3. Least square means and standard errors for the measured traits of the different genotypes.

Trait	LSM±SE			Additive effect (a)	Dominance effect (d)
	AA	*AB*	*BB*		
Birth weight (kg)	3.65±0.13	3.64±0.13	3.55±0.25	0.05	0.04
1-month body weigh (kg)	8.60±0.43	8.57±0.45	7.34±0.75	0.63	0.60
Weaning weight (kg)	18.48±1.04	18.17±1.08	15.34±1.94	1.57	1.26

Values with same or no letters within the same row means no significant difference (P > 0.05); a = (*AA* - *BB*)/2; d = *AB* - (*AA* + *BB*)/2.

the genetic polymorphism of *myogenin* gene is associated with the growth performance in the goats, it still remains unknown. In the present study, the genotypic effects of the *myogenin* on the birth weight, 1-month body weight and the weaning weight in goats were first analyzed. Results indicated that significant differences were not found among the *myogenin* genotypes for the three growth traits. This may be the reason for the number of the experimental goats, especially the number of *BB* gaots which was too small. These results suggest that the *myogenin* genotype has some effects on the partial growth traits of goat, and selecting the individuals with *A* allele could be favorable to the birth weight, 1-month body weight and the weaning weight. With the limited number of the experimental goats in this study, analyzing more goats may be necessary to further confirm the genotypic effects of the *myogenin* gene.

ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of Hebei Province (C2007000743), Doctor Foundation of Hebei Normal University of Science and Technology (2010YB006) and the National Key Technology R&D Program (2008BADB2B04-8-4).

REFERENCES

Anton I, Zsolnai A, Komlósi I, Király A, Fésüs L (2006). Effect of MyoG genotypes on growth rate and production traits in Hungarian large white pigs. Acta. Vet. Hung. 54(3): 393-397.

Campher JP, Hunlun C, Van zyl GJ (1998). South African Livestock Breeding. Bloemfontein: South African Stud Book and Livestock Improvement Association, pp. 106-107.

Ernst CW, Vaske DA, Larson RG, Rothschild MF (1993). Rapid communication: MspI restriction fragment length polymorphism at the swine myogenin locus. J. Anim. Sci. 71(12): p. 3479.

Ernst CW, Mendez EA, Robic A, Rothschild MF (1998). Rapid communication: myogenin (MyoG) physically maps to porcine chromosome 9q2.1-q2.6. J. Anim. Sci. 76(1): p. 328.

Gao GY, Wang J, Wang Y, Fu CX, Zhou GM, Zhou LX,Wang CX (2009). Cloning and sequence analysis of myogenin of Chengdu Grey Goats. J. Southwest University for Nationalities. 35(1): 84-88.

Gong YF, Li XL, Liu ZZ, Dong JQ, Zhang H (2002). Meat performances of upgraded offspring of Boer goat and Tangshan Diary goat. Journal of Hebei Vocation-Technical Teachers College. 16(4): 30-33.

Goonewardene LA, Day PA, Patrick N, Scheer HD, Patrick D, Suleiman A (1998). A preliminary evaluation of growth and carcass traits in Alpine and Boer goat crosses. Can. J. Anim. Sci. 78(2): 229-232.

Hasty P, Bradley A, Morris JH, Edmondson DG, Venuti JM, Olson EN, Klein WH (1993). Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. Nature, 364(6437): 501-506.

Ke X, Collins A, Ye S (2001). PIRA PCR designer for restriction analysis of single nucleotide polymorphisms. Bioinformatics, 17(9): 838-839.

Nabeshima Y, Hanaoka K, Hayasaka M, Esumi E, Li S, Nonaka I, Nabeshima Y (1993). Myogenin gene disruption results in perinatal lethality because of severe muscle defect. Nature, 364(6437): 532-535.

Olson EN (1990). MyoD family: a paradigm for development? Genes Dev. 4(9): 1454-1461.

Soumillion A, Erkens JH, Lenstra JA, Rettenberger G, te Pas MF (1997). Genetic variation in the porcine myogenin gene locus. Mamm. Genome, 8(8): 564-568.

Te Pas MF, Soumillion A, Harders FL, Verburg FJ, van den Bosch TJ, Galesloot P, Meuwissen TH (1999). Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs. J. Anim. Sci. 77(9): 2352-2356.

Waldron DF, Huston JE, Willingham TD, Oman JS, Savell JW (1995). Growth rate, feed consumption and carcass measurements in Spanish and Boer × Spanish goats. J. Anim. Sci. 73 (1): 253.

Wang Q, Liu YP, Jiang XS, Yang CW, Du HR, Qiu MH, Zhu Q (2007). Correlation analysis of relationships between polymorphisms of high quality chicken myogenin gene and slaughter and meat quality traits. Yi Chun, 29(9): 1089-1096.

Weintraub H, Davis R, Tapscott S, Thayer M, Krause M, Benezra R, Blackwell TK, Turner D, Rupp R, Hollenberg S, Zhuang Y, Lassar A (1991). The MyoD gene family: nodal point during specification of the muscle cell lineage. Science, 251(4995): 761-766.

Wright WE, Sassoon DA, Lin VK (1989). Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. Cell, 56(4): 607-617.

Xue HL, Zhou ZX (2006). Effects of the MyoG gene on the partial growth traits in pigs. Yi Chuan Xue Bao. 33(11): 992-997.

Xue HL, Zhou ZX, Bo W (2007). PCR-SSCP genetic polymorphism and its genetic effect of 3′side of pig MyoG gene. Chin. J. Ecol. 26(4): 505-508.

Zhao J, Nie GJ, Zhang JZ, Guo XL, Xu NY (2005). Influences of MyoG gene on reproductive traits in Jinhua pig. Yi Chuan. 27(6): 893-897.

Zhu L, Li XW, Shuai SR, Li MZ, Chen L, Gu YR, Zhang K (2010). The phylogeny analysis of MyoG gene in different pig breeds. Interdiscip. Sci. 2(2): 175-179.