Pakistan J. Zool., vol. 47(6), pp. 1605-1610, 2015.

Analysis of Association Between Polymorphism of *TFB2M* Gene and Meat Quality, Growth and Slaughter Traits in Guizhou White Goat, a Well-known Chinese Indigenous Goat Breed

Yanyan Sun, Weixing Luo, Haiqiang Xie, Yiyu Zhang and Huifen Cai *

Key Laboratory of Animal Genetics, Breeding and Reproduction in the Plateau Mountainous Region, Ministry of Education, College of Zoology, Key Laboratory of Animal Genetics, Breeding and Reproduction in Guizhou Province, Guizhou University Guiyang, Guizhou 550025, People's Republic of China

Abstract.- The present study aims to determine the useful SNPs in *TFB2M* gene and explore their effects on the meat quality in Guizhou White Goat. In this study, the technologies of DNA pool, direct sequencing and PCR-SSCP were -adopted to screen the SNPs of *TFB2M* gene in 29 experimental Guizhou White Goats. We analyzed the association between the polymorphism of *TFB2M* gene and the growth traits, meat quality traits and slaughter traits in experimental goats. It is shown from the results that A17110C locus was detected in exon 8, and three genotypes (AA, AC and CC) were discovered by SSCP. The association analysis showed that there was no significant difference in growth traits ($P>0.05$), meanwhile SNPs (A17110C) made a certain influence on bone weight in Guizhou White Goat, and the individuals of genotype AA were superior than genotype AC in bone weight ($P<0.05$). According to the results in this study, it is suggested that *TFB2M* gene might be regarded as a candidate gene affecting bone weight in goats.

Key words: Guizhou white goat; *TFB2M,* gene polymorphism, meat quality traits, growth traits, slaughter traits

INTRODUCTION

Guizhou white goat is a breed of Chinese goat whose origin and its history could be traced back to the Han Dynasty. The breed is known as its crude feed tolerance, higher disease resistance, delicious and tender meat, which are superior to normal goats. Because of the enormous maket of goat, there are many researches on goats(Zhang et al., 2014).However, the researches on Guizhou White Goat mainly focused on growth performance and fecundity, basing on the purpose for the pursuit of economic effect (Chen *et al.*, 2013; Song *et al.*, 2013; Huang *et al.*, 2010).

TFB2M (mitochondrial transcription factor B2) encoded by the nuclear genome is one of the important factors, and it regulates the mitochondrial gene expression, and the expression of TFB2M is strictly regulated by nuclear transcription factors, such as NRF-1 (nuclear respiratory factor-1) and NRF-2 (Yakubovskaya *et al.*, 2010; Rebelo *et al*, 2011). TFB2M, TFB1M, TFAM (mitochondrial transcription factor A, TFAM) and POLRMT (mitochondrial RNA polymerase) comprise the mitochondrial transcription basic machine together (Falkenberg *et al*., 2002; Fisher and Clayton, 1988). In recent years, a large number of studies have demonstrated that two nearer SNPs (single nucleotide polymorphism) situated in the promoter region of *TFAM* gene made significant impacts on marbling and subcutaneous fat depth by the association analysis of Wagyu and Limousin cattle. In addition, the two SNPs affected TFBS (transcription factor binding site) which related to energy metabolism (Jiang *et al.*, 2006; Jiang and Kunaj, 2010). On basis of the above results, it was inferred that *TFB2M* gene played a critical role in meat quality and growth traits. In this study, we used polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and direct sequencing to screen the SNPs in *TFB2M* gene for their association with meat quality traits in Guizhou white goat.

MATERIALS AND METHODS

Experimental materials

We obtained 29 healthy commodity Guizhou white goats with the same feeding conditions from Yanhe County, Guizhou province. We collected the blood and recorded the body weight, body height,

* Corresponding author: fengfeng6111@163.com
0030-9923/2015/0006-1605 $ 8.00/0

Table I.- Primer sequence, annealing temperature, amplified DNA fragment and amplified region.

Primer	Primer sequence(5'→3')	Annealing temperature (°C)	Amplified DNA fragment(bp)	Amplified region
TFB2M-1	F:AGGGCAGACGACCATAGAGT R: TCTTGTGTTCCCATACGGTCT	59.9	812	E1
TFB2M-2	F: AAGTGACTGTCTAATCGCCG R: CTGTTCCATCCACTGAGGGT	54.5	416	E2
TFB2M-3	F: CCTTCTGTTGCTGGCTCTGA R: CACAAATCCCAACAAGCCTGG	61.6	343	E3
TFB2M-4	F: TCAGAGCAGTTTTGTAGGGGC R: TGAGCCCAAATTAAAATCATGCAG	62.5	416	E4
TFB2M-5	F: TGAGCCCATCAGCTCGTATATT R: GTGACACAAAACATCAGCACAG	55.1	442	E5
TFB2M-6	F: TAAGAGCAACTGGGGCCTTG R: CGAAAACATGGTCCCTCACTG	63	446	E7
TFB2M-7	F: TGCAGGCAAGCCATGAAAC R: CACCAAAGAAATCCAACAGAGGAG	64	1199	E8
TFB2M-8	F: TTGCACAACTAGGCAGATCATTTC R: TTTATATACACTCTTCTGCTCCCC	61.5	301	SNP

body length, hucklebone width and chest girth of total individuals separately. The experimental goats were slaughtered and the longissimus dorsi muscle were taken. The data of slaughter and meat quality traits was also recorded at the same time. The samples were later taken to the laboratory and intramuscular fat (IMF) of longissimus dorsi muscle was estimated by Soxhlet extraction method in triplicate. The DNA was extracted from blood of Guizhou White Goat by using the genomic DNA kit (Sangon Biotech (Shanghai) Co., Ltd), then it was preserved in refrigerator at -20°C.

PCR amplification of TFB2M

Prime 5.0 was used to design 8 pairs of specific primers (Table I), on the basis of *TFB2M* gene sequence (GenBank accession No: NC_019469) in NCBI database. Primers above were synthesized by Sangon Biotech (Shanghai) Co., Ltd.

PCR reaction mixture: 10.0 μl 2xEs Taq MasterMix, 1.5 μl 10 pmol/μl upstream primer, 1.5 μl 10 pmol/μl reverse primer, 2.0 μl DNA template, and add ddH_2O to a final volume 20.0 μl. PCR thermal cycle comprised initial denaturation for 5 min, followed by 35 cycles each of denaturation at 95°C for 30 s, annealing at 59.9/54.5/61.6/62.5/ 55.1/63/64°C for 30 s, extension at 72°C for 30s, and one final extension at 72°C for 10 min. The PCR products were examined with 1% agarose gel electrophoresis (Fig. 1).

PCR-SSCP reaction

PCR reaction mixture: 5.0 μl 2xEs Taq MasterMix,0.75 μl 10 pmol/μl upstream primer, 0.75 μl 10 pmol/μl reverse primer, 1.0 μl DNA template, and add 2.5 μl ddH_2O. PCR thermal cycle comprised initial denaturation for 5 min, followed by 35 cycles each of denaturation at 95°C for 30 s, annealing at 61.5°C for 30 s, extension at 72°C for 30 s, and one final extension at 72°C for 10 min (The primer was *TFB2M*-8).

The PCR product (10 μl) was mixed with 10 μl denaturing buffer (98% formamide, 0.025% bromophenol blue, 25mmol EDTA and 0.2% xylene cyanol), heated at 100°C for 15 min and chilled at -20°C for 15 min and then loaded on 10% neutral polyacrylamide gels (acrylamide: bisacrylamide= 29:1). Polyacrylamide gel electrophoresis was performed at 110 V/15 h/4°C. After electrophoresis, the DNA bands were stained by silver staining. Different electrophoresis patterns were sent to conduct bidirectional sequence.

Statistical analysis

The genotype frequency was recorded, and the model of the least square method was built by

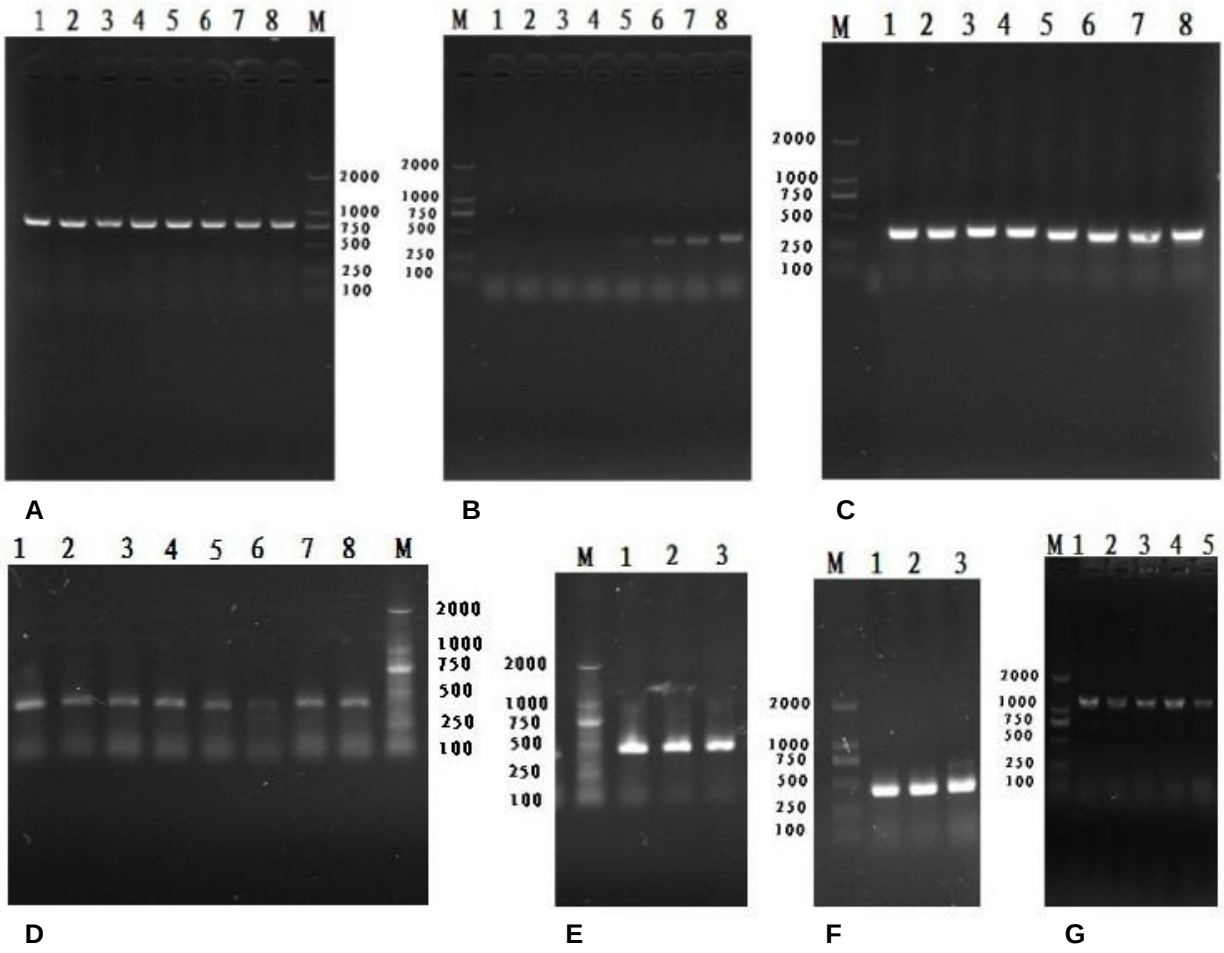

Fig.1. PCR product of TFB2M gene using TFB2M-1 (A), TFB2M-2 (B), TFB2M-3 (C), TFB2M-4 (D), TFB2M-5 (E), TFB2M-7 (F) and TFB2M-8 (G) primers.

Chi-Square Goodness-of-Fit Test. Phenotypic value of Individual traits (Yijk) was calculated by the formula:

$$Y_{ijk} = \mu + marker_k + e_{ijk.}$$

Note μ represented the general mean; markerk represented the effect of marker genotype; eijk was the random error.

The associations between different genotypes and traits were analyzed. SPSS 19.0 GLM (general liner model) software was applied for statistical treatment using the least square method. The statistical results were presented as means ± standard error.

RESULTS AND DISCUSSION

Identification of SNP site A17110C

Target fragments of *TFB2M* gene were amplified with 8 pairs of specific primers (Figs. 1, 2).

The high-level products and clear single DNA pool products were sequenced by SionGene Mox (Beijing) Co., Ltd. Target sequences were blasted by the software of MegAlign and SeqMan. Figure 2 shows one SNP site (A17110C) in exon 8 was in the PCR products of *TFB2M*. The first site in exon 1 of *TFB2M* gene was set as NO.1. One pair of primers (*TFB2M*-8) in exon 8 of *TFB2M* gene were synthesized for PCR-SSCP. The genotypes of AA,

AC and CC were only detected in SSCP pattern of exon 8 (Fig.3).

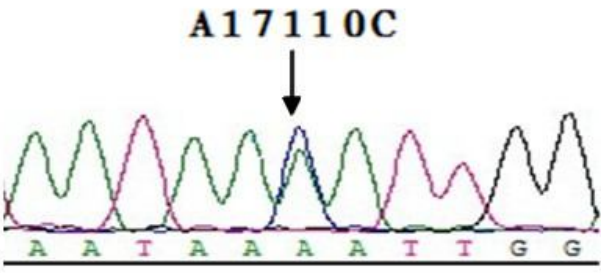

Fig.2. Sequencing atlas of PCR product in DNA pool.

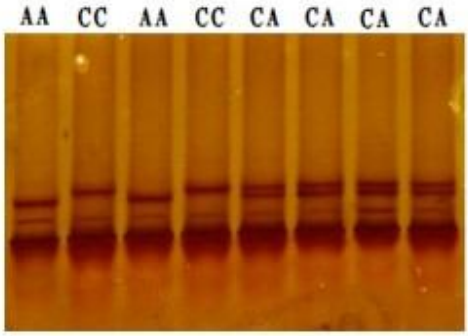

Fig.3. Electrophoresis pattern for PCR-SSCP of A17110C locus.

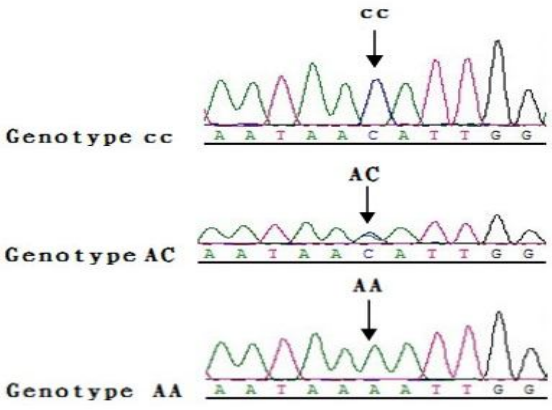

Fig. 4. Sequencing atlas of A17110C locus.

By comparing the sequences with the known sequences listed in the GenBank database (No: NC_019469), genotype AA was defined as wild type, genotype AC was defined as heterozygous type, and genotype CC was defined as mutant type (Fig.4).

Allele and genotype frequencies of TFB2M gene

Statistical analysis was conducted between A17110C locus and the genetic parameters in the experimental Guizhou White Goat (Table II). There were three genotypes of A17110C in commodity goats, and the amount of these three genotypes were sequenced as AC>AA>CC, and the genotype AC could be seen as dominant. Effective number of alleles (Ne) of the experimental population was close to 2, and it is suggested that the allele distributed evenly in the population. Regarding polymorphic information content, two experimental populations achieved moderate polymorphism (0.25<PIC<0.5). Hardy-Weinberg equilibrium for each population was analyzed with Chi-Square Goodness-of-Fit Test. Meanwhile the SNP locus was in Hardy-Weinberg disequilibrium in Guizhou White Goat (P>0.05).

Association of polymorphism with growth, meat quality and slaughter traits

There were no significant differences in growth traits (Table III), and of meat quality traits (Table IV). The association analysis between the genotypes of A17110C locus and evaluation indexes of slaughter traits showed that the adult individuals of AC were lower than AA in bone weight (P<0.05) (Table V).

TFB2M was a key factor in regulating the expression of mammalian chondriogene, and it took effect after it is transferred into mitochondria. TFB2M not only formed a network of coactions with DNA near the transcription initiation site and facilitated promoter melting (Sologub *et al*., 2009), but also took possession of the methyltransferase effects (Seidel-Rogol *et al*., 2002; Cotney and Shadel, 2006), during the process of transcription. *TFB2M* gene could regulate the mitochondrial transcription and translation and had effects on the formation of marbling (Cotney *et al*., 2007), as an important factor of mtDNA transcription machinery. Meanwhile, the main function of mitochondrial was to supply power for organism, and it was significant to explore the relationship between *TFB2M* gene and growth performance in goats.

It appears that *TFB2M* gene makes less impact on growth and meat quality performance, though the SNPs A17110 C influences the bone

Table II.- Genotype frequency, allele frequency and genetic diversity of A17110C locus (Mean±SEM).

Site	Month	Genotype	Genotype frequency	Allele	Allele frequency	Ne	Ho	He	PIC	χ^2
A 17110C	24-48	AA(10)	0.3448	A	0.6034	1.9183	0.5213	0.4787	0.3642	0.1891
		AC(15)	0.5172							
		CC(4)	0.1379	C	0.3965					

Note: df=2, X20.05(2)=5.99，X20.01(2)=9.21. PIC >0.50 means high diversity, 0.25<PIC<0.50 means moderate diversity, PIC<0.25 means low diversity. The number inside the brackets stands for the number of individuals with genotype.

Table III.- Association analysis between different genotypes and growth traits of A17110C locus (Mean±SEM).

Site	Month	Genotype	Growth traits				
			Body weight/kg	Body height/cm	Body length/cm	Chest girth/cm	Hucklebone width/cm
A17110C	24-48	AA(10)	43.20±2.97	58.49±1.79	67.45±1.64	82.30±2.19	9.30±0.39
		AC(15)	39.80±2.42	56.93±1.46	65.95±1.34	82.70±1.79	9.15±0.32
		CC(4)	34.44±4.69	56.50±2.83	65.13±2.59	77.75±3.47	8.75±0.62

Note: Numbers in brackets are defined as the numbers tested individuals of each genotype. Any data containing different superscripts within the same rank mean differ significantly (P<0.05).

Table IV.- Association analysis between different genotypes and meat quality traits of A17110C locus (Mean±SEM).

Site	Month	Genotype	Meat quality traits				
			Water holding capacity	Water loss rate	Cooked meat percentage	Shear force	IMF
A17110C	24-48	AA(10)	AA(10)	2.48±0.35	36.67±3.09	53.83±1.44	3.94±0.45
		AC(15)	AC(15)	2.98±0.28	33.78±2.52	52.49±1.18	4.46±0.37
		CC(4)	CC(4)	2.88±0.55	31.57±4.89	52.89±2.28	3.94±0.72

Note: Numbers in brackets are defined as the numbers tested individuals of each genotype. Any data containing different superscripts within the same rank mean differ significantly (P<0.05).

Table V.- Association analysis between different genotypes and Slaughter Traits of A17110C locus (Mean±SEM).

Site	Month	Genotype	Slaughter Traits				
			Carcass weight/kg	Dressing percentage	Net meat weight/Kg	Bone weight/Kg	Fur weight/ Kg
A17110C	24-48	AA(10)	19.13±1.61	44.86±1.82	15.15±1.46	3.98±0.29^a	2.82±0.18
		AC(15)	17.70±1.31	44.21±1.48	14.51±1.19	3.17±0.24^b	2.55±0.15
		CC(4)	14.98±2.54	43.93±2.87	11.81±2.30	3.17±0.46	2.43±0.29
Site	**Month**	**Genotype**	**Lumbar muscle thickness/cm**	**Net meat percentage**	**Fur percentage**	**Bone percentage**	**Meat-bone ratio**
A17110C	24-48	AA(10)	1.85±0.08	35.31±1.80	15.25±1.01	21.71±1.37	3.91±0.40
		AC(15)	1.77±0.07	36.15±1.47	14.98±0.82	18.22±1.12	4.72±0.33
		CC(4)	1.73±0.13	34.47±2.84	16.26±1.60	21.29±2.17	3.80±0.64

Note: Numbers in brackets are defined as the numbers tested individuals of each genotype. Any data containing different superscripts within the same rank mean differ significantly (P<0.05).

weight in Guizhou White Goat, such as individuals of genotype AA are superior than genotype AC in bone weight ($P<0.05$).

CONCLUSION

In conclusion, the findings in the present research have demonstrated that the SNPs can reduce bone weight by affecting *TFB2M* gene in Guizhou White Goat. However, more goat breeds and data of Slaughter Traits need to be collected for further studies to confirm its function.

ACKNOWLEDGEMENTS

The research was funded by the agricultural science and technology research projects of Guizhou province (#[2012]3006), and the research of molecular mechanisms on nuclear-encoded mitochondrial gene and mutton quality[Z132024].

REFERENCES

CHEN, Z., LUO, W.X., LIU, R.Y., CAI, H.F., CHENG, J., SONG, T.W. AND ZHANG, Y.Y., 2013. Study on the relationship between polymorphism of RERG gene and growth traits of three goats breeds in Guizhou. *Scient. Agric. Sin.*, **46**: 1915-1922.

COTNEY, J. AND SHADEL, G.S., 2006. Evidence for an early gene duplication event in the evolution of the mitochondrial transcription factor B family and maintenance of rRNA methyltransferase activity in human mtTFB1 and mtTFB2. *J. Mol. Evolut.*, **63**: 707-717.

COTNEY, J., WANG, Z. AND SHADEL, G.S., 2007. Relative abundance of the human mitochondrial transcription system and distinct roles for h-mtTFB1 and h-mtTFB2 in mitochondrial biogenesis and gene expression. *Nucl. Acids Res.*, **35**: 4042-4054.

FALKENBERG, M., GASPARI, M., RANTANEN, A., TRIFUNOVIC, A., LARSSON CLAES, M. AND GUSTAFSSON, N.G., 2002. Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. *Nat. Genet.*, **31**: 289-294.

FISHER, R.P. AND CLAYTON, D.A., 1988. Purification and characterization of human mitochondrial transcription factor 1. *Mol. Cell Biol.*, **8**: 3496-3509.

HUANG, Q.H., ZHANG, C., HUANG, W.L., WANG, T.M. AND LIU, R.Y., 2010. Study on LHβGene Polymorphism and Relationship between LHβGene Polymorphism and Litter Size in Guizhou Black Goat. *Guizhou Agric. Sci.*, **38**:162-164.

JIANG, Z. AND KUNEJ, T., 2010. Polymorphisms in mitochondrial transcription factor A ("TFAM") gene and their associations with measures of marbling and subcutaneous fat depth in beef cattle. *Google Patents*, 1-54.

REBELO, A.P., DILLON, L.M. AND MORAES, C.T., 2011. Mitochondrial DNA transcription regulation and nucleoid organization. *J. Inherit. Metab. Dis.*, **34**: 941-951.

SEIDEL-ROGOL, B.L., MCCULLOCH, V. AND SHADEL, G.S., 2002. Human mitochondrial transcription factor B1 methylates ribosomal RNA at a conserved stem-loop. *Nat. Genet.*, **33**: 23-24.

SOLOGUB, M., LITONIN, D., ANIKIN, M., MUSTAEV, A. AND TEMIAKOV, D., 2009. TFB2 is a transient component of the catalytic site of the human mitochondrial RNA polymerase. *Cell*, **139**: 934-944.

SONG, T.W., LUO, W.X., CAI, H.F., LIU, B. AND SUN, Y.Y., 2013. Polymorphism of GHSR Gene and Its Relationship with Body Weight and Body Size Traits in Goat. *Acta Vet. Zootech. Sin.*, **44**:1717-1723.

YAKUBOVSKAYA, E., MEJIA, E., BYRNES, J., HAMBARDJIEVA1, E. AND GARCIA, D.M., 2010. Helix unwinding and base flipping enable human MTERF1 to terminate mitochondrial transcription. *Cell*, **141**: 982-993.

ZHANG, S., ZHANG T., CHANG, G. J., YAN, R., MUHAMMAD, N., NASIR, R. AND SHEN, X.Z., 2014. The metabolism of bolatile fatty acids by portal-drained viscera and liver of goats fed diets with different forage to concentrate ration. *Pakistan J. Zool.*, **46**: 391-400.

(Received 30 December 2014, revised 21 April 2015)