Journal of Animal and Veterinary Advances 13 (13): 792-796, 2014
ISSN: 1680-5593

Genetic Variants of the *A-FABP* Gene are Associated with Meat Production Traits in Different Cattle Breeds

[1]Qianfu Gan, [2]Lupei Zhang, [1]Jiaqin Chen, [1]Haile Yu, [2]Huijiang Gao, [2]Xue Gao and [2]Junya Li
[1]College of Animal Science, Fujian Agricultural and Forestry University,
350002 Fuzhou, Fujian, China
[2]The Key Laboratory for Farm Animal Genetic Resources and Utilization,
Chinese Academy of Agricultural Sciences, Ministry of Agriculture of China,
Institute of Animal Science, 100193 Beijing, China

Abstract: This study was aimed to search new genetic variants in the bovine *A-FABP* gene as molecular markers for meat production traits. One SNP (A2989G with) was genotyped by PCR-SSCP Methods. Sequence analysis revealed that SNP was located in exon 2 (A2989G) of *A-FABP* gene. The gene-specific SNP marker association analysis indicated that the A2989G was significantly associated with marbling score ($p<0.05$) and loin muscle area ($p<0.01$). Results from this study suggest that *A-FABP* gene-specific SNP may be a useful marker for meat quality with traits in future marker assisted selection programs in beef cattle.

Key words: SNP, marker, genetic, production, China

INTRODUCTION

FABP4 (Fatty Acid Binding Protein 4) also known as A-FABP (Adipocyte Fatty Acid Binding Protein), ALBP (Adipocyte Lipid Binding Protein) or ap2 (adipocyte protein-2) is a member of the tissue-specific FABP family which possesses high affinity for fatty acids and is involved in the transport of long-chain fatty acids within the adipocyte (Ockner *et al*., 1972). Studies suggest that FABP4 is directly related to fatness traits and may be a potential marker for beef tenderness and intramuscular fat content (Michal *et al*., 2006; Jurie *et al*., 2007; Hoashi *et al*., 2008; Barendse *et al*., 2009; Lee *et al*., 2010; Mannen, 2011; Narukami *et al*., 2011; Zhao *et al*., 2012).

Intramuscular Fat (IMF) content is a desirable trait in the selection criteria of commercial breeding plans. Intramuscular Fat (IMF) also known as marbling is correlated positively with meat tenderness, juiciness and flavour of cooked meat (Shahidi, 2002; Thompson, 2004). In addition, IMF contents are considered as major factors affecting the sensory meat quality (Gerbens *et al*., 2001, 1998).

The importance of A-FABP for IMF deposition has been demonstrated by several independent studies (Jurie *et al*., 2007; Wang *et al*., 2005). For example, the relevant small effects of genetic variants of the *A-FABP* gene on meat quality traits in pigs and cattle have been reported (Chmurzynska, 2006; Gerbens *et al*., 2001; Hoashi *et al*., 2008). Michal *et al*. (2006) searched for polymorphisms in the bovine *FABP4* gene in DNA pools of animals with high and low marbling scores and identified significant associations between AAFC_01136716.1:g.7516GNC SNP genotypes and backfat thickness and marbling in a Wagyu x Limousin F2 population (*B. taurus*). Recently, Hoashi *et al*. (2008) and Barendse *et al*. (2009) identified new gene polymorphisms that have potential application to beef cattle breeding. However, many studies failed to assure significant associations between genotypes of the *A-FABP* gene and marbling score using several cattle breeds. These problems could be solved if significant effects of genetic variants for the *A-FABP* gene on marbling score be verified with many animals as well as breeds.

The objective of present study was to search new alleles of the *A-FABP* gene to evaluate the effect of genotypes on meat quality traits in several cattle breeds.

MATERIALS AND METHODS

Animals and carcass data: A total of 318 animals including Angus (n = 48), Jinnan (n = 23), Limousin

Corresponding Author: Qianfu Gan, College of Animal Science, Fujian Agricultural and Forestry University, 350002 Fuzhou, Fujian, China

(n = 22), Luxi (n = 29), Qinchuan (n = 27), Simmental (n = 110), Hereford (n = 30) and Charolais (n = 29) were randomly selected from commercial populations and used in the association analysis which were reared in the province of Inner Mongolia and Hebei Province, respectively. The animals (405±50.5 kg; 30±2 months of age) were housed in a concrete-floored cowshed (in a single pan for each animal) and fed 195 days. Carcass and meat quality traits were measured according to the criterion GB/T 17238-1998 Cutting Standard of Fresh and Chilled Beef in China (China Standard Publishing House). Blood samples were taken from the jugular and put into test tubes containing ACD anticoagulant and stored at -80°C until analysed. The following traits, Live Weight (LW), Carcass Weight (CW), Dressing Percentage (DP), Meat Weight (MW), Meat Percentage (MP), Backfat Thickness (BF), Marbling Score (MS), Average Daily Gain (ADG), Loin Muscle Area (LMA), Thigh Meat Thickness (TMT) and Waist Muscle Thickness (WMT) were measured or calculated. BF and LMA were measured between the 12 and 13th rib. All experimental procedures were performed according to authorization granted by the Chinese Ministry of Agriculture.

PCR amplification and SNP genotyping: DNA samples were extracted from blood samples according to (Mullenbach *et al*., 1989) and detected using 10% agarose gel electrophoresis. Primer sets focusing on all exons and partial introns of the *A-FABP* gene were designed based on a GenBank Accession No. (NC_007312.5) using a web-based Software Primer4.0 with options of an optimal Tm, amplification size in genomic regions (Table 1). The primers were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. Polymerase Chain Reaction (PCR) amplifications were performed in 32 μL volume containing 100 ng of genomic DNA, 5 pmol L^{-1} of each primer, dNTPs (0.25 mmol L^{-1}) $MgCl_2$ (1.5 mmol L^{-1}) and 1 U Taq DNA polymerase (Tiangen Biotech, Beijing, China). Amplification conditions were as follows: denaturation at 94°C for 5 min, 35 cycles of amplification at 94°C for 30 sec, 60°C for 40 sec, 72°C for 30 sec and an extension step at 72°C for 7 min. The products were purified using Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology Co., Ltd. P.R. China) and sequenced in both directions (Beijing Aolaibo Biotechnology Co., Ltd. P.R. China) (Applied Biosystems 3730×l DNA sequencer, Foster city, CA, USA).

Statistical analysis: The association between single SNP marker genotypes of the *A-FABP* gene and meat quality traits was analyzed by the Least-Squares Method as applied in the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). According to the following statistical linear model:

$$Y_{abc} = \mu + G_a + CR_b + M_c + e_{abc}$$

Where:

Y_{abc} = Stands for observed value
μ = Overall mean for each trait
G_a = The effect of genotype
CR_b = The effect of cohort
M_c = The effect of cth month of slaughtering
e_{abc} = Random error

Table 1: Primer sequence of *A-FABP* gene

Names	Sequences	Fragment	Size (bp)	Tm (°C)
AF-E1	F: 5'-ACCATTTGCCAAGGAGAGC-3'	-175-167	343	60
	R: 5'-ATAAGCCCAGCCATTCAAC-3'			
AF-E2	F: 5'-AGGCGTGGGCTTTGCTAC-3'	2843-3109	267	60
	R: 5'-GAATGGCTTTCCTCCTTCTACA-3'			
AF-E3	F: 5'-TTATCCCCACAGAGCATCGT-3'	3598-3933	336	58
	R: 5'-GCACAATACAATACGGTTCACA-3'			
AF-E4	F: 5'-TCCCTCTCAATGTGAACCGTAT-3'	3902-4213	312	54
	R: 5'-ATAAACTCTGGTGGCAGTGACA-3'			
AF-N1	F: 5'-CAGTCAGACAGCCCTACCCA-3'	825-1208	384	61
	R: 5'-AGGAAGCCCAAACGCAAT-3'			
AF-N2	F: 5'-AGCAAGAGGAGAGTGTGATGT-3'	1561-1792	231	58
	R: 5'-GAATAGTGGGGAGGCGATA-3'			
AF-N3	F: 5'-TATCGCCTCCCCACTATT-3'	1774-2106	333	56
	R: 5'-TAAAGAGGGCTTAGGCATC-3'			
AF-N4	F: 5'-AAAAAAGAGGAGTTTGCCAC-3'	1991-2330	340	54
	R: 5'-TTTAATAGGATTCAGGGGAC-3'			
AF-N5	F: 5'-AACCCCTATGATGCTATTCC-3'	3358-3626	269	57
	R: 5'-TCTAAGTTTACGATGCTCTGTG-3'			
AF-N6	F: 5'CCCCCAGCAGTATTTTCTTATC-3'	3358-3626	332	58
	R: 5'-ATAAACTCTGGTGGCAGTGACA-3'			

The primer sequences and nucleotide positions were based on a GenBank sequences (Ref.:: NC-007312.5)

RESULTS AND DISCUSSION

Genetic polymorphism of the bovine *A-FABP* gene and the χ^2-test: PCR-SSCP and DNA sequencing technologies were used to investigate the detailed genetic variations within the bovine *A-FABP* gene. By comparing the sequencing results with the DNA sequence of *A-FABP* gene published in GenBank, the results revealed one variation: A2989G. The identified genetic variant was located in exon 2 (A2989G) (Fig. 1 and 2).

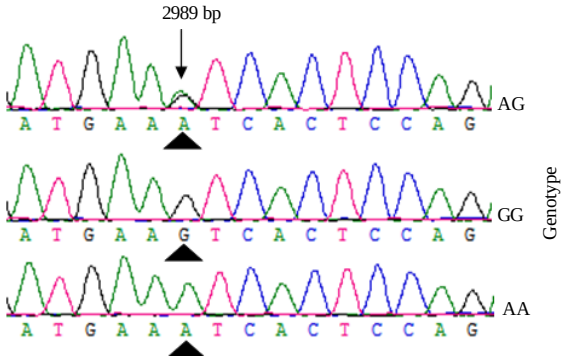

Fig. 1: The sequence alignment result of AF-1 fragment

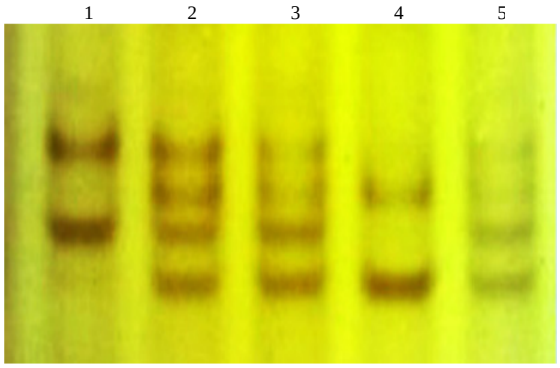

Fig. 2: SSCP analysis of PCR product of AF-1 fragment. 1 = AA genotype; 4 = GG genotype; 2, 3, 5 = AG genotype

The genotype frequencies of SNP (A2989G) was shown in Table 2. According to Nei and Roychoudhury's methods, the population genetic indexes (namely, gene homozygosity, gene heterozygosity, effective allele Numbers (Ne) and Polymorphism Information Content (PIC)) were calculated (Table 3). The PIC values were range from 0.25-0.5 at locus A2989G. This reflected that the locus A2989G was median polymorphism with in the 8 populations. The genotype distributions at locus A2989G displayed deviation from Hardy-Weinberg equilibrium in Simmental and Qinchuan populations (χ^2>5.991) while the remaining six populations agreed with Hardy-Weinberg equilibrium.

Association analyses: The relationship between genotypes of 318 individuals' and meat quality traits were evaluated. The least squares mean and standard error for each trait of different *A-FABP* genotypes were given in Table 4. The gene-specific SNP marker association analysis indicated that the SNP (A2989G) was significantly associated with marbling score with ($p<0.05$) and loin muscle area ($p<0.01$). As shown in Table 4, multiple comparisons results indicated that individuals with genotype AG and GG were significantly higher than those of individuals with genotype AA in marbling score ($p<0.01$). Moreover, there was a tendency that genotype AG and GG individuals had better performance in other aspects such as loin muscle area than genotype AA individuals ($p<0.01$) but there was no significant difference between the AG and GG genotypes ($p>0.05$). The results may imply that animals with alleles G have a great impact to commercial markets when animal breeders consider selecting sires with alleles.

A number of potential candidate genes containing genetic variants have been selected to identify associations with meat quality traits based on physiological and biochemical mechanisms. Therefore, genetic variations in the animal genome are major factors accounting for phenotypic variations of Quantitative Traits (QTL) that have a massive impact to commercial

Table 2: Genotype frequencies within different breeds for the SNP (A2989G) in the *A-FABP* gene

		Genotype distribution			Genotypic frequency			Allelic frequency	
Breeds	No.	AA	AG	GG	AA	AG	GG	A	G
Angus	48	18	27	3	0.3750	0.5625	0.0625	0.6563	0.3438
Hereford	30	8	17	5	0.2667	0.5667	0.1667	0.5500	0.4500
Jinnan	23	3	12	8	0.1304	0.5217	0.3478	0.3913	0.6087
Limousin	22	1	14	7	0.0455	0.6364	0.3182	0.3636	0.6364
Luxi	29	1	19	9	0.0345	0.6552	0.3103	0.3621	0.6379
Qinchuan	27	17	4	6	0.6296	0.1481	0.2222	0.7037	0.2963
Simmental	110	21	68	21	0.1909	0.6182	0.1909	0.5000	0.5000
Charolais	29	5	18	6	0.1724	0.6207	0.2069	0.4828	0.5172
Total	318	74	179	65	0.2327	0.5629	0.2044	0.5142	0.4858

Table 3: Associations of A2989G of *A-FABP* gene with meat production traits in different cattle

Breeds	PIC	Ho	He	Ne	χ^2
Angus	0.3494	0.5488	0.4512	1.8221	2.9226
Hereford	0.3725	0.5050	0.4950	1.9802	0.6288
Jinnan	0.3629	0.5236	0.4764	1.9097	0.2086
Limousin	0.3557	0.5372	0.4628	1.8615	3.0938
Luxi	0.3553	0.5380	0.4620	1.8586	5.0737
Qinchuan	0.3301	0.5830	0.4170	1.7153	11.2235**
Simmental	0.3750	0.5000	0.5000	2.0000	6.1455*
Charolais	0.3747	0.5006	0.4994	1.9976	1.7104

$\chi^2_{0.05} = 5.991$, $\chi^2_{0.01} = 9.21$, χ^2-value with ** and ** differ significantly at $p<0.01$ and $p<0.05$, respectively. Different small letters mean significant difference at $p<0.05$ and different capital letters mean remarkable significant different at $p<0.01$; *Means effect was significant at $p<0.05$; **Means effect was remarkable significant at $p<0.01$

Table 4: Genetic diversity of locus A2989G in 8 bovine population

	Genotypes (Mean±SE)			
Traits	AA	AG	GG	p-values
Live weight (kg)	564.56±9.82	562.15±7.93	549.92±9.63	0.2635
Carcass weight (kg)	317.95±6.12	315.56±4.91	305.42±5.89	0.0862
Dressing percentage	55.39±0.50	55.60±0.46	55.12±0.49	0.5270
Meat percentage	48.57±0.39	48.39±0.31	48.08±0.38	0.4587
Marbling score (1-5)	$1.95±0.19^{B}$	$2.38±0.15^{Aa}$	$2.40±0.19^{Aa}$	0.0202*
Loin muscle area (cm^2)	$65.93±2.19^{B}$	$72.36±1.73^{Aa}$	$70.73±2.06^{Aa}$	0.0027**
Backfat thickness (cm)	1.18±0.07	1.09±0.06	1.18±0.07	0.1425
Meat tenderness (kg)	4.29±0.22	4.40±0.18	4.42±0.22	0.8175
Thigh meat thickness (cm)	14.35±0.28	14.54±0.23	14.38±0.27	0.7273
Waist muscle thickness (cm)	7.73±0.20	8.11±0.16	7.98±0.19	0.0739
Carcass length (cm)	136.37±1.06	138.34±0.86	137.24±1.03	0.0687
Average daily gain (kg)	0.58±0.04	0.60±0.04	0.64±0.04	0.4321

industry. The QTL for marbling scores between markers BMS740 and BMS1304 on the bovine chromosome 14 containing many functional candidate genes related to fat synthesis in both daily and beef cattle (Cattle QTL Database) have been verified with A-FABP. This is an important step for the successful application of the *A-FABP* gene to Marker Assisted Selection (MAS) programs in commercial beef populations.

The effect of A-FABP polymorphisms on IMF accretion could be due to differences in intracellular fatty acid transport by the A-FABP protein (Coe and Bernlohr, 1998). A-FABP, acting as a key mediator of intracellular transport and metabolism of fatty acids is expressed in a differentiation-dependent fashion in adipocytes (Amri *et al*., 1991; Gregoire *et al*., 1998). Different alleles of A-FABP could enable IMF to accrete by a more efficient influx of fatty acids in fatty tissue or by differentially regulating intracellular fatty acid trafficking. However, variation at A-FABP has not always been associated with IMF, marbling or even subcutaneous fat, either in pigs or in cattle (Gerbens *et al*., 2001; Chmurzynska *et al*., 2004; Hoashi *et al*., 2008) which suggests that it may be relatively small in effect and that most studies are underpowered to detect the effect.

Many studies had been researched in *A-FABP* gene. Michal *et al*. (2006) reported that bovine *A-FABP* genotype significantly affected the marbling score and subcutaneous fat depth in a Wagyu x Limousine F2 population. Some researchers had obtained consistent or similar results with Michal. Hoashi *et al*. (2008) have identified A-FABP I74V that was significantly associated with C16:1 (A-FABP I74V) in intramuscular fat. Similarly, Shin *et al*. (2012) reported genotypes GG of g.3691G>A locus presented significantly higher values of MS and MG than other genotypes, reflecting an economically important candidate SNP that can be used in commercial are as to maximize economic benefits.

In this study, researchers identified one SNP in exon 3 of *A-FABP* gene by sequencing and evaluated their effects on carcass and meat quality traits in Chinese cattle. A nucleotide position at 2989 was revealed an amino acid substitution (isoleucine to Valine). The functional significance of genes with the substitution of amino acids by SNPs may change protein structures, resulting from altered mRNA translation and functional mechanisms. Also, researchers found that the SNP (A2989G) had a significant association with MS and LMA. The result was consistent or similar with previous studies (Michal *et al*., 2006; Hoashi *et al*., 2008; Shin *et al*., 2012).

CONCLUSION

The results provide evidence that the SNP in exon 3 of *A-FABP* gene has potential effects on carcass or growth traits such as MS and LMA. However, the Simmental and Qinchuan populations were not in Hardy-Weinberg disequilibrium ($\chi^2>5.991$) while other breeds were in Hardy-Weinberg (H-W) equilibrium ($\chi^2>5.991$). Therefore, further research is necessary to test the SNP in larger population and whether the *A-FABP* gene plays a role in cattle meat production traits.

ACKNOWLEDGEMENTS

This study was supported by Natural Science Foundation of Fujian Province of P.R. China (2010J05042), Outstanding Young Foundation of Fujian Agriculture and Forestry University (xjq201211) Major Projects Science and Technology of Fujian Province (2014N51010116) Scientific Research Foundation of Fujian Provincial Education Department (K8011048).

REFERENCES

Amri, E.Z., B. Bertrand, G. Ailhaud and P. Grimaldi, 1991. Regulation of adipose cell differentiation. I. Fatty acids are inducers of the aP2 gene expression. J. Lipid Res., 32: 1449-1456.

Barendse, W., R.J. Bunch, M.B. Thomas and B.E. Harrison, 2009. A splice site single nucleotide polymorphism of the fatty acid binding protein 4 gene appears to be associated with intramuscular fat deposition in longissimus muscle in Australian cattle. Anim. Genet., 40: 770-773.

Chmurzynska, A., 2006. The multigene family of Fatty Acid-Binding Proteins (FABPs): Function, structure and polymorphism. J. Applied Genet., 47: 39-48.

Chmurzynska, A., M. Mackowski, M. Szydlowski, J. Melonek and M. Kamyczek *et al*., 2004. Polymorphism of intronic microsatellites in the *A-FABP* and *LEPR* genes and their association with productive traits in the pig. J. Anim. Feed Sci., 13: 615-624.

Coe, N.R. and D.A. Bernlohr, 1998. Physiological properties and functions of intracellular fatty acid-binding proteins. Biochim. Biophys. Acta Lipids Lipid Metab., 1391: 287-306.

Gerbens, F., A. Jansen, A.J.M. van Erp, F. Harders and T.H.E. Meuwissen *et al*., 1998. The adipocyte fatty acid-binding protein locus: characterization and association with intramuscular fat content in pigs. Mammalian Genome, 9: 1022-1026.

Gerbens, F., F.J. Verburg, H.T. van Moerkerk, B. Engel, W. Buist, J.H. Veerkamp and M.F. te Pas, 2001. Associations of heart and adipocyte fatty acid-binding protein gene expression with intramuscular fat content in pigs. J. Anim. Sci., 79: 347-354.

Gregoire, F.M., C.M. Smas and H.S. Sul, 1998. Understanding adipocyte differentiation. Physiol. Rev., 78: 783-809.

Hoashi, S., T. Hinenoya, A. Tanaka, H. Ohsaki and M. Taniguchi *et al*., 2008. Association between fatty acid compositions and genotypes of FABP4 and LXR-α in Japanese Black cattle. BMC Genet., Vol. 9. 10.1186/1471-2156-9-84.

Jurie, C., I. Cassar-Malek, M. Bonnet, C. Leroux and D. Bauchart *et al*., 2007. Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. J. Anim. Sci., 85: 2660-2669.

Lee, S.H., J.H.J. van der Werf, E.W. Park, S.J. Oh, J.P. Gibson and J.M. Thompson, 2010. Genetic polymorphisms of the bovine fatty acid binding protein 4 gene are significantly associated with marbling and carcass weight in Hanwoo (Korean Cattle). Anim. Genet., 41: 442-444.

Mannen, H., 2011. Identification and utilization of genes associated with beef qualities. Anim. Sci. J., 82: 1-7.

Michal, J.J., Z.W. Zhang, C.T. Gaskins and Z. Jiang, 2006. The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses Anim. Genet., 37: 400-402.

Mullenbach, R., P.J. Lagoda and C. Welter, 1989. An efficient salt-chloroform extraction of DNA from blood and tissue. Trends Genet., 5: 391-391.

Narukami, T., S. Sasazaki, K. Oyama, T. Nogi, M. Taniguchi and H. Mannen, 2011. Effect of DNA polymorphisms related to fatty acid composition in adipose tissue of Holstein cattle. Anim. Sci. J., 82: 406-411.

Ockner, R.K., J.A. Manning, R.B. Poppenhausen and W.K. Ho, 1972. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium and other tissues. Science, 177: 56-58.

Shahidi, F., 2002. Lipid-Derived Flavors in Meat Products. In: Processing: Improving Meat Quality, Kerry, J., J. Kerry and D. Ledward (Eds.). Woodhead Publishing Limited, Cambridge, pp: 105-121.

Shin, S.C., J.P. Heo and E.R. Chung, 2012. Genetic variants of the *FABP4* gene are associated with marbling scores and meat quality grades in Hanwoo (Korean cattle). Mol. Biol. Rep., 39: 5323-5330.

Thompson, J.M., 2004. The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness. Aust. J. Exp. Agric., 44: 645-652.

Wang, Y.H., K.A. Byrne, A. Reverter, G.S. Harper and M. Taniguchi *et al*., 2005. Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. Mammalian Genome, 16: 201-210.

Zhao, C., F. Tian, Y. Yu, J. Luo and Q. Hu *et al*., 2012. Muscle transcriptomic analyses in Angus cattle with divergent tenderness. Mol. Biol. Rep., 39: 4185-4193.