African Journal of Biotechnology Vol. 10(63), pp. 13714-13718, 17 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.338
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Association of *Calpain 1* (*CAPN1*) and *HRSP12* allelic variants in beef cattle with carcass traits

Guanyu Hou[1,2], Meng Huang[2], Xue Gao[2], Junya Li[2], Huijiang Gao[2], Hongyan Ren[2] and Shangzhong Xu[2]*

[1]Tropical Crops Genetic Resources Institute, CATAS, Danzhou Hainan, 571737
[2]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Accepted 25 April, 2011

***Calpain 1* (*CAPN1*) and its activator *HRSP12* are evaluated as candidate gene for quantitative trait loci (QTLs) affecting meat tenderness. In this study, SNPs were detected by sequencing in 323 cattle from 9 breeds. The association results showed that the A3553G and T824C loci individually related with marbling and tenderness, and *CAPN1*/*HRSP12* double homozygote and heterozygote/homozygote pairs (AA/TT, AA/CC, AG/TT and GG/TT) had higher marbling score than the other groups. Our findings suggest that polymorphisms in *CAPN1* and *HRSP12* might be the important genetic factor influencing meat quality in carcass trait.**

Key words: Cattle, *Calpain 1, HRSP12*, association, carcass trait.

INTRODUCTION

Meat tenderness is an important component of palatability, whereas the difficulty in obtaining phenotypic data makes it hard for it to be selected for this trait. Establishing the genetic basis for variation in meat tenderness would likely aid in the development of selection criteria for improving meat tenderness in cattle.

Protein hydrolysis is closely related to muscle growth during the development and meat tenderness after slaughter. *Calpain* I (*CAPN1*) is an important protease that hydrolyzes proteins in myofibrils (Koohmaraie, 1996). The micromolar calcium-activated neutral protease (*CAPN1*) gene encodes a cysteine protease, μ-calpain, which degrades myofibrillar proteins under postmortem conditions and appears to be the primary enzyme in the postmortem tenderization process (Koohmaraie, 1992, 1994). Hundreds of allelic variants (SNPs) of *CAPN1* investigated as potential candidate gene on BTA29 affecting meat tenderness are identified in bovine (Smith et al., 2000; Casas et al., 2003; Page et al., 2002), and some of them are found to have significant effects on tenderness (Casas et al., 2003; Juszczuk-Kubiak et al., 2004; Page et al., 2004; White et al., 2005; Morris et al., 2006; Rincon et al., 2006; Van et al., 2007). Current studies have shown that *CAPN1* is a potential gene or linked with a major gene which controls meat tenderness (Casas et al., 2005, 2006). HRSP12 (UK114) is a *Calpain1* activator of low molecular weight that is distinct from CaM. This heat responsive protein stimulates the hydrolysis activity of *Calpain1* (Melloni et al., 1998). Under low calcium concentration, *HSRP12* can activate *Calpain1* that participates in muscle growth (Fox et al., 1985). *HRSP12* gene is located at BTA 19 and the relationship between its polymorphisms and carcass traits in bovine has not been reported yet.

In order to identify the candidate genetic markers for commercial traits in cattle, we investigated polymerphisms in two carcass trait candidate genes *CAPN1* and *HRSP12*, and explored the relationship between genotypes and carcass traits.

MATERIALS AND METHODS

The samples were collected from Inner Mongolian Sanyuan Cattle Farm (46 head of Angus sires, 30 head of Hereford sires and 59 head of Simmental sires), Hebei Dachang Cattle Farm (24 head of Jinnan sires, 25 head of Limousin sires, 29 head of Qinchuan sires, 28 head of Charolais sires and 30 head of Luxi sires) and Inner Mongolian Baolongshan cattle farm (52 head of Simmental ×

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

Menggu crossbreds). Carcasses were stored in refrigerated rooms at a temperature of 0 to 4°C for 24 h. Then, all the carcass traits were measured based on the criterion of GB/T17238-1998 cutting standard of fresh and chilled beef of U.S. (U.S. Standard Publishing House). Blood samples (10 ml each) were collected from the vein with anticoagulant ACD and stored in -70°C. DNA was extracted from 1 ml whole blood with the Tiangen DNA Blood Kit (Tiangen Biotech Co., Ltd., Beijing) by the manufacturer's directions.

SNPs detecting and genotyping

Alignment of DNA sequences for *CAPN1* and *HRSP12* (GenBank accessions: NW_001494538.2, 297609-303340(166-5897); NC_007317.4, range 51579483 -51580438) was amplified and sequenced respectively from 24 samples of the population for SNPs detecting. Primer pairs for genotyping were synthesized by Invitrogen Co. (Invitrogen Co. Ltd., Shanghai, China). *CAPN1* gene primer sequences are: Forward: 5' CCGAGGGATCTCAAAGCAG 3'; Reverse: 5' TGGGCTGAGTAGAGAGAAGG 3'. *HRSP12* gene primer sequences are: Upstream: 5' ACTTGTATTGCTGGATGTGGG 3'; Downstream: 5' GTGGTGGTCTTTGGGGAGGT 3'.

The 20 µl PCR mixture contained 0.2 µl (5 U/µl) of Taq, 2.5 µl of 10 × buffer, 1. 5 µl of 25 mM $MgCl_2$, 0.8 µl of 0.01 mM primers, 2.5 µl of 2.0 mM dNTPs, 2 µl of 50 ng/l genomic DNA. PCR protocol was as follows: predenaturation at 94°C for 3 min; 38 cycles of amplification at 94°C for 30 s, 58°C for 30 s and 72°C extension for 40 s; final extension at 72°C for 10 min.

Data analysis

Genotype and allele frequencies were calculated for all the SNPs of the *CAPN1* and *HRSP12* gene, and the overall sample was set with Genepop (Raymond et al., 1995).

Evaluation of association between the two SNP genotypes at each polymorphic locus and carcass traits were carried out with the general linear model (GLM) in SAS (Version 9.1, SAS Inst., Inc., Cary, NC, 2002 to 2003). The linear model is as follows: $Y_{ijK} = \mu + BF_i + Month_j + G_k + e_{ijk}$, where, Y_{ijk} is the observed value; μ is the overall mean for each trait; BF_i is the fixed effect of i^{th} breed and farm; $Month_j$ is the fixed effect of j^{th} month of slaughtering; G_k is the fixed effect of k^{th} single SNP marker genotype and e_{ijk} is the random error.

RESULTS

Genotyping and frequencies

Sequencing result showed that there was A/G substitution at position 3553 of *CAPN1* and missense T/C substitution at position 824 of *HRSP12* which replaced lysine acid with argnine acid. Among the 9 breeds of cattle, G allele was predominant at A3553G locus, while genotype AA was not found in Hereford, Limousin, Luxi and Charolais cattle. For T824C locus, allele T was predominant in all breeds except Jinnan and Luxi cattle, whereas no genotype TT was observed in these two breeds (Table 1).

Association of SNPs with carcass traits

Correlations of the *CAPN1* and *HRSP12* SNPs genotypes with 14 carcass traits were analyzed with GLM procedure in SAS, and *F* test was used to generate *P* value. Table 2 indicated trait values for each genotype in the 9 breeds. For locus A3553G, genotype AA had higher marbling score than genotypes AG and GG ($P<0.05$); genotype AA had higher tenderness value than genotype GG ($P<0.01$). There were no significant differences among genotypes at locus A3553G in all other traits. For locus T824C, genotype CC was heavier in live weight and meat weight than genotype TC ($P<0.05$), and the same circumstances emergence in carcass weight ($P<0.01$), while genotype TT has higher marbling score than genotypes TC and CC ($P<0.01$). No distinct difference was found between *HRSP12* genotypes and other meat parameters.

When both *CAPN1* and the *HRSP12* genotypes of each sample were considered together as a compound genotype (AA/TT, AA/TC, AA/CC, AG/TT, AG/TC, AG/CC, GG/TT, GG/TC and GG/CC), double homozygote alleles samples (AA/TT and AA/CC) were found to have significantly higher marbling scores ($P<0.01$, Table 3). Those who were *CAPN1* heterozygous and *HRSP12* homozygous (AG/TT) or other double homozygous (GG/TT) also had significantly higher marbling score ($P<0.05$). No significant association was found between the compound *CAPN1*/*HRSP12* genotypes and tenderness ($P>0.10$).

DISCUSSION

Meat quality is of commercial importance for the animal husbandry industry. It is affected by genetic background, management, nutrition and meat processing. Calpain proteases participate in protein metabolism during animal development and muscle maturation after slaughter; therefore the genes encoding these enzymes have been studied widely as candidates for meat tenderness. In cattle, the relationship between *CAPN1* and carcass traits is investigated intensively. More and more studies demonstrates that *CAPN1* is remarkably correlated to beef tenderness (Koohmaraie, 1992, 1994; Smith et al., 2000; Casas et al., 2003; Page et al., 2002; Juszczuk-Kubiak et al., 2004).

Population genetic analysis indicated that allele G was predominant for A3553G locus among 9 breeds. Genotype GG was predominant in all breeds except Angus and Simmental. Genotype AA was not found in Hereford, Limousin, Luxi and Charolais populations, which may result from genetic background and sample size analyzed. For the T824C locus, allele T sample frequently existed in Angus, Hereford, Simmental and Charolais populations; sample with genotype TT was the highest in these breeds. Allele C emergence was more frequent in local breeds (Jinnan and Luxi populations). The results illustrated that the foreign breeds were opposite to the local breeds in the predominant genotype at T824C locus, which might be related to the origin and

Table 1. Genotype frequencies of A3553G and T824C loci in 9 cattle breeds.

Locus	Breed	Number of cattle	Genotype frequency			Allele frequency	
			AA/TT	GG/CC	AG/TC	A/T	G/C
A3553G T824C	Angus	46	0.19(9)	0.32(19)	0.39(18)	0.48	0.52
			0.47(22)	0.26(12)	0.26(12)	0.61	0.39
	Hereford	30	0.00(0)	0.66(20)	0.33(10)	0.17	0.83
			0.63(19)	0.36(11)	0.00(0)	0.63	0.37
	Simmental	59	0.16(10)	0.38(23)	0.44(26)	0.39	0.61
			0.59(35)	0.11(7)	0.28(17)	0.74	0.26
	Jinnan	24	0.04(1)	0.75(18)	0.20(5)	0.15	0.85
			0.00(0)	0.37(9)	0.62(15)	0.31	0.69
	Limousin	25	0.00(0)	0.52(13)	0.48(12)	0.24	0.76
			0.32(8)	0.32(8)	0.36(9)	0.50	0.50
	Luxi	30	0.00(0)	0.63(19)	0.36(11)	0.18	0.82
			0.00(0)	0.80(24)	0.20 (6)	0.10	0.90
	Qinchuan	29	0.13(4)	0.58(17)	0.27(8)	0.28	0.72
			0.41(12)	0.10(3)	0.48(14)	0.66	0.34
	Charolais	28	0.00(0)	0.78(22)	0.21(6)	0.11	0.89
			0.71(20)	0(0)	0.28(8)	0.86	0.14
	Simmental×Menggu	52	0.17(9)	0.48(25)	0.34(18)	0.35	0.65
			0.59(31)	0.09(5)	0.30(16)	0.75	0.25

breeding.

The marbling property was directly linked to meat tenderness and texture, which was a very important indicator of meat quality. Our results showed that A3553G locus was correlated to marbling and tenderness. Genotype AA was different from AG and GG genotype in marbling trait (P<0.05), and markedly different from GG in tenderness (P<0.01). These results are consistent with other reports ((Koohmaraie, 1992, 1994; Smith et al., 2000; Casas et al., 2003; Page et al., 2002; Juszczuk-Kubiak et al., 2004; Morris et al., 2006). Significant differences were also found among different genotypes at T824C locus in marbling index (P<0.01).

Therefore, both *CAPN1* and *HRSP12* are correlated to marbling trait. On the other hand, the homozygous/ homozygous (AA/TT and AA/CC) and heterozygous/ homozygous (AG/TT and GG/TT) compound *CAPN1*/*HRSP12* SNP genotypes that were associated with marbling parameters presented higher score than samples with other genotypes. The two genes may be potentially functional genes influencing carcass traits, and the identification of such susceptibility genes may therefore be important marks in beef cattle breeding projects.

Conclusion

An experimental analysis in our study indicated that polymorphism in *CAPN1* and *HRSP12* was moderate and the genotype distributed in nine breeds was found to have marked differences in meat tenderness and marbling properties. This study would also be a foundation for further research of the beef cattle molecular assistant selection.

ACKNOWLEDGEMENTS

This work was supported by the New Variety of Transgenic Organism Great Breeding Program (grant no. 2008ZX08007-002; 2009ZX08009-157B), the Eleventh “Five-Year” National Science and Technology Support Project (grant no. 2006BAD04A16; 2007BAD56B04), the National 863 Program of China (grant no. 2008AA10Z146) and the Public-welfare Profession Agro-industry

Table 2. Analysis of the relationship between genotypes and productive performances using the least square method.

Loci	Trait	Genotype (LSM±SE)			*P* value		
		AA/TT	GG/CC	AG/TC	AA-AG TT-TC	AG-GG TC-CC	AA-GG TT-CC
A3553G T824C	Live weight	547.20±13.73 561.47±7.07	544.84±7.49 573.25±8.84	546.00±6.24 535.01±11.92	0.8802 0.6597	0.9051 0.0298*	0.9369 0.4826
	Carcass weight	297.94±8.33 309.07±4.68	300.97±4.54 319.63±5.85	301.64±3.79 297.26±7.89	0.7505 0.0647	0.9096 0.0086**	0.6868 0.0579
	Meat weight	253.78±7.37 264.41±3.59	257.34±4.02 274.41±4.49	258.94±3.35 251.54±6.05	0.6717 0.0632	0.7611 0.0348*	0.5248 0.0597
	Dressing (%)	54.24±0.51 34.60±2.57	55.19±0.28 36.69±3.22	55.10±0.23 38.68±4.34	0.1077 0.6248	0.8066 0.6312	0.1308 0.4951
	Meat (%)	46.94±0.75 47.374±0.35	47.25±0.41 47.73±0.44	47.34±0.34 46.72±0.59	0.7195 0.9248	0.8626 0.8479	0.6282 0.9147
	Bone weight	33.36±2.48 32.62±1.29	32.57±1.36 33.71±1.61	31.93±1.13 33.71±2.17	0.7796 0.7154	0.7179 0.9426	0.6005 0.7161
	Marbling score	2.70±0.21 2.49±0.10	2.16±0.11 1.92±0.12	2.15±0.09 1.98±0.16	0.0273* 0.0078**	0.967 0.7598	0.0202* 0.0082**
	Eye muscle area	72.08±2.62 69.71±1.27	70.22±1.43 72.76±1.59	69.69±1.19 70.26±2.15	0.5354 0.6487	0.7753 0.7102	0.4083 0.7548
	Meat color	4.48±0.14 4.43±0.07	4.42±0.07 4.39±0.09	4.44±0.06 4.52±0.12	0.7569 0.8511	0.8957 0.7568	0.8132 0.8124
	Fat color	1.40±0.07 1.30±0.03	1.27±0.04 1.31±0.04	1.31±0.03 1.32±0.06	0.1609 0.4556	0.4284 0.3421	0.3453 0.4677
	Backfat thickness (cm)	0.96±0.09 0.97±0.04	1.02±0.05 1.11±0.05	1.07±0.04 1.08±0.07	0.5466 0.2648	0.4655 0.7895	0.2731 0.5647
	Tenderness	4.69±0.28 3.96±0.14	4.13±0.15 3.99±0.17	3.89±0.13 3.86±0.23	0.0849 0.7597	0.2380 0.5641	0.0091** 0.6653
	Daily gain	0.63±0.05 0.68±.025	0.61±0.03 0.67±0.03	0.65±0.02 0.62±0.04	0.7155 0.8479	0.3178 0.7576	0.7884 0.7142
	Initial weight	382.64±10.44 361.55±5.09	366.15±5.69 367.47±6.37	364.49±4.74 368.00±8.59	0.1672 0.6548	0.1152 0.8972	0.8232 0.3148

Data are least square means ± standard error. Values with different lower case scripts in the same row are significantly different at $P<0.05$, with extremely different at $P<0.01$. *: $P<0.05$, **: $P<0.01$.

Table 3. Significance of association of marbling score and tenderness with combined genotypes.

Genotype	Trait (LSM±SE)	
	Marbling score	**Tenderness**
AA/TT	2.97±0.35**	4.26±0.49
AA/TC	1.88±0.61	4.81±0.87
AA/CC	3.93±0.74**	2.64±1.04
AG/TT	2.30±0.26*	3.32±0.38
AG/TC	2.07±0.28	3.68±0.40
AG/CC	2.00±0.38	3.72±0.54
GG/TT	2.36±0.26*	3.37±0.37
GG/TC	2.20±0.30	3.22±0.42
GG/CC	1.66±0.32	3.37±0.45
P value	0.0095	0.3010

Data are least square means ± standard error. *: $P<0.05$, **: $P<0.01$.

Research Program (grant no. nyhyzx07-035-07).

REFERENCES

Casas E, Shackelford SD, Keele JW, Koohmaraie M, Smith TP, Stone RT (2003). Detection of quantitative trait loci for growth and carcass composition in cattle. J. Anim Sci. 81(12): 2976-2983.

Casas E, White SN, Riley DG, Smith TPL, Brenneman RA, Olson TA, Johnson DD, Coleman SW,Bennett GL, Chase CC (2005). Assesment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in Bos indicus cattle. J. Anim. Sci. 83: 13-19.

Casas E, White SN, Wheeler TL (2006). Effects of calpastatin and μ-calpain markers in beef cattle on tenderness traits. J. Anim. Sci. 84: 520-525.

Fox JEB, Goll DE, Reynolds CC (1985). Identification of two proteins (actin-binding protein and P235) that are hydrolyzed by endogenous Ca^{2+}-dependent protease during platelet aggregation. J. Bio. Chem. 260 (2): 1060-1066.

Juszczuk-Kubiak E, Sakowski T, Flisikowski K, Wicinska K, Oprzadek J, Rosochacki SJ (2004). Bovine mu-calpain (CAPN1) gene: new SNP within intron 14. J. Appl. Genet. 45(4): 457-460.

Koohmaraie M (1996). Biochemical factors regulating the toughening and tenderization process of meat. Meat Sci. 43(Suppl.): 193-201.

Koohmaraie, M (1992). The role of Ca^{2+}-dependent proteases (calpains) in postmortem proteolysis and meat tenderness. Biochemie. 74: 239-245.

Koohmaraie, M (1994). Muscle proteinases and meat aging. Meat Sci. 36:93-104.

Melloni E, Michetti M, Salamino F (1998). Mechanism of action of a new component of the Ca2+- dependent proteolytic system in rat brain: the calpain activator. Biochem. Biophys. Res. Commun. 249: 583-588.

Morris CA, Cullen NG, Hickey SM, Dobbie PM, Veenvliet BA, Manley TR, Pitchford WS, Kruk ZA, Bottema CD, Wilson T (2006). Genotypic effects of calpain 1 and calpastatin on the tenderness of cooked M. longissimus dorsi steaks from Jersey x Limousin Angus and Hereford-cross cattle. Anim. genet. 37(4): 411-414.

Page BT, Casas E, Heaton MP, Cullen NG, Hyndman DL, Morris CA, Crawford AM, Wheeler TL, Koohmaraie M, Keele JW, Smith TP (2002). Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. J. Anim. Sci. 80(12): 3077-3085.

Page BT, Casas E, Quaas RL, Thallman RM, Wheeler TL, Shackelford SD, Koohmaraie M, White SN, Bennett GL, Keele JW, Dikeman ME,

Smith TP (2004). Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbred populations that sample influential industry sires. J. Anim Sci. 82(12): 3474-3481.

Raymond M, Rousset F (1995). Genepop (version 1.2): population genetics software for exact tests and ecumenicism. Heredity. 86: 248-249.

Rincon G, Medrano JF (2006). Assays for genotyping single nucleotide polymorphisms in the bovine CAPN1 gene. Anim. genet. 37(3): 294-295.

Smith TP, Casas E, Rexroad CE 3rd, Kappes SM, Keele JW (2000). Bovine CAPN1 maps to a region of BTA29 containing a quantitative trait locus for meat tenderness. J. Anim Sci. 78(10): 2589-2594.

Van Eenennaam AL, Li J, Thallman RM, Quaas RL, Dikeman ME, Gill CA, Franke DE, Thomas MG (2007). Validation of commercial DNA tests for quantitative beef quality traits. J. Anim Sci. 85(4): 891-900.

White SN, Casas E, Wheeler TL, Shackelford SD, Koohmaraie M, Riley DG, Chase CC, Johnson DD, Keele JW, Smith TP (2005). A new single nucleotide polymorphism in CAPN1 extends the currenttenderness marker test to include cattle of Bos indicus, Bos taurus, and crossbred descent. J. Anim. Sci. 83(9): 2001-2008.