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Full Length Research Paper

A novel missense mutation of bovine lipase maturation factor 1 (*LMF1*) gene and its association with growth traits

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Lipase maturation factor 1 (*LMF1*) gene is a novel candidate gene in severe hypertriglyceridemia. To detect the polymorphism in *LMF1* gene in 804 Chinese cattle, we firstly described the polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP), DNA sequence and PCR-RFLP methods for detecting mutations of *LMF1* gene, which affected growth and development in animal. The results showed a novel mutation in exon 4: NC_007326.3: g.27154 T>C, which resulted in a missense mutation within *LMF1* protein (p.197 Trp>Arg). Genotype TT was dominant in four breeds, and genotypic frequencies of *LMF1* exon 4 *Ava*I locus were calculated in four populations which agreed with Hardy-Weinberg equilibrium (p > 0.05). The association analysis showed that individuals with genotype TC had greater body weight than those with genotype TT at 12, 18 and 24 months of age in Nanyang cattle (P < 0.05).

Key words: Bovine, polymorphism, growth traits, *LMF1* gene.

INTRODUCTION

Lipase maturation factor 1 (*LMF1*) protein is encoded by the *LMF1* gene, which is located in chromosome 16p on human, and it has 11 exons and covers 84 kb on mouse chromosome 17 (Péterfy et al., 2007). The *LMF1* protein is located in the endoplasmic reticulum (ER), as demonstrated by colocalization with calnexin, a membrane-bound protein of the ER (Helenius and Aebi, 2004). The maturation of lipases into a catalytically active enzyme are believed to occur only after they have been transported from the ER to the Golgi apparatus (Ben-Zeev et al., 2002, 2004), where *LMF1* finish their maturation. After maturation, the lipases secede from the endoplasmic reticulum and are secreted to extracellular sites, where they carry out a number of functions related to lipid metabolism (Attie, 2007; Doolittle and Peterfy, 2010).

LMF1 plays an important role in lipoprotein lipase (LPL) and hepatic lipase (HL) maturation. Yin et al. (2009) demonstrated that *LMF1* mutations have a wide range of effect on *LMF1* function and protein expression. The mutations of *LMF1* gene cause the deficiency of lipoprotein lipase (LPL) and hepatic lipase (HL) (Ben-Zeev et al., 2002, 2004). Besides, Pe´terfy et al. (2007) used a mouse which has severe hypertriglyceridemia to prove the function of *LMF1* gene. In addition, hypertriglyceridemia is a hallmark of many disorders, including metabolic syndrome and obesity (Austin et al., 1998; Ginsberg and Stalenhoef, 2003; Pejic et al., 2006). *LMF1* is a new reported gene which leads to hypoleptinemia (Doolittle and Peterfy, 2008). However, to date, few polymorphisms of *LMF1* gene have been reported. In human, Pe´terfy et al. (2007) reported a common nonsense mutation in exon 9 (Y439X), which results in a premature stop codon. Angelo also found a mutation of G>A substitution in exon 9, and the mutation led to a premature stop codon (W464X) (Cefalu et al., 2009). Human carrying the mutations shows severe hypertriglyceridemia, metabolic syndrome and obesity. In view of *LMF1's* important regulatory role of fat metabolism and growth in animal, *LMF1* gene is suggested to be possibly

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associated with economic traits in animal production.

However, there was no published study about the association of mutations of the *LMF1* gene in bovine. So, we first described the genetic variation of this gene in four Chinese cattle breeds and assessed the possible association of mutations with production traits, to further provide a scientific basis for selection of molecular breeding and genetics in animal.

MATERIALS AND METHODS

In order to evaluate the effect of polymorphism on economically important traits genomic DNA, samples were obtained from 804 individuals belonging to four breeds: Nanyang cattle (NY, n = 268), Qinchuan cattle (QC, n = 300), Jiaxian cattle (JX, n = 141) and Chinese Holstein (CH, n = 95). All four cattle breeds represent the main breeds of China and are reared in the provinces of Henan and Shaanxi. Records of growth traits and body sizes (body height, body length, chest girth, body weight, hucklebone width and average daily gain) for different growth periods (birth, 6, 12, 18 and 24 months old) in 268 NY cattle were collected for statistical analysis. Genomic DNA of the 804 animals were isolated from 2% heparin-treated blood samples and stored at -80℃, following standard procedures (Sambrook and Russell, 2001).

According to the sequence of *LMF1* gene (GenBank accession number: NC_007326.3), one pair of PCR primers was designed to amplify the coding region of bovine *LMF1* gene exon 3 *(F: 5'-CTTTAGCAGGTAAAGCGATGC-3', R: 5'-GGAGACGAGACCC GTTGG-3')* exon 4 *(F: 5'-CATCCTGCCTGGGCTCTG-3'* R: *5'-TCACGGGCTCAGAAACAGGT-3')* and exon 5 *(F: 5'-AGTTGCC CTTCTGCGTCAC-3', R: 5'-CCTGCTCACCTCATAGTGGAAG-3')*. The 25 µl volume contained: 50 ng genomic DNA, 0.5 µM of each primer, 1×buffer (including 1.5 mM $MgCl_2$), 200 µM dNTPs and 0.625 units of Taq DNA polymerase (MBI). The cycling protocol was 5 min at 95℃, 35 cycles of 94℃ for 30 s, 61℃ (exon 3, exon 5) and 66℃ (exon 4) annealing for 35 s, and 72℃ for 30s, with a final extension at 72℃ for 10 min.

Aliquots of 5 µl PCR products were mixed with 5 µl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), were heated for 10 min at 98℃ and chilled on ice (Sun et al., 2002). Denatured DNA was subjected to PAGE (80 × 73 ×0.75 mm) in 1×TBE buffer and constant voltage of 200 V for 2.5 h. The gel was stained with 0.1% silver nitrate (Zhang et al., 2007). After polymorphism was detected, the PCR products of different electrophoresis patterns were sent to sequence in both directions in ABI PRIZM 377 DNA sequencer (Perkin-Elmer) and the sequences were analyzed with BioXM software (version 2.6).

Comparison sequences of exon 4 at the *LMF1* gene revealed a single nucleotide polymorphism (SNP). The novel SNP could be genotyped by *Ava*I endonuclease. Aliquots of 10 µl PCR products were digested with 6 units *Ava*I (MBI fermentas) for 5 h at 37℃ following the supplier's directions. The digested products were detected by electrophoresis in 10% polyacrylamide gel stained with ethidium bromide.

Differences in genotypic and haplotypic frequencies in the bovine *LMF1* gene exon 4 among Chinese populations were analyzed by χ^2 test, which were performed by SPSS software (version 16.0). Population genetic indexes: gene heterozygosity (He), gene polymorphism information content (PIC) was calculated by Botstein's methods (Botstein et al., 1980).

Statistical analysis was performed on records of growth traits in NY cattle (n = 268). The growth traits of NY cattle were analyzed with the linear model by the use of PROC MIXED in the SAS system (version 8.0) according to the methods of the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). This procedure implements random effects in the statistical model and permits modeling of the covariance structure of the data. The linear homozygosity (Ho) and effective allele numbers (Ne) were calculated using the PopGen software (version 3.2), and model was: $Y_{ijkl} = \mu + S_i + A_j + G_k + (AG)_{jk} + E_{ijkl}$, where, Y_{ijkl} was the l^{th} measurement on the ijk^{th} animal, S_i was the fixed effect associated with the i^{th} sire, A_j was the fixed effect due to the j^{th} age class, G_k was the fixed effect associated with the k^{th} genotype, $(AG)_{jk}$ was the interaction between the j^{th} age and the k^{th} genotype, and E_{ijkl} was random error. Effects associated with the different cattle or age of dam and sire were not included in the linear model, because preliminary statistical analysis indicated that these effects had no significant influence on variability of the traits in female populations. The least square means (LSMs) estimates with standard errors and multiple range tests for two *LMF1* genotypes (TT n = 155 and TC n = 105) and traits were used.

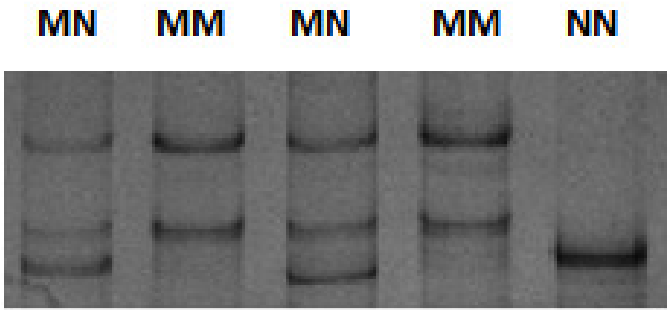

Figure 1. The PCR-SSCP patterns of exon 4 within the Chinese bovine *LMF1* gene. Three unique SSCP banding patterns (MM, MN and NN) were observed in Chinese cattle.

RESULTS AND DISCUSSION

So far, no paper about polymorphisms of *LMF1* gene in bovine has been reported. In this paper, polymorphisms in the bovine *LMF1* gene exon 3, exon 4 and exon 5 were scanned. Three unique SSCP banding patterns were observed in four Chinese bovine populations in exon 4 only (Figure 1). In order to better understand the detailed genetic variation within the bovine *LMF1* gene, DNA sequencing was used. In comparison with nucleotide sequence of the bovine *LMF1* gene (GenBank accession number NC_007326.3) a novel missense mutation: NC_007326.3: g.27154 T>C was revealed (Figure 2). The mutation resulted in a change in LMF1 protein (p.197 Trp>Arg). Interestingly, the novel mutation could be detected by *Ava*I endonuclease. The T>C mutation created a new *Ava*I endonuclease restriction site (CCGG). Therefore, digestion of the PCR fragment of *LMF1* exon 4 with *Ava*I resulted in fragment lengths of 307 bp for genotype TT, 161 and 146 bp for genotype CC and 307, 161 and 146 bp for genotype TC (Figure 3). It is clear that 161 and 146 bp fragments could exactlyclassify the genotypes by 10% PAGE.

Frequencies of T allele in the analyzed populations were 0.77, 0.94, 0.84 and 1.00 on NY, QC, JX and CH breeds, respectively. Genotype TT was dominant in the

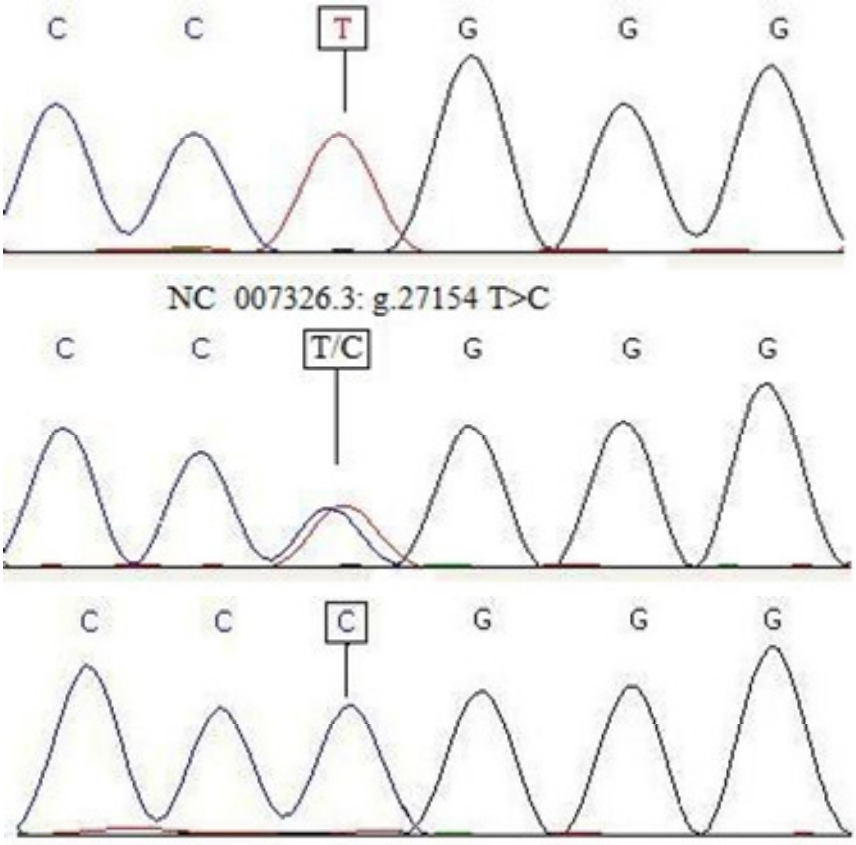

Figure 2. The sequencing map of a missense mutation selected in exon 4 region of the bovine *LMF1* gene.

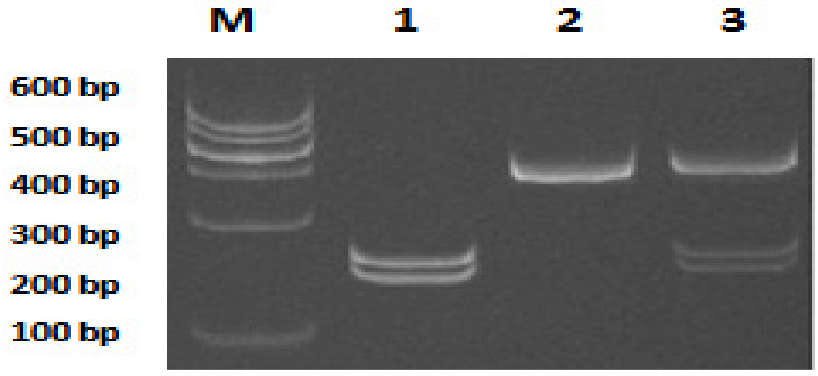

Figure 3. The 10% polyacrylamide gel electrophoresis patterns of 307 bp PCR products of *LMF1* gene exon 4 digested with *Ava*I endonuclease in four Chinese cattle breeds. M: marker I; Lane 1: genotype CC (161 and 146 bp); Lane 2: genotype TT (307 bp); Lane 3: genotypeTC (307, 161 and 146 bp).

four breeds. The different genotypes distributed in the four breeds all agreed with Hardy-Weinberg equilibrium, respectively (Table 1, $p > 0.05$). Genetic indices He, Ne and PIC of bovine *LMF1* exon 4 locus in the Chinese populations varied from 0.00 (CH) to 0.39 (NY), 1.00 (CH) to 1.54 (NY) and 0.00 (CH) to 0.29 (NY), respectively (Table 2).

Significant statistical differences in genotypic frequencies for E4 *Ava*I locus of *LMF1* implied that this locus was significantly associated with bovine breeds by

Table 2. Genetic indexes of *LMF1* gene E4 *Aval* locus in four Chinese cattle populations.

Breeds (E4 *Aval* locus)	Ne	PIC	He (Obs)	H_O
NY	1.54	0.29	0.39	0.61
QC	1.13	0.11	0.12	0.88
JX	1.37	0.23	0.26	0.74
CH	1.00	0.00	0.00	1.00

He, gene heterozygosity; Ho, gene homozygosity; Ne, effective allele numbers; PIC, polymorphism information; Obs, observed.

Table 3. χ^2 and P values differences for genotypic frequencies between four Chinese breeds at bovine *LMF1* gene E4 *Aval* locus.

Breeds	NY	QC	JX	CH
NY		$\chi^2 = 70.37$	$\chi^2 = 7.09$	$\chi^2 = 78.38$
QC	$P < 0.001$***		$\chi^2 = 21.90$	$\chi^2 = 12.54$
JX	$P < 0.05$*	$P < 0.001$***		$\chi^2 = 42.16$
CH	$P < 0.001$***	$P < 0.001$***	$P < 0.001$***	

χ^2 and P values allelic differences for frequencies between two breeds are shown in the up-triangle and the down-triangle of this table, respectively (total: $\chi^2 = 120.866$, df = 6), *represent significance at $P < 0.05$, *** represent significance at $P < 0.001$.

the χ^2-test ($\chi^2 = 120.87$, df = 6) (Table 3). As the analyzed breeds represented bovine breeds with different types of utility (dairy, farming and beef), the genotypic distribution possibly had significant effects on the utility type and breeds. This result may be caused by long-term selection for different purposes. When compared with the dairy breed (CH), the farming and beef breeds (NY, QC and JX) possessed higher frequencies of allelic C, which implied that allelic C is possibly associated with meat production.

In this paper, we revealed for the first time, the association of the polymorphism of *LMF1* gene with growth traits in the NY Breed. Because of the low frequency of the genotype CC (n = 8), only one contrast was estimated: the difference between genotypes TT (n = 155) and TC (n = 105). Growth traits (birth weight, body weight, body height, body length, heart girth, hucklebone width and average daily gain) were analyzed in NY cattle at 6, 12, 18 and 24 months of age. Individuals with TC genotype had greater body weight than individuals with TT genotype at 12, 18 and 24 months of age ($P < 0.05$). In hucklebone width at 24 months of age, the animals with the TC genotype were significantly longer than those with genotype TT ($P < 0.05$; Table 4). The rest of the records of growth traits had no significant association ($P > 0.05$). The SNP (27154 T>C) reported here in the exon 4 coding region of bovine *LMF1* is a missense mutation, which led to amino acid alterations from Trp to Arg. According to Majewski and Ott (2003), Trp is one of the least mutable amino acids; when substitutions of this amino acid do occur, protein structure and function can be affected, and then will affect the phenotype of the animals.

LMF1 plays an important role of lipase maturation. The mutations of *LMF1* gene may cause the deficiency of LPL and HL, and this situation would lead to severe hypertriglyceridemia, and hypertriglyceridemia is a hallmark of many disorders, including metabolic syndrome and obesity in human and animals (Attie, 2007; Péterfy et al., 2007). Therefore, *LMF1* gene seems to be a promising candidate for a genetic marker as it affects growth traits in cattles. However, this study is the first report on polymorphism of *LMF1* gene in bovine. It appears clearly that there should be further research on *LMF1* gene in livestock.

Taken together, the mutations of *LMF1* gene significantly affected the growth traits in the Chinese cattle, but the association analysis with more animals is needed before this gene can be considered as a genetic marker.

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REFERENCES

Austin MA, Hokanson JE, Edwards KL (1998). Hypertriglyceridemia as

Table 4. Least square analysis between genotypes of E4 *Ava*I locus in *LMF1* and growth traits of NY cattle.

Ages	Growth traits	Genotypes at *LMF1* gene E4 *Ava*I locus		P value
		TT (mean ± SE)	TC (mean ± SE)	
Birth	BW (kg)	29.83±0.37	30.50±0.47	0.266
Six months	BW (kg)	158.14±2.80	161.50±3.58	0.468
	HW (cm)	18.09±0.19	18.65±0.25	0.080
Twelve months	BW (kg)	220.06^{a}±3.28	231.03^{b}±4.19	0.042
	HW (cm)	20.58±0.23	21.00±1.13	0.944
Eighteen months	BW (kg)	300.61^{a}±3.28	313.20^{b}±4.20	0.021
	HW (cm)	23.15±0.27	23.25±0.34	0.943
Twenty-four months	BW (kg)	357.71^{a}±5.34	377.03^{b}±6.83	0.029
	HW (cm)	24.93^{a}±0.30	25.98^{b}±0.39	0.035

BW, Body weight; HW, hucklebone width. LSM in a column with no common superscripts differ significantly, low-case character represents significance at $P < 0.05$.

a cardiovascular risk factor. Am. J. Cardiol. 8: 17B-12B.
Attie AD (2007). High-maintenance proteins and hypertriglyceridemia. Nat. Genet. 39: 1483-1487.
Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.
Ben-Zeev O, Mao HZ, Doolittle MH (2002). Maturation of lipoprotein lipase in the endoplasmic reticulum-Concurrent formation of functional dimers and inactive aggregates. J. Biol. Chem. 277: 10727-10738.
Ben-Zeev O, Doolittle MH (2004). Maturation of hepatic lipase - Formation of functional enzyme in the endoplasmic reticulum is the rate-limiting step in its secretion. J. Biol. Chem. 279: 6171-6181.
Cefalu AB, Noto D, Arpi ML (2009). Novel LMF1 Nonsense Mutation in a Patient with Severe Hypertriglyceridemia. J. Clin. Endocr. Metab. 11: 4584-4590.
Doolittle MH, Peterfy M (2008). Lipase maturation factor 1 (Lmf1), a new gene in hypertriglyceridemia. Future. Lipidol. 2: 119-122.
Doolittle MH, Peterfy M (2010). Mechanisms of lipase maturation. Clinical. Lipidology. 1: 117-130.
Ginsberg HN, Stalenhoef AF (2003). The metabolic syndrome: targeting dyslipidaemia to reduce coronary risk. J. Cardiovasc. Risk. 10: 121-128.
Helenius A, Aebi M (2004). Roles of N-linked glycans in the endoplasmic reticulum. Annu. Rev. Biochem. 73: 1019-1049.
Majewski J, Ott J (2003). Amino acid substitutions in the human genome: evolutionary implications of single nucleotide polymorphisms. Gene, 305: 167-173.
Pejic RN, Lee DT (2006) Hypertriglyceridemia. J. Am. Board. Fam. Med. 19: 310-316.
Péterfy M, Ben-Zeev O, Mao HZ (2007). Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. Nat. Genet. 39: 1483-1487.
Sambrook J, Russell DW (2001). Molecular cloning. 3nd. Edn. NY: Cold Spring Harbor Laboratory Press.
SAS Institute (1999). Statistical Analysis Systems User's Guide (Version 8.0) SAS Institute Inc Cary NC USA.
Sun HS, Andersona LL, Yu TP (2002). Neonatal Meishan pigs show POU1F1 genotype effects on plasma GH and PRL concentration. Anim. Reprod. Sci. 69: 223-237.
Yin F, Doolittle MH, Peterfy M (2009). A quantitative assay measuring the function of lipase maturation factor 1. J. Lipid. Res. 50: 2265-2269.
Zhang CL, Wang YH, Chen H, Lan XY, Lei CZ (2007). Enhance the efficiency of single-strand conformation polymorphism analysis by short polyacrylamide gel and modified silver staining. Anal. Biochem. 365: 286-287.