Association between PCR-RFLP Polymorphism of the Fifth Intron in Lipoprotein Lipase Gene and Productive Traits in Pig Resource Family

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ABSTRACT : The study was aimed at detecting polymorphism of the fifth intron in lipoprotein lipase (LPL) gene and analyzing association between the polymorphism and productive traits. A pair of primers was designed for amplifying the fifth intron. Sequence analysis indicated that a G1171C substitution existed in Large White breed. The mutation was detected by PCR-*Afa*I-RFLP. Polymorphism analysis in a pig resource family showed that there existed significant effects on carcass and meat quality traits. Thorax-waist fat thickness of *BB* genotype was significantly higher (14.2%, $p<0.05$) than that of *AA* on carcass traits, while *BB* genotype was significantly lower (3.6% $p<0.01$, 4.1% $p<0.01$; 2.3% $p<0.01$, 1.9% $p<0.01$; 1.8% $p<0.01$, 1.4% $p<0.05$) than *AA* and *AB* genotype in pH of *m. Longissimus Dorsi* (LD), *m. Biceps Femoris* (BF), *m. Semipinali Capitis* (SC). The allelic frequencies were also significantly different between indigenous Chinese breeds and exotic breeds. Data analyzed revealed that the mutation locus affected production traits mostly by additive effects. Based on these results, it is necessary to do more studies on LPL gene before making the LPL locus into the application of marker-assisted selection (MAS) programs. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 4 : 458-462)***

Key Words : Association, Productive Traits, Polymorphism, Lipoprotein Lipase Gene, Pigs

INTRODUCTION

Lipoprotein lipase (LPL) is a major enzyme responsible for the hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL) (Chamberlain et al., 1989; Ahn et al., 1993; Zhang et al., 1996). It also promotes the exchange of lipids between VLDL and high density lipoproteins (HDL) and is an essential enzyme in the formation of low density lipoprotein (LDL) particles (Hokanson et al., 1999). So LPL plays a key role in lipid metabolism. The chylomicrons are not broken down properly if the lipoprotein lipase enzyme is deficient, which would result in a fat-laden chylomicrons in blood. Many studies have demonstrated significant associations between changes in coronary heart disease (CHD) risk factors and allelic variants in the LPL gene in human (Dichek et al., 1991; Hokanson et al., 1997). However, studies on the association of productive characteristics with LPL gene polymorphism are scanty. Hence, the purpose of this study was to determine whether there was any evidence of genetic correlation between productive traits in pig resource family and the polymorphisms of the fifth intron in porcine LPL gene.

MATERIALS AND METHODS

Experimental animals

The pig resource family used in this study was established by mating three Large White boars to seven Meishan sows. Five males and twenty-three females in the F_1 generation were selected for intercrossing randomly. One hundred and forty F_2 individuals were slaughtered for test. Carcass traits and meat quality traits were recorded according to the method of Xiong and Deng (1999). For each trait (Table 1), one hundred and forty phenotypic records were available.

DNA preparation

Genomic DNA was isolated from blood white cells and DNA extraction procedure was described by Sambrook et al. (1989). After isolation, DNA pellet was dissolved in TE buffer and was stored at -20°C.

Cloning and sequencing of the amplified fragment of intron 5

The intron 5 was amplified with genomic DNA from two pig breeds (Qingping and Large White). The primer pair 1 (forward: 5`-CCAACCAGGGTGTAACAT-3`; reverse: 5`-GGATTTTCTTCATTCAACAGA-3`) was designed according to cDNA sequence of Landrace breed in Genbank (X62984) for cloning and sequencing. Polymerase chain reaction (PCR) was performed in 25 μl reaction volume containing: 1×PCR buffer, 1.5 mM $MgCl_2$, 250 μmol/l dNTP, 5 pm of each PCR primer, 2 units *Taq* DNA polymerase (Biostar International, Canada), 200 ng genomic DNA as template. PCR was run in the GeneAmp PCR system 9600 (Perkin-Elmer Co., USA) thermocycler as follows: initial denaturation at 94°C for 4 min, 35 cycles of 94°C for 1 min, 63°C for 1 min, 72°C for 1.5 min, and a final extension for 10 min at 72°C. The purified PCR

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Table 1. Abbreviations of names for traits in pig resource family

No.	Abbreviations	Names	Unit
1	WLR	Water lost rate	%
2	WHC	Water holding capacity	%
3	MC1	Meat color (LD)	-
4	pH (LD)	pH of muscle for longissimus Dorsi, (LD)	-
5	pH (BF)	pH of muscle for Biceps Femoris, (BF)	-
6	pH (SC)	pH of muscle for Semipinali Capitis, (SC)	-
7	RFT	6-7 rib fat thickness	mm
8	TFT	Thorax-waist fat thickness	mm
9	BFT	Buttock fat thickness	mm
10	ABT	Average backfat thickness	mm
11	DR	Dressing percentage	%
12	FR	Fat percentage	%

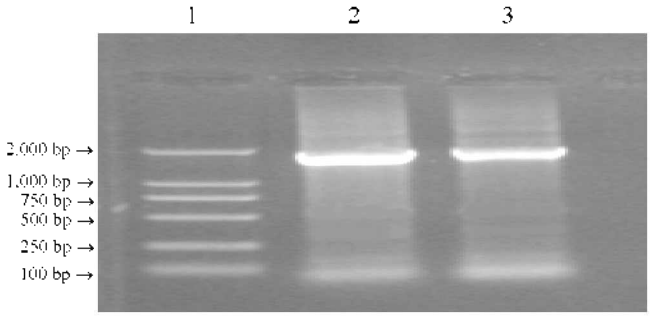


Figure 1. The amplified products of intron 5 of LPL gene. Lane 1: DL 2,000 markers; Lane 2: PCR product in Large White pig, 1,902 bp; Lane 3: PCR product in Qingping pig, 1,916 bp.

products were cloned into the pGEM-T vector and were sequenced by Sangon Company. The sequencing results of different pig breeds were compared by using BLAST (http://www.ncbi.nlm.nih.gov).

Detection of PCR-*Afa*I-RFLP

According to the BLAST results of intron 5 in Large White and Qingping breeds, Primer pair 2(forward: 5'-CAGGGAACTTTCCATACTTTGTG-3'; reverse: 5'-AGC ATGTAATACTTCCAGAGGCT-3') was designed for detection of PCR-*Afa*I-RFLP. PCR amplification (20 μl final volume) was performed using 250 ng genomic DNA, 200 μmol/l dNTP, 5 pm of each primer, and 1 U *Taq* DNA polymerase with the reaction buffer supplied by the manufacturer. The thermocycler profile was 94°C for 4 min, 35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 45 s, followed by a final extension step at 72°C for 10 min. For the PCR-RFLP assays, PCR products were digested with 3 units *Afa*I (TaKaRa Company) in 1×buffer with 1×BSA added in a total volume of 10 μl, following digestion for 4 h at 37°C. Digested products were separated by electrophoresis on a 3% agarose gel in 1×TAE buffer and stained with 0.5 μg/mL ethidium bromide.

Statistical analysis

The association between genotype and production traits was performed with the least square method (GLM procedure, SAS version 8.0). Both additive and dominance effects were also estimated using REG procedure of SAS version 8.0, where the additive effect was denoted as -1.0 and 1 for *AA*, *AB* and *BB*, respectively, and the dominance effect represented as 1, -1 and 1 for *AA*, *AB* and *BB*, respectively (Liu, 1998). The model used to analyze the data was assumed to be:

$$Y_{ijk} = \mu+S_i+F_j+G_k+b_{ijk}X_{ijk}+e_{ijk}$$

Where, Y_{ijk} is the observation of the trait; μ is the least square mean; S_i is the effect of its sex; F_j is the effect of its family; G_k is the effect of its genotype; b_{ijk} is the regression coefficient of the slaughter age and e_{ijk} is the random residual.

RESULTS

Amplification and cloning of intron 5

The primer pair 1 produced two fragments with genomic DNA from two pig breeds. Product of PCR (5 μl) was observed through 1% agarose gel electrophoresis. The results were displayed in Figure 1.

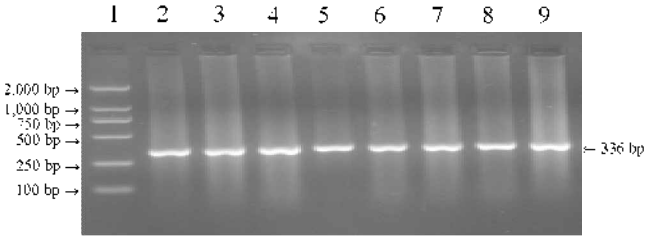
Sequencing analysis

Sequence analysis of PCR products revealed that a new *Afa*I polymorphism was identified at 1,171th position where the nucleotide G was substituted by nucleotide C. The sequence has been deposited in the GenBank database under the accession number AY 566861.

Restriction site analysis

A 336 bp fragment was amplified with primer pair 2. PCR was performed on DNA of nine pig breeds and one hundred and forty F_2 offspring. Results of amplification were shown in Figure 2.

The G/C substitution was detected by PCR-*Afa*I-RFLP. The PCR products were digested with restriction enzyme



Figue 2. The amplified products of intron 5 with primer pair 2. Lane 1: DL 2,000 marker; Lane 2-9: PCR products.

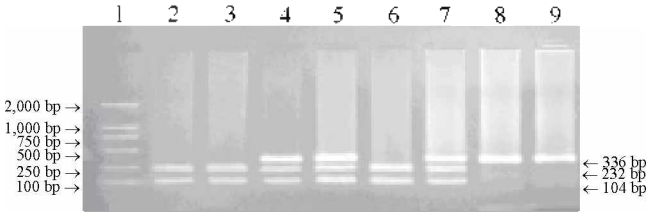


Figure 3. The PCR-RFLP results of intron 5 with primer pair 2. Lane 1: DL 2,000 marker; Lane 2, 3, 6: genotype *BB*; Lane 4, 5, 7: genotype *AB*; Lane 8, 9: genotype *AA*.

(*Afa*I) and the fragments were separated by 3% agarose gel electrophoresis. Three genotypes were presented: *AA* (336 bp), *AB* (336 bp+232 bp+104 bp), *BB* (232 bp+104 bp) (Figure 3).

Frequencies of allele and genotype of different pig breeds

Allele frequencies for the PCR-*Afa*I-RFLP were studied in 9 different populations (Table 2). Allele *A* was the predominant allele in Chinese indigenous pig breeds, such as, frequencies of allele *A* was 1.00 in Huainan pigs, while in Western pig breeds, allele *B* was predominant. There were three genotypes (*AA*, *BB* and *AB*) existed in all pig populations. Standard t testing showed highly significant difference in genotype frequencies between Chinese indigenous pig populations and Western pig breeds ($p<0.01$) (Table 3), indicating clearly genetic differentiation at the locus between Chinese and Western pigs, whereas the differences among Chinese indigenous pig populations were slighter.

Analysis of phenotype value about carcass traits and meat quality traits

SAS package was conducted for statistical analyses on carcass traits and meat quality traits on F_2 offspring (Large White×Meishan). Here significant tests and multi-comparison were performed. The results of tests for the genotypes and carcass traits were given in Table 4. Pigs with *BB* genotype had more TFT (+0.28 mm), RFT (+0.09 mm), BFT (+0.21 mm) and ABT (+0.15 mm) than pigs with *AA* genotype, however, pigs with *BB* genotype had fewer DR (1.94%) than pigs with *AA* genotype. Statistically significant associations with only DR and TFT were found. P-value of DR and TFT was 0.026 and 0.014, respectively. No significant differences were observed in RFT, BFT and ABT. Data (Table 5) revealed that an allele (*A*) was associated with a desirable effect on meat quality traits. Pigs with *AA* genotype had more pH (LD) (+0.23, $p<0.01$), pH (BF) (+0.15, $p<0.01$), pH (SC) (+0.12, $p<0.01$), and WHC (+5.35, $p<0.01$), than pigs with *BB* genotype. Another trend that homozygotes (*BB*) had significantly higher ($p<0.01$)

Table 2. Genotype and allele frequencies at *Afa*I locus of intron 5 in different pig breeds

Breeds	Number of pigs	Genotype and frequency			Allele frequency	
		AA	*AB*	*BB*	*A*	*B*
Large White	20	2 (0.10)	10 (0.50)	8 (0.40)	0.35	0.65
Landrace	23	9 (0.39)	12 (0.52)	2 (0.09)	0.65	0.35
Huainan	25	25 (1.00)	0 (0.00)	0 (0.00)	1.00	0.00
Qingping	23	13 (0.57)	10 (0.43)	0 (0.00)	0.78	0.22
Meishan	24	19 (0.79)	5 (0.21)	0 (0.00)	0.90	0.10
Tongcheng	23	14 (0.61)	7 (0.30)	2 (0.09)	0.76	0.24
Duroc	20	3 (0.15)	14 (0.70)	3 (0.15)	0.50	0.50
Bamei	12	4 (0.33)	6 (0.50)	2 (0.17)	0.58	0.42
Erhualian	19	13 (0.68)	6 (0.32)	0 (0.00)	0.84	0.16

Table 3. T test results for the allele frequency distribution among different pig breeds

Breeds	Huainan	Landrace	Qingping	Meishan	Tongcheng	Duroc	Bamei	Erhualian
LargeWhite	7.69**	3.97**	4.16**	5.83**	4.26**	1.00	1.95*	4.75**
Huainan		4.57**	4.40**	2.91**	4.31**	6.94**	5.73**	3.63**
Landrace			0.22	2.14*	0.33	3.03**	1.56	1.11
Qingping				1.93*	0.11	3.23**	1.75	0.90
Meishan					1.82	4.96**	3.42**	0.94
Tongcheng						3.33**	1.84	0.80
Duroc							1.07	3.88**
Bamei								2.44*

* $p<0.05$; ** $p<0.01$.

Table 4. The effects of *Afa*I locus in intron 5 of LPL gene on carcass traits

Genotype	FP	DR	TFT	RFT	BFT	ABT
AA	18.5±10.63	69.80±0.59^{ab}	1.67±0.07^{a}	2.44±0.08	1.45±0.08	2.16±0.07
BB	19.65±0.78	70.71±0.73^{a}	1.95±0.09^{b}	2.53±0.10	1.66±0.09	2.31±0.09
AB	18.96±0.68	71.74±0.62^{b}	1.79±0.07^{ab}	2.38±0.09	1.45±0.08	2.17±0.08
Additive	0.57±0.51	0.45±0.47	0.14±0.06	0.05±0.07	0.11±0.06	0.07±0.06
Effect	(p=0.26)	(p=0.34)	(p=0.014)	(p=0.49)	(p=0.08)	(p=0.20)
Dominance	0.06±0.42	-0.74±0.39	0.01±0.05	0.05±0.05	0.05±0.05	0.03±0.05
Effect	(p=0.89)	(p=0.06)	(p=0.84)	(p=0.36)	(p=0.32)	(p=0.48)

Data in the table are least square means±standard error.
Values in each line in different lower case superscripts are significantly different at $p<0.05$.
Additive effect (a) = (*BB*-*AA*)/2; Dominance effect (d) = *AB*-(*AA*-*BB*)/2.

Table 5. Effect of different genotype in intron 5 of LPL gene on meat quality traits

Genotype	pH (LD)	pH (BF)	pH (SC)	WLR	WHC	MC1
AA	6.38±0.027^{A}	6.47±0.020^{A}	6.49±0.021^{A}	5.89±0.67^{A}	92.07±0.93^{A}	21.29±0.52^{A}
BB	6.15±0.034^{B}	6.32±0.026^{B}	6.37±0.026^{aB}	9.46±0.86^{B}	86.72±1.15^{B}	24.25±0.65^{Ba}
AB	6.41±0.029^{A}	6.44±0.022^{A}	6.46±0.022^{b}	5.95±0.72^{A}	91.95±0.99^{A}	21.45±0.56^{b}
Additive	-0.12±0.02	-0.08±0.02	-0.06±0.02	1.78±0.54	-2.67±0.74	1.48±0.42
Effect	(p=0.0001)	(p=0.0001)	(p=0.0005)	(p=0.0012)	(p=0.0005)	(p=0.0006)
Dominance	-0.07±0.02	-0.02±0.01	-0.01±0.01	0.86±0.45	-1.28±0.62	0.66±0.35
Effect	(p=0.0002)	(p=0.074)	(p=0.29)	(p=0.057)	(p=0.041)	(p=0.062)

Data in the table are least square means±standard error.
Values in each line in different lower case superscripts are significantly different at $p<0.05$; and with capital superscripts different at $p<0.01$.
Additive effect (a) = (*BB*-*AA*)/2; Dominance effect (d) = *AB*-(*AA*+*BB*)/2.

WLR and MC_1 than that of homozygotes (*AA*) and heterozygotes (*AB*) was observed.

DISCUSSION

In the present study, frequencies of allele *A* and *B* were distributed in nine pig breeds. Significant difference was revealed (Tables 2 and 3). Higher frequencies of allele *A* were found in indigenous Chinese breeds, suggesting that allele *A* could be associated with fat deposition. It is also in agreement to the fact that Chinese pig populations have a strong tendency to accumulate fat. Therefore, it is a strong possibility that enzymatic activity of lipoprotein lipase changed because of the substitution from G to C.

Backfat thickness would be an important factor for carcass traits (Archibald, 1994; Zuo et al., 2003). It should also be closely correlated with fat deposition. A mutation was also found in 5'-untranslated regions (UTR) of LPL gene, which could be related with backfat thickness (Wu, 1998). However, no significant effect on backfat thickness was observed except for TFT in present study, which might be due to the limited numbers of individuals analyzed. More individuals are necessary to confirm the present results.

Ultimate pH of pork is most commonly used to assess pork quality. A higher level of acidity within the muscle (lower pH) causes muscle protein to denature and lose their ability to hold water. Therefore, meat with relatively higher pH will tend to have more desirable characteristics (Simpson et al., 1989; Gu et al., 1992; Zuo et al., 2003). Furthermore, pigs with *BB* genotype presented greater drip loss and meat color values, which indicated the pigs with *BB* genotype have a worse meat quality compared to pigs with *AA* genotype, and eventually resulted in pale, yellow meat, similar to PSE meat rejected by consumers (Minkema et al., 1976).

The polymorphism analyzed in porcine *LPL* gene was located in intron 5. Although the single nucleotide polymorphism (SNP) in introns do not directly alter any functionally important amino acid residue, they may play a role in regulating gene expression and thus their constituent SNPs may be directly related to functional variation. Other researchers have also reported that introns may play a role in splicing of mRNA and its stability (Padma et al., 2004).

It has not been reported that association exits between the polymorphisms in *LPL* gene and production traits. The polymorphism could be a potential genetic marker for production traits, especially for meat quality. In addition, data analyzed revealed that the mutation locus controlled production traits mostly by additive effects (Tables 4 and 5). The porcine *LPL* gene has been assigned to chromosome $14^{q12\text{-}q14}$ (Gu et al., 1992). Other closely linked genes on porcine chromosome 14 might affect the observed results. Further investigation is required among more populations of pigs to verify whether the substitution could be utilized as a genetic marker in MAS programs.

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REFERENCES

Ahn, Y. I., C. Kamboh and R. F. Hamman. 1993. Two DNA polymorphisms in the lipoprotetin lipase gene and their association with factors related to cardiovascular diease. J. Lipid Res. 34:421-428.

Archibald, A. L. 1994. Livestock genetics: fat pigs can blame their genes. Current Biology 4:728-30.

Chamberlain, J. C., J. A. Thorn, K. Oka and Galton. 1989. DNA polymorphisms at the lipoprotein lipase gene: associations in normal and hypertriglyceridaemic subjects. Atherosclerosis 79:85-91.

Dichek, H. L., S. S. Fojo and O. U. Beg. 1991. Identification of two separate allelic mutations in the lipoprotein lipase gene of a patient with the familial hyperchylomicronemia syndrome. J. Biochem. Chem. 266(1):473-477.

Gu, F. and I. Harbitz. 1992. Mapping of the porcine lipoprotein lipase (LPL) gene to chromosome $14^{q12\text{-}q14}$ by *in situ* hybridization. Cytogenet Cell Genet 59(1):63-64.

Harbitz, I., M. Langset and A. G. Ege. 1999. The porcine hormone-sensitive lipase gene: sequence, structure, polymorphisms and linkage mapping. Anim. Gene. 30:10-15.

Hokanson, J. E. 1997. Lipoproteinlipase gene variants and risk of coronary disease: a quantitative analysis of population-based studies. J. Clinical Lab. Res. 27:24-34.

Hokanson, J. E., J. D. Brunzell, G. P. Jarvik and E. M. Wijsman. 1999. Linkage of low-density lipoprotein size to the lipoprotein lipase gene in heterozygous lipoprotein lipase deficiency. J. Human Gene. 64:608-618.

Liu, B. H. 1998. Statistical genomics: linkage, mapping, and QTL analysis. CRC Press, LLC.

Minkema, D., G. Eikelenboom and P. Eldik. 1976. Inheritance of MH-susceptibility in pigs. In: Proceedings of the 3rd International Conference on Production Disease in Farm Animals. pp. 203-207.

Padma, B., P. Kumar, V. Choudhary, S. K. Dhara, A. Mishra, T. K. Bhattacharya, B. Bhushan and A. Sharma. 2004. Nucleotide sequencing and PCR-RFLP of insulin-like growth factor binding protein-3 gene in Riverine Buffalo (Bubalus bubalis). Asian-Aust. J. Anim. Sci. 17(7):910-913.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning: A laboratory manual. Ed 2nd, Vol 3. Cold Spring Harbor Laboratory Press. New York.

Simpson, S. P. and A. J. Webb. 1989. Growth and carcass performance of British Landrace pigs heterozygous at the halothane locus. Anim. Prod. 49:503-509.

Wu, Z. F. 1998. Molecular analyzing of porcine lipoprotein lipase and hormone-sensitive lipase gene. Ph.D. Thesis, Hua zhong Agricultire University, Wuhan.

Xiong, Y. Z. and C. Y. Deng. 1999. Technical testing rule of lean pig. Chinese Agricultural Press, Beijing.

Zhang, H., H. Henderson and S. E. Gagne. 1996. Common sequence variants of lipoprotein lipase: standardized studies of *in vitro* expression and catalytic function. Biochim. Biophys. Acta. 1302:159-166.

Zuo, B., Y. Z. Xiong, Y. H. Su, C. Y. Deng, R. Zheng and S. W. Jiang. 2003. Mapping quantitative trait loci meat quality on pig chrosomone 3, 4 and 7. Asian-Aust. J. Anim. Sci. 16:320-324.