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New SNPs of the Duck *LPL* Gene are Associated with Body Weight, Fatness and Carcass Traits

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Abstract: Lipoprotein Lipase (LPL) is a multifunctional protein that plays a major role in the hydrolysis of triglycerides present in chylomicrons and very low-density lipoproteins. This study was designed to investigate the effects of Single Nucleotide Polymorphisms (SNPs) of the duck *LPL* gene on fatness and carcass and growth traits. A White Kaiya x White Liancheng F_2 population with a total of 1069 individuals was used in the present study, 440 healthy ducks randomly selected from this population were slaughtered at 80 days of age. PCR-Single Strand Conformation Polymorphism (PCR-SSCP) and sequencing methods were used to detect SNPs in the duck *LPL* gene. Two new SNPs (C645T and G726A) were discovered in exons 5 and genotyped using the PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) Method. Genotyping results showed that the genotype distribution differed between males and females, thus the association analyses were performed separately for males and females. Association analyses indicated that the SNPs were significantly associated with body weight, fatness and carcass traits ($p<0.05$ or $p<0.01$). We concluded that LPL is a major gene or is linked with a major gene that influences body weight, fatness and carcass traits and that C645T and G726A could be used as candidate molecular genetic markers for breeding selection.

Key words: Duck, LPL, SNP, body weight, fatness, carcass, PCR-SSCP, PCR-RFLP

INTRODUCTION

Duck breeding for the harvesting of meat has advanced considerably in recent decades, resulting in reduced slaughter age and greatly increased feed efficiency, meat production and growth rate. The intensive selection for growth rate however has led to increased body fat deposition which has become one of the main problems facing the duck breeding industry. Excessive fat deposition affects duck carcass appearance and meat quality and it also causes low feeding efficiency, environmental pollution and food safety problems.

Lipoprotein Lipase (LPL) plays a central role in normal lipid metabolism. It is the key enzyme involved in the hydrolysis of triglycerides present in chylomicrons and very low-density lipoproteins. LPL is related to adipose tissue fat deposition (Hermier *et al*., 1989, 1991) and Frayn *et al*. (1995) reported that it mediates fat deposition and mobilization in white adipose tissue. Sato *et al*. (1999) successfully reduced chicken body fat by injecting anti-LPL antibody into chickens, thereby illustrating that LPL plays an important role in fat deposition. Many studies have used the *LPL* gene to detect polymorphisms associated with adipose traits (including abdominal fat weight, subcutaneous fat weight, thickness of subcutaneous fat and intermuscular fat width) in chickens, pigs and other animals (Harbitz *et al*., 1992; Lei *et al*., 2004; Liu *et al*., 2006). In ducks, Wu *et al*. (2008) discovered a single nucleotide polymorphism (SNP; C/T alteration) in exon 7 of the *LPL* gene and found that it was associated with abdominal fat weight in native and Cherry Valley Peking duck however, no association was found between the SNP and subcutaneous fat plus skin weight and carcass traits.

To further study the *LPL* gene and to find a molecular marker that could be used for marker-assisted selection in duck breeding, we screened the polymorphisms of the duck *LPL* gene and studied the associations of polymorphisms with body weight, carcass and fatness traits in the study population.

MATERIALS AND METHODS

Animals and samples: In collaboration with Jingwu Food Industrial Garden Ltd., (Wuhan, China), a White Kaiya x White Liancheng F_2 population with a total of 1069

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Table 1: Primers designed for amplification of the *LPL* gene

Primer name	Primer sequence (5'-3')	Annealing temp (°C)	Product locus	Product size (bp)
Lexon2F	AGCACGAAGCTGAGACGAAT	58	Exon 2	170
Lexon2R	CGTCCATCCATGGATGACCA			
Lexon3F	GTGACAGGCATGTATGAAAGTT	56	Exon 3	180
Lexon3R	CTCCATCCAGTCAATAAACATAG			
Lexon5F	GGGCCCACCTTTGAGTAC	58	Exon 5	219
Lexon5R	TGCAAGGCCTTTTTCAGC			
Lexon6F	GTGGATCAGCTGGTGAAATG	57	Exon 6	238
Lexon6R	TGTAGGGCATCTGAGCACG			
Lexon8F	GCCAGAAGTCTCCTCAAACA	56	Exon 8	183
Lexon8R	TTTTTCTGAGTTTCGCCTGA			

individuals was used in the present study. All birds were raised in floor pens and fed standard commercial corn-soybean diets. Body weight was recorded every 2 weeks from birth to the 12th weeks. Individual blood samples were collected and genomic DNA was extracted using a standard phenol; chloroform extraction method these samples then were stored at -20°C. At 80 days of age, 440 healthy ducks randomly selected from this population were slaughtered. Three kinds of fatness traits were measured; abdominal fat weight, subcutaneous fat plus skin weight and thickness of subcutaneous fat. The following conventional carcass traits were measured as well: body weight (before slaughter), carcass weight, eviscerated with giblet weight, eviscerated weight, breast muscle weight (right side) and leg muscle weight (right side). In China, people also eat the head, neck, wings, palma and some of the organs. Thus, based on the characteristics of the Chinese duck products market, head weight, neck weight, wing weight (right side), shank plus palma weight, heart weight, liver weight and muscular stomach weight also were measured.

SNP identification and PCR-RFLP analysis: The cDNA sequence of the duck *LPL* gene that we obtained from a normalized cDNA library was submitted to the GenBank database under the accession no. FJ859348. Using this sequence, five pairs of primers were designed using the software Primer Premier 5.0 (Premier, Palo Alto, CA, USA) (Table 1).

All PCRs were performed in Mastercycler ep gradient S (Eppendorf, Hamburg, Germany) using the following cycling parameters; 94°C initial denaturation for 7 min, 32 cycles of 94°C denaturing for 40 sec, 56-58°C (Table 1) annealing for 40 sec and 72°C extension for 40 sec, followed by a 7 min extension at 72°C.

PCR-Single Strand Conformation Polymorphism (PCR-SSCP) was used to detect polymorphisms with the following procedure; PCR products were denatured at 98°C for 10 min and rapidly chilled on ice. Next, 4 μL of PCR product mixed with 10 μL loading buffer were loaded on 10% PAGE gels (39:1) at 4°C at an initial voltage of 300 V for 10 min then 110 V for 16 h. Results were visualized by silver staining. Based on the different band patterns, the PCR products were isolated and purified then cloned into pGEM-T vectors (Promega, Madison, WI, USA) and sequenced.

Two new SNPs were discovered in exons 5 and genotyped using the PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) Method. PCR products of exon 5 were digested respectively with 4U of Sac II and 4U of Mva I (MBI, Vilnius, Lithuania) in 10 μL reaction solutions overnight at 37°C following the supplier's directions. The digested products were detected by electrophoresis on 2.0% agarose gel stained with ethidium bromide and visualized using a gel imaging system (BIO-RAD, Hercules, CA, USA).

Statistical analysis: The distribution of genotypes differed between sexes in this study and the traits analyzed also differed significantly between sexes ($p<0.05$). Moreover, the *LPL* gene might be a *z-linked* gene. Therefore, the association analyses between the SNPs and traits were performed separately for males and females using the t statistic and the SPSS 13.0 statistical software package (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Discovery of new SNPs in the *LPL* gene: All fragments were successfully amplified. Two new SNPs, C645T and G726A were discovered in exon 5 through sequence comparison (Fig. 1). The SNPs were synonymous and caused restriction enzyme sites change (SacII and MvaI) and did not result in an amino acid change.

SNP genotyping using PCR-RFLP: The presence of three genotypes, CC (132 and 87 bp), CT (219, 132 and 87 bp) and TT (219 bp) was revealed by a PCR-RFLP Method using the SacII restriction enzyme at C645T (Fig. 2a). Three genotypes, AA (168 and 51 bp), AG (219, 168 and 51 bp) and GG (219 bp) were revealed using the PCR-MvaI-RFLP method at G726A (Fig. 2b).

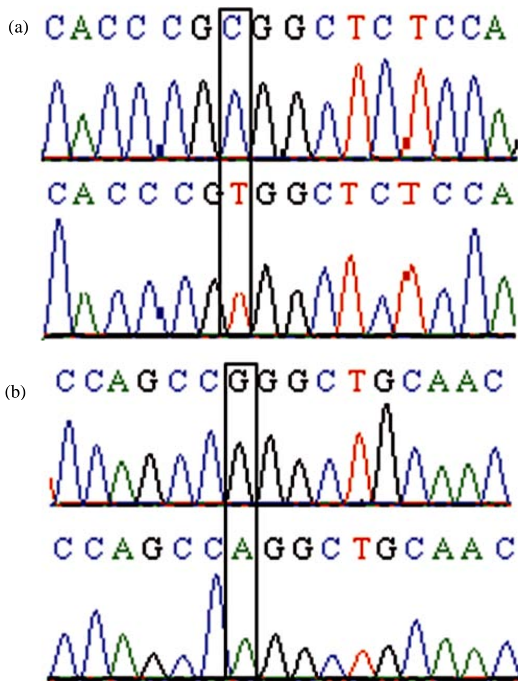

Fig. 1: Sequence analysis revealed two new SNPs in the *LPL* gene; a) C/T mutation in exon 5 at position 645; b) G/A mutation in exon 5 at position 726

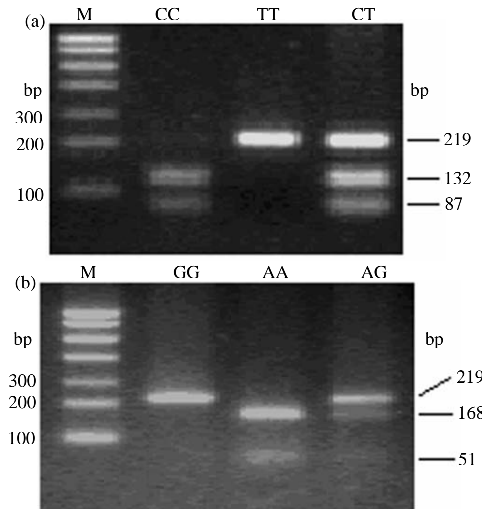

Fig. 2: PCR-RFLP patterns of the *LPL* gene resolved by agarose gel electrophoresis; a) Genotyping of the *LPL* gene at C645T using the PCR-SacII-RFLP Method. M: DNA marker; CC: CC genotype; TT: TT genotype; CT:CT genotype; b) Genotyping of the *LPL* gene at G726A using the PCR-MvaI-RFLP Method. M: DNA marker; GG: GG genotype; AA: AA genotype; AG: AG genotype

Table 2: Genotype distribution of SNPs differed between males and females

	Genotype			
Sex	CC (GG)	CT (AG)	TT (AA)	Total
Male	389	80	0	469
Female	589	0	11	600

Genotyping results showed that the genotypes of the two SNP loci completely corresponded to each other with CC corresponding to GG, TT corresponding to AA and CT corresponding to AG (Fig. 2). Therefore, the genotypes CC and GG were combined into one genotype CC (GG), TT and AA were combined into TT (AA) and CT and AG were combined into CT (AG). The distribution of genotypes differed between males and females (Table 2). Only two genotypes, CC (GG) and CT (AG) were detected in males whereas only CC (GG) and TT (AA) were detected in females. Thus, the association analyses were performed separately for males and females.

Association and effect of SNPs on body weight, fatness and carcass traits: The association analysis result between SNPs and body weight is shown in Table 3. In males, the body weight of genotype CT (AG) was significantly higher than that of CC (GG) from 8-12 weeks ($p<0.01$). In females, the body weight of genotype TT (AA) was significantly higher than that of CC (GG) from 4-12 weeks ($p<0.05$ or $p<0.01$).

The association analyses results of SNPs and fatness and carcass traits are shown in Table 4. In males, the fatness and carcass trait values of genotype CT (AG) were significantly higher than those of CC (GG) ($p<0.05$ or $p<0.01$), except for thickness of subcutaneous fat ($p = 0.225$). In females, the fatness and carcass trait values of genotype TT (AA) were significantly higher than those of CC (GG) ($p<0.05$ or $p<0.01$) except for head weight, abdominal fat weight and thickness of subcutaneous fat ($p>0.05$).

These results indicate that these two SNPs (C645T and G726A) of the duck *LPL* gene are significantly associated with body weight, fatness and carcass traits; the effect of the duck *LPL* gene on body weight appears earlier in females than in males and trait values for genotypes CT (AG) and TT (AA) are all higher than those of the CC (GG) genotype in both males and females and the alleles T (A) were associated with an increase in the trait values.

LPL is an important marker for adipocyte differentiation (Bjorntorp *et al*., 1978) and LPL expression increases in parallel with cellular triglyceride accumulation as preadipocytes differentiate (Semenkovich *et al*., 1989). Although, adipose tissue can synthesize free fatty acids de novo, free fatty acids for lipid storage are preferentially

Table 3: Association analysis of SNPs with body weight

	Male			Female		
	Genotype (μ±SD, g)			Genotype (μ±SD, g)		
Weeks	CC (GG) (n = 389)	CT (AG) (n = 80)	p-value	CC (GG) (n = 589)	TT (AA) (n = 11)	p-value
Birth	44.9±4.900	44.9±4.400	0.991	45.8±4.600	47.2±3.400	0.340
2	330.8±69.50	345.8±71.80	0.090	340.1±68.80	350.9±49.30	0.605
4	883.3±151.1	899.5±159.6	0.412	882.9±144.9	973.0±68.30	0.050
6	1393.2±247.3	1416.8±238.4	0.437	1389.7±227.4	1563.6±130.8	0.012
8	1748.4±244.5	1868.5±281.0	0.000	1694.3±240.3	1965.5±160.8	0.000
10	1848.7±242.0	2032.8±265.2	0.000	1805.2±227.8	2121.5±98.30	0.000
12	1815.7±243.1	1999.7±270.7	0.000	1849.5±214.0	2205.5±234.1	0.020

Table 4: Association analysis of SNPs with fatness and carcass traits

	Male			Female		
	Genotype (μ±SD)			Genotype (μ±SD)		
Traits	CC (GG) (n = 206)	CT (AG) (n = 40)	p-value	CC (GG) (n = 187)	TT (AA) (n = 7)	p-value
Body weight (before slaughter) (g)	1638.8±218.8	1856.2±239.0	0.000	1637.8±198.5	1986.6±143.1	0.000
Carcass weight (g)	1432.9±189.1	1621.7±209.6	0.000	1443.2±175.9	1733.2±159.2	0.000
Eviscerated with giblet weight (g)	1302.2±182.1	1476.9±207.5	0.000	1324.9±162.1	1586.6±137.9	0.000
Eviscerated weight (g)	1196.3±167.0	1357.3±189.3	0.000	1213.3±148.0	1437.9±104.5	0.000
Leg muscle weight (right side) (g)	95.4±12.3	105.9±14.2	0.000	93.0±11.6	105.0±7.0	0.007
Breast muscle weight (right side) (g)	81.1±14.9	94.9±16.4	0.000	82.2±13.6	102.4±9.8	0.000
Head weight (g)	87.9±8.5	94.2±9.3	0.000	80.6±7.9	85.4±5.9	0.112
Neck weight (g)	95.2±12.8	106.1±14.4	0.000	88.0±11.6	102.7±8.9	0.001
Wing weight (right side) (g)	62.7±8.7	69.7±8.7	0.000	60.9±8.1	71.1±5.8	0.001
Shank plus palma weight (g)	20.1±2.6	22.9±3.3	0.000	19.6±2.6	21.6±1.6	0.050
Heart weight (g)	12.6±1.9	14.1±1.9	0.000	12.6±1.7	15.0±1.8	0.000
Liver weight (g)	32.8±7.8	37.1±9.0	0.002	33.5±6.8	51.1±20.7	0.000
Muscular stomach weight (g)	45.7±7.7	52.6±7.1	0.000	44.2±7.1	54.77.6	0.000
Abdominal fat weight (g)	13.2±7.4	16.1±8.6	0.029	21.4±9.9	28.7±13.9	0.060
Subcutaneous fat plus skin weight (g)	244.1±56.9	280.4±67.4	0.000	295.2±55.4	374.9±77.9	0.000
Thickness of subcutaneous fat (cm)	0.397±0.113	0.421±0.101	0.225	0.456±0.110	0.507±0.084	0.229

provided by LPL-mediated hydrolysis of plasma TG-rich lipoproteins (Hollenberg, 1966). Thus, LPL is considered to be a gatekeeper enzyme that plays an important role in the initiation and/or development of obesity and that relates to energy balance insulin action and body weight regulation (Wang and Eckel, 2009). The candidate gene approach is a powerful method to investigate associations between gene polymorphisms and economically important traits in farm animals (Rothschild and Soller, 1997). Many studies have examined the effect of polymorphisms of the *LPL* gene on fatness traits in different species (Ginzinger *et al*., 1999; Harbitz *et al*., 1992; Hata *et al*., 1992; Lei *et al*., 2004; Liu *et al*., 2006; Peterson *et al*., 2002; Reymer *et al*., 1995; Wu *et al*., 2008). Genetic markers associated with fat deposition are used to assist in selection for meat quality and production efficiency in chickens (Liu *et al*., 2006). In this study, the *LPL* gene was selected as a candidate gene to investigate associations of gene polymorphisms with body weight, fatness and carcass traits in a White Kaiya x White Liancheng F_2 population.

A ~1.3 kb cDNA sequence of the duck *LPL* gene was generated from a normalized cDNA library that we constructed previously (GenBank accession no. FJ859348). We screened this sequence and detected two new SNPs in exons 5 using PCR-SSCP and DNA sequencing methods. The SNPs were genotyped by PCR-RFLP using SacII and MvaI, respectively. The genotypes of the two SNPs completely corresponded to each other indicating that these two SNPs were completely linked.

However, no localization information exists for the *LPL* gene in the duck. The *LPL* gene is located on chromosome Z in *Gallus gallus* and on chromosome Z in another bird, *Taeniopygia guttata*. Much research has shown strong chromosome homology between macrochromosomes in different bird species (Masabanda *et al*., 2004). Therefore, on the basis of the chromosome location of the *LPL* gene in *G. gallus* and *T. guttata*, the strong chromosome homology among different bird species and the lack of a heterozygote in females in the study, we suspect that the duck *LPL* gene also might be localized on chromosome Z.

In this study, two new SNPs from exon 5 were genotyped. Association analyses of these SNPs with body weight, fatness and carcass traits were performed separately for males and females. The results showed significant differences in body weight, fatness and carcass traits between different genotypes ($p<0.05$ or $p<0.01$).

CONCLUSION

This finding indicates that the SNPs (C645T and G726A) in the *LPL* gene were significantly associated with these traits. Thus, the *LPL* gene may be a major gene or may be linked to a major gene that affects duck body weight, fatness and carcass traits. These SNPs can be used in molecular marker-assisted selection as a genetic marker for duck breeding.

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