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PCR-SSCP Analysis of Avian Lipoprotein Lipase Gene (Intron 8) and its Relation with Body Weight and Abdominal Fat

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Abstract: The genetically fat Anka and lean Rugao chicken breeds were used for this study, both are reared in the same environment and management system. Body weight and abdominal fat weight were determined at 12 weeks of age. A polymerase chain reaction single strand conformation polymorphism technique was applied to detect mutation in intron 8 of lipoprotein lipase gene. Hardy Weinberg Equilibrium (HWE) test shows that allele frequency was significantly ($p<0.01$) different in Rugao population in locus LPL10 and non significantly different in Anka population. In addition chi-square test shows that populations were significantly ($p<0.01$) differed from each other. Body weight in fat breed was significantly ($p<0.05$) different between LPL genotypes in LPL9 and LPL10 loci. However, the abdominal fat was significantly ($p<0.05$) different in LPL10 locus. In addition lean breed shows non significant difference on body weight and fat weight. Genotype effect within two breeds showed that body weight and fat weight in LPL9 and LPL10 loci were significantly ($p<0.05$) higher in heterozygous AC compared with homozygous AA.

Key words: PCR-SSCP, mutation, lipoprotein lipase, body weight, fat

INTRODUCTION

In the 1970s, apolipoprotein CII (apoCII) was found in both human (Havel *et al*., 1973) and rat (LaRosa *et al*., 1970) serum that activated LPL. Inhibition of LPL by other serum apolipoproteins such as apoCIII and apoCI was also noted (Brown and Baginsky, 1972). ApoCIII-deficient subjects were shown to have rapid plasma VLDL catabolism (Ginsberg *et al*., 1986). The physiological actions of LPL in catabolism of chylomicrons and VLDL and in the production of much of the lipids and apolipoproteins that form High Density Lipoprotein (HDL) have been appreciated for more than a decade (Goldberg, 1996). The major tissues thought to control the circulating levels of plasma lipoproteins are the adipose tissues and muscles. However, LPL is expressed in other sites, including the nervous system, heart, liver, adrenals, macrophages, proximal tubules of the kidneys, pancreatic islet cells and lungs. In these organs, LPL may have specialized functions. LPL is normally not made in adult liver; however, it is expressed in the liver of newborn animals. In fed mammals, LPL activity is enhanced in adipose tissue but is low in muscle, which results in fat storage. The opposite is seen in unfed animals. In birds, LPL regulation in the adipose tissue seems to be less sensitive to the nutritional state (Hermier *et al*., 1984). There is a paucity of information on the hormonal regulation of LPL in birds. Very high concentrations of insulin stimulate LPL activity in chicken adipose tissue (Borron *et al*., 1979). Total LPL activity in abdominal fat was significantly correlated ($r = 0.5$) with fat pad weight, but there was no correlation between specific activity of the enzyme and fat weight. LPL activity in post-heparin plasma showed no correlation with either abdominal fat or total body fat content (Guo *et al*., 1988). Heterozygous LPL mutations associated with a reduction or loss of LPL activity and this may increase the risk of Familial Combined Hyperlipidemia (FCHL) and premature atherosclerosis. It is noted that a reduction of LPL activity should lead to increased triglycerides, decreased HDL and therefore premature atherosclerosis. Mutations in the LPL gene have been linked to other diseases such as Alzheimer's disease (Baum and Chen, 1999), Hypertension (Sprecher *et al*., 1996), severe hypertriglyceridemia and pancreatitis can occur during pregnancy and with diabetes (Henderson *et al*., 1998). Also mutations may have an increased risk of pre-eclampsia (Hubel *et al*., 1999). The objectives of this experiment were to detect mutation in lipoprotein lipase gene and study its relation with body weight and fat weight in genetically fat and lean chicken.

MATERIALS AND METHODS

Experimental animals: A total of 120 birds from fat type (Anka) and lean type (Rugao) were used in the present study. Both were reared under the same environment and

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Table 1: Primers sequences, location, PCR product and annealing temperature of chicken Lipoprotein lipase gene

Primers name	Sequences (5-3 flanking region)	Direction product	Location temperature	PCR	Annealing
LPL-9	GACGAAACATGGAAAACAG	Upstream	14486-14843	357 bp	54.4°C
LPL-9	CAACTCCCAAGAAAACTCA	Downstream			
LPL-10	GCTGAGTTTTCTTGGGAGTTGGG	Upstream	17822-15217	395 bp	59.8°C
LPL-10	GCCTTGCTCCCTTGAATGTTTG	Downstream			

management in Jaingsu Poultry institute, Yangzhou, China. At 12 weeks of age 0.5 mL blood samples were taken for DNA isolation. Thereafter, chickens were weight, slaughtered and carcasses were dissected manually and then abdominal fat weight was estimated. The percentage of abdominal fat weight was expressed as a ratio of body weight.

DNA extraction and primers design: DNA was isolated from the whole blood collected using exactly the saturated salt method previously described by (Sambrook *et al*., 1989). Primers of lipoprotein lipase gene were designed by Oligo 6.0 software, according to chicken genomic sequence in GenBank database (accession number X60547) Table 1.

PCR-SSCP and gene Sequencing: PCR-SSCP analysis was carried out in total volume 20 μL of PCR reaction, contain 100 ng of template DNA, 13.3 μL of sterilized distilled water, 0.5 μL (5pmol) of each primer, 1.5 μL 10X PCR Buffer (Mg2plus), 2.5 μL of 2.5 mM dNTP Mixture and (5 U uL^{-1}) of Taq polymerase (TakaRa Biotechnology Dalian Co., Ltd.). The PCR conditions determined was initially denatured at 94°C for 3 min, followed by 30 cycles at 94°C for 30s and annealed for 30 sec and 72°C for 30 sec, finally PCR product was extended at 72°C for 8 min. The annealing temperature for Lipoprotein lipase gene primers were ranged between 54.4 to 59.8°C Table 1. PCR product was mixed with 5 μL of loading buffer, denatured at 98°C for 10 min and then quickly place in ice box and incubated 5 min at -20°C. Ten microliter PCR products were loaded in to12% (39:1) polyacrylamide under 150 V for 9 h, the results of electrophoresis were silver stained.

Statistical analysis: PCR-SSCP data obtained from chicken populations was used for the measurement of genetic parameters. The chi-square test was used to test if a sample of data came from a population with a specific distribution using chi-Square calculator V 1.51. All values are presented as means±Standard Error of Mean (SEM). The following model was fitted for association of each genotype with body weight and abdominal fat. Yij = μ+Mi+eij where Yij is phenotypic value of (body weight and abdominal fat), μ is population mean, Mi is the fixed effect of the ith genotype and eij is random error effect of each observation, it was determined by ANOVA using general linear model GLM, all analysis was performed by SAS 9.0 software.

RESULTS

PCR-SSCP genotype and hardy-Weinberg equilibrium test: Mutation detected by PCR-SSCP in lipoprotein lipase was presented in Fig. 1 and 2. Allele frequency was obtained by calculating the number of different size allele's outcome for lipoprotein lipase loci. It was significantly different ($p<0.01$) for Rugao breed in LPL10 locus. The genotype distribution of each locus in lipoprotein lipase gene between fat and lean chicken indicated that Anka and Rugao populations were significantly different ($p<0.01$) (Table2).

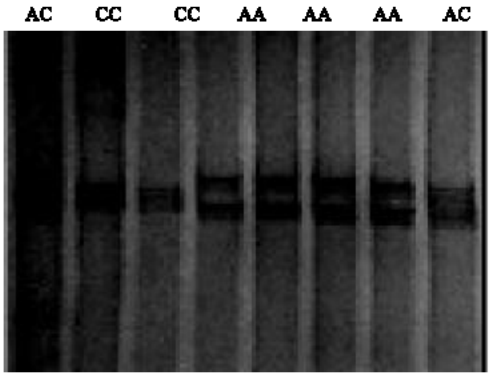

Fig. 1: PCR-SSCP of LPL9 primer

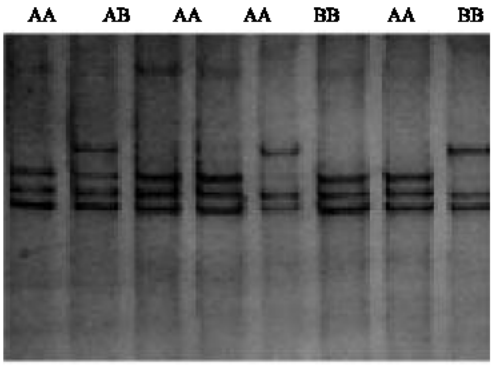

Fig. 2: PCR-SSCP of LPL10 primer

Table 2: Genotype and allele frequencies of lipoprotein lipase loci in different chicken populations

		LPL 9				LPL 10		
Population	No.	AA	AB	BB	X^2	A	B	X^2
LPL9								
Anka	59	0.475	0.389	0.136	11.50**	0.669	0.331	0.53
Rugao	59	0.763	0.136	0.102	0.831	0.169	5.53	
LPL10								
Anka	59	0.254	0.271	0.475	17.09**	0.389	0.611	5.49
Rugao	59	0.627	0.169	0.203	0.712	0.288	9.38**	

** Chi-square value was significance at ($p<0.01$)

Table 3: Relation between breed, lipoprotein lipase genotype, body weight and abdominal fat weight

Loci	Genotype	n	Body weight	Fat weight	Fat weight (%)
LPL9					
Anka	AA	28	3249.710±89.102a	54.674±1.848	1.818±0.074
	AC	23	3614.740±98.311b	58.630±2.039	1.703±0.082
	CC	8	3284.250±166.695	52.725±3.458	1.683±0.139
Rugao	AA	45	1113.93±26.299	16.582±0.453	1.657±0.031
	AC	8	1106.880±62.373	16.675±1.075	1.681±0.074
	CC	6	1162.330±72.023	18.200±1.241	1.727±0.086
LPL10					
Anka	AA	15	3111.670±111.571a	51.727±2.368a	1.815±0.102
	AB	16	3786.810±108.028b	62.211±2.292b	1.736±0.099
	BB	28	3326.460±81.662ac	54.639±1.733ac	1.734±0.075
Rugao	AA	37	1110.460±28.044	16.368±0.483	1.639±0.034
	AB	10	1211.800±53.943	18.780±2.317	1.723±0.066
	BB	12	1062.580±49.243	16.283±0.847	1.705±0.060

Genotype with different subscript in column was significant different at ($p<0.05$)

Table 4: Relation between lipoprotein lipase genotype, body weight and adipose tissue weight

Loci	Genotype	n	Body weight	Fat weight	Fat weight (%)
LPL9					
	AA	73	1933.140±131.737a	31.193±2.327a	1.719±0.037
	AC	31	2967.550±202.157b	47.803±3.571b	1.697±0.057
	CC	14	2374.860±300.820	37.929±5.313	1.701±0.085
LPL10					
	AA	52	1687.730±152.288a	26.567±2.666a	1.690±0.044
	AB	26	2796.420±215.367b	45.507±3.770b	1.731±0.062
	BB	40	2647.300±173.635bc	43.132±3.040bc	1.725±0.050

Genotype with different subscript in column was significant different at ($p<0.05$)

Relation between lipoprotein lipase polymorphism, body weight and abdominal fat: The relation between breed and lipoprotein lipase genotype and body weight, abdominal fat weight and percentage of abdominal fat weight were presented in (Table 3). In Anka breed body weight was significantly ($p<0.05$) different between AA and AC genotype in LPL9 locus. However, in LPL10 locus body weight and abdominal fat weight were significantly ($p<0.05$) differed in lipoprotein lipase genotypes. Genotype effect within two breeds showed that body weight and fat weight in LPL9 locus were significantly ($p<0.05$) higher in heterozygous AC compared with homozygous AA, similar results were also observed in LPL10 loci. In addition the homozygous genotype BB in LPL10 locus was significantly ($p<0.05$) observed high body weight and adipose tissue weight compared with AA genotype. When the fat weight estimated as a percentage of body weight no significant effect was observed within breed and lipoprotein lipase genotypes (Table 4).

DISCUSSION

In this study we used PCR-SSCP to detect mutation in intron8 of lipoprotein lipase gene. Lipoprotein lipase allele frequency was significantly ($p<0.01$) different in Rugao population at locus LPL10. It is known that allele frequency which shows Hardy Weinberg Equilibrium is inherited randomly in the population. The genotype distribution of lipoprotein lipase gene between chicken populations tested by chi-square was in consistent with the phenotypic information of the breeds. It is difficult to separate genetic and environmental factors and assign the relative contribution of each to the development of obesity. Body weight in Anka population was significantly ($p<0.05$) different between homozygous AA and heterozygous AC genotype in LPL9 and LPL10 loci. However, the abdominal fat weight was significantly ($p<0.05$) differed between homozygous and heterozygous genotypes in LPL10 locus. In addition Rugao population shows non significant different in body weight and fat

weight with in LPL loci. The major tissues thought to control the circulating levels of plasma lipoproteins are the adipose tissues and muscles. Therefore, body fat content was highly correlated with rate of secretion of plasma triglyceride-rich lipoprotein (Griffin *et al*., 1991). From experimental animals studies it is clear that many animals that develop adipose tissue are energetically more efficient than lean animals; that is for a given amount of food, the obesity-prone animals deposit more fat than do the obesity-resistant animals. In fact, when genetically obese rodents are not allowed to overeat from birth, they are still considerably fatter than their lean brothers and sisters (Greenwood and Johnson, 1993).

Lipase genotype within two breeds showed that body weight and fat weight in LPL9 and LPL10 loci were significantly ($p<0.05$) higher in heterozygous AC than the homozygous AA. Chicken LPL plays a crucial role in fat accumulation in adipose tissue and that the reduction of LPL protein in adipose tissue by some nutritional means may be effective in retarding fatness in broiler chickens (Sato and Akibal, 2002). In addition, Griffin *et al.* (1987) reported that LPL activity in chicken adipose tissue gradually increases from 4 week up to 6 week of age. These results suggest that LPL mRNA expression may also be dependent on the age of chickens, as well as on nutritional states. The rate of lipoprotein hydrolysis is dependent not on LPL mRNA expression but, rather on the biochemical characteristics of plasma lipoproteins, the substrate of LPL (Sato and Akibal, 2002). The rates of lipoprotein hydrolysis and of trapping into adipose tissue are the major factors determining fatness in broiler chickens. Thus, nutritional manipulation of broiler chickens to retard fatness could be better achieved by changing the fatty acid profile of the plasma lipoproteins rather than by regulating LPL mRNA expression in the adipose tissue (Sato and Akibal, 2002). As mentioned, body fat deposition depends on the net balance among absorbed fat, endogenous fat synthesis and fat catabolism. Because abdominal fat was positively correlated with total body fat (Becker *et al*., 1979). In fed mammals, LPL activity is enhanced in adipose tissue but is low in muscle, which results in fat storage, the opposite is seen in unfed animals. In birds, LPL regulation in the adipose tissue seems to be less sensitive to the nutritional state (Hermier *et al*., 1984). Very high concentrations of insulin stimulate LPL activity in chicken adipose tissue (Borron *et al*., 1979). In abdominal adipose tissue of obese functionally castrated hens, LPL activity per gram of tissue or milligram protein was lower than in obese (Jaccoby *et al*., 1996). In genetically fat chickens, higher LPL activity in adipose tissue is a result of cell hyperplasia because LPL activity for a single cell did not differ from that of a normal chicken (Hermier *et al*., 1989). Beside plasma activity, estrogen also depresses LPL activity in adipose tissue of young chicks (Hasegawa *et al*., 1980). Hepatic lipogenesis or LPL activity in adipose tissue did not differ between lean and fat lines and therefore they did not appear to be limiting factors of susceptibility to fattening. In contrast Bosello *et al.* (1984) indicate that obese individuals have a higher adipose tissue LPL activity than lean persons, even when expressed relative to fat cell size. An increase in LPL activity could subsequently affect changes in body fat storage and lipid metabolism (Barbara *et al*., 2000).

REFERENCES

Baum, L. and L. Chen, 1999. Lipoprotein lipase mutations and Alzheimer's disease. Am. J. Med. Genet., 88: 136-139.

Barbara, J.N., M.R. Ellen, M.B. Dora, E.D. Karen and P.G. Andrew, 2000. Responses of adipose tissue lipoprotein lipase to weight loss affect lipid levels and weight regain in women Am. J. Physiol. Endocrinol. Metab., 279: 1012-1019.

Becker, H., B.W. Smith, N.E. White, S.S. Holt, E.A. Boldt, R.F. Mushotzky and P.J. Serlemitsos, 1979. X-Ray Spectrum of Cassiopeia A Measured with the Einstein SSS. A.P. J., 234: L73.

Borron, D.C., L.S. Jensen, M.G. McCartney and W.M. Britton, 1979. Comparison of lipoprotein lipase activities in chickens and turkeys. Poult. Sci., 58: 659-662.

Bosello, O., M. Cigolini, A. Battaggia, F. Ferrari, R. Micciolo, R. Olivetti and M. Corsato, 1984. Adipose tissue lipoprotein lipase activity in obesity. Int. J. Obes., 8: 213-220.

Brown, W.V. and M.L. Baginsky, 1972. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. Biochem. Biophys. Res. Commun., 46: 375-382

Greenwood, M.R.C. and P.R. Johnson, 1993. Genetic differences in adipose tissue metabolism and regulation. Ann. N.Y. Acad. Sci., 676: 253-269.

Ginsberg, H.N., N.A. Le, I.J. Goldberg, J.C. Gibson, A. Rubinstein, P. Wang-Iverson, R. Norum and W.V. Brown, 1986. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase *in vivo*. J. Clin. Invest., 78: 1287-1295.

Griffin, H.D., S.C. Butterwith and C. Goddard, 1987. Contribution of lipoprotein lipase to differences in fatness between broilers and layer-strain chicks. Br. Poult. Sci., 28:197-206.

Griffin, H.D., D. Windsor and C.C. Whitehead, 1991. Changes in lipoprotein metabolism and body composition in chickens in response to divergent selection for plasma very low density lipoprotein concentration. Br. Poult. Sci., 32: 195-201.

Goldberg, I.J., 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J. Lipid Res., 37: 693-707.

Guo, K., H.D. Griffin and S.C. Butterwith, 1988. Biochemical indicators of fatness in meat-type chicken: lack of correlation between lipoprotein lipase activity in post-heparin plasma and body fat. Br. Poult. Sci., 29: 343-350.

Hasegawa, S., T. Nimora, K. Sato, Y. Hikami and T. Mizano, 1980. Effects of estrogen on triglyceride metabolism in the chick liver. J. Zootech. Sci., 53: 699-706.

Havel, R.J., C.J. Fielding, T. Olivecrona, V.G. Shore, P.E. Fielding and T. Egelrud, 1973. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoproteins lipase from different sources. Biochem., 12: 1828-1833.

Henderson, H., F. Leisegang, F. Hassan, M. Hayden and D.A. Marais, 1998. Novel Glu421Lys substitution in the lipoprotein lipase gene in pregnancy-induced hypertriglyceridemic pancreatitis. Clin. Chim. Acta, 269: 1-12.

Hermier, D., M.J. Chapman and B. Leclercq, 1984. Plasma lipoprotein profile in fasted and refed chickens of two lines selected for high or low adiposity. J. Nutr., 114: 1112-1121.

Hermier, D., A. Quignard-Boulangé, I. Dugail, G. Guy, M.R. Salichon, L. Brigand, B. Ardouin and B. Leclercq, 1989. Evidence of enhanced storage capacity in adipose tissue of genetically lean and fat chickens. J. Nutr. ,119: 1369-1375.

Hubel, C.A., J.M. Roberts and R.E. Ferrell, 1999. Association of pre-eclampsia with common coding sequence variations in the lipoprotein lipase gene. Clin. Genet., 56: 289-296

Jaccoby, R.F., D.J. Marshall, M.A. Newton, K. Novakovic, K. Tutsch, C.E. Cole, R.A. Lubet, G.J. Kelloff, A. Verma, A.R. Moser and W.F. Dove, 1996. Chemoprevention of spontaneous intestinal adenomas in the Apc^{Min} mouse model by the nonsteroidal anti-inflammatory drug piroxicam. Cancer Res., 56: 710-714.

LaRosa, J.C., R.I. Levy, P. Herbert, S.E. Lux and D.S. Fredrickson, 1970. A specific apoprotein activator for lipoprotein lipase. Biochem. Biophys. Res. Commun., 41: 57-62.

Sambrook, J., E.F. Fristch and T. Manistais, 1989. Molecular cloning: A laboratory manual: Vol.3. Cold Spring Harbor Laboratory Press. Cold Spring Harbor. USA.

Sprecher, D.L., B.V. Harris, E.A. Stein, P.S. Bellet, L.M. Keilson and L.A. Simbartl, 1996. Higher triglycerides, lower high-density lipoprotein cholesterol and higher systolic blood pressure in lipoprotein lipase-deficient heterozygotes. A preliminary report. Circulation, 94: 3239-3245.

Sato, K. and Y. Akiba1, 2002. Lipoprotein Lipase mRNA Expression in Abdominal Adipose Tissue Is Little Modified by Age and Nutritional State in Broiler Chickens. Poult. Sci., 81: 846-852.