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Full Length Research Paper

The effect of interaction between Lipoprotein Lipase and ApoVLDL-II genes on fat and serum biochemical levels

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Body weight, abdominal fat weight and serum biochemical levels were determined from lean and fat chicken breeds at 12 weeks of age. Single nucleotide polymorphism (SNP) in apoVLDL-II and lipoprotein lipase genes was screened by PCR-SSCP and detected by direct sequencing. Lipoprotein lipase gene frequency was found to be significantly different (P < 0.01) in lean chicken whereas it was non-significantly different in fat chicken. SNP in apoVLDL-II and lipoprotein lipase genes significantly (P < 0.05) affected body weight and fat weight. Similarly their interaction significantly (P < 0.05) affected body weight and fat weight. However, no significant difference was observed in the percentage of abdominal fat. SNP in apoVLDL-II and lipoprotein lipase genes significantly (P < 0.05) affected total cholesterol and high density lipoprotein. More likely, the interaction of apoVLDL-II and lipoprotein lipase significantly affect total cholesterol, triglyceride, high density lipoprotein, very low density lipoprotein and low density lipoprotein.

Key words: Interaction, lipoprotein lipase, apoVLDL-II, fat, serum biochemical levels.

INTRODUCTION

The increase in physiological disorders such as obesity has attracted the attention of most scientists. The reduction in fitness is mainly because of a tremendous increase in body mass without parallel improvements in the internal organs, vascular system, and skeleton to support such a rapidly growing and large body mass (Dunnington and Siegel, 1996). Growth and meat yield traits in poultry, as well as fitness traits, are controlled by many genes quantitative trait loci (QTL). The total effect of QTLs influenced by many genes might interact with each other, and the environment might interact with the genotype (Cahaner, 1990). The negative genetic correlation between growth and fitness is not absolute, it is possible to select for genotypes with high fitness characteristics and high yield using marker assist selection (MAS). However, the relative advantage of MAS over phenotypic selection depends on the heritability of the traits and the cost of phenotyping versus genotyping (Deeb and Lamont, 2002).

ApoVLDL-II is estrogen dependent, absent from plasma of immature hens and inhibits lipoprotein lipase (LPL) activity (Nimpf and Schneider, 1991). Highly significant differences are found in the plasma triglyceride concentration of genetically lean and fat chickens (LeClercq, 1983). Similarly very low density lipoprotein (VLDL) and high density lipoprotein (HDL) concentration are higher in the fat birds whether fed or starved. High plasma levels of VLDL or triglycerides are frequently associated with genetic or nutritional factors that induce excessive hepatic lipogenesis (Leclercq, 1984). In contrast Behr et al. (1981) noted that increased VLDL concentrations were not associated with fatness. The last stage in the control of adipose tissue accretion is the removal of triglycerides from the plasma; this process depends mainly on lipoprotein lipase activity. In birds, LPL regulation in the adipose tissue seems to be less sensitive to the nutritional state (Hermier et al., 1984). Total LPL activity in abdominal fat was significantly corre-

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Table 1. Primers sequences, location, product size and annealing temperature of candidates (LPL and apoVLDL-II) genes.

Gene	Sequences (5-3 flanking region)	Location	Product size	Annealing temp
LPL	FGCTGAGTTTTCTTGGGAGTTGGG R GCCTTGCTCCCTTGAATGTTTG	14822-15217	395bp	59.8°C
ApoVLDL-II	F ATTGACTAGCGTGAGATTCC R ATGATGGTGCAGTTCTTCTT	2788-3071	303 bp	57°C

F and R refers to forward and reverse primers

Table 2. Genotype and gene frequency of lipoprotein lipase and apoVLDL-II genes in chicken populations.

Population No.		AA	AB	BB	X^2	A	B	X^2
Lipoprotein lipase								
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**
ApoVLDL-II								
Anka	59	0.644	0.322	0.034	16.58**	0.805	0.195	0.02
Rugao	59	0.271	0.644	0.085		0.593	0.407	3.86

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

lated with fat pad weight in chicken and turkey (Kouba et al., 1995; Griffin et al., 1987). Hepatic lipogenesis or LPL activity in adipose tissue did not differ between lean and fat lines. In contrast, hepatic secretion and plasma concentration of VLDL were always higher in fat chicken than lean chickens (Hermier et al*.,* 1984). Adipose tissue growth in birds depends mainly on the availability of triglycerides transported by VLDL. The VLDL concentration is a good indicator of the degree of fatness (Griffin and Whitehead, 1985). More likely, LPL activity increases with the number of adipocytes and thus reflects the degree of hyperplasia, rather than an increase in the fat-storing ability of individual cell (Hermier, 1997). The objective of this experiment was to investigate the interaction of single nucleotide polymorphisms (SNPs) in lipoprotein lipase and apoVLDL-II genes on fat and serum biochemical levels in genetically lean and fat chickens.

MATERIALS AND METHODS

Animals and genetic analysis

The data of abdominal fat and serum biochemical concentrations were obtained from 120 chickens at 12 weeks of age includes Anka as fat type and Rugao as lean type breeds described by Musa et al. (2006). Total serum cholesterol and triglycerides were assayed using a commercial enzymatic kit supplied by (Zhe jiang Dongou Biological Engineering Co., Ltd.) according to the manufacturer recommendations. High-density lipoprotein cholesterol was detected enzymatically after precipitation of (LDL and VLDL) by heparin and manganese. Very low-density lipoprotein cholesterol and low-density lipoprotein cholesterol are estimated using the Friedewald equation (Friedewald et al., 1972). DNA was isolated from the whole blood according to the methods described by Sambrook et al. (1989). ApoVLDL-II and Lipoprotein lipase genes primers were design by Oligo 6.0 software (Table 1). Thereafter, polymerase chain reaction single strand conformation polymerphism technique (PCR-SSCP) as described by Orita et al. (1989) was developed for mutation detection. Then the genomic DNA of individuals with variant single strand conformers were directly sequenced.

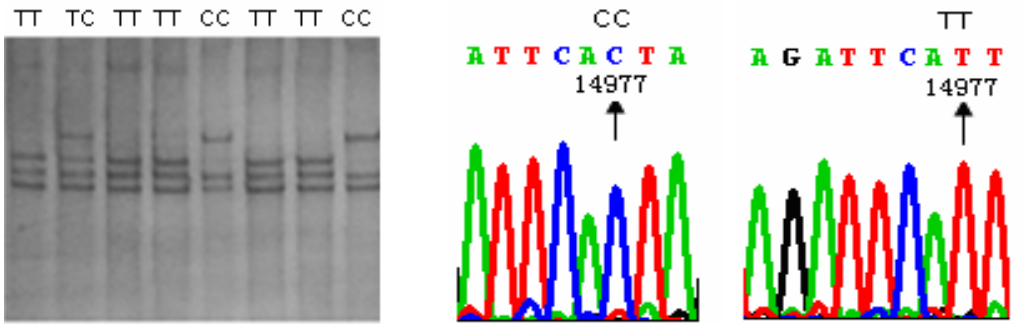
Statistical analysis

Genotype and gene frequency were estimated according to Cerit et al. (2004). Agreement of the genotype frequencies with the Hardy-Weinberg equilibrium expectations was tested using a chi square goodness of-fit test using Chi-Square calculator V 1.51. The associations of abdominal fat and lipoprotein concentration with apoVLDL-II and lipoprotein lipase SNPs genotype were determined using the following model: $Yij = \mu + Mi + eij$. The following model was also fitted to study the interaction effect of apoVLDL-II and lipoprotein lipase genes genotype on abdominal fat and serum biochemical concentrations: $Yij = \mu + Mi + breed + LPL + ApVLDL\text{-}II + [LPL \times apoVLDL\text{-}II] + eij$ where Yij is phenotypic value of (abdominal fat and/or serum biochemical concentrations), μ is population mean, Mi is the fixed effect of the ith genotype and eij is random error effect of each observation. All models were determined by ANOVA using general linear model GLM performed by SAS 9.0 software. Data was presented as mean and standard error of mean.

RESULTS

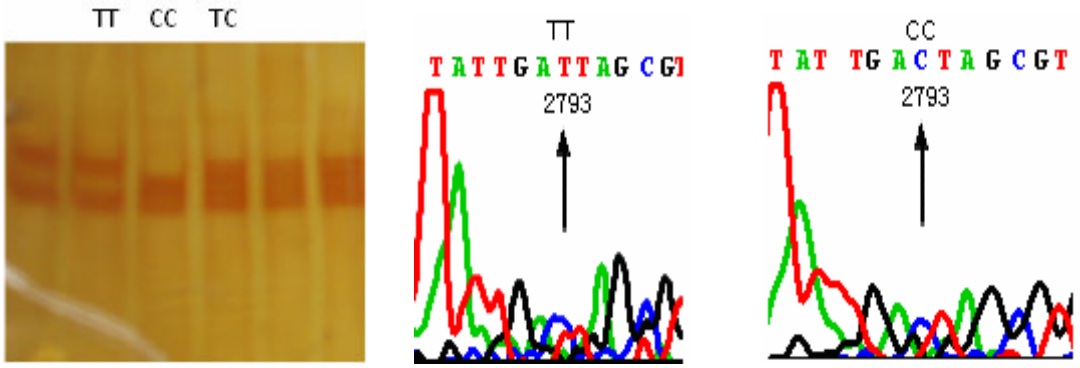
Single nucleotide polymorphism

Single nucleotide polymorphisms screen by PCR-SSCP and detected by direct sequencing in lipoprotein lipase and apoVLDL-II genes were presented in Figures 1 and 2. Genotype and gene frequency of candidate Lipoprotein lipase and apoVLDL-II genes were estimated (Table 2).



A. PCR-SSCP genotype B. Sequence analysis

Figure 1. Polymorphisms of LPL10 primer in intron 8 of lipoprotein lipase gene; (A) PCR-SSCP genotype and (B) sequence analysis of the mutation.



A. PCR-SSCP genotype B. Sequence analysis

Figure 2. Polymorphisms of VLDL10 primer in intron1 of apoVLDL-II gene, (A) PCR-SSCP genotype, (B) Sequence analysis

Table 3. Effect of SNP genotypes on body weight and fat weight.

Loci	Genotype	n	Body weight	Fat weight	Fatweight (%)
Lipoprotein lipase	TT	52	1669.067±198.079a	25.792±3.462a	1.681±0.062
	TC	26	2337.833±280.126b	37.772±4.896b	1.727±0.088
	CC	40	2972.837±358.979bc	46.244±6.274bc	1.650±0.112
ApoVLDL-II	TT	54	2792.583±141.545a	46.000±2.474a	1.759±0.044
	TC	57	2025.320±148.365b	32.166±2.593b	1.682±0.046
	CC	7	2161.833±452.244ab	31.642±7.904ac	1.617±0.142

Different superscripts means significant difference (P<0.05).
n=Samplesize.

Agreement of the genotype frequencies with the Hardy-Weinberg equilibrium expectations tested indicated that lipoprotein lipase gene frequency was significantly different (P < 0.01) in Rugao population, while it was non-significantly difference in Anka population. In addition, gene frequency in apoVLDL-II gene was non-significantly different between populations. On the other hand, fat breed was significantly (P < 0.01) differed from lean in both genes.

Interaction of LPL and apoVLDL-II genes and its effect on body and fat weight

The effect of lipoprotein lipase and apoVLDL-II genes on body weight, fat weight and percentage of fat weight are depicted in Table 3 and their interaction in Table 4. In

Table 4. Effect of the interaction of SNP genotypes on body weight and fat weight.

LPL	ApoVLDL-II	n	Body weight	Fat weight	Fat weight (%)
TT	TT	20	2038.450±229.330	33.405±4.008a	1.768±0.072
	TC	28	1463.250±193.819	22.396±3.387b	1.643±0.061
	CC	4	150.500±512.797	21.575±8.962	1.633±0.160
TC	TT	14	3191.000±274.102a	52.205±4.791a	1.754±0.086
	TC	10	2548.500±324.321	41.260±5.668	1.698±0.101
	CC	2	1274.000±725.204b	19.850±12.674b	1.730±0.227
CC	TT	20	3148.300±229.330a	52.390±4.008a	1.757±0.072
	TC	19	2064.211±235.289b	32.842±4.112b	1.705±0.074

Different superscripts means significant difference (P<0.05); n, sample size.

Table 5. Effect of SNP genotypes on serum biochemical levels.

Loci	Genotype	Sample size	TCH	TG	HDL	VLDL	LDL
Lipoprotein lipase	TT	52	151.585±6.193a	19.730±1.279	114.153±5.455a	3.946±0.256	33.294±6.486
	TC	26	166.719±8.758	20.424±1.808	109.590±7.715	4.086±0.362	53.043±9.173
	CC	40	146.094±11.223b	19.908±2.317	95.304±9.886b	3.985±0.463	46.805±11.755
ApoVLDL-II	TT	54	140.280±4.425a	18.452±0.914	97.449±3.898a	3.692±0.182	38.948±4.635
	TC	57	153.444±4.638 b	21.221±0.958	110.349±4.086b	4.247±0.192	38.849±4.858
	CC	7	170.673±14.139	20.392±2.919	111.249±12.455c	4.078±0.584	55.345±14.808

n = Sample size.

lipoprotein lipase gene, body weight and fat weight were significantly (P < 0.05) higher in individuals with homozygous CC SNP genotype compared with individuals with homozygous TT SNP genotype. However, in apoVLDL-II gene homozygous TT SNP genotype has significantly (P < 0.05) higher body weight and fat weight than homozygous CC SNP genotype.

The interaction of lipoprotein lipase and apoVLDL-II genes indicated that individuals with homozygous (TT) LPL and apoVLDL-II SNP show significantly (P < 0.05) higher fat weight than those homozygous (TT) LPL and heterozygous (TC) apoVLDL-II. In addition, individuals with heterozygous (TC) LPL and homozygous (TT) apoVLDL-II observed significantly (P < 0.05) high body and fat weight than those with homozygous (TT) LPL and homozygous (CC) apoVLDL-II. On the other hand individuals with homozygous (CC) LPL and homozygous (TT) apoVLDL-II showed significantly (P < 0.05) higher estimates of body and fat weight compared with those homozygous (CC) LPL and heterozygous (TC) apoVLDL-II.

Interaction of LPL and apoVLDL-II genes and its effect on serum biochemical levels

Serum biochemical concentrations in Table 5 indicated that TT LPL genotype shows significantly high total cholesterols and high density lipoprotein than CC genotype. In apoVLDL heterozygous CC genotype observed significantly high total cholesterol and high density lipoprotein than homozygous TT genotypes. The interaction effect of LPL and apoVLDL-II indicated that the homozygous TT LPL and apoVLDL SNP genotypes had significantly (P < 0.05) lower total cholesterol and high density lipoprotein levels compared to those have TT LPL and TC apoVLDL-II genotype. However, the individuals with TC LPL and TT apoVLDL observed significantly (P < 0.05) lower triglycerides and very low density lipoprotein than that with TC LPL and TT apoVLDL-II. In addition individuals with CC LPL and TT apoVLDL-II showed lower high density lipoprotein levels than individuals with CC LPL and TC apoVLDL-II genotype (Table 6).

DISCUSSION

Agreement of the SNPs genotype frequencies with the Hardy-Weinberg equilibrium expectations tested indicated that lipoprotein lipase gene frequency was significantly different (P < 0.01) in Rugao population, whereas in apoVLDL-II gene the gene frequency was non-significantly different between populations, this may be due to the high selection program done in Anka as meat chicken with similar gene frequency. Therefore, the populations differed significantly. Most chicken growth and fitness traits are known controlled by multiple genes (Deeb and Lamont, 2002). The effect of each gene on body weight and fat weight was different between individuals with homozygous and heterozygous SNP genotype. In fact,

Table 6. Effect of the interaction of SNP genotypes on serum biochemical levels.

LPL	ApoVLDL-II	n	TCH	TG	HDL	VLDL	LDL
TT	TT	20	143.408±7.170a	20.412±1.408	99.757±6.316a	4.081±0.296	38.993±7.509
	TC	28	169.899±6.060b	19.904±1.251	119.786±5.338b	3.980±0.250	46.135±6.346
	CC	4	141.448±16.032	18.883±3.310	122.918±14.123ad	3.775±0.662	14.755±16.791a
TC	TT	14	140.806±8.569a	17.132±1.76a	103.239±7.549	3.427±0.354a	34.140±8.975
	TC	10	149.970±10.140	22.996±2.094b	102.700±8.932	4.602±0.419b	42.668±10.620
	CC	2	209.380±22.673c	21.996±4.681	122.830±19.972	4.230±0.936	82.320±23.746b
CC	TT	20	136.627±7.170b	17.811±1.480	89.350±6.316 ac	3.566±0.296ac	43.711±7.509
	TC	19	140.464±7.356	20.764±1.519ac	108.562±6.480abde	4.158±0.304	27.744±7.704ac

Different superscripts means significant difference ($P<0.05$).
TCH, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; n, sample size.

positive correlation was found between adipose tissue LPL activity and growth of fat depots in broilers, but this does not proves that fatness results from higher LPL activity (Griffin et al., 1987). However, LPL deficiency has no obvious defect in adipose tissue (Greenwood, 1985), because both humans and mice without adipose tissue LPL still have some essential fatty acid in the adipose tissue (Jaccoby et al., 1996).

The interaction of lipoprotein lipase and apoVLDL-II genes is associated with body weight and fat weight. Fatness is quite highly heritable in birds; the highest heritability (up to 0.73) was obtained for abdominal fat weight (Zerehdaran et al., 2004). In contrast leaner broilers have been produced by selection for low abdominal fat or indirectly for feed efficiency (Bordas and Mérat, 1984) or low plasma VLDL (Whitehead and Griffin, 1984). Adipose tissue growth in birds depends mainly on the availability of triglycerides transported by VLDL (Griffin and Whitehead, 1985). In general, body fat accumulation may be considered the net result of the balance among dietary absorbed fat, endogenous fat synthesis (lipogenesis) and fat catabolism via ß-oxidation (lipolysis). Thus, if the amount of absorbed fat is the same, lower body fat deposition may be attributed to increased fat catabolism or diminished endogenous fatty acid synthesis or to both processes (Sanz et al., 2000).

In this study we conclude that the interaction between SNPs in apoVLDL-II and lipoprotein lipase genes was significantly ($P < 0.05$) associated with total cholesterol, triglyceride, high density lipoprotein, very low density lipoprotein and low density lipoprotein levels in genetically fat and lean chickens. LPL catalyzes the hydrolysis of TG contained in VLDL and chylomicrons leading to the release of free fatty acids and their subsequent uptake by myocytes for energy production or adipocytes for reesterification and storage (Hermier, 1997). The adipose tissue accretion is controlled by the removal of triglycerides from the plasma; this process depends mainly on lipoprotein lipase activity (Rudas et al., 1972). Thus, LPL inhibition results in a decrease in the esterified cholesterol percentage and an increase in triglycerides. Schneider et al. (1990) indicated that no appreciable hydrolysis of TG occurs during transport of

TG-rich lipoproteins. The divergent genetic studies carried out in chicken and turkey indicated that the hepatic secretion of VLDL, VLDL-triglycerides and total triglycerides was always higher in chicken than in turkey in both young and old birds, but the rate of secretion was decreased in both species with age (Kouba et al., 1995). Nutrition experiments indicated that high fat low carbohydrate diets inhibit fatty acid synthesis; fatty acid synthesis also may contribute to elevated levels of plasma triyglycerols and therefore, to development of atherosclerosis (Bradley et al., 1996). Mutations in the LPL gene prevent lipoprotein lipase from breaking down chylomicrons effectively. As a result, this substance accumulates in the blood, leading to the signs and symptoms of familial lipoprotein lipase deficiency. Many fundamental studies have carried out to determine which hormones are responsible for lipolysis in birds. It was shown very early that epinephrine, norepinephrine and adrenocorticotropin stimulate lipolysis in birds only at very high concentrations (Leclercq, 1984). The effect of insulin on triglyceride synthesis was also demonstrated *in vivo* (Vives et al., 1981). Probably, the insulin and glucagon balance is a better explanation of the control of liver lipogenesis. Liver is a main organ responsible for fatty acid synthesis in birds (Evans, 1972).

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