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Full Length Research Paper

Association of polymorphisms in avian apoVLDL-II gene with body weight and abdominal fat weight

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To investigate the association of avian apoVLDL-II gene polymorphism with body weight and fat, exactly 120 genetically fat (Anka) and lean (Rugao) chicken reared under the same environment and management were selected. Blood samples from the respective populations were taken for DNA extraction, and then slaughter for fat determination. Polymorphism was detected by PCR-RFLP and PCR-SSCP techniques. Gene frequency was non significantly different between population at VLDL6 and VLDL10 loci. However, in VLDL9 and VLDL17 loci the gene frequency was differed significantly (P<0.01) between populations. Polymorphism in apoVLDL-II gene was significantly (P<0.05) associated with body weight and fat weight at VLDL9 and VLDL17 loci in lean chicken. In addition, polymorphism of apoVLDL-II gene at VLDL6, VLDL9 and VLDL10 loci was significantly (P<0.05) associated with body weight and fat weight.

Key words: Polymorphism, apoVLDL-II, body weight, fat, avian.

INTRODUCTION

In various cells, including adipocytes, lipoprotein lipase (LPL) enhances cellular binding and uptake of lipoproteins (Schneider et al., 1990). Like chylomicrons, very low density lipoprotein (VLDL) undergoes constant changes in the plasma. At onset of egg-laying in the chicken, plasma levels of apolipoprotein VLDL-II (apoII) increase dramatically (Nimpf et al., 1988; Schneider et al., 1990).

The *de novo* fatty acid synthesis in birds takes place mainly in the liver, adipose tissue growth and subsequent fattening associated with the availability of plasma triglycerides, which are transported as components of lipoproteins. VLDL genes specify the most abundant mRNA species present in livers of hens or estrogen-treated roosters (Hache et al., 1983). In growing birds, VLDL is the major transporter of triglycerides, and attempts to reduce excessive fatness in poultry have involved the control of VLDL metabolism.

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Therefore, lean and fat chicken lines have been divergently selected for adipose tissue weight (Leclercq et al., 1980) and for VLDL plasma concentration (Whitehead and Griffin, 1984). Studies performed in lean and fat line by (Leclercq et al., 1980) indicated that the difference in adiposity between lines was not the result of a difference in food consumption or in metabolic utilization of energy. Stearoyl-CoA desaturase activity and plasma VLDL concentration were found to be higher in the fat line, suggesting a higher lipogenesis rate in this line (Legrand and Hermier, 1992).

The low density lipoprotein (LDL) and very low density lipoprotein (VLDL) particles in meat type cockerel chickens occur in much smaller proportion compared to high density lipoprotein (HDL), with LDL exceeding that of VLDL (Peebles et al., 2004). In the mature egg-laying hens, VLDL particles are the most predominant lipoprotein (Walzem et al., 1994). In Obese functionally castrated hens, where plasma level of apo-VLDL-II was low, plasma LPL activity was elevated, because obese functionally castrated hen had lower ovarian weight and plasma apoVLDL-II and higher post heparin plasma LPL activity than obese laying hen (Jaccoby et al., 1996).

The objective of this experiment was to detect polymer-

Table 1. Primers sequences, location, PCR product and annealing temperature of chicken ApoVLDL-II gene.

Primers	Sequences (5′-3′ flanking region)	Direction	Location	PCR product	Annealing temperature
VLDL6	CCTCTATGACATGGT TGCCT	Sense	1549-2041	492 bp	58°C
VLDL6	ATGGGTTTGACCCTGCTATG	Antisense			
VLDL9	CACCTTTCTAAATGCACAGT	Sense	2490-2759	289 bp	53.4°C
VLDL9	GCAATGATCTTCTGAATGAC	Antisense			
VLDL10	ATTGACTAGCGTGAGATTCC	Sense	2788-3071	303 bp	57°C
VLDL10	ATGATGGTGCAGTTCTTCTT	Antisense			
VLDL17	ACTGCCTATTCCTGCCTTCT	Sense	4199-4479	280 bp	56°C
VLDL17	CACCGACTTTTCTTCCAACT	Antisense			

phisms in apoVLDL-II gene and study its association with body weight and fat in chicken.

MATERIALS AND METHODS

Experimental stocks

The genetically fat (Anka) and lean (Rugao) chicken were reared under the same environment and management. 0.5 ml blood samples were taken from the wing vein of respective populations for DNA extraction. Chickens were slaughtered, carcasses were eviscerated and dissected manually, and abdominal fat weight was estimated. The percentage of abdominal fat weight was expressed as a ratio of body weight (Musa et al., 2006).

DNA Isolation and primers design

Genomic DNA was isolated from the whole blood using saturated salt method (Sambrook et al., 1989). Primers were designed based on the published sequences in Gene bank, accession number (J00810). One pair of ApoVLDL-II primer for PCR-RFLP was chosen based on the primer design by (Li et al., 2005). PCR-SSCP primers were design using Primers Primers 5.0, Oligo 6.0 and DNA star softwares, their related information was presented in (Table 1).

PCR-RFLP

PCR-RFLP amplification of VLDL6 primer was carried out in total volume of 25 µl containing 100 ng of template DNA, 5 pmol of each primer, 10X PCR Buffer (Mg2plus), 2.5 mM dNTP mixture and (5 U/µl) of Taq DNA polymerase (TakaRa Biotechnology Dalian Co., Ltd). The amplification cycle entailed 3 min of denaturation at 94^{O}C, followed by 35 cycles of 1 min at 94^{O}C, 1 min at 58^{O}C and 1 min at 72^{O}C. This was followed by 10 min of extension at 72^{O}C. PCR products were checked in 0.8% agarose gels stained with ethidium bromide to determine the presence of product. Thereby, 10 ul of each PCR products were digested overnight at 60^{O}C by 3 U of *Sfcl* restriction enzyme (Sangon, China). The digested fragments were electrophorsed using 10% polyacrilamide gels in 1XTBE buffer at a constant voltage of 120 V for 4 h. DNA was visualized by staining the gel with silver and their photo were taken by Olympus digital camera.

PCR-SSCP

Single strand conformation polymorphism analysis was performed as described by (Orita et al., 1989). The analysis was carried out in 20 µl of PCR product for all primers. The total volume used included 100 ng of template DNA, 5 pmol of each primer, 10X PCR Buffer (Mg2plus), 2.5 mM dNTP mixture and (5 U/µl) of Taq DNA polymerase (TakaRa Biotechnology Dalian Co., Ltd.). PCR condition was carried out by thermal cycle. Initial denaturation for 3 min at 94^{O}C, was followed by 30 cycles of denaturation at 94^{O}C for 30 s, annealing for 30 s, plus extension at 72^{O}C for 30 s, with final extension at 72^{O}C for 8 min. The 20 µl of PCR product was mixed with 5 µl loading buffer, denatured at 98^{O}C for 10 min and then quickly placed into ice for 5 min. 10 µl of mixture was applied into (poly acrylamide: bisacrylamide 49:1) electrophoresed in 1X TBE buffer for 8 – 12 h at 140 to 160 voltage. The gel was stained with sliver according to standard protocol.

Statistical analysis

Allele frequency of the genetic data obtained by PCR-RFLP and PCR-SSCP was determined using Cerit et al. (2004) formula. 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 following model was fitted for association of each genotype with body weight and adipose tissue $Yij=\mu+Mi+eij$ where Yij is phenotypic value of (body weight or fat weight), 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-RFLP and PCR-SSCP analysis

In this study allele frequencies were performed by PCR-RFLP and PCR-SSCP (Figure 1) and determined by direct gene counting for each locus in each chicken population. In Anka population allele frequency was significantly different ($P<0.01$) at VLDL17 locus. However, in Rugao the gene frequency differed significantly ($P<0.01$) at VLDL9 and VLDL 17 loci Table 2.

Association of polymorphism in apoVLDL-II gene with body and fat weight

Polymorphisms at VLDL9 and VLDL17 loci were significantly ($P<0.05$) associated with body weight in Rugao population; it was high in the homozygous geno-

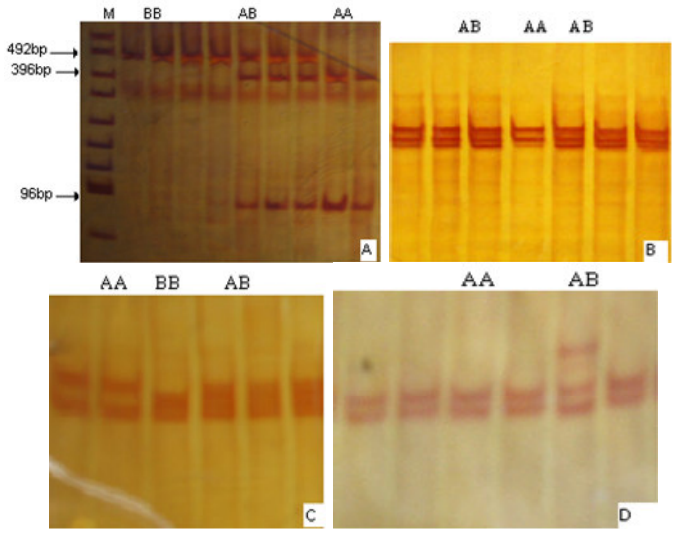

Figure 1. Polymorphisms of avian apoVLDL-II gene in genetically fat and lean chickens. **A.** PCR-RFLP analysis of VLDL6 locus; **B.** PCR-SSCP analysis of VLDL9 locus; **C.** PCR-SSCP analysis of VLDL10 locus; and **D.** PCR-SSCP analysis of VLDL17 locus). M is pUC19DNA/MspI (HpaII) marker.

Table 2. Allele frequencies of apoVLDL-II gene in chicken populations.

Populations	Allele frequencies					
	Anka			Rugao		
	G	A	X^2	A	C	X^2
VLDL6	0.932	0.068	0.06	0.983	0.017	0.00
VLDL9	0.653	0.348	3.59	0.525	0.475	29.76**
VLDL10	0.805	0.195	0.02	0.593	0.407	3.86
VLDL17	0.542	0.458	12.66**	0.712	0.288	37.05**

**Chi-square (X^2) value was significant at (P<0.01).

type compared with the heterozygous. In addition, polymorphism in VLDL17 locus was associated significantly (P<0.05) with abdominal fat weight in Rugao breed. It was observed significantly high in homozygous genotype (Table 3). On the other hand, heterozygous genotype in VLDL6 locus observed significantly (P<0.05) higher body weight and fat weight, whereas, in VLDL9 locus body weight and fat weight were significantly (P<0.05) higher in homozygous genotype (Table 4). In locus VLDL10 body and fat weight were significantly (P<0.05) different between apoVLDL-II genotypes.

DISCUSSIONS

A candidate gene research was a powerful method to investigate QTL that is responsible for genetic variation in the traits of interest in animals breeding (Rothschild and Soller, 1997). In this study the polymorphism of apoVLDL-II gene was detected by PCR-RFLP and PCR-SSCP techniques, most of polymorphisms detected were located in non-coding region of the gene, and in exon4.The apo-VLDL-II is a yolk protein, is inducible by estrogen Lin et al. (1986), absent from plasma of immature hens, and inhibits lipoprotein lipase activity (Nimpt et al., 1988). Thus, estrogen may reduce lipid deposition in fat cells of mature hens. The apoVLDL-II gene contains sequences within its first intron that increase transcription (Berkowitz and Evans, 1992). The variation in allele frequencies between populations will reflect the distribution of genetic diversity within and amongst populations. The primary objective is to maximize the conservation of the genetic diversity available for potential future use. In this study we used chi-squared goodness of fit test to detect the allele variation. This statistical method quantifying the extent to which the observed and expected proportions of genotype counts for a specific DNA locus in a population, agree or disagree. The variations in allele frequencies in our study may be as a result of different breeding criteria for Chin-

Table 3. Effect of breed and ApoVLDL-II gene polymorphisms on body weight and fat weight.

Loci	Genotype	N	Body weight	Fat weight	Fat weight (%)
VLDL6					
Anka	AA	51	3384.57±69.87	55.84±1.39	1.67±0.06
	AB	8	3474.00±176.42	56.69±3.52	1.67±0.14
Rugao	AA	57	1121.00±23.14	16.82±0.40	1.52±0.03
	AB	2	1029.50±123.55	15.00±2.15	1.46±0.15
VLDL9					
Anka	AA	18	3421.11±117.78	57.36±2.34	1.68±0.09
	AB	41	3385.98±78.04	55.34±1.55	1.66±0.06
Rugao	AA	3	1335.00±96.96a	18.73±1.74	1.40±0.12
	AB	56	1106.27±22.44b	16.65±0.40	1.53±0.03
VLDL10					
Anka	AA	38	3428.47±81.51	57.29±1.59	1.70±0.06
	AB	19	3334.89±115.27	54.15±2.25	1.63±0.09
	BB	2	3380.00±355.28	47.50±6.93	1.40±0.28
Rugao	AA	16	1132.94±44.21	16.84±0.77	1.55±0.05
	AB	38	1113.50±28.69	16.71±0.50	1.50±0.03
	BB	5	1103.20±79.08	16.90±1.38	1.55±0.09
VLDL17					
Anka	AA	5	3402.00±223.59	63.54±4.33	1.89±0.17
	AB	54	3396.20±68.04	55.25±1.32	1.65±0.05
Rugao	AA	4	1319.50±83.29a	19.93±.1.46a	1.51±0.11
	AB	55	1103.24±22.46b	16.53±0.39b	1.52±0.03

Means in a column that are followed by the different letter are significant at (P<0.05).

Table 4. Effect of apoVLDL-II polymorphisms on body weight and fat weight.

Loci	Genotype	N	Body weight	Fat weight	Fat weight (%)
VLDL6					
	AA	108	2189.91±114.21a	35.25±1.99a	1.71±0.03
	AB	10	2985.10±375.33b	48.36±6.56b	1.67±0.10
VLDL9					
	AA	21	3123.10±248.18a	51.84±4.32a	1.75±0.07
	AB	97	2069.86±115.48b	33.00±2.01b	1.70±0.03
VLDL10					
	AA	54	2748.32±152.87a	45.31±2.65a	1.76±0. 04
	AB	57	1853.97±148.79b	29.19±2.57b	1.67±0.04
	BB	7	1753.71±424.59bc	25.64±7.35bc	1.64±0.12
VLDL17					
	AA	9	2476.44±402.03	44.16±6.98	1.85±0.10
	AB	109	2239.20±115.52	35.71±2.01	1.69±0.03

Means in a column that are followed by the different letter are significant at (P<0.05)

ese chicken such as meat quality, growth rate and or improved egg performance Zhang et al. (2002), it may also be due to reproductive isolation (Chenyambuga et al., 2004).

Polymorphism of apoVLDL-II gene at VLDL9 and VLDL17 loci were significantly associated with body weight and abdominal fat weight in genetically lean chicken. It is known that VLDL is the major transporter of triglycerides, and attempts to reduce excessive fatness in poultry have involved the control of VLDL metabolism. Therefore, lean and fat chicken lines have been divergently selected for adipose tissue weight (Leclercq

et al., 1980) and for VLDL plasma concentration (Whitehead and Griffin, 1984). The combine analysis of fat and lean chicken shows apoVLDL-II gene was significantly ($P<0.05$) associated with body weight and fat weight at VLDL6, VLDL9 and VLDL10 loci. Previous studies carried in obese functionally castrated hens, observed that when plasma level of apo-VLDL-II was low, plasma LPL activity was elevated, because obese functionally castrated hen had lower ovarian weight and plasma apo-VLDL-II and higher post heparin plasma LPL activity than obese laying hen (Jaccoby et al., 1996). Plasma level of apo-VLDL-II was negatively correlated with postheparin plasma LPL activity. Zollitsch et al. (1997) reported that the fatty acid pattern of the abdominal fat was significantly influenced by the dietary fatty acid. Studies performed in lean and fat line by Leclercq et al. (1980) indicated that the difference in adiposity between lines was not the result of a difference in food consumption or in metabolic utilization of energy. Stearoyl-CoA desaturase activity and plasma VLDL concentration were found to be higher in the fat line (Legrand and Hermier, 1992), suggesting a higher lipogenesis rate in this line. Body Selection for high VLDL has increase the proportion of circulating VLDL- triglyceride taken up by the abdominal fat pad by over 2 fold, but there was no difference between high and low VLDL lines in the proportion of VLDL triglyceride taken up by tissues and oxidized to 14C –carbon dioxide (Griffin et al., 1991).

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