

Single strand conformation polymorphism in intron I of the chicken apoVLDL-II gene, and its relationship with triglyceride and very low density lipoprotein concentrations

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Abstract: Genetically fat and lean chickens reared identically in the same environment were used to investigate the effect of the apoVLDL-II gene (intron I) on total cholesterol, triglyceride, and lipoprotein concentrations. The overall allele frequency in the 2 populations was 0.59 for allele A and 0.41 for allele B. The allele frequency in the Rugao breed was 0.52 for allele A and 0.48 for allele B; however, in the Anka breed it was 0.65 for allele A and 0.35 for allele B. The apoVLDL-II genotype in the genetically fat Anka breed was associated with a significant increase in serum triacylglyceride concentration in sexually immature birds.

Key Words: PCR-SSCP, Apo VLDL-II, serum biochemical, chicken

Apo lipoprotein B-100 (1) and apo A-I (2) are the major apolipoproteins of chicken very low lipoproteins (VLDLs) and high-density lipoproteins (HDLs), respectively. They are the 2 main classes of lipoprotein particles that are synthesized and secreted by the liver. The triacylglyceride-rich lipoproteins travel from the liver, primarily to growing oocytes, and no appreciable hydrolysis of triacylglyceride occurs during this transport (3). At egg-laying onset in the chicken, plasma levels of the apolipoprotein VLDL-II (apoVLDL-II) increase dramatically, suggesting a function of apoVLDL-II in depositing triacylglyceride-rich lipoproteins in the yolk (3). When circulating levels of estrogen increase during egg laying the liver-specific estrogen-dependent very low-density apoVLDL-II gene is

expressed (4). Similarly, Yen et al. (5) reported that apoVLDL-II mRNA was highly expressed in laying ducks. A high cholesterol diet alters the composition of VLDLs, with cholesteryl esters substituting for triacylglycerols as the primary constituent of the lipid core. Like chylomicrons, VLDLs undergo constant change in the plasma. Our aim was to study the effect of polymorphisms within the apoVLDL-II gene on serum total cholesterol (TC), triacylglycerol (TAG), and HDL cholesterol (HDL-C) concentrations in sexually immature fat and lean chicken breeds.

The study included genetically fat (Anka) and lean (Rugao) chicken breeds (n = 118) that were reared under the same environment and management conditions at the Institute of Poultry

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Table. Effect of breed and apoVLDL-II genotype on cholesterol, triglyceride, and lipoprotein concentrations (mg/dl).

Population		(n)	TCH ¹	TG ²	HDL ³	VLDL ⁴	LDL ⁵
Anka	AA	18	147.91 ± 25.18	22.58 ± 10.38 ^a	92.69 ± 16.15	4.52 ± 2.07 ^a	50.69 ± 30.54
	AB	41	137.79 ± 27.91	17.60 ± 5.45 ^b	94.15 ± 23.75	3.52 ± 1.09 ^b	39.89 ± 31.15
Rugao	AA	3	193.86 ± 11.32	20.71 ± 6.35	135.22 ± 49.90	4.14 ± 1.27	54.50 ± 55.08
	AB	56	156.25 ± 38.53	20.27 ± 5.46	117.19 ± 30.29	4.05 ± 1.09	34.98 ± 35.63
Both populations	AA	21	154.62 ± 28.69	22.31 ± 9.80 ^a	98.76 ± 26.52	4.47 ± 1.96 ^a	51.24 ± 33.14
	AB	97	148.45 ± 35.48	19.14 ± 5.59 ^b	107.45 ± 29.86	3.83 ± 1.12 ^b	37.05 ± 33.73

^{a,b}Significant difference (P < 0.05) within a column.

¹Total cholesterol; ²triglycerides; ³high-density lipoproteins; ⁴very low-density lipoproteins; ⁵low-density lipoproteins.

Science of Jiangsu province, Yangzhou city, China. Each breed sample group contained 59 birds (30 male and 29 female). At 12 weeks of age the birds were subjected to overnight feed withdrawal to facilitate gut clearance, and then 5-ml blood samples were taken for DNA extraction and biochemical analysis of serum components. Total cholesterol (free cholesterol + cholesteryl esters) and TAG were assayed using a commercial enzymatic kit (Zhe Jiang Dongou Biological Engineering Co., Ltd.). HDL-C was detected enzymatically after precipitation of LDLs and VLDLs by heparin and manganese (6). VLDL cholesterol was estimated as triglycerides/5 and LDL cholesterol was estimated using the Friedewald equation, [LDL cholesterol = total cholesterol-HDL-C-triglycerides/5] (7).

Genomic DNA was isolated from whole blood using the saturated salt method (8). Oligo 6.0 software was used to design the primers for the apoVLDL-II gene at intron 1 based on its GenBank sequence (J00810). The forward primer was 5'CAC CTT TCT AAA TGC ACA GT3' and the reverse primer was 5'GCA ATG ATC TTC TGA ATG AC3'. PCR-SSCP analysis was carried out in a total volume of 20 µl of PCR reaction, containing 100 ng of template DNA, 13.3 µl of sterilized distilled water, 0.5 µl (5 pmol) of each primer, 1.5 µl 10× PCR buffer (Mg2plus), 2.5 µl of 2.5 mM dNTP mixture, and 5 U/µl of Taq polymerase (TakaRa Biotechnology Dalian Co., Ltd.). The PCR samples were initially denatured at 94 °C for 3 min, followed by 30 cycles at 94 °C for 30 s, annealing for 30 s at 72 °C, and final

extension at 72 °C for 8 min. The PCR products were electrophoresed in a 12% (39:1) polyacrylamide gel for 9 h at 150 V and then subsequently silver stained. Allele frequency was obtained according to the method described by Cerit et al. (9). The association between measured serum lipid concentrations and the apo VLDL-II genotype was determined with the general linear model (GLM) using SPSS v.11.5.

Two genotypes were detected with PCR-SSCP in each of the 2 chicken breeds. The overall allele frequency in the combined population was 0.59 for allele A and 0.41 for allele B. Allele frequency varied between the 2 chicken breeds. The Rugao breed possessed an allelic frequency of 0.52 for allele A and 0.48 for allele B, whereas in the Anka breed the allelic frequency was 0.65 for allele A and 0.35 for allele B.

It is known that the plasma lipoprotein profile of birds does not resemble that of mammals. In humans LDLs are the largest component of plasma lipoproteins, whereas in growing birds the largest component is HDLs (10,11). In the present study the AA apoVLDL-II genotype was detected in 30.5% of the Anka breed chickens and was associated (P < 0.05) with a 28% increase in serum TAG concentration at 12 weeks of age (Table). This same AA genotype was not associated with elevated serum TAG in the Rugao breed (2.3% increase over AB), albeit only 5% of the chickens studied had this genotype (Table). When genotype was used as the grouping variable, the AA genotype was again associated (16.6% increase, P < 0.05) with elevated

serum TAG when compared to the AB genotype; 18 of the 21 AA birds were the Anka breed (Table). In laying hens VLDLs are the most predominant lipoproteins, followed by HDLs and LDLs (12). This change is due to estrogen secreted by follicles stimulating liver lipogenesis (13,14). The combined analysis of Anka and Rugao breeds indicated that the AA genotype had a significantly ($P < 0.05$) higher level of TAG than did the AB genotype. The A allele in the Anka breed, which was selected as a fat

chicken breed in the present study, had a significant ($P < 0.05$) effect on serum TAG concentration, perhaps due to increased lipoprotein lipase activity. Hermier et al. (15) and Legrand and Hermier (16) reported higher plasma concentrations of TAG and VLDLs in fat chickens than in lean birds. In addition, SNP in the lipoprotein lipase gene was reported to be significantly ($P < 0.01$) different in Anka and Rugao chicken populations (17).

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