

Polymorphism in Intron-1 of ApoVLDL-II Gene and its Association with Meat Quality and Fatness Traits in Duck

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Abstract: Very Low Density apolipoprotein-II (apoVLDL-II) is a major constituent of Very Low Density Lipoprotein (VLDL) and is involved in lipid transportation in bird species. The current study was designed to investigate the associations of an apoVLDL-II gene intron-1 polymorphism on duck meat quality and fatness traits. The results showed that there were two alleles (A and B) and three genotypes (AA, AB and BB) in the experimental populations. AA genotype and A allele frequency were highest. Eight SNPs (T667C, C669G, T673C, G674A, G683A, G688A, C708G and T715G) and inserted/deleted GT after position 764 bp were found by sequencing. The apoVLDL-II gene intron-1 polymorphism was associated with multiple traits of meat quality and fatness in the 170 individuals, including pH-value, tenderness, water holding capacity, IMF, serum total cholesterol, serum triglyceride and abdominal fat. This research suggests that apoVLDL-II intron-1 has broad effects on meat quality and fatness traits in duck.

Key words: Duck, apoVLDL-II gene, polymorphism, meat quality, fatness traits, association analysis

INTRODUCTION

Although, intensive selection in duck has made great improvement in growth rate and feed efficiency, modern strains of ducks exhibit excessive body fat deposition. That is one of the main problems encountered by the duck industry today, because it has significant negative effects on feed efficiency and causes great economic loss to processing plants. The economic concern and recognition of consumer aversion to excess fatty tissue deposition have led commercial breeders to incorporate significant selection for reduced body fatness in their breeding programs. Therefore, considerable research effort has been applied to study factors associated with fat deposition and methods to reduce it (Wawro *et al.*, 2004). The candidate gene approach is a powerful method for finding the QTL responsible for genetic variation in traits of interest in agricultural animal species (Lamont *et al.*, 1996; Bai *et al.*, 2006). Similar to other economically important traits, most duck meat quality and fitness traits are controlled by multiple genes. Understanding the genetic control of meat quality and fitness traits in duck will provide an opportunity for genetic enhancement of production performance (Deeb and Lamont, 2002). The combination of traditional genotic and

breeding methods and modern molecular methods may be preferred for genetic improvement of duck in the future.

Very Low Density apolipoprotein-II (ApoVLDL-II) is a small phospholipid-binding protein (Binder *et al.*, 1990). It is a major polypeptide component of Very Low Density Lipoprotein (VLDL) and is involved in lipid transportation in avian. Its function is the transport of neutral lipids in the form of very low density lipoprotein in the plasma. The apoVLDLII gene is normally dormant in males but can be activated by the administration of estrogen and specifically expressed in liver (Pinchasov *et al.*, 1994; Tejada *et al.*, 1999). The expression of ApoVLDL-II protects ApoVLDL-II containing very low density lipoprotein so that it can be transported to the ovary and not hydrolyzed by lipoprotein lipase in other peripheral tissues in laying birds. Laying may affect particular aspects of lipid metabolism, especially biochemical pathways in which ApoVLDL-II and HMG-CoA reductase are involved (Yen *et al.*, 2005). Thus, ApoVLDL-II is a candidate gene for traits related to fat deposition and then it affect on meat quality possibly. However, up to now, the research about SNPs of the ApoVLDL-II gene in duck was not reported. The objectives of the present study were to identify SNPs in the duck ApoVLDL-II gene intron-1 and to evaluate associations between the polymorphisms and meat quality and fatness traits in three duck populations.

MATERIALS AND METHODS

Samples collection and preparation: Blood samples were collected from 170 individuals belongs to three duck populations: Cherry Valley duck, (CV) (55), Jinding Duck, (JD) (55) and Hybrid duck (CV x JD) (HB) (60). The blood samples were collected from College of Jiangsu Animal Husbandry and Veterinary. Ducks were reared in the same management system. The 170 ducks were slaughtered at the end of 70 days. The meat quality and fatness traits such as pH, tenderness (shear value) (kg cm⁻²), meat colour (OD₅₄₀), water holding capacity (%), Intramuscular Fat (IMF) (%), serum total cholesterol (mmol L⁻¹), serum triglyceride (mmol L⁻¹) and abdominal fat (%) in these ducks were collected for statistical analysis. Genome DNA was obtained by phenol and chloroform (1:1) extraction and stored at -20°C.

Primer design and PCR amplification: Based upon duck ApoVLDL-II gene sequences (Accession no. GQ180104), four pair primers (Table 1) were designed using the Oligo 6.0 program to amplify the duck ApoVLDL-II gene intron-1 and detect the single nucleotide polymorphisms. Following optimization, PCR reactions were carried out in a total volume of 25 µL with 100 ng of genomic DNA, 5 pmol of each of forward and reverse primer, 2.5 µL of 10 × buffer, 1.5 mM of MgCl₂, 0.16 mM of dNTP and 1 U of Taq DNA polymerase (Fermentas). PCR program: initial denaturation for 10 min at 95°C, 30 cycles each 45 sec at 94°C, 45 sec at 58°C and 45 sec at 72°C and 10 min final extension at 72°C.

Single Stranded Sonformation Polymorphism (SSCP) and sequencing: Aliquots of 5 µL PCR products were mixed with 10 µL denaturing solution (98% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and cooled on ice. Denatured PCR products were subjected to 10% acrylamide: bisacrylamide (39:1) gels in 1× TBE buffer and constant voltage (130 V) for 12-14 h. The gel was stained with 0.1% silver nitrate. PCR fragments from different

SSCP patterns in different populations were sub-cloned to T-vector (Takara, China). All fragments were sequenced in both directions.

Statistical analysis: χ²-tests were used to determine if the individual variant was in Hardy-Weinberg equilibrium. The genotype and allele frequency distributions were compared by χ²-test. The association between apoVLDL-II gene intron-1 and meat quality and fatness traits was evaluated according to follow model:

$$Y = \mu + G + B + G \times B + e$$

Where:

- Y = Dependent variable (analyzed traits)
- μ = Overall mean
- G = Genotype of apoVLDL-II intron-1 (AA, AB and BB)
- B = Duck population
- G × B = Interactions between genotype and duck population (all fixed effects)
- e = Random error

Difference between genotypes was determined by least square analysis.

RESULTS AND DISCUSSION

SSCP in intron-1 of the apoVLDL-II gene: Amplicons of the expected size were obtained from duck DNA using different primers. These were identified by PCR-SSCP method. The results of primer A1, A3 and A4 showed no polymorphism. The amplicons of primer A2 were approximately 285bp in size and exhibited polymorphism in different individuals and populations. Three band patterns could be identified in intron-1 of apoVLDL-II gene (Fig 1), which were the products of two alleles (A and B).

DNA sequencing: The sequencing results were compared with the intron-1 sequence of duck (Accession no. GQ180104) and showed: BB genotype sequences and GQ180104 was identical was defined as mutant-type, AA genotype as wild-type. Alignment sequences results

Table 1: Primers for ApoVLDL-II gene intron-1 and PCR analysis parameters established for duck

Primers	Primer sequence (5'→3')	Fragment length (bp)	Annealing temperature (°C)
A1	F: GGACCTTTGACCCCTCACTA R: GCCCTCCTATGAGTGTCCCTATG	260	58
A2	F: GCCACTACCTGGAAACAGGATT R: CAGCCTCATCCAAACATCCTT	285	55
A3	F: GCAATGTCATCTTTTCGATG R: TTGCAAAATGGCTTAACGAA	280	58
A4	F: TGTAACCTGCCACAGATTCCCTG R: ATGGTTGCGGTACCAGTTC	287	56

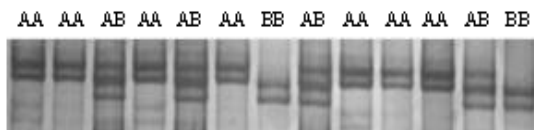


Fig. 1: PCR-SSCP band pattern on a 10% non-denaturing polyacrylamide gel

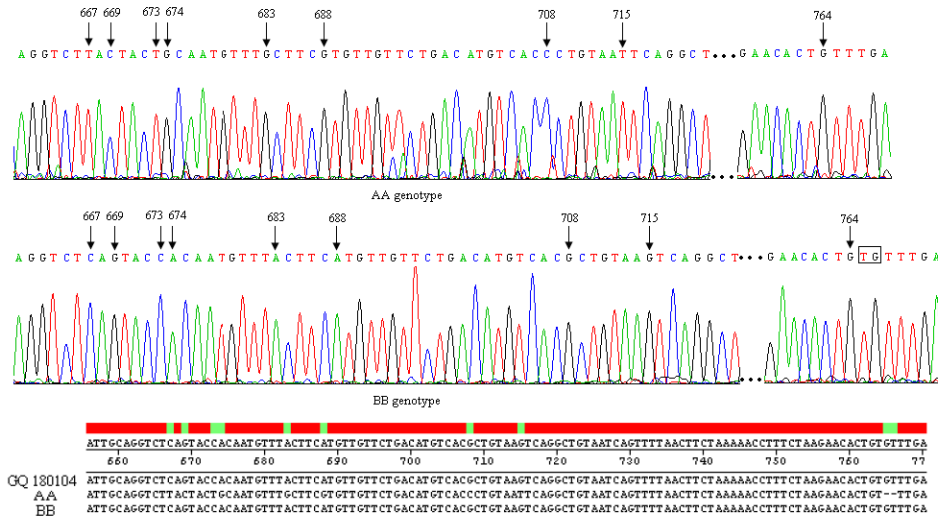


Fig. 2: The sequencing result of AA and BB genotypes (The arrows indicated mutation positions, the inserted/deleted fragments were bold with the frame)

Table 2: Allele and genotype frequencies of the SNP in intron-1 of apoVLDL-II gene in three duck populations

Population	Genotype frequencies			Allele frequencies					
	AA	AB	BB	A	B	h	Ne	PIC	χ^2 (HWE)
CV	0.8182	0.0545	0.1273	0.8455	0.1545	0.2612	1.3535	0.2270	12.93**
JD	0.4909	0.0727	0.4364	0.5273	0.4727	0.4986	1.9944	0.3744	25.43**
HD	0.5500	0.4500	0.0000	0.7750	0.2250	0.3488	1.5356	0.2880	3.88

CV = Cherry Valley duck; JD = Jinding Duck; HB = Hybrid duck of CV x JD; h = Heterozygosity; Ne = Effective number of alleles; PIC = Polymorphism Information Content; χ^2 (HWE) = Hardy-Weinberg Equilibrium χ^2 value, **($p < 0.01$)

Table 3: Least square analysis between intron-1 of apoVLDL-II and meat quality and fatness traits in duck

Traits	Genotype AA (n = 105)	Genotype AB (n = 34)	Genotype BB (n = 31)
pH-value	6.00±0.03 ^a	5.96±0.04 ^a	6.21±0.04 ^b
Meat colour (OD ₅₄₀)	1.04±0.04	1.00±0.06	1.15±0.06
Shear value (kg cm ⁻²)	1.99±0.07 ^a	2.26±0.11 ^b	2.09±0.11 ^{ab}
Water holding capacity (%)	18.82±0.62 ^a	15.20±0.96 ^b	14.42±0.98 ^b
Intramuscular Fat (IMF) (%)	8.80±0.30 ^a	7.23±0.45 ^b	7.48±0.50 ^b
Serum total cholesterol (mmol L ⁻¹)	5.75±0.27 ^a	4.87±0.43 ^b	6.75±0.42 ^c
Serum triglyceride (mmol L ⁻¹)	1.06±0.27 ^a	1.09±0.43 ^a	1.39±0.42 ^b
Abdominal fat (%)	1.27±0.30 ^a	0.77±0.48 ^b	0.92±0.52 ^{ab}

abc: Significantly different (LSD test, $p < 0.05$) in genotypes AA, AB and BB; n: Number of the genotype in the population

showed that eight SNPs, T667C, C669G, T673C, G674A, G683A, G688A, C708G and T715G and one insertion/deletion (the inserted/deleted GT after position 764 bp) were found in intron-1 in 170 duck from three populations (CV, JD and HD) (Fig. 2). Therefore, there are multiple mutations in the region from 667-764 bp.

Allele and genotype distribution: In three duck populations, genotype frequency of AA was highest. The A allele frequencies for the intron-1 of apoVLDL-II gene in the analyzed populations were 0.8455, 0.5273 and 0.7750 for CV, JD and HD populations, respectively. The genotype distributions of CV and JD in disagreement with Hardy-Weinberg equilibrium ($p < 0.05$) except HD

population (Table 2). The locus belonged to medium polymorphism in JD and HD populations ($0.25 < PIC < 0.5$). For CV population, it was low polymorphism ($PIC < 0.25$).

Allelic effect of the apoVLDL-II gene intron-1 on some meat quality and fatness traits: The allelic effects of the apoVLDL-II Gene intron-1 on some meat quality and fatness traits in 170 samples of three duck populations were shown in Table 3. For meat quality and fatness traits with significant differences between genotypes, the allelic effect of pH-value, shear value, Water holding capacity, IMF, serum total cholesterol, serum triglyceride and abdominal fat acted in a dominant mode. The bird with homozygote (AA) had significant higher than

Table 4: Effects (P-value) of polymorphism of apoVLDL-II gene intron-1 on duck meat quality and fatness traits

Trait	Genotype	Population	Genotype x Population
pH-value	<0.001	<0.001	<0.001
Meat colour (OD ₅₄₀)	0.303	0.113	0.041
Shear value (kg cm ⁻²)	0.022	<0.001	<0.001
Water holding capacity (%)	<0.001	<0.001	<0.001
Intramuscular fat (%)	0.004	<0.001	<0.001
Serum total cholesterol (mmol L ⁻¹)	<0.001	<0.001	<0.001
Serum triglyceride (mmol L ⁻¹)	0.002	<0.001	<0.001
Abdominal fat (%)	0.015	<0.001	<0.001

heterozygotes (AB) and homozygotes (BB) for waterholding capacity and IMF. However, for shear value, the birds with heterozygote (AB) had significant higher than homozygotes (AA). The birds with homozygotes (BB) had significant higher than homozygotes (AA) and heterozygote (AB) for pH-value, serum triglyceride and serum total cholesterol. For abdominal fat and serum total cholesterol, the homozygotes (AA) had significant higher than heterozygotes (AB). Furthermore, no significant association of different genotypes with other traits were detected ($p>0.05$). The results indicated that the locus linked strongly with the QTL controlling meat quality and fatness traits. Therefore, it was presumed that it may have a QTL controlling fatness and meat quality in the region.

Interaction between the apoVLDL-II gene intron-1 and genetic background of three duck populations: The interactions between the genotypes of apoVLDL-II gene intron-1 and genetic background of three duck populations were showed in Table 4. Only the meat colour had not genotype and population effect ($p>0.05$) and then other traits were significant genotypes and populations and genotype x population effects ($p<0.05$).

The apoVLDL-II is a member of the avian yolk protein gene family (Wiskocil *et al.*, 1981). The apoVLDL-II gene promoter contains many regulatory elements that have been previously related with gene expression. The apoVLDL-II gene also contains sequences within its first intron that increase transcription (Schippers *et al.*, 1994). By using in vitro DNaseI footprinting, 6 protein-binding sites were revealed throughout the first intron and those protein-binding sites effect expression of apoVLDL-II gene and other correlative gene (Shuler *et al.*, 1998). At the same time, Li *et al.* (2005) found a G634A mutation (GenBank accession no: V00448) in the first intron of the apoVLDL-II gene and the G/A mutation has broad effects on growth and development in the chicken. Cheng *et al.* (2008) reported four mutation (G950 A, A 2522 C, A2705 C and C2793T) in intron-1 and the 4 SNPs have remarkable genetic effect for the meat quality (tenderness and pH) in chicken. In this study, we found

8 SNPs, T667C, C669G, T673C, G674A, G683A, G688A, C708G and T715G and one insertion/deletion (the inserted/deleted GT after position 764 bp) in intron-1 of apoVLDL-II gene in three duck populations (170 individuals), but only which resulted in 3 genotypes: AA, AB and BB. In three duck populations, AA genotype and A allele frequency were highest. The genotype distributions of CV and JD in disagreement with Hardy-Weinberg equilibrium ($p<0.05$). The locus belonged to medium polymorphism in JD and HD populations ($0.25<PIC<0.5$). For CV population, it was low polymorphism ($PIC<0.25$). One explanation could be they were suffered intensive selection during the long term commercial breeding. In selected populations, deviations of genotype frequencies from the Hardy-Weinberg equilibrium should be expected for loci with impact on traits under selection.

Poultry meat quality and fatness traits are very important to keep up with consumer demand. Therefore, different methods have been used to reduce fatness and improve meat quality in poultry (Mullen *et al.*, 2006). The candidate gene approach is a very powerful method to investigate associations of gene polymorphisms with economically important traits in farm animals. Many studies showed that apoVLDL-II gene play a important role in lipid transportation in poultry (Zsigmond *et al.*, 1995; Berkowitz and Evans, 1992; Schippers *et al.*, 1994). In this study, the apoVLDL-II gene was selected as a candidate gene to investigated associations of gene polymorphisms with some meat quality and fatness traits in three duck populations. The results indicated that breed and genotype has significant effect in these metrical indexes except meat colour by using fixed model. For pH-value, water holding capacity, Intramuscular Fat (IMF), serum total cholesterol and serum triglyceride, there had significant difference between AA and BB genotype. The polymorphism locus linked strongly with the QTL controlling meat quality and fatness traits. This result was consistent with the study in chicken (Cheng *et al.*, 2008). So, we conjecture the apoVLDL-II gene may be a major candidate gene or linked to major candidate gene that impact duck lipid metabolism

and the SNPs could be used in Molecular Assistant Selection (MAS) as a genetic marker for duck fatness traits. However, an explanation for the results was also probably due to the low animal numbers. We will further test and verify to enlarge the number of samples in the future.

CONCLUSION

In this study, eight SNPs, T667C, C669G, T673C, G674A, G683A, G688A, C708G and T715G and one insertion/deletion (the inserted/deleted GT after position 764 bp) in apoVLDL-II gene intron 1 was found in three duck populations (CV, JD and HD), which resulted in three genotypes (AA, AB and BB).

There have been significant breed and genotype effect on pH-value, shear value, water holding capacity, IMF, serum total cholesterol, serum triglyceride and abdominal fat except meat colour by using fixed model. This results suggests that apoVLDL-II intron-1 has broad effects on meat quality and fat metabolism in duck.

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