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Research Article
Association of ApoVLDLII Gene Polymorphism with Body Composition Traits in Kampung Chicken

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Abstract

Background and Objective: Very low density lipoproteins (VLDLs) is a major class of lipoprotein particles that is synthesized and secreted by the liver. Selection for economic traits based on molecular marker assisted selection are required to increase production performance. The present study was designed to analyze associations of very low density Apo lipoprotein II (ApoVLDLII) gene polymorphisms with body composition traits. Kampung chicken, a native chicken in Indonesia, is slow-growing chicken. **Materials and Methods:** A total of 76 male Kampung chickens were used in the current study. Body compositions were measured in 12 and 26 weeks of age. Primers for intron 1 region were designed from genomic chicken sequence. A G634A SNP of the ApoVLDLII gene intron 1 region was detected and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was then used for genotyping of Kampung chicken population. **Results:** The ApoVLDLII polymorphism was significantly associated with body, carcass, breast, thigh, back and thigh muscle weight in 26 weeks old Kampung chicken population ($p<0.05$). In 12 weeks old Kampung chicken, ApoVLDLII polymorphism was not significantly associated with body composition traits. **Conclusion:** It is concluded that the ApoVLDLII gene could be a candidate gene that affects growth and body composition traits in chicken.

Key words: Kampung chicken, body composition, ApoVLDLII gene, polymorphism

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Kampung chicken is a native chicken in Indonesia. Kampung chickens spread out in all regions in Indonesia. Most of Kampung chickens are frequently bred with traditional management. Kampung chickens have an important role in supplying of egg and meat. Commonly, Kampung chickens are slow growing and lean meat type chickens[1]. The meat and eggs taste of Kampong chickens are appreciated by public and the production cost is relatively cheaper than another chicken. In addition, Kampong chickens have a good adaptation to the tropical climate in Indonesia[2].

Identifying candidate genes related to economic traits will provide an opportunity for genetic improvement in breeding programs. Recent studies in molecular genetics and genomic technologies have led to the discovery of genes associated with growth and meat quality traits[3]. A polymorphic locus associated with growth and body composition traits of animals can be used as genetic markers. These markers can be used as a criterion of selection for genetic improvement[4].

Very low density lipoproteins (VLDLs) and high density lipoproteins (HDLs) are the 2 major classes of lipoprotein particles that are synthesized and secreted by the liver[5]. The regulation of the assembly and secretion of VLDL comprises a complexity appropriate for the creation of a large, the multimolecular transport vehicle that help to maintain nutrient homeostasis in the liver[6]. The proportion of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) particles in meat type cockerel chickens is smaller than high density lipoprotein (HDL), with LDL exceeding that of VLDL[7]. Very low density apolipoprotein (ApoVLDLII) is a major component of VLDL fraction of hen serum. This protein binds lipoprotein and forms an outer polar shell surrounding the water-insoluble lipid core[8]. Association of polymorphisms in avian ApoVLDLII with growth, body composition and meat quality had been reported[9-11].

The objectives of the current study were to identify an SNP in ApoVLDLII gene intron 1 using PCR-RFLP and evaluate associations between ApoVLDLII SNP and body composition traits on different ages of Kampung chickens.

MATERIALS AND METHODS

Experimental populations and management: Kampung chicken, a native chicken in Indonesia, is slow-growing chicken. A total of 48 males at 26 weeks of age and 28 males at 12 weeks of age were used in the current study. All chickens had access to feed and water *ad libitum*. From hatch to 8 weeks of age, Kampung chickens received a starter feed (4,080 kcal of gross energy kg^{-1} and 19.03% of crude protein) and from 9-24 weeks of age, Kampung chickens were fed a grower diet (4,001 kcal of gross energy kg^{-1} and 17.42% of crude protein).

Phenotypic measurements: Body weight and body composition traits were recorded at 12 and 26 weeks of age. These measurements included body weight (BW), carcass weight (CW), breast weight (BrW), breast muscle weight (BrMW), thigh weight (ThW), drumstick weight (DrW), back weight (BcW), wings weight (WW), thigh muscle weight (ThMW) and drumstick muscle weight (DrMW).

DNA isolation and PCR amplification: Blood samples were collected from Vena axillaris. Genomic DNA were isolated according to Sambrook *et al.*[12].

The PCR primers of ApoVLDLII gene intron 1 used in this study were based on previous study conducted by Li *et al.*[9]. This primers (5' CCT CTA TGA CAT GGT TGC CT 3' and 5' ATG GGT TTG ACC CTG CTA TG 3)' were designed to amplify a 492 bp fragment by oligo 5 according to the chicken genomic sequence in the GenBank database (accession number V00448). The PCR reaction conditions were 94°C for 5 min, 35 cycles of 94°C for 10 sec, 60°C for 20 sec, 72°C for 30 sec and an extension at 72°C for 5 min. The 25 μL reaction volume included 50 ng of DNA template, 1×reaction buffer, 5 pmol of each primer, 0.16 mM of deoxyribonucleotide triphosphate, 1.5 mM of $MgCl_2$ and 1 U of *Taq* polymerase.

Screening of the population for restriction enzyme-detectable SNP: A single nucleotide polymorphism (SNP) of the ApoVLDLII gene was detected by digesting 7 μL of the 492 bp PCR product with 3 U of the *Sfc*I enzyme (Thermo Fisher Scientific Inc.) at 37°C overnight. The restriction digests were electrophoresed for 45 min at 100 V on a 2.0% agarose gel with ethidium bromide. Individual PCR-RFLP fragment sizes for the gene were determined by visualizing the band pattern under UV Transilluminator (AlphaImager® EP).

Statistical analysis: The association between the polymorphism and body composition traits were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC). The model was fitted with the genotype (G, 2 levels) as fixed effects, as follows:

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

where, Y is the dependent variable for trait measured in the population, μ is the overall population mean for traits, G is the fixed effects and *e* is the random error. Significant differences between means of the different genotypes were calculated using the t-test. Significance was determined as $p<0.05$, unless otherwise specified.

RESULTS AND DISCUSSION

In this study, one pair of specific primers was used to amplified 492 bp of DNA fragment of ApoVLDLII intron 1. There was a G/A SNP at base 634 (accession number V00448). The PCR-RFLP method was used successfully for genotyping the SNP in intron 1 of the chicken ApoVLDLII gene. Two genotypes were detected and defined as GG and AG (Fig. 1). The 492 bp fragment was digested with *Sfc*I restriction enzyme. The digested PCR products had fragment sizes of 396 and 96 bp for the GG genotype and a combination of 492, 396 and 96 bp for AG genotype.

According to previous study[9], the ApoVLDLII could be used as a criterion of selection in poultry breeding program to increase growth and body composition traits. Recent advances in molecular genetics and genomic technologies have led to the discovery of genetic markers associated with growth and body composition traits[13,14].

The ApoVLDLII is a small phospholipid binding protein synthesized in the liver[15]. This protein is also detectable in plasma and liver of normal young cockerels[16]. This protein contains 82 amino acid residues with a single cysteine at residue number 75[17]. The particle size of ApoVLDLII is 46.8±8.6 nm[18]. The ApoVLDLII gene is comprised of four exons and three introns which span 2.9 kilobases of DNA[19].

The genotype and allele frequencies were calculated in two groups of Kampung chicken (Table 1). Genotype AA was not found and there were only two genotypes (AG and GG) found in these population. The G allele was more frequent than A allele in two groups of kampung chicken. This was similar to the results of Seyedabadi *et al.*[20], in Iranian commercial broiler lines.

The association of ApoVLDLII gene polymorphism and body composition in 12 and 26 weeks old kampong chicken is summarized on the Table 2 and 3, respectively. The ApoVLDLII polymorphism was generally significantly associated with body composition in 26 weeks old chicken. There were significant association exist between the ApoVLDLII polymorphism and BW, CW, BrW, ThW, BcW and ThMW in 26 weeks old chicken. No significant difference was observed in DrW, WW, BrMW and DrMW. The heterozygous (AG) were significantly higher than the homozygous (GG) in BW, CW, BrW, ThW, BcW and ThMW.

Table 1: Genotype and allele frequencies of ApoVLDLII intron1 in two groups of Kampung chicken

		Genotype frequency			Allele frequency	
Population	Number	AA	AG	GG	A	G
12 weeks old chicken	28	0.000	0.250	0.750	0.125	0.875
26 weeks old chicken	48	0.000	0.208	0.792	0.172	0.828

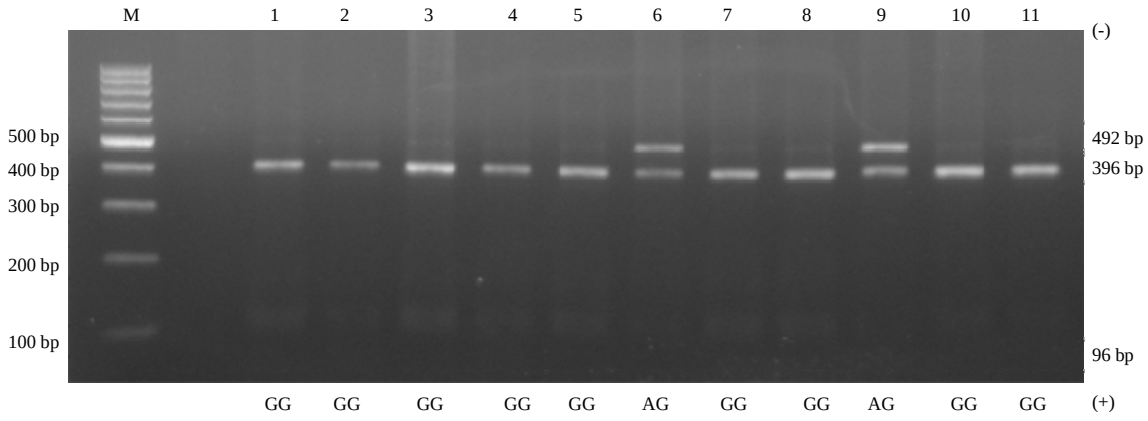

Fig. 1: PCR-RFLP pattern for ApoVLDLII gene intron 1 region with *Sfc*I restriction enzyme
M = 100 bp markers, 1-5,7,8,10,11 = GG genotype, 6, 8 = AG genotype

Table 2: Effect of the ApoVLDLII genotype on body composition traits in 12 weeks old male Kampung chicken

	Genotype	
Traits[1]	GG (21)[2]	AG (7)
BW[3]	769.43±23.05	771.57±44.20
CW	443.76±20.15	448.29±27.81
BrW	113.67±5.44	111.29±7.28
ThW	82.57±3.47	85.29±6.19
DrW	80.19±3.39	82.14±5.36
WW	72.43±2.65	75.14±2.41
BcW	111.95±4.9	111.29±9.36
BrMW	65.43±3.57	64.57±4.42
ThMW	48.00±2.80	47.43±4.09
DrMW	44.29±1.94	44.00±2.53

[a,b]Means within a row with no common superscript are different (p<0.05). [1]BW: Body weight, CW: Carcass weight, BrW: Breast weight, ThW: Thigh weight, DrW: Drumstick weight, WW: Wings weight, BcW: Back weight, BrMW: Breast muscle weight, ThMW: Thigh muscle weight, DrMW: Drumstick muscle weight, [2]Numbers shown in parentheses are the number of individuals with the specified genotype, [3]All of traits are in grams, Values are expressed as Mean±SE

Table 3: Effect of the ApoVLDLII genotype on body composition traits in 26 weeks old male Kampung chicken

	Genotype	
Traits[1]	GG (38)[2]	AG (10)
BW[3]	1566.20±13.16[a]	1662.25±50.96[b]
CW	988.76±12.34[a]	1076.10±44.61[b]
BrW	251.61±3.41[a]	273.20±10.91[b]
ThW	192.66±3.32[a]	212.10±11.59[b]
DrW	181.08±2.57	194.50±9.60
WW	128.76±1.63	131.10±4.12
BcW	242.89±5.85[a]	269.90±10.50[b]
BrMW	175.79±3.58	188.45±9.80
ThMW	140.52±3.39[a]	159.85±9.51[b]
DrMW	120.14±2.75	130.45±7.11

[a,b]Means within a row with no common superscript are different (p<0.05). [1]BW: Body weight, CW: Carcass weight, BrW: Breast weight, ThW: Thigh weight, DrW: Drumstick weight, WW: Wings weight, BcW: Back weight, BrMW: Breast muscle weight, ThMW: Thigh muscle weight, DrMW: Drumstick muscle weight. [2]Numbers shown in parentheses are the number of individuals with the specified genotype, [3]All of traits are in grams, Values are expressed as Mean±SE

No significant association was found between the ApoVLDLII gene polymorphism and body compositions in 12 weeks old chicken. The possible reason may be due to the fact that this gene is not expressed in 12 weeks old chicken. According to the expression gene study in chicken[21], ApoVLDLII was not expressed in chicken adipose tissue at 7 weeks old chicken. In the study, although this gene was not detected in chicken adipose, it may regulate lipid metabolism indirectly. In addition, ApoVLDLII gene expression was also investigated in chicken hepatocytes[22].

The association of ApoVLDLII gene polymorphism in intron 1 with growth and body composition traits in Iranian commercial broiler lines was investigated by Seyedabadi *et al.*[20]. The polymorphism of ApoVLDLII gene intron 1 was significantly associated with body weight at 6 weeks, carcass weight, breast muscle weight, drumstick weight and wing weight. The effect of breed, ApoVLDLII gene polymorphism and metabolic biochemical markers on growth and body composition traits in four breeds of commercial broiler was reported by Ghanem *et al.*[4]. The restriction enzyme *Bfm*I was used to disgest PCR products. The SNP was significantly associated with weight, gain, feed intake, feed efficiency, dressing percentage and giblets weight. Both of their results showed that the heterozygous were significantly higher than the homozygous. This was similar to the result in this study.

In another study, Musa *et al.*[23] reported the significant relationship of ApoVLDLII gene polymorphism intron 1 and triglyceride and VLDL concentration using single strand conformation polymorphism (SSCP) in Rugao and Anka chickens. Additionally, another SNP in exon 4 of ApoVLDLII gene was associated with meat tenderness in Anka and Rugao chickens[11].

CONCLUSION

The ApoVLDLII gene polymorphism in two populations of Kampung chicken were polymorphic. The genotype AA was not found in two different ages of Kampung chicken. The ApoVLDLII gene polymorphism intron 1 was significantly associated with body composition traits in 26 weeks old Kampung chicken. The SNP can be used as a potential marker to enhance the genetic improvement for breeding program of Kampung chicken.

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