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Association between polymorphism in the 5' UTR region of the ACACB gene and LDL concentration in layer chickens

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Abstract

This study aimed to identify polymorphisms in the 5' untranslated region (UTR) of the Acetyl-CoA Carboxylase Beta (ACACB) gene and assess their association with low-density lipoprotein (LDL) levels in the IWI line of White Leghorn layer chickens. A total of 250 birds were analyzed using the single-strand conformation polymorphism (SSCP) technique. The findings demonstrated a significant relationship between ACACB gene polymorphisms and serum LDL concentration. Among the identified haplogroups, birds with the h7h7 haplogroup showed the highest LDL levels (373.30 ± 2.21), while those with the h3h3 haplogroup had the lowest levels (51.41 ± 1.02). Overall, the results indicate that the promoter region of the ACACB gene is highly polymorphic and plays a significant role in influencing serum LDL concentration in White Leghorn layer chickens.

Keywords: ACACB gene, Polymorphism, SSCP, LDL concentration

1. Introduction

Excessive fat accumulation negatively impacts body composition as well as the quality of meat and eggs in poultry. In chickens, fat is mainly deposited beneath the skin, around internal organs, and in the abdominal area near the gonads. An increased amount of white adipose tissue lowers both the nutritional and economic value of poultry products. Furthermore, excessive fat deposition is often linked to unfavorable traits arising from selection for rapid growth, such as reduced fertility and weakened immune function, resembling conditions observed in obesity in humans^[1]. High fat content in poultry products may also pose potential health risks to consumers². Obesity is a significant global public health issue that elevates the risk of numerous chronic conditions, including type 2 diabetes, cardiovascular diseases, metabolic syndrome, hypertension, and certain forms of cancer^{3,4}. However, excessive fat deposition in modern poultry strains remains a major challenge for the poultry industry⁵. For example, studies have shown that modern broiler strains possess approximately 15–20% body fat, of which over 85% is not required for normal physiological functions⁶. Therefore, the poultry industry needs effective strategies to reduce fat accumulation in chicken meat and eggs, which would enhance economic returns for producers while improving the health and welfare of both birds and consumers. Additionally, laying hens are prone to excessive fat deposition, which can negatively impact their reproductive performance⁷. Genes and molecular pathways involved in regulating abdominal fat deposition and adipose tissue development can be identified through a range of genomic approaches^{8,9,10,11,12,13}. ACACB was initially identified in rat heart tissue¹⁴ and is located on chromosome 15, according to NCBI. The ACACB protein (approximately 280 kDa) is primarily expressed in non-lipogenic tissues such as skeletal and cardiac muscle, with lower levels of expression observed in the liver^{15,16,17,18}. Malonyl-CoA generated by ACACB functions as an inhibitor of carnitine palmitoyltransferase 1 (CPT1), thereby controlling the transport of fatty acyl groups into the mitochondria through the carnitine shuttle system for β -oxidation^{19,20,21}. This leads to decreased fatty acid oxidation

and enhanced synthesis of fatty acids and triglycerides, often at the expense of glucose utilization. Studies have demonstrated that mice deficient in ACC2 show increased fatty acid oxidation along with reduced fat accumulation²².

2. Materials and Methods

2.1 Location

The present study was carried out at the ICAR–Directorate of Poultry Research (DPR), Hyderabad, Telangana, India. Hyderabad is situated in the central part of Telangana and spans an area of about 260 km². The city is geographically located at 17.366° N latitude and 78.476° E longitude. It experiences a tropical wet and dry climate, with distinctly hot summers and mild winters. The average daily temperature ranges from 30 °C to 36 °C during April to June and from 20 °C to 24 °C during December to January.

2.2 Experimental birds

The present study was carried out on the IWI lines of the White Leghorn chicken breed. Birds from the selected pure lines were developed through pedigree mating using artificial insemination. Day-old chicks were vent-sexed, wing-banded, and reared in an open-sided deep litter system up to 16 weeks of age. During the brooding period, they were fed a layer chick starter diet containing 2,600 kcal/kg metabolizable energy (ME) and 18% crude protein (CP) until 8 weeks of age, followed by a grower diet with 2,500 kcal/kg ME and 16% CP from 9 to 16 weeks. After 16 weeks, the birds were shifted to individual cages for recording body weights and were provided with a layer ration containing 2,600 kcal/kg ME and 16% CP from the onset of egg production until the conclusion of the experiment. A 16-hour lighting regimen, including natural daylight, was maintained throughout the laying period. All birds were reared under uniform management conditions with unrestricted access to water. On the day of hatch, chicks were vaccinated against Marek's disease and later immunized against major diseases such as Ranikhet disease (RD), infectious bursal disease (IBD), and fowl pox as per the standard vaccination schedule. During the summer months, cooling was provided through roof water sprinkling, and adequate lighting was maintained within the poultry house. In addition, all other management practices, including strict biosecurity measures, were diligently followed in and around the sheds to ensure a conducive environment for optimal bird performance.

2.3 Collection of samples

2.3.1 Blood samples

A total of 250 birds from the IWI line were used in the present study to investigate polymorphisms in the promoter

region of the acetyl-CoA carboxylase beta (ACACB) gene. About 0.5–1.0 ml of blood was aseptically collected from the wing vein of each bird into sterile 2 ml polypropylene centrifuge tubes containing 2.7% ethylenediaminetetraacetic acid (EDTA) (60–70 µl per 1 ml of blood) as an anticoagulant. Following collection, the tubes were securely capped and gently inverted several times to ensure thorough mixing of blood with the anticoagulant. The samples were immediately placed in an icebox with ice and gel packs and transported to the laboratory without delay. Upon arrival, all samples were stored at –20°C until DNA extraction.

2.3.2 Serum samples

Approximately 1–2 ml of blood was aseptically collected from the wing veins of 250 IWI line birds into sterile 2 ml polypropylene centrifuge tubes. The collected samples were subsequently used for the association study.

2.4 PCR-SSCP

The 5' upstream region of the ACACB gene (534 bp) located near the transcription start site was divided into two non-overlapping fragments, designated ACACB-S1 and ACACB-S2. These fragments were analyzed using the single-strand conformation polymorphism (SSCP) technique. The sensitivity of SSCP is inversely proportional to fragment size, with single base-pair variations detectable in approximately 99% of cases for fragments of 100–300 bp and in more than 80% of cases for fragments around 400 bp, as reported previously^[23]. Primers for amplification of these regions were designed using the chicken ACACB gene sequence (Accession No. NC_006102) (Table 1).

Table 1: Details of primers used for cloning of ACACB gene promoter.

Primer name	Primer Sequences (5'-3')	Amplicon Size (bp)
ACACB-P1	F: AGATCTAGGTGAGCTCCATGGCACTG R: GAATTCCTGTGGGACAAAGGTGCCATG	534
ACACB-P2	F: AGATCTTCTGCTGCTGTCAACTTGATG R: GAATTCCTGTGGGACAAAGGTGCCATG	1518

Polymerase chain reaction (PCR) amplification was carried out in 0.2 ml PCR tubes. Each reaction mixture contained 1 µl of 10X DreamTaq buffer, 0.5 µl of 2.5 mM dNTP mix, 1 µl each of forward and reverse gene-specific primers (20 ng/µl), 0.2 µl of Taq DNA polymerase (5 U/µl), and 1 µl of genomic DNA as the template, with nuclease-free water added to achieve a final volume of 10 µl. The thermal cycling conditions employed are summarized in Table 2.

Table 2: PCR amplification conditions followed for ACACB gene promoter fragment.

Fragment	Thermal cycling conditions			
	Initial denaturation at 95 ^[0] C for 10 minutes	Cycles (35)		
		Denaturation	Annealing	Final Extension
ACACB-P1	95 ^[0] C for 10 minutes	95 ^[0] C for 30 seconds	62 ^[0] C for 40 seconds	72 ^[0] C for 40 seconds
ACACB-P2		95 ^[0] C for 40 seconds	64 ^[0] C for 40 seconds	72 ^[0] C for 1 minute 30 sec

2.5 Genotyping

Genotyping by single-strand conformation polymorphism (SSCP) analysis was employed to detect polymorphisms in the promoter region of the chicken ACACB gene. A 12% polyacrylamide gel was prepared using 9.6 ml of acrylamide: bisacrylamide (49:1), 28.3 ml of 1X Tris-borate-EDTA (TBE) buffer, 1.5 ml glycerol, 100 µl of 10% ammonium persulfate

(APS), and 75 µl tetramethyl ethylenediamine (TEMED). PCR products were first denatured at 95°C for 5 minutes and immediately chilled on ice for 15 minutes to maintain single-stranded DNA. The denatured samples were then loaded onto the gel, and electrophoresis was performed at 4 °C for 12 hours at 200 V. Following electrophoresis, the gel was stained with silver nitrate to reveal the fragment banding patterns^[24].

2.6 Statistical analysis

Allelic and genotypic frequencies for each fragment were estimated using POPGENE software²⁵. Associations between the different traits of the birds were analyzed using a univariate General Linear Model (GLM) in the Statistical Package for Social Sciences (SPSS) software, version 17.0.

2.7 Estimation of Low density lipoprotein (LDL) concentration in serum

Approximately 1–2 ml of blood samples without anticoagulant were aseptically collected from 250 birds of the IWI layer line into sterile 2 ml tubes. The samples were kept in a slanting position at 37 °C for 2 h to allow clotting. They were then centrifuged at 1000 rpm for 5 min, and the separated serum was carefully collected into 1.5 ml tubes. The serum samples were used for the estimation of low-density lipoprotein (LDL) levels. LDL content was measured using a commercial LDL test kit with a Thermo Chem 100 automatic blood analyzer.

3. Results

3.1 To ascertain polymorphism in ACACB gene promoter and its association with Low Density Lipoprotein

PCR products corresponding to the 301 bp and 233 bp promoter fragments of the ACACB gene were analyzed using single-strand conformation polymorphism (SSCP) followed by silver staining, which identified polymorphisms in both fragments of the IWI lines. The ACACB-S1 (301 bp) fragment exhibited six distinct banding patterns within the IWI population (Fig. 1), In contrast, the ACACB-S2 (233 bp) fragment displayed three distinct banding patterns (Fig.2).

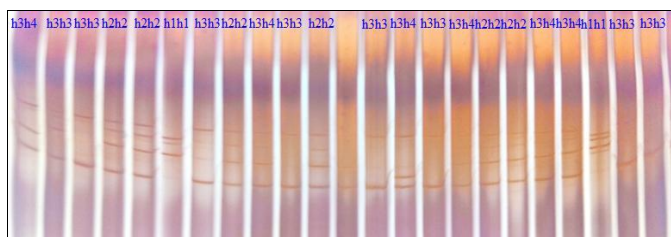


Fig 1: Polyacrylamide gel electrophoresis of 301bp fragment showing representative SSCP patterns in IWI lines of ACACB gene promoter



Fig 2: Polyacrylamide gel electrophoresis of 233bp fragment showing representative SSCP patterns in IWI lines of ACACB gene promoter

The combined analysis of both fragments resulted in the identification of twelve haplotypes (h1–h12) and eighteen haplogroups (h1h1, h1h2, h2h2, h3h3, h3h4, h4h4, h5h5, h5h6, h6h6, h7h7, h7h8, h8h8, h9h9, h9h10, h10h10, h11h11, h11h12, and h12h12). These ACACB promoter haplogroups were characterized in 250 birds of the IWI White Leghorn line. Serum LDL concentrations were also measured in these birds, and the association between different haplogroups and this trait was subsequently evaluated in the IWI population.

3.2 Association of haplogroups with serum LDL concentration

The association between haplogroups and serum LDL concentration in the IWI line of White Leghorn chickens was evaluated using a univariate General Linear Model (GLM), and the findings are presented in Table 3. Significant associations between different haplogroups and Table 3. Haplogroups -wise serum concentration of LDL in IWI lines.

Haplo group	Serum LDL concentration (mg/dL)
	IWI
h3h3	51.41±1.02
h6h6	172.13±5.97
h7h7	373.30±2.21
h8h8	202.27±1.36
h9h9	307.27±2.24
h9h10	195.00±1.83
h10h10	210.75±4.12
h11h11	168.83±3.11
h12h12	229.39±2.16

Serum LDL levels were observed in the population. Haplogroups with very low representation were excluded from the analysis. The results showed that birds belonging to the h7h7 haplogroup had the highest serum LDL concentration (373.30 ± 2.21), while the lowest level was recorded in the h3h3 haplogroup (51.41 ± 1.02). Overall, a significant ($P \leq 0.05$) effect of haplogroups on serum LDL concentration was detected within the IWI line.

4. Discussion

The acetyl-coenzyme A carboxylase beta (ACACB) gene has been implicated in several metabolic and clinical conditions, including nephropathy²⁶, obesity, diabetes²⁷, and end-stage renal disease²⁸. The enzyme plays a key role in fatty acid metabolism by catalyzing the production of malonyl-CoA, which is involved in fatty acid synthesis and regulation of fatty acid oxidation²². Mice deficient in the ACACB gene have been reported to be resistant to obesity and diabetes²⁹. Efforts have also been made to elucidate the role of the ACACB gene in livestock species, where promoter region mutations in cattle have been associated with variations in milk production traits in dairy animals^[30]. Although putative mutations in the ACACB gene or its promoter with functional significance have not yet been identified in animals, including chickens, the enzyme's key role in regulating fatty acid oxidation suggests that this gene may influence serum LDL concentration. A better understanding of genetic polymorphisms and functional regions within the gene could facilitate future genetic interventions aimed at improving body fat composition. The present study was undertaken to investigate polymorphisms in the ACACB gene and their association with serum LDL concentration in IWI lines of White Leghorn chickens.

4.1 Genetic polymorphism of ACACB gene promoter region in chicken

Genetic polymorphisms in promoter regions can influence gene function, leading to variation in their associations with different traits in chickens^[31, 32]. In general, such polymorphisms can be detected using techniques such as direct sequencing, SSCP, and PCR-RFLP. However, considering the cost-effectiveness and suitability of these methods, genetic variation in the ACACB gene in the present study was identified using PCR-SSCP analysis. The minimal promoter region identified was further divided into two non-

overlapping fragments of 301 bp (ACACB-S1) and 233 bp (ACACB-S2).

4.2 Association of haplogroups with serum concentration of LDL

A review of the literature indicates a scarcity of studies examining associations between genetic polymorphisms in the promoter, coding, or intronic regions of the ACACB gene and production traits in chickens. In the present study, an effort was made to assess the effect of SSCP variants on serum LDL concentration in IWI chickens. In particular, the association between haplogroups and serum LDL levels was evaluated in the IWI genetic group selected for high egg production. Given the role of the ACACB gene in fatty acid oxidation, the association between different haplotypes and serum LDL concentration was evaluated. In the IWI line, mean serum LDL levels ranged from 51.41 ± 1.02 to 373.30 ± 2.21 . A significant relationship was observed between haplogroups and serum LDL concentration in this population. The underlying biological mechanism of this association may provide valuable insights for future genetic interventions aimed at modulating serum LDL levels in chickens.

5. Conclusion

The findings of this study suggest that the associations observed in serum LDL levels are likely driven by underlying biological mechanisms, given the central role of the ACACB gene in fatty acid synthesis. This indicates that variations within the gene may influence lipid metabolism, thereby affecting serum LDL concentrations in chickens.

6. Declaration of interests

The authors declare that there are no competing interests. All authors have reviewed and approved the final version of the manuscript.

Conflict of Interest

Not available

Financial Support

Not available

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