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Novel variants associated with adiponectin-related traits in Awassi ewes

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Abstract

Background: Adipose tissue secretes adiponectin (ADIPOQ), a hormone related to fat oxidation, glucose metabolism, and reproduction. The polymorphism of adiponectin is associated with productive traits in domestic animals. Thus, this study investigated the association of adiponectin gene polymorphism with lipid profile and reproductive hormones in Awassi ewe. In this study, 200 ewes between the ages of 2.5 and 5 years, neither pregnant nor lactating, were included. To determine the lipid profile and reproductive hormones, sera were separated from the blood. DNA extraction, genotyping, and sequencing reactions were used to verify the variants in the amplified fragments (exon 1).

Results: Three genotypes, CC, CA, and AA, were identified from 368 bp amplicons (exon 1). A sequencing reaction revealed a novel mutation, c.198473337C > A, in the CA genotype. The results revealed significant differences ($P \leq 0.05$) in cholesterol and HDL levels in the AA genotype than CC and CA genotypes. The AA genotype had higher estradiol and progesterone levels (50.52 ± 0.64) (pg/ml) and (7.10 ± 0.04) (ng/ml), respectively, than those with the CC and CA genotypes.

Conclusions: These results conclude that the *ADIPOQ* gene affects lipid profiles and sex hormone levels in Awassi sheep. Choosing sheep that are polymorphic for the *ADIPOQ* gene should be a future study, as this gene could be linked to high prolificacy.

Keywords: Adiponectin gene, Awassi sheep, Fertility, Lipid profile, Polymorphism

1 Background

Adiponectin gene (*ADIPOQ*) is positioned on chromosome 1 (BTA) in cattle [1], and chromosome 1q27 in sheep with three exons and two introns (NCBI Reference Sequence NC_019480.2). This gene-encoded adiponectin protein mainly controls the endocrine function of adipose tissues and plays a central role in energy homeostasis [2, 3]. Moreover, Hadley et al. [4] report that it stimulates the production of ovarian steroidogenesis in granulosa and theca cells and has been found in ovarian cells in various species, including dairy cows [5]. Furthermore, adiponectin reduces lipid accumulation and is negatively correlated with triglycerides, while good cholesterol (HDL-c) is positively related [6, 7]. Adiponectin performs these functions by mediating several tissue-specific signaling pathways. Through its effects on the AMP-activated protein kinase (AMPK), adiponectin enhanced fatty acid oxidation and glucose uptake in skeletal muscles, thus contributing to carcass traits [8]. Moreover, adiponectin activates the AMPK pathway to affect oocyte nutrition and reproductive traits [9]. Polymorphisms of adiponectin have been documented in domestic animals with economic traits. Adiponectin gene polymorphism has been confirmed to affect ribeye muscle area, fat thickness, and marbling in Angus cattle [10]. In addition, the adiponectin gene is found to be highly associated with carcass and meat quality traits in Qinchuan cattle with CD genotypes exhibiting higher slaughter weight, subcutaneous fat thickness, and back fat thickness [11]. Genetic variants (g.81966235CNT, g.81966377 TNC, and g.81966364DNI) of the *ADIPOQ* significantly affected

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marbling score and carcass traits in Hanwoo cattle [12]. A novel BC140488:m.832T > A polymorphism in the 3'UTR within the goat adiponectin gene has been associated with growth traits [13]. Moreover, in New Zealand Romney lambs, haplotypes of the adiponectin gene have been associated with growth and carcass traits [14], suggesting that this gene is a potential candidate for animal productive traits. A limited amount of research has been conducted on the genetic polymorphism of the *ADIPOQ* and its association with livestock reproductive traits. One study examined polymorphisms of the *ADIPOQ* concerning reproductive performance and litter size in domestic porcine. A novel SNP (c. 1138G > A) is associated with litter size in the Wannan Black pig, a breed of pig found in China [15]. Further, the ADIPOQ/TasI genotypes revealed that TT genotype showed a shorter calving interval, a longer lactation period, and a higher milk yield in comparison with CC and CT genotypes [1].

In light of the aforementioned studies, only a few studies have investigated the relationship between genetic polymorphisms of the *ADIPOQ* gene and productive traits in sheep, and no studies have investigated their correlation with lipid profile and reproductive hormones in Awassi sheep. Therefore, this study evaluated the genetic diversity and polymorphism of the *ADIPOQ* gene and examined how *ADIPOQ* polymorphism influences the levels of lipids and reproductive hormones in Awassi ewes.

2 Methods

2.1 Animals

The study was conducted Al-Qasim Green University between July 2020 and March 2021 according to international guidelines for animal care and use, with approval number Agri, No. 020,7,18. A total of 200 Awassi ewes that were sexually mature, not pregnant or lactating, between the ages of 2.5 and 5 years were included in this study. A random sampling of sheep stations was conducted in Babylon and Karbala. Animals were fed seasonal grass and concentrate food (2.5% of their live body weight) daily, which consisted of barley (59%), bran (40%), salt (1%), and freshwater. Blood was collected from the jugular vein of the sheep before it was fed in the morning. Serum was stored at − 20 °C and then used in biochemical analysis.

2.2 Biochemical analysis

A serum sample was separated at 2000 × *g* for 15 min at room temperature to measure the lipid profile and hormonal levels. RANDOX Laboratories' kits were used to measure triglycerides, HDL-c, and total cholesterol in serum. LDL-c was calculated using Friedewald et al.'s equation [16]. ELISA kits were used to measure reproductive hormones (catalog numbers E0047Sh, E0015Sh, E0105Sh, and E0106Sh) from Bioassay Technology Laboratory.

2.3 DNA extraction and PCR amplification

A rapid salting-out technique was used to extract genomic DNA from whole blood [17]. DNA extracts were analyzed with a Nanodrop (Biodrop, UK) for DNA amplification. An amplification reaction contains 10 pmols of each primer, 50 ng of genomic DNA, 50 mM Tris-HCl (pH 9), 30 mM KCl, 1.5 mM $MgCl_2$, and one unit of top DNA polymerase. Based on the GenBank ovine sequence (NC_019480.2), primers were designed for the *ADIPOQ* gene (exon 1, 368 bp). The sequences of the forward and reverse primers were 5′-CCTGTATCTCTCCCC ACCCT-3′ and 5′-GTGTGATGCCTGCAGCTCTA-3′, respectively. An initial denaturation of the PCR product was carried out at 94 °C for 4 min, an annealing step at 57 °C for 30 s, an extension step at 72 °C for 30 s, and finally a final extension step at 72 °C for 10 min [18]. PCR products were visualized using a 2% agarose gel and UV light [19].

2.4 Genotyping and sequencing reaction

The genotyping step was performed following PCR using PCR-SSCP [20]. Denaturing-loading buffer SSCP was applied equally to each amplification product. The denaturated sample was loaded onto neutral polyacrylamide gels after seven minutes of denaturation and 10 min of cooling on wet ice using a 0.5 TBE buffer. A constant current and voltage of 200 mA and 100 V were used during electrophoresis for 4 h at room temperature. The gels were stained using the method described by Byun et al. [21]. Using Macrogen Geumcheon (Korea) and BioEdit 7.1 (DNASTAR, Madison), polymorphisms in each genotype were sequenced, edited, and visualized using SnapGene Viewer 4.0.4 (http://www.snapgene.com). Additionally, the Ensemble genome browser 96 checked the *ADIPOQ* gene for new variants.

2.5 Statistical analysis

PopGen32, version 1.31, was used for genetic analysis [22]. The following model was used to analyze the relationships between *ADIPOQ* genotypes and traits of interest using SPSS (version 23.0), which was compared with a Tukey–Kramer test:

$$Y_{ijk} = \mu + G_i + P_j + A_k + e_{ijk}$$

where Y_{ijk} = traits studied, μ = overall mean, G_i = fixed effect of ith genotypes (i = CC,CA,AA), P_j = fixed effect of jth parity (j = 1, 2, 3, 4), A_k = fixed effect of kth age group (2.5–3.5, > 3.5–5), and e_{ijk} = random error. A preliminary statistical analysis showed no significant impact

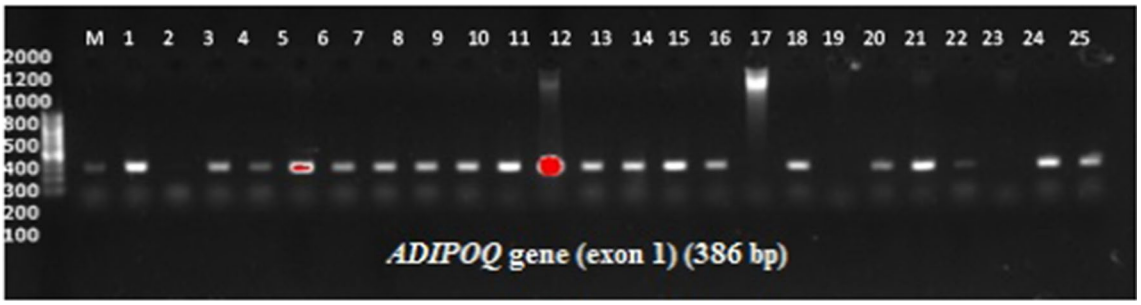

Fig. 1 Agarose gel electrophoresis for PCR products amplification of the *ADIPOQ* gene. Lane M: 100 bp DNA ladder; lanes 1–25 indicate samples of ewes. Electrophoresis conditions: 1.5% agarose concentration, electrophoresis time 25 min. The buffer used TBE pH 8.3

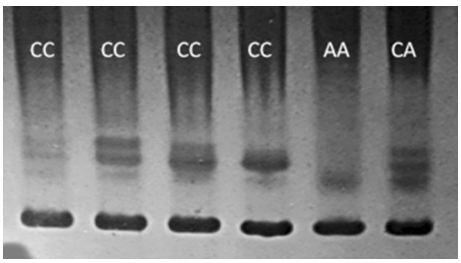

Fig. 2 SSCP non-denaturing polyacrylamide gel electrophoresis of ovine *ADIPOQ* gene (exon 1) PCR fragments, which appeared three PCR-SSCP banding patterns, CC, CA, and AA in Awassi ewes

of interactions between variables, season, and nutrition on the studied traits.

3 Results

All 200 samples were investigated with PCR (Fig. 1). Based on PCR-SSCP banding patterns, three distinct genotypes CC, CA, and AA were identified (Fig. 2). Sequence analysis revealed that the c.198473337 C > A SNP was present in only one SSCP variant, indicating heterogeneity in exon 1. *ADIPOQ* genetic diversity indicated the most common genotype was CC with a total frequency of 0.48 ($n = 97$). The CA genotype was detected with a frequency of 0.33 ($n = 66$), followed by the AA genotype with a frequency of 0.19 ($n = 37$) (Fig. 3).

Concerning the association analysis, the results refer to the significant differences ($P \leq 0.05$) in cholesterol and HDL levels in the AA genotype than CC and CA genotypes, as shown in Table 1. Regarding the effects of *ADIPOQ* genotypes on reproductive hormones, the three genotypes showed significant differences ($P \leq 0.05$) in sex hormone levels (Table 2). The AA genotype had higher estradiol and progesterone levels (50.52 ± 0.64) (pg/ml) and (7.10 ± 0.04) (ng/ml), respectively, than CC and CA genotypes.

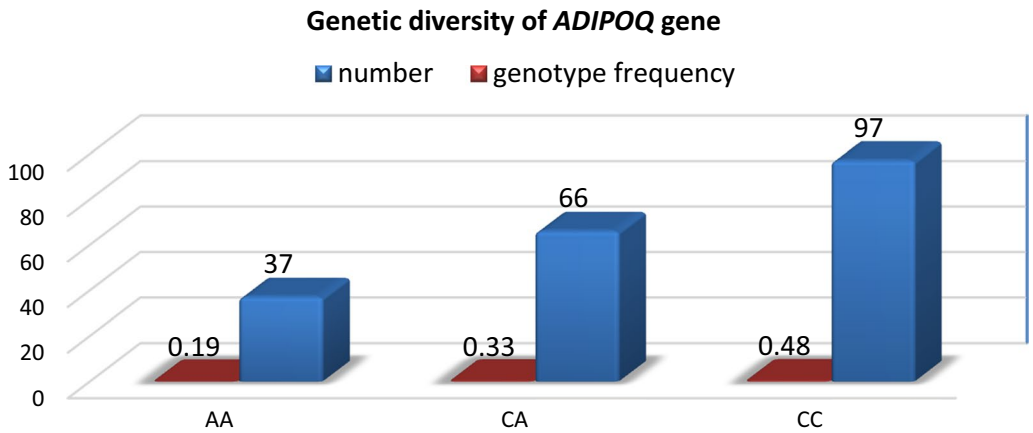

Fig. 3 Genetic diversity of the *ADIPOQ* gene exhibited the most preponderant genotype was CC genotype

Table 1 Association of *ADIPOQ* genotypes and lipid profile of Awassi ewes

Indices	*ADIPOQ* genotype (LSM ± SE)			*P* value
	CC	CA	AA	
Cholesterol (mg/dl)	**50.76 ± 1.12^{b}**	**59.74 ± 1.62^{ab}**	**65.32 ± 1.72^{a}**	**0.03**
Triglyceride (mg/dl)	4.01 ± 0.61	4.64 ± 0.67	5.24 ± 0.91	0.44
HDL (mg/dl)	**29.11 ± 0.84^{b}**	**31.75 ± 0.42^{ab}**	**37.31 ± 0.85^{a}**	**0.04**
LDL (mg/dl)	29.53 ± 0.67	30.21 ± 0.46	31.62 ± 0.34	0.62

LSM ± SE least square means ± standard error. Different superscript in the same raw within each classification indicates significant differences ($P \leq 0.05$), Bold numbers indicates the *P* value with statistical significance. *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

4 Discussion

Varieties of regions of the *ADIPOQ* gene have been reported to contain genetic polymorphisms. Genetic polymorphism has been observed in the promoter region of *ADIPOQ* in Angus cattle, as reported by Morsci et al. [10]. Shin and Chung [23] identified three genotypes of the *ADIPOQ* gene promoter in the Hanwoo (Korean) cattle population using PCR–RFLP: CC, CT, and TT. Lan et al. [12] identified 13 SNPs within exon 1 of the *ADIPOQ* gene in New Zealand Romney lambs. In light of the above studies, there have been no studies conducted on the genotyping of the *ADIPOQ* gene in Awassi sheep. Marker-assisted selection programs can benefit from these genotypic data and associations.

The AA genotype was significantly ($P \leq 0.05$) associated with higher cholesterol and HDL levels as compared to the CC and CA genotypes. This indicates that this genotype is related to lipid metabolism. It has been shown that adiponectin has several functions, such as controlling energy metabolism, insulin sensitivity, and regulating lipid levels [24]. Moreover, ADIPOQ levels are associated with lipoprotein metabolism, in particular, triglyceride and HDL metabolism [7]. Adiponectin increases HDL levels and lowers TG levels [25]. This hormone may raise HDL levels by increasing lipoprotein lipase (LPL) and the transporter of ATP while lowering hepatic lipase. By increasing LPL activity and VLDL receptor, as well as decreasing apo-CIII, VLDL catabolism might be increased, resulting in a reduction in serum TG [7]. Furthermore, adiponectin exhibits distinct anti-atherosclerosis properties, including reducing macrophage scavenger receptors and increasing cholesterol clearance [26]. According to Tang et al. [27], the *ADIPOQ* gene is implicated in lipid metabolism by inhibiting lipid synthesis and promoting fatty acid oxidation. Most research on the *ADIPOQ* gene has focused on humans and rodents, but fewer studies have examined livestock and poultry [28, 29]. Liu et al. [29] found that *ADIPOQ* gene expression levels correlated positively with intramuscular fat content during early fattening and negatively with late fattening in Shandong black cattle and Luxi cattle. This suggests that *ADIPOQ* appears to be an effective candidate gene for intramuscular fat accumulation and adipogenesis.

As it relates to reproductive hormones, adiponectin regulates the hypothalamus–pituitary–gonadal axis [30]. By its role in regulating hypothalamic–pituitary axis activity, adiponectin has been identified as important for FSH and LH secretion [31]. The release of progesterone (P4) and estradiol (E2) by luteal and follicular cells has been observed in response to adiponectin [32]. Moreover, the expression of adiponectin and its receptors has been demonstrated in the reproductive organs of many animals, which suggests that this hormone could affect follicular development and reproductive [5, 33]. Ovarian function is disrupted by adiponectin gene mutations that affect GnRH immunoreactive neurons [34]. Three SNPs (c. 178G > A, c. 1165A > G, and c. 1138G > A) in the porcine *ADIPOQ* gene have been shown to influence litter size [15]. A recent study by [1] reported that SNP 1431C > T in the *ADIPOQ* gene promoter has also been associated with reproductive traits in Indian dairy cattle.

Although the Awassi breed is known for its hardiness in unfavorable conditions [35], its reproduction rates are low compared to nearby breeds similar to Karakul and Assaf in the Middle East [36, 37]. Based on these findings,

Table 2 The association of *ADIPOQ* genotypes with reproductive hormones assay of Awassi ewes

Indices	*ADIPOQ* genotype (LSM ± SE)			*P* value
	CC	CA	AA	
Follicle-stimulating hormone (ng/ml)	18.22 ± 1.72	19.37 ± 1.40	19.78 ± 1.23	0.44
Luteinizing hormone (ng/ml)	20.98 ± 0.32	21.11 ± 0.61	22.53 ± 0.43	0.32
Estradiol (pg/ml)	**34.97 ± 0.78^{b}**	**42.87 ± 0.70^{ab}**	**50.52 ± 0.64^{a}**	**0.03**
Progesterone(ng/ml)	**3.41 ± 0.03^{b}**	**5.22 ± 0.07^{ab}**	**6.10 ± 0.04^{a}**	**0.01**

LSM ± SE least square means ± standard error. Different superscript in the same raw within each classification indicates significant differences ($P \leq 0.05$), Bold numbers indicates the *P* value with statistical significance

genetic variation within the *ADIPOQ* gene could contribute to breeding improvements in Awassi sheep. It is possible that the c.198473337 C > A SNP is responsible for the higher levels of reproductive hormones in the AA genotype. As the AA genotype exhibited higher levels of reproductive hormones, it tended to be more prolific than the AC or CC genotypes.

5 Conclusions

A polymorphism in the *ADIPOQ* gene affects the lipid profile and levels of sex hormones in Awassi sheep. The AA genotype showed a higher lipid profile and sex hormone levels, which made them more productive. This suggests that *ADIPOQ* is a potential candidate gene for traits associated with fat deposition in livestock. Future studies should investigate *ADIPOQ* gene polymorphisms and their relationship with high prolificacy in sheep.

Abbreviations

ADIPOQ: Adiponectin; AMPK: AMP-activated protein kinase; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; PCR: Polymerase chain reactions; SSCP: Single-strand conformation polymorphism; LPL: Lipoprotein lipase; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; P4: Progesterone; E2: Estradiol; GnRH: Gonadotropin-releasing hormone.

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Author contributions

All authors contributed equally. In addition, all authors reviewed and approved the final manuscript.

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Availability of data and materials

Data and materials are available.

Declarations

Ethics approvals and consent to participate

The study was conducted by Al-Qasim Green University between July 2020 and March 2021 according to international guidelines for animal care and use, with approval number Agri, No. 020,7,18.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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