Association of *STAT5A* Gene Polymorphisms and Ewe Age with Wool Production Traits in Local Iraqi Sheep

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Abstract

Signal transducer and activator of transcription 5A (*STAT5A*) is an important gene that plays vital roles in regulating several physiological processes related to growth, cell differentiation, and gene expression. Its genotypes have been associated with several productive traits in farm animals. Despite the growing interest in this gene, information regarding its potential role in wool traits remains limited. Therefore, this study evaluated the effect of *STAT5A* gene genotypes on wool traits, accounting for the influence of non-genetic factors, including sheep age. A PCR-Sanger sequencing protocol was utilized to assess the polymorphism of *STAT5A* PCR products with wool traits in Iraqi sheep. A silent single nucleotide polymorphism (SNP) was identified in exon 12 at position 311 of the *STAT5A* gene. This mutation resulted in the substitution of the third nitrogenous base, G, with A in the amino acid threonine. This mutation showed a significant association with both greasy and clean fleece weight. The results indicated that ewes with the AA genotype exhibited higher wool production traits compared to ewes with the GG and GA genotypes, respectively. This apparent effect on wool traits may be due to indirect effects on cell growth regulation and fiber formation. The age of the ewes also showed a highly significant effect on fiber diameter, favoring 3-year-old ewes. To the best of our knowledge, this study is among the first in Iraq to address the relationship between genetic variation in the *STAT5A* gene and wool traits in local ewes. The results suggest that the *STAT5A* gene could be considered a candidate gene worthy of further investigation to understand its potential role in wool traits. Larger studies are needed to verify this relationship and its potential application in genetic selection programs.

Key words: Local sheep, wool traits, genotypes, *STAT5A*.

Introduction.

Iraq is recognized as one of the historically significant regions in the origin and development of sheep husbandry (2). Sheep have been raised in the region for centuries to provide essential products, including meat, milk, hides, and wool (4). Historical evidence from clay tablets and Assyrian artifacts further documents early practices of sheep grazing and herd management in ancient Iraq (30, 22).

Iraqi sheep are of Asian origin and are generally classified as fat-tailed sheep (13). Moreover, they possess considerable genetic diversity, which enhances their value as important animal genetic resources (7). The importance of local sheep also stems from their ability to provide multiple animal products, with growth performance, meat and milk production, and wool characteristics representing major indicators of their productive potential (14). Among these products, wool represents a valuable animal resource with considerable economic importance (3). Wool production and quality are primarily determined by physical characteristics such as fiber diameter, staple length, and

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fiber length, which influence wool processing performance and its suitability for different end uses (26, 17, 6). A complex interaction between genetic and non-genetic factors, including breed, age, nutrition, and environmental conditions, influences these traits. Recent advances in molecular genetics have provided valuable tools for investigating the genetic basis of wool and fiber traits. Different approaches, including candidate gene studies, transcriptome analyses (29; 18), and genome-wide association studies (GWAS) (32), have contributed to the identification of genes and genomic regions associated with fiber growth and characteristics. Furthermore, DNA-based genetic marker technologies have facilitated the identification of genomic regions and quantitative trait loci (QTLs) associated with complex traits, including fiber diameter and other economically important wool characteristics (27).

Several studies have shown that genetic variation contributes to phenotypic differences in wool production and quality traits (1, 27). Given the important role of genetic factors in regulating productive traits in sheep, the present study focused on the *STAT5A* gene to explore potential associations between its variants and wool traits. The *STAT5A* (Signal Transducer and Activator of Transcription 5A) gene belongs to the *STAT* gene family and has the Gene ID 113046928 in the National Center for Biotechnology Information (NCBI) database. In sheep, the gene is located on chromosome 11, whereas in cattle and goats, it is located on chromosome 19 (15). *STAT5A* is involved in several essential biological processes, including cell proliferation and differentiation, regulation of the cell cycle, apoptosis, and transcriptional regulation of genes involved in diverse physiological functions (10, 19, 23). In addition, *STAT5A* contributes to the regulation of immune responses and cellular homeostasis (31), and its genetic polymorphisms have been associated with several productive traits in farm animals (9, 24, 28). Despite growing interest in STAT5A as a candidate gene associated with economically important traits, information on its potential relationship with wool production and physical wool characteristics remains limited. Therefore, the present study was conducted to identify genetic variations in the *STAT5A* gene and investigate their potential associations with wool production and physical wool traits in sheep.

Materials and Methods

The study was conducted at the farm of a breeder in Abi Gharaq Subdistrict, western Babylon Governorate, Iraq, with a sample of 85 local sheep. Genetic analyses were conducted at the Molecular Genetics Laboratory of the Marshes Research Center/University of Thi-Qar to extract genetic material (DNA) and determine genotypes. For the *STAT5A* gene, blood samples were drawn from the jugular vein of ewes, placed in tubes containing EDTA (anticoagulant), and then stored at refrigerated temperatures. The laboratories of the Faculty of Veterinary Medicine at Al-Qasim Green University were also used to measure wool traits, wool production and physical traits. The sheep were sheared in early April and weighed immediately. Before shearing, a sample of 10 x 10 cm of wool was taken from the center of the right side of the animal as the best sampling area according to (25). The samples were weighed with a sensitive balance and then washed. They were washed in three troughs to remove dust and dirt by stirring and squeezing, then dried at room temperature for 48 hours. The clean wool samples were weighed to calculate the percentage of clean wool and the weight of clean fleece, as follows: Clean wool percentage = (clean sample weight / greasy sample weight) x 100. Clean weight of the fleece = percent of clean wool x greasy weight of the fleece. Staple length was measured after taking three staples randomly from each sample, and their lengths were measured using the usual ruler without pulling or pulling, from the base of the tuft to its end. Fiber length was measured manually using a ruler for up to 50 randomly selected fibers from the sample, and the mean was calculated after fixing both ends

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with special clamps to make the fiber tight and straight to obtain its true length. The diameter of the fiber was measured using a microscope with an Axio cam in the Histology and Anatomy Laboratory of the College of Veterinary Medicine / Al-Qasim Green University; ten fibers were taken from each sample, and the mean diameter was calculated. The fiber was placed under the microscope and appeared on the computer screen connected to the microscope, showing the fiber diameter in microns.

DNA extraction and electrophoresis

DNA was extracted from the blood, according to the instructions of the kit (Kit) provided by the Korean company Geneaid, and the success of the extraction was confirmed by using electrophoresis technology on an agarose gel (20)

Selection of primers and polymerase chain reaction (PCR)

Primers were selected to determine the genotype of the *STAT5A* gene:

5`- CTGCAGGGCTGTTCTGAGAG -3`

5`- CCAGAGCAACCAGATGGGAG -3`

Then, the polymerase chain reaction (PCR) protocol designed to amplify the specific fragment of the *STAT5A* gene included 95°C for 5 minutes for initial denaturation and 35 cycles. Each cycle included 95°C for 30 seconds for denaturation, followed by 58°C for 1 minute for annealing and 72°C for 30 seconds for extension. After the 35th cycle, the protocol concluded with 72°C for 7 minutes for final extension. The PCR mixture was 25 µL, containing 13 µL of 2x Amplicon master mix and 1 µL of forward and reverse primers, 4 µL of medium-concentration DNA template (approximately 50 ng/L), and 6 µL of free nuclease water (FNW). After PCR amplification, the STAT5A gene was selected by electrophoresis on a 1.5% agar gel in TBE solution, using a DNA standard for 100- 3000 bp and a safe green dye (SafeView dye ID: G108 from Canadian AMP company). The PCR products for all samples were sent to Macrogen, Korea, for sequencing analysis. Immediately after receiving the results, multiple sequence alignment analysis was performed using BioEdit v7.2.5 software (16)

The data were statistically analyzed using the Statistical Analysis System-SAS (2018) (21) software to study the effect of the genetic makeup of specific regions of the *STATSA* gene, as well as the mother's age at birth, on the wool characteristics of a sample of sheep. The following model was used:

Yijlm = µ + Ai + Sj + eijmn Where: µ= overall mean Ai= the effect of age of Ewe (i=3) Sj= the effect of *STAT5A* gene (j=3) eijmn= the effect of the experimental error, which is distributed randomly and naturally with an average of zero and variance ϭ 2 e.

Significant differences between the means were compared using Duncan's (11) multiple tests by applying the least squares method. The (Chi-Square- $\chi 2$) test was also used to compare the percentage distribution of genotypes for the gene in the studied sheep sample.The following formula was also applied to calculate the allelic frequency according to Hardy-Weinberg's rule (Weinberg Equilibrium) according to (12)

Results and Discussion

The results of agarose gel electrophoresis showed successful amplification of the *STAT5A* gene fragment at a size of 446 bp (Figure 1).

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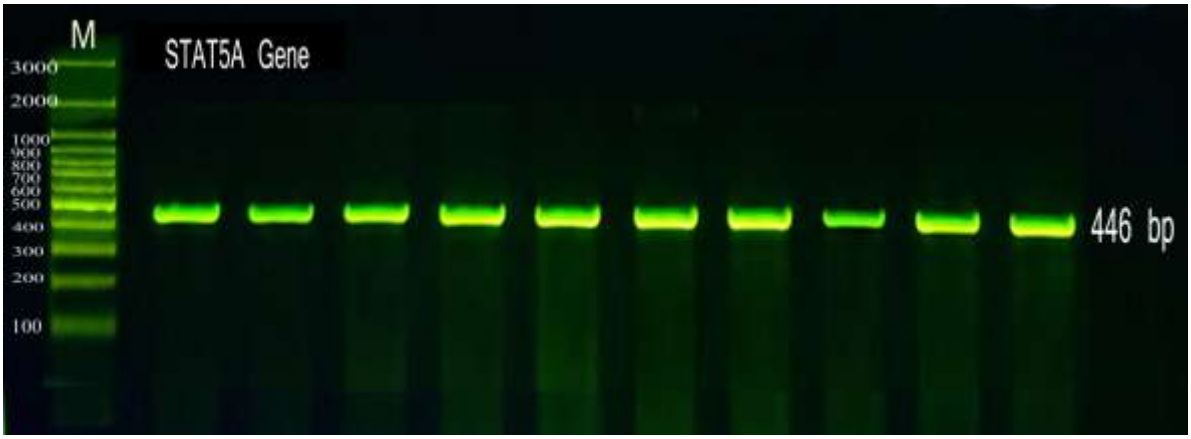


Figure 1) The amplified fragment of the STAT5A gene, with a size of 446 bp, was electrophoresed on a 1.5% agarose gel at 70 V and 85 mA for 45 min.

The multiple alignment analysis of the studied *STAT5A* gene fragment revealed a genetic variation at position 311 (G>A). This mutation produced three distinct genotypes, as shown in Table (1) and Figure (2). The nucleotide change occurred at position 436 of the polypeptide chain within the studied region and at position 794 of the complete polypeptide chain of the gene, according to the *STAT5A* protein accession number NP_001009402. The mutation was located in the ACG codon, which encodes the amino acid threonine. With the A genetic change, the codon changed from ACG to ACA. This alteration did not lead to an amino acid substitution, since both ACG and ACA encode threonine. The statistical analysis of genetic variation in the 311 G>A mutation showed that the frequency of the GA genotype was highest at 42.35, compared to 37.65 for the homozygous GG genotype and 20 for the homozygous recessive AA genotype. In addition, the Chi-square test for genotype distribution yielded a value of 7.282, which was statistically significant at the 0.05 level.

Table (1) Frequencies of genotypes and alleles for the *STAT5A* gene of local sheep

Genotype	(%)	count	Chi-square
GG	37.65	32	* 7.282
GA	42.35	36	
AA	20.00	17	
Alelle	(%)		
G	0.59		
A	0.41		
Signification	*P≤0.05.		

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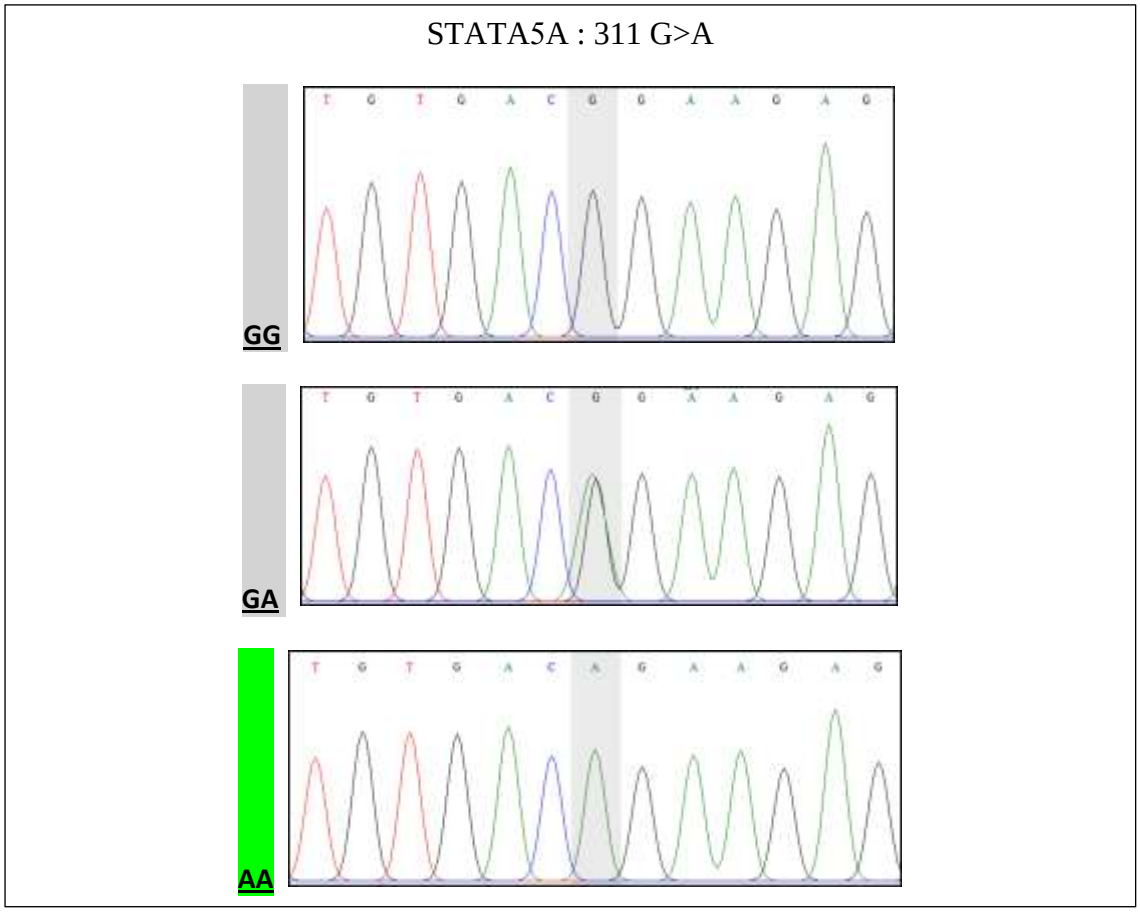


Figure (2) shows the 311G>A mutation in the *STAT5A* gene of local sheep .

Efect of ewe age and genotypes of the *STAT5A* gene on greasy and clean fleece weight and percentage of clean wool.

Table (2) shows that Ewe age has no significant effect on Greasy and clean fleece weight, despite clear numerical differences. Four-year-old ewes yielded the highest Greasy and clean fleece weights, averaging 2.16 kg and 1.27 kg, respectively. Five-year-old ewes followed with averages of 2.07 kg and 1.20 kg, while three-year-old ewes recorded the lowest Greasy and clean fleece weights, averaging 1.98 kg and 1.16 kg, respectively. The lack of a significant effect of Ewe age on wool characteristics may be explained by genetic, nutritional, and management factors that minimized the differences between clean and Greasy fleece yields and the percentage of clean fleece across the three age groups.

The *STATSA* gene genotypes significantly influenced the weight of greasy and clean fleece. Ewes with the AA genotype significantly outperformed those with the GG genotype (averages 2.36 and 1.39 kg, respectively) compared to ewes with the GG genotype (averages 2.01 and 1.1 kg, respectively). Ewes with the GA genotype had the lowest averages (1.98 and 1.19 kg, respectively). Genotype did not significantly affect the percentage of clean fleece, which averaged 56.31% for the GG genotype, 59.83% for the GA genotype, and 59.06% for the AA genotype. This finding is consistent in principle with the findings of (1, 32), which demonstrated a significant correlation between the genetic variants of the growth hormone gene and fleece weight.

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Table (2) Effect of ewes' age and genotypes of the *STAT5A* gene on the mean weight of greasy and clean fleece and the percentage of clean wool ± standard error

influencing factors		Number of animals	Greasy fleece weight (kg)	Clean fleece weight (kg)	Clean wool percentage (%)
Age of Ewe	3	18	0.08± 1.98	0.05± 1.16	1.07± 58.58
	4	31	0.07± 2.16	0.05± 1.27	1.17± 58.61
	5	36	0.11± 2.07	0.08± 1.20	1.70± 57.44
The genotype of the *STAT5A* gene	GG	32	0.08± 2.01 b	0.06± 1.14 b	1.27± 56.31
	GA	36	0.07± 1.98 b	0.05± 1.19 b	0.98± 59.83
	AA	17	0.07± 2.36a	0.05± 1.39a	1.45± 59.06

The averages with different letters within the same column are significantly different (P<0.05).

Effect of ewes' age and genotypes of the *STAT5A* gene on staple length and fiber length and diameter.

The age of the ewe had a significant effect (P < 0.05) on fiber diameter, as shown in Table (3). The youngest ewes (3 years old) produced wool with the smallest mean fiber diameter (23.83 µm). Fiber diameter increased progressively with advancing age, reaching 26.03 µm in 4-year-old ewes and attaining its highest value (28.64 µm) in the oldest ewes (5 years old). This positive association between ewe age and fiber diameter may reflect age-related changes in fiber growth characteristics, resulting in increased wool coarseness. This finding is consistent with results reported by (1, 5) but contrasts with the findings of (8), which reported no significant effect of ewe age on fiber diameter. In contrast, ewe age had no significant effect on staple length or fiber length, as the differences among age groups did not reach statistical significance. This lack of significance may be associated with the physiological, nutritional, and management conditions of the ewes, in addition to other factors influencing these traits. With respect to the genetic variants of the *STAT5A* gene, Table (2) indicates that the different genotypes had no significant effect on the physical characteristics of wool. The mean fiber lengths were 13.06, 12.26, and 13.46 cm for the AA, GA, and GG genotypes, respectively. Similarly, fiber diameter did not differ significantly among the *STAT5A* genotypes, despite the apparent numerical variation. The GG genotype exhibited the lowest mean fiber diameter (25.03 µm), compared with the GA and AA genotypes, which showed mean values of 27.94 and 27.06 µm, respectively.

Table (3) Effect of ewe age and genotypes of the *STAT5A* gene on staple length, fiber length, and fiber diameter ± standard error

influencing factors		Number of animals	Staple length (cm) fiber	Fiber length (cm)	Fiber diameter (micron)
Age of Ewe	3	18	0.41± 12.57	0.49± 15.37	0.76± 28.64a
	4	31	0.48± 13.19	0.59± 16.70	0.98± 26.03ab
	5	36	0.54± 12.58	0.67± 15.85	1.18± 23.83b
The genotype of the *STAT5A* gene	GG	32	0.42± 13.06	0.49± 15.99	1.14± 25.03
	GA	36	0.44± 12.26	0.55± 15.51	0.69± 27.94
	AA	17	0.55± 13.46	0.79± 16.84	1.06± 27.06

The averages with different letters within the same column are significantly different (P<0.05).

Conclusions:

This study reveals physical characteristics of wool that improved in younger ewes, as shown by a decrease in fiber diameter compared with older ewes. It also shows the existence of a relationship between the *STAT5A* gene and wool production. Consequently, it is considered genetically possible to improve wool production and its physical traits in local Iraqi sheep.

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