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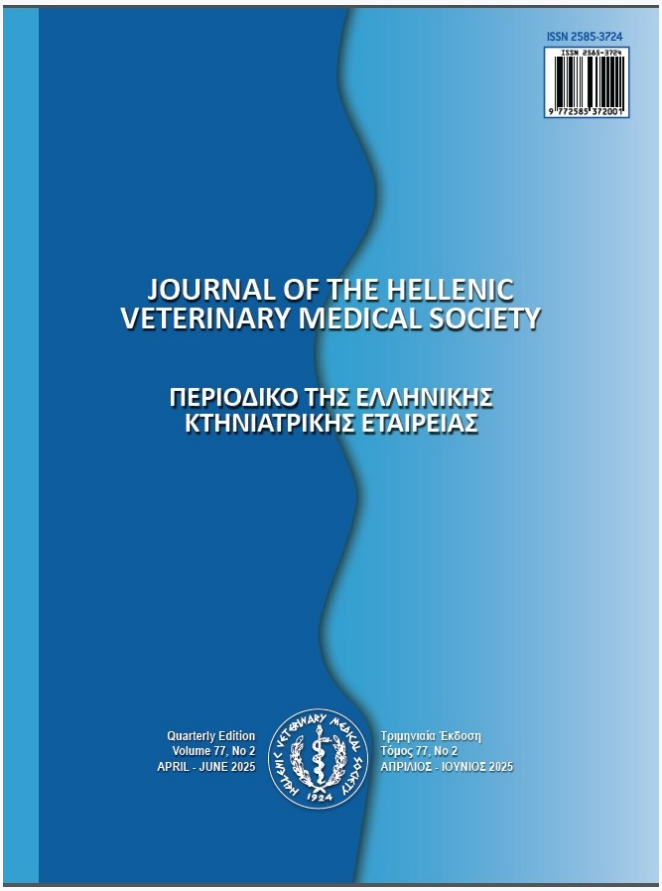

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Genetic diversity and phylogenetic analysis of ADRB2 gene in Awassi and Karakul sheep

Zaid M. Jassim, Thamer R. S. Aljubouri, Mohammed Baqur S. Al-Shuhaib*

Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil, Iraq.

ABSTRACT: Due to the importance of the *ADRB2* (adrenergic receptor beta 2) gene in a wide range of metabolic activities, it was aimed to evaluate its potential to differentiate between the Iraqi Awassi (n=88), and the Iranian Karakul (n=84) sheep. Twenty-seven variants were identified, including fourteen insertion variants, which constituted 51.9% of the total polymorphisms. Additionally, nine silent SNPs (33.3%) and four missense SNPs (14.8%) were detected. The analysis of genetic diversity revealed that Karakul showed a higher number of haplotypes (h=16) and variable sites (v=11) compared with Awassi (h=13, v=9). The assessment of the evolutionary relationship among the examined populations was conducted utilizing molecular variance analysis, which indicated that the predominant diversity observed stemmed from variations within populations (76.55%), while variations between populations accounted for a mere 23.45%. The median-joining network illustrated that Karakul harbored 11 haplotypes and Awassi had 8 haplotypes, segregated into distinct groups based on their respective breeds, with 5 haplotypes showing shared localizations. Extensive phylogenetic information unveiled that the Chinese origin was the sole precursor to all identified haplotypes. Considering the ADRB2 gene's capacity to distinguish between the crucial Middle Eastern breeds, it is strongly advised that it be utilized as a robust biological marker for tracing biological diversity across broader ovine sequences.

Keyword: Awassi; Biological diversity;Evolution; Genetic variation; Karakul.

Correspondence author:
Mohammed Baqur S. Al-Shuhaib,
Department of Animal Production, College of Agriculture,
Al-Qasim Green University, Al-Qasim, Babil, Iraq
E-mail address (MBSA):
mohammed79@agre.uoqasim.edu.iq

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INTRODUCTION

Biological diversity, or biodiversity, encompasses the variety of all life forms on Earth ranging from the genetic makeup of microorganisms to the complex ecosystems they inhabit. Through employing various tools related to a wide range of biological diversity routes, species are enabled to adapt to changing environments, resist diseases, and contribute to ecosystem stability and productivity (Khush, 2023). One of the critical aspects of the genetic foundation in biological diversity is attributed to the presence and functionality of a wide range of multifunctional genetic loci. Genetic factors play a crucial role in determining the biological diversity of sheep, influencing their adaptation to environmental changes and disease resistance. Studies on various sheep breeds worldwide have highlighted the significance of genetic diversity in maintaining indigenous breeds and preserving unique traits (Ozerov et al., 2020). It has been shown that genetic variation in sheep populations is influenced by factors such as geographical latitude, temperature, and historical breeding practices, leading to distinct genetic structures within different breeds (Huang et al., 2017). Structural variations, including insertions, deletions, and multiallelic variations, contribute significantly to genetic diversity, with some variations linked to specific phenotypic traits like tail length (Li et al., 2023; Machová et al., 2023).

Despite the importance of genetic factors in determining the biological diversity of sheep, some genetic loci remain unexplored. One such critical locus is the *ADRB2* gene. It is one of the three recognized beta-adrenergic receptors (*ADRB1, ADRB2*, and *ADRB3*), which are part of the G protein-coupled receptor class(Ali et al., 2023; Zavala et al., 2017). These genes are widely known to mediate a broad spectrum of metabolic effects, including lipolysis in adipose tissue, insulin resistance, and energy balance(Burguete-García et al., 2014; Dahlman and Arner, 2007). The *ADRB2* gene plays a crucial role in providing genetic diversity among mammals, especially in livestock animals, due to its involvement in various physiological processes and its impact on responses to external stimuli like catecholamines and synthetic agonists (Sponenberg, 2020). Genetic polymorphisms within the ADRB2 gene, including single-nucleotide polymorphisms (SNPs) and missense polymorphisms, influence receptor function and cellular responses, contributing to genetic diversity within populations (Litonjua et al., 2010; Notter, 1999). The ADRB2 gene, located on chromosome 5q32, encodes a protein with seven transmembrane domains, and specific polymorphisms like the T164I substitution and the R16G and Q27E polymorphisms have been identified to alter receptor function and down-regulation processes, affecting clinical and pharmacologic responses in individuals (Lucas et al., 2004). Understanding the genetic variations within the *ADRB2* gene is essential for comprehending the genetic diversity present in mammalian populations, including livestock animals, and their responses to environmental and therapeutic stimuli.Despite the acknowledged significance of these G-protein-coupled receptors in various metabolic processes(Wu et al., 2012; Yang et al., 2011), there is a lack of literature associating *ADRB2* polymorphism with economic traits in sheep. Considering its pivotal role in regulating sheep reproduction, it is valuable to investigate the *ADRB2* gene polymorphism in two distinct breeds in the Middle East to identify the potentially causative SNP(s) underlying the observed phenotypic and productive variations.

In the Middle East, the Karakul and Awassi sheep breeds are renowned for their unique fat tails. Karakul sheep, native to Iran, exhibit medium size and distinctive black and grey coloration, and pendulous ears (Obeidat and Obeidat, 2022). On the other hand, both breeds have been studied for genetic associations with growth traits, revealing significant polymorphisms in various genetic loci that influence body weight, length, and other physical characteristics (Moradi et al., 2022). Various studies have comparedreproductive traits between Awassi and Karakul sheep and found the presence of a wide range of significant differences in growth rates, and feed efficiency. The findings of these studieshave suggested the presence of remarkable differences between the two compared breeds (Hassan, et al., 2020).Recent genetic investigations have shown that the Karakul sheep display distinct mitochondrial genetic diversity patterns, with a lower number of haplotypes compared to Awassi sheep, indicating differences in nucleotide polymorphism and haplotype diversity. Additionally, microsatellite marker analyses have revealed genetic diversity within and between local Iraqi sheep breeds, including the Awassi breed, highlighting the presence of dominant allele frequencies specific to each breed (Hadi et al., 2020). Moreover, assessments of genetic diversity in sheep breeds within Arab nations have revealed that the Awassi sheep constitutes a delineated ge-

netic cluster. This observation underscores the distinctive genetic composition and diversity inherent in these breeds within the region (Al-Atiyat et al., 2018; Fadhil and Al-Shuhaib, 2022). Moreover, other genetic studies have shown that the Awassi sheep breed is genetically closer to the other breeds than to the Karakul breed, emphasizing the distinct genetic relationships and diversity among these breeds in the Middle East (Al-Barzinj and Ali, 2013). Given the unique characteristics of Karakul and Awassi sheep, the objective of this research was to examine the potential genetic impact of the *ADRB2* gene on the well-documented variations in the biological characteristics of the Awassi and Karakul breeds. Due to the lack of research focusing on *ADRB2* in the evolutionary history of sheep, the present study represents the pioneering effort to investigate the diversity of *ADRB2* within two distinct breeds exhibiting differing geographical, morphological, and productive traits in order to evaluate their interconnectedness and to determine their evolutionary relationships with other relevant species.

MATERIALS AND METHODS

Animal resources

This investigation concentrated on two distinct Middle Eastern breeds that displayed notable disparities in growth attributes. The research encompassed two cohorts of sheep: 88 Awassi and 84 Karakul breeds. The experimental phase of the inquiry took place at the Barakat Abu al Fadhl Al-Abbas Sheep Station (BAFAS), the rearing site for both breeds. Positioned at 32.6027° N and 44.0197° E, the BAFAS facility sits at an elevation of 32 meters above sea level. BAFAS station is characterized by warm summers and mild to freezing winters. All animal trials were executed in compliance with ethical protocols for the handling and utilization of farm animals (Vaughn, 2012). Both sets of sheep under scrutiny were propagated at the same breeding establishment and subjected to identical environmental conditions.

Genomic DNA extraction

Aseptic extraction of genomic DNA from blood samples of both Awassi and Karakul sheep was performed using EDTA anticoagulant tubes. During blood collection, special care was taken to prevent any discomfort observed in the animals. A salting-out technique was used to manually isolate genomic DNA(Al-Shuhaib, 2018). Agarose Gel electrophoresis of 0.8% (w/v in 1x TAE buffer) was applied to determine the integrity of the isolated genomic DNA. The quality and quantity of genomic DNA were validated with a Nanodrop spectrophotometer following the recommended protocols of the manufacturers (BioDrop LITE; Biodrop, UK).

PCR primers' designing

The *ADRB2* gene was included for only one exon, leading to the generation of a pair of PCR-specific primers through the utilization of default settings of the freely available online tool NCBI's primer BLAST (Ye et al., 2012). Based on the NCBI gene annotation tool (GenBank NC_056079.1), adjustments were made to the length of the designed amplicons to encompass the largest possible fragment without compromising the accuracy of PCR outcomes. The length of the PCR fragment generated was specifically set at 1166 bp, covering 379 amino acids, which accounts for approximately 91% of the total length of the protein consisting of 418 amino acid residues. Consequently, the sequences for the forward (5′-GGGCATCCTCATGTCGCTTA-3′) and reverse (5′-AGTAGAAAAACCTGCATTACAGC-3′) primers were acquired.

PCR conditions

PCR amplifications were performed on the specified 1166 bp PCR amplicons using a gradient thermocycler (Nexus, Eppendorf, Germany). In order to carry out the PCR procedure, a 20µL PCR-ready PreMix (Catalog number K-2012, Bioneer, Seoul) was employed according to the manufacturer's guidelines. The PCR experiments were executed under the following conditions: an initial denaturation step at 94 °C lasting 5 minutes, 30 cycles consisting of denaturation at 94 °C for 60 sec, primer annealing at 60 °C for 60 sec, polymerase extension at 72 °C for 60 sec, and a final extension step at 72 °C for 5 minutes. Prior to sequencing, the expected sizes of the PCR products (1166 bp) were confirmed through 1.5% (w/v in 1x TAE buffer) agarose gel electrophoresis.

DNA Sequencing

Upon confirmation of the specificity of all PCR amplicons, they underwent Sanger dideoxy-sequencing in accordance with the prescribed protocols outlined by Macrogen laboratories in Geumchen, Seoul. The identification of nucleic acid variations was meticulously carried out using SnapGene Viewer (http://www.snapgene.com) by examining the original electropherogram. As per the recommended technical parameters for visualized electropherograms(Al-Shuhaib and Hashim, 2023), only the distinct nucleotide

peaks corresponding to the representative genotypes were included for alignment with the genomic sequences of the *ADRB2* gene (GenBank accession no. NC_056079.1), while less reliable readings were excluded from further analysis.

Genetic diversity analysis

The examination of the *ADRB2*variation of identified haplotypes was carried out utilizing the standaloneDnaSPtool(Librado and Rozas, 2009). A comparison of the obtained ovine haplotypes was executed through a median-joining based network that was created by Popart software (French et al., 2014). Following this, the association between the identified nucleic acid haplotypes and the corresponding ADRB2 sequences was determined using the NCBI-blast tool (Mahram and Herbordt, 2015). Upon translation into their respective amino acid sequences, the amino acid sequences of the haplotypes, together with their associated sequences, were aligned with the corresponding NCBI-Blastp-retrieved sequences utilizing the Clustal Omega suite (Sievers and Higgins, 2014). Subsequently, a neighbor-joining tree was constructed, and the evolutionary information of the resultant tree was annotated utilizing the iTOL online server (Letunic and Bork, 2019). The Molecular Variance Analyses (AMOVA), incorporating the haplotype frequencies, number of haplotypes (h), number of variable sites (v), average indices of nucleotide differences (k), nucleotide diversity (Pi), and haplotype diversity (Hd) values. As well, Tajima's D test statistic (D) was also calculated between and within the two examined ovine breedsusing Arlequin tool (Excoffier et al., 2005).

RESULTS

SNPs detection

Upon analysis of the PCR products derived from the *ADRB2* sequences, a subsequent direct sequencing assay was conducted on the entire cohort of 172 samples under investigation. The outcomes of the sequencing analysis showed that the amplified segment of 1166 base pairs exhibited a notable level of genetic diversity, which was supported by the identification of twenty-seven SNPs. Through the examination of sequencing data and subsequent alignment with known genomic DNA sequences, it was observed that fourteen insertion SNPs were present, while the remaining SNPs were classified as substitution SNPs. The identified insertion SNPs were distributed evenly throughout the amplified section with varying frequencies across the studied ovine groups. Subsequent to the insertion SNPs, thirteen substitution SNPs were detected within the 1166 base pair fragments. The analysis of substitution SNPs into amino acid sequences revealed that nine of these SNPs had a synonymous effect on the protein. Conversely, only four substitutions resulted in non-synonymous mutation on the *ADRB2* protein (Table 1). The sequencing analysis indicated that the detected insertion SNPs constituted the majority of identified SNPs, comprising approximately 51.9% of the total polymorphism. In contrast, synonymous and non-synonymous SNPs represented about 33.3% and 14.8% of the overall SNPs, respectively. Sequencing results showed that fourteen SNPs of these variants, which accounted for about 51.8% of the total twenty-seven SNPs, were detected in both populations.

Genetic diversity of the detected SNPs

Based on the identified genetic variations, various tools were employed to assess genetic diversity and haplotyping potentials for both sheep populations. DnaSP tool showed the presence of various numbers of haplotypes between Awassi and Karakul sheep were compared in terms of genetic diversity. The Awassi breed displayed a lower number of haplotypes (h = 13) in comparison to Karakul (h = 16), leading to a slightly decreased Hd value in Awassi (0.6635) as opposed to Karakul (0.6414). Nucleotide diversity assessments revealed a lower Pi value in Awassi (Pi = 0.00093) when contrasted with Karakul (Pi = 0.00132). Moreover, Karakul demonstrated higher Pi indices and a greater average number of nucleotide differences (k = 1.54446) than Awassi (k = 1.08673). Both Awassi and Karakul breeds displayed non-significant Tajima's D test indices, indicating no significant departure from neutrality in terms of genetic variation. For Awassi sheep, the Tajima's D value is -0.99321 and is non-significant with a p-value greater than 0.05. This indicates that the observed value is not statistically different from what would be expected under neutrality. For Karakul sheep, the Tajima's D value is -0.79661 and is non-significant with a p-value greater than 0.01 (Table 2).

Similar to the Awassi, this suggests that the genetic variation observed is consistent with neutral evolution, without strong evidence of selection or demographic changes. The genetic variation within and between these populations was assessed using AMOVA calculations between the Awassi and Karakul breeds and within each population. Within between population scales, the observed variance

Table 1. Genetic polymorphism that detected in the *ADRB2* gene of Awassi and Karakul breeds.

No.	Polymorphism of nucleic acid	Position in amino acid	Effect of variant	Variant name	Breed
	100C>T	Asn29	Silent SNP	p.Asn29=	Both
	105-106insT	Phe31	Insertion SNP	g.58017536-58017537insT	Both
	115C>A	Ser35	Silent SNP	p.35Ser=	Both
	176-177insC	Leu56	Insertion SNP	58017607-58017608insC	Both
	230-231insG	Asp74	Insertion SNP	58017661-58017662insG	Awassi
	334G>A	Lys108	Silent SNP	p.Lys108=	Both
	390-391insT	Phe127	Silent SNP	p.Phe127=	Awassi
	478G>A	Thr156	Silent SNP	p.Thr156=	Karakul
	569G>C	Ala226	Silent SNP	p.Ala226=	Both
	756C>G	Pro249	Missense SNP	p.Pro>Arg249	Karakul
	808G>C	Lys266	Missense SNP	p.Lys>Asn266	Both
	829C>A	Asn273	Missense SNP	p.273Asn>Lys	Both
	838C>G	Gly276	Silent SNP	p.Gly276=	Karakul
	976-977insG	Phe282	Insertion SNP	58018407-58018408insG	Awassi
	981-982insA	Asp324	Insertion SNP	58018413-58018414insA	Awassi
	988G>A	Gly327	Silent SNP	p.Gly327=	Both
	992-993insG	Gly327	Insertion SNP	58018422-58018423insGA	Awassi
	993-994insA	Gly327	Insertion SNP	58018422-58018423insGA	Awassi
	998-999insA	Ser330	Insertion SNP	58018429-58018430insA	Awassi
	999G>A	Ser330	Missense SNP	p.Ser>Asn330	Both
	1003-1004insA	Gly331	Insertion SNP	58018434-58018435insA	Awassi
	1010-1011insC	His333	Insertion SNP	58018440-58018441insC	Awassi
	1016-1017insG	Glu336	Insertion SNP	58018447-58018448insG	Both
	1024-1025insA	Lys338	Insertion SNP	58018455-58018456insA	Both
	1029G>C	Ser340	Silent SNP	p.Ser340=	Karakul
	1033-1034insA	Asp339	Insertion SNP	58018457-58018458insA	Both
	1065-1066insA	Asn352	Insertion SNP	58018496-58018497insA	Both

Table 2. The analysis of genetic diversity of Awassi and Karakul breeds based on the ADRB2 gene sequencing.

Breed	N	h	v	Hd	Pi	k	D
Awassi	88	13	9	0.6635	0.00093	1.08673	-0.99321 non-significant (p> 0.05)
Karakul	84	16	11	0.6414	0.00132	1.54446	-0.79661 non-significant (p> 0.01)

Abbreviations: n– Sampling size; v – number of variable sites; h – number of haplotypes, Hd– haplotype diversity; Pi – nucleotide diversity; k – average number of nucleotide differences; D – Tajima's D test statistic for Awassi and Karakul sheep.

component of 1.25 Vb indicated the presence of noticeable genetic differentiation between the Awassi and Karakul breeds. The percentage of variation (23.45%) showed that a significant portion of the total genetic variation was attributed to differences between these two populations. Within each population scale, the variance component of 121.25 Vw showed that the greater amount of genetic variation was attributed to the genetic differences in each breed. The percentage of variation of 76.55% demonstrated that the majority of the genetic variation was within populations rather than between them. While 67.89% of the variation was within the Awassi population, 45.67% was detected within the Karakul population (Table 3).

Haplotypes distributions

Based on the analysis of sequencing reactions, the number of haplotypes was categorized for both investigated breeds. The distribution and frequency

Table 3. Analysis of Molecular Variance (AMOVA) of theADRB2 gene of Awassi and Karakul breeds.

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	*p* - value
Between populations	1	12.34	1.25 Vb	23.45%	< 0.05
Within populations	170 (87 for Awassi and 83 for Karakul)	91.34 (56.78 for Awassi and 34.56 for Karakul)	121.25Vw (for both Awassi and Karakul)	76.55% (67.89% for Awassi and 45.67% for Karakul)	< 0.05
Total	171	103.68	122.5	100.00	< 0.05

Abbreviation:Vb= variance between populations, Vw= variance within populations

Table 4. Haplotype distributions and frequencies for the studied ADRB2 gene in 172 samples of Awassi (n=88) and Karakul (n=84) sheep.

Awassi sheep (haplotype no. =13)		Karakul sheep (haplotype no. =16)	
Haplotype (n)	**Sample no.**	**Haplotype (n)**	**Sample no.**
Awa-1: 2	[1-2]	Kara-1: 50	[1-8 13-24 31-32 37-38 51-52 55-60 63-66 69-72 75-84]
Awa-2: 50	[3-4 9-12 15-24 29-42 45-46 49-56 65-68 79-80 83-86]	Kara-2: 2	[9-10]
Awa-3: 8	[5-6 27-28 57-58 81-82]	Kara-3: 2	[11-12]
Awa-4: 2	[7-8]	Kara-4: 4	[25-26 43-44]
Awa-5: 2	[13-14]	Kara-5: 2	[27-28]
Awa-6: 4	[25-26 87-88]	Kara-6: 2	[29-30]
Awa-7: 4	[43-44 69-70]	Kara-7: 2	[33-34]
Awa-8: 2	[47-48]	Kara-8: 2	[35-36]
Awa-9: 2	[59-60]	Kara-9: 2	[39-40]
Awa-10: 2	[61-62]	Kara-10: 2	[41-42]
Awa-11: 6	[63-64 73-74 77-78]	Kara-11: 2	[45-46]
Awa-12: 2	[71-72]	Kara-12: 2	[47-48]
Awa-13: 2	[75-76]	Kara-13: 2	[49-50]
		Kara-14: 2	[53-54]
		Kara-15: 4	[61-62 67-68]
		Kara-16: 2	[73-74]

of haplotypes in the *ADRB2* gene among 172 sheep samples, comprising 88 Awassi sheep and 84 Karakul sheep are presented in Table 4. DnaSP tool showed the presence of 13 haplotypes identified in Awassi sheep (assigned Awa-1 to Awa-13) and 16 in Karakul sheep (assigned Kara-1 to Kara-16). It was found that the Awa-2 is the most frequent haplotype due to its presence in 50 out of 88 samples (approximately 56.8%). Similarly, Kara-1 is the predominant haplotype in Karakul sheep due to its detection in 50 out of 84 samples (approximately 59.5%). The remaining haplotypes in both breeds are distributed among a smaller number of samples that ranged from two in most of them to eight samples.

The differences among the haplotypes between Awassi and Karakul were reflected in their relative percentages between Awassi and Karakul breeds. Awa-2 scored the highest relative percentage with a frequency of 0.5682 compared with the other twelve haplotypes that constituted the remaining percentage. Similarly, Kara-1 exhibited the utmost degree of relative percentage at a frequency of 0.5952 in comparison to the fifteen other haplotypes that made up the rest of the percentage (Table 5). However, not all the detected haplotypes exerted exclusive availability in a particular breed since five shared haplotypes between both investigated populations were identified (Fig. 1, A). The resultant median-joining network depicted the true relationships among the identified haplotypes, showcasing the distribution and connections of haplotypes within these populations. Within the majority of haplotypes' distributions, a distinct differentiation was noted between the two scrutinized breeds owing to the evident segregation of both breeds into two discrete clusters, namely the Awassi group and the Karakul group. This produced network revealed that a total of eight haplotypes belonging to Awassi (Awa-1, Awa-4, Awa-6, Awa-7, Awa-9, Awa-10, Awa-12, and Awa-13) were exclusively present in the Awassi region. Likewise, eleven haplotypes of Karakul (Kara-2, Kara-3, Kara-4, Kara-5, Kara-6, Kara-8, Kara-9, Kara-11, Kara-12, Kara-13, and Kara-16) were also exclusively found in the Karakul region as well. From both investigated breeds, five haplotypes were found to exhibit intermediate positioning between the haplotypes of Awassi and Karakul (Fig. 1, B).

Table 5. Relative frequencies of the ADRB2 gene-based haplotypes detected in Awassi and Karakul populations.

Haplotype	n	Awassi	Karakul
Awa-1	2	0.0227	-
Awa-2	50	0.5682	-
Awa-3	8	0.0909	-
Awa-4	2	0.0227	-
Awa-5	2	0.0227	-
Awa-6	4	0.0455	-
Awa-7	4	0.0455	-
Awa-8	2	0.0227	-
Awa-9	2	0.0227	-
Awa-10	2	0.0227	-
Awa-11	6	0.0455	-
Awa-12	2	0.0227	-
Awa-13	2	0.0227	-
Kara-1	50	-	0.5952
Kara-2	2	-	0.0238
Kara-3	2	-	0.0238
Kara-4	4	-	0.0476
Kara-5	2	-	0.0238
Kara-6	2	-	0.0238
Kara-7	2	-	0.0238
Kara-8	2	-	0.0238
Kara-9	2	-	0.0238
Kara-10	2	-	0.0238
Kara-11	2	-	0.0238
Kara-12	2	-	0.0238
Kara-13	2	-	0.0238
Kara-14	2	-	0.0238
Kara-15	4	-	0.0476
Kara-16	2	-	0.0238

Phylogenetic analysis

Upon evaluating the phylogenetic connections existing among the haplotypes under investigation, an assessment was conducted in conjunction with the sequences of related ovine and caprine organisms. This evaluation involved the development of a comprehensive tree. The majority of the haplotypes examined were observed to be distributed across different neighboring phylogenetic clusters within this tree. This particular arrangement of phylogenetic clusters provided compelling evidence of the close phylogenetic relationships between the two analyzed breeds. The constructed tree featured three primary haplotype groups that were specific to Awassi (Awa-haplotypes), Karakul (Kara-haplotypes), and

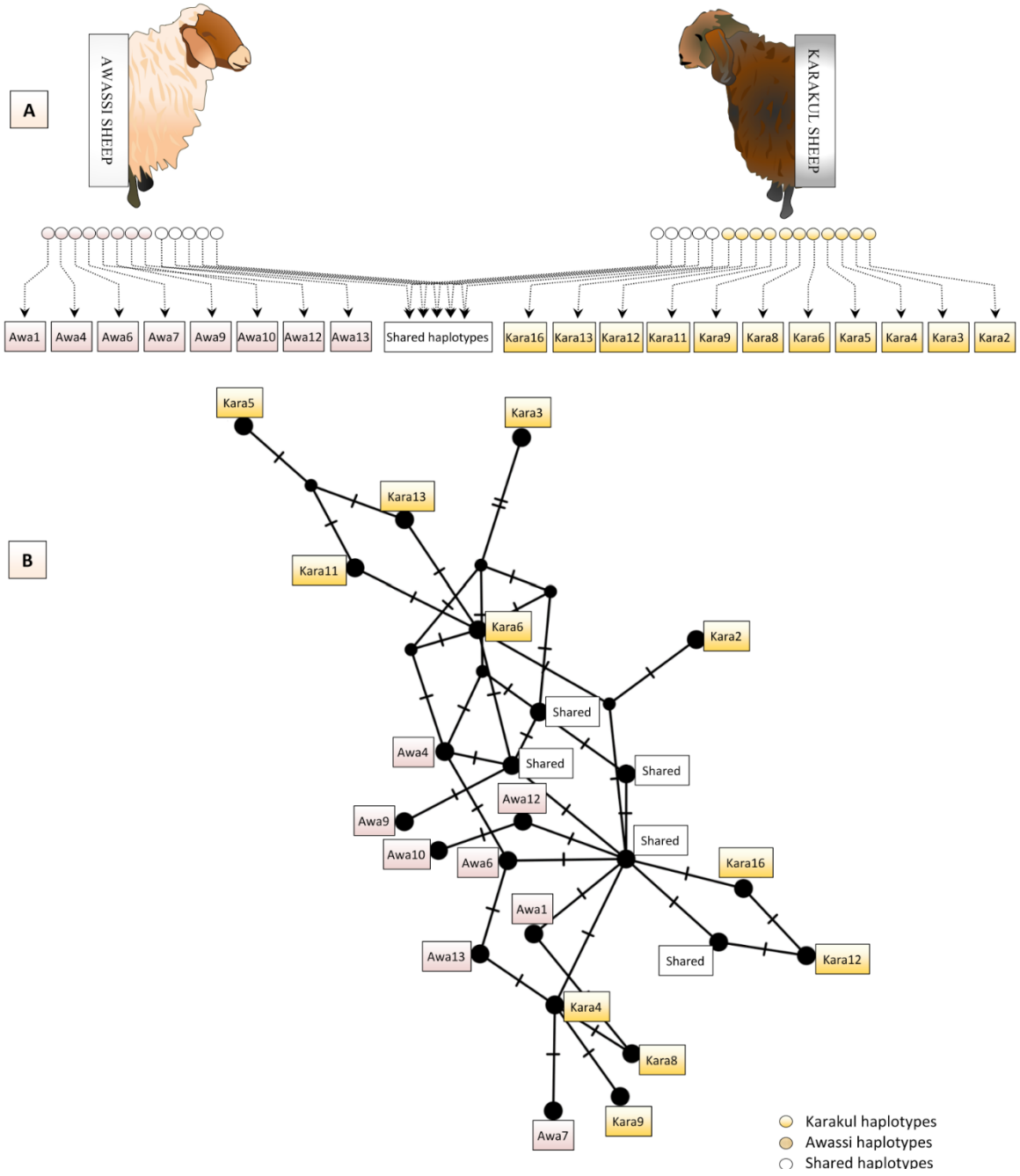

Figure 1. Nucleic acids networking in the ADRB2 gene between Awassi and Karakul sheep. (A) The total number of haplotypes in both investigated populations. (B) The median-joining network generated for the identified haplotypic distributions. Yellow and pink colors respectively refer to Karakul and Awassi breeds.

Shared-haplotypes. The ovine breeds displayed distinctive phylogenetic placements, with the shared haplotypes occupying proximate positions. Notably, among these haplotypes, Kara-2 exhibited the most extreme positioning relative to the others. In contrast, Kara-3 and Kara-4 displayed less extreme placements in the tree, which were descended from Kara-5 and Kara-6. Other than these haplotypes, the positioning of the other haplotypes was determined to display identical rooting values as observed for all ovine haplotypes. The inclusive tree revealed that the haplotypes of the scrutinized Awassi and Kara-

kul sheep were derived from Chinese breeds. These registered breeds were linked to the Chinese Merino (GenBank ACD85812.1), the Chinese Hazakh breeds (GenBank NP_001123626.1), and the Chinese Tibetan breed (GenBank KAG5209382). Nonetheless, it was noted that all these specified breeds originated from Asian nations, with no indication of non-Asian origins. Apart from the ovine species, several outgroup species have been incorporated to establish the tree's root and offer an evolutionary framework. These include sequences from species of *Sus scrofa, Mus musculus, Camelus ferus, Equus caballus, Mirounga angustirostris, Bos taurus,* and *Capra hircus*. Among these outgroup sequences, the *C. hircus* sequences showed the presence of the highest level of amino acid similarity with the investigated ovine sequences (Fig. 2). The highest level of similarity showed the presence of highly-related ovine - caprine species in the investigated sequences than that observed with the other out-group species.

DISCUSSION

The primary rationale behind conducting an association analysis between *ADRB2* and growth characteristics in sheep of the Karakul and Awassi breeds lies in the existing knowledge of distinct growth traits exhibited by these breeds (Aljubouri et al., 2021). The quest for identifying a causal SNP or SNPs responsible for the documented phenotypic differences is indeed a thought-provoking endeavor. Consequently, an examination of the disparities between these significant sheep breeds was undertaken, focusing on the variability of the ADRB2 gene and its potential correlations with both breeds. The concise nature of the *ADRB2* gene, encompassing only one exon, necessitated the design of a singular PCR fragment to encompass the majority of its coding sequences.

The observed differences in ADRB2 polymorphisms between Awassi and Karakul reflect the level of genetic biodiversity underlying the distinct pheno-

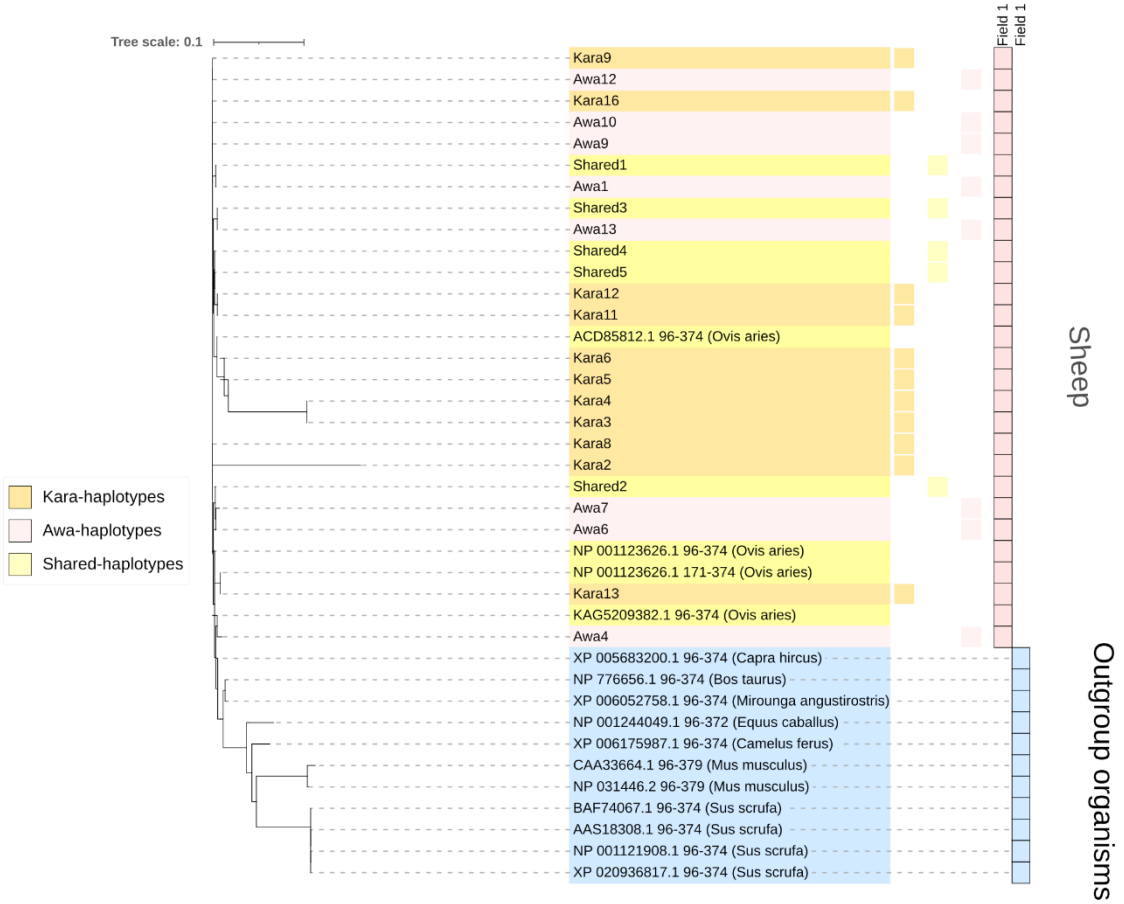

Figure 2. ADRB2 amino acid sequences-based neighbor-joining phylogenetic tree for Awassi and Karakul populations.

typic traits of these breeds. These observations align with studies indicating that both breeds exhibit high levels of genetic diversity, with significant polymorphisms detected across various loci (Yalmaz et al., 2014). While the Awassi breed is well known for its resilience in arid climates and enhanced growth traits across Asian and African regions (Galal et al., 2008; Khazaal et al., 2023), the Karakul breed is recognized for its superior adaptability and unique fleece characteristics (Aljubouri and Al-Shuhaib, 2023). When contrasting Awassi and Karakul sheep with other ovine breeds such as Merino or Suffolk, several key differences emerge. Studies have shown a significant genetic distance between Awassi, Karadi, and Jaff breeds, reflecting moderate levels of differentiation (Yousif et al., 2023). In contrast, Merino sheep exhibit lower genetic diversity compared to Awassi (Parsons et al., 1996), likely due to selective bottleneck breeding practices that have reduced genetic variability (Diez-Tascón et al., 2000). The observed SNPs of the ADRB2 gene further contribute to this understanding by identifying novel haplotypes and breed-specific polymorphisms, providing valuable insights into the genetic mechanisms driving phenotypic and adaptive differences across ovine breeds.

Notably, a total of twenty-seven SNPs were detected within the two scrutinized populations, with the majority being ascribed to insertions. The notable genetic diversity observed within *ADRB2* is not surprising, given the extensive diversity previously noted in the *ADRB3* gene, which has demonstrated significant associations with various growth traits in ovine populations (Yang et al., 2009).As a result of the considerable genetic diversity, the ADRB2 amplicons under examination displayed unique distributions that differed between the Awassi and Karakul breeds. Among the twenty-nine haplotypes identified, Karakul sheep demonstrated a greater capacity for haplotyping with 16 haplotypes, in contrast to Awassi, which presented only 13 haplotypes. The higher haplotyping potential of Karakul sheep was reflected in the higher nucleotide variations and diversity. In contrast with our study, it has been shown that the mitochondrial D-loop genetic diversity highlighted higher nucleotide polymorphism in Awassi sheep compared to Karakul sheep, emphasizing the genetic differences among these breeds(Aljubouri and Al-Shuhaib, 2021). However, this study was conducted on mitochondrial genetic diversity and may not be directly related to the nuclear genetic polymorphisms of the *ADRB2* gene. Other than this study, no sufficient literature was found to support or refute our findings since the majority of comparison studies have been confined only to the assessment of the genotype-phenotype associations between both breeds. However, these studies have confirmed that the superiority of Karakul over Awassi in various growth traits has been correlated with the polymorphism of various genetic loci that performed variable metabolic activities (Alwan et al., 2023). Due to the complexity of the genetic makeup in mammals, the *ADRB2* gene may be one of these genetic loci that might contributeto the possible association with one or several of these differences between both breeds.

For both breeds, Tajima's D values are negative but not statistically significant, suggesting a possible excess of low-frequency polymorphisms, which could be indicative of population expansion or purifying selection, but the non-significant p-values imply that these results are not strong enough to reject the null hypothesis of neutral evolution. Though both breeds showed non-significant Tajima's D values, the Awassi breed has a slightly more negative Tajima's D value compared to the Karakul breed. These data suggest a marginally stronger indication of potential population expansion or purifying selection in Awassi than in Karakul. These data are in agreement with other reports that showed that this breed has widely been subjected to selective pressures favoring population expansion and genetic improvement (Ali et al., 2020). Studies on Awassi sheep in Middle Eastern regions have focused on estimating heritability and genetic trends for growth traits, indicating a need for controlled breeding to enhance desirable characteristics (Al-Moman et al., 2020). The genetic potential of Awassi sheep, demonstrated through factors like birth weight, weaning weight, and wool production, showcases their resilience and productivity, suggesting a history of selective pressures for favorable traits(Al-Atiyat et al., 2021).

The median-joining network analysis revealed that five shared haplotypes were present in both examined sheep populations, indicating that the *ADRB2* variation exhibits multiple homologies across the two investigated breeds. The shared presence of these haplotypes may indicate either gene flow between the populations or a shared ancestral genetic reservoir. The genetic overlap between Awassi and Karakul sheep may be linked to historical interactions between these populations, or it could indicate that *ADRB2* sequences play a role in establishing a shared genetic background for both breeds. Never-

theless, the extent of these shared haplotypes did not exceed 5 out of 13 in Awassi(about 38.5%) and 16 (31.2%) in Karakul sheep. Whereas the other eight and nine haplotypes in Awassi and Karakul sheep exerted unique genetic constituents throughout the amplified genetic fragments. Among the unique Karakul haplotypes, the Kara2, Kara3, Kara4, Kara5, Kara6, Kara8, Kara9, Kara11, Kara12, Kara13, and Kara16 showed specific distribution to the Karakul sheep population. Their distinct positions in the network indicate unique genetic variations that are not shared with the Awassi population or other haplotypes. The presence of these unique haplotypes suggests a level of genetic differentiation specific to the Karakul population. This can be indicative of unique evolutionary pressures, breeding practices, or historical lineage that have shaped the genetic makeup of the Karakul sheep. On the other hand, Awa1, Awa4, Awa6, Awa7, Awa9, Awa10, Awa12, and Awa13 haplotypes were found to be unique to the Awassi sheep population due to their variations that are specific to this population. However, both Kara-1 and Awa-2, the two haplotypes that were observed to exhibit the highest frequencies in Karakul (0.5952) and Awassi (0.5682) were found to be shared for both breeds. These highly frequent areas of overlap can suggest the presence of a potential common ancestor for both breeds. To assess the accuracy of this suggestion, a phylogenetic tree was generated to find out to what extent the claimed common ancestor is present for both populations.

Our extensive dataset revealed close proximity between the two breeds under study and suggested that both populations investigated shared ancestry with various sheep breeds originating from a single Asian source. The proximate phylogenetic connections noted between Awassi and Karakul may possibly be clarified by a notable genetic similarity existing between them. Although sequences of *ADRB2* for Awassi and Karakul sheep were found to exert various numbers of haplotype differences, the comprehensive tree constructed in this study indicated that most identified haplotypes clustered near three Chinese ovine strains, specifically Merino, Hazakh, and Tibetan breeds. Despite the Kara-2 haplotype deviating from others, this difference did not extend beyond the species level. The prevalence of Merino, Hazakh, and Tibetan breeds in the tree constructed could be due to factors other than a strong similarity to the sequences under investigation, possibly linked to the limited *ADRB2* database in NCBI. The deficiency of *ADRB2* sequences led to the absence of other breeds in close proximity to our sequences. Nonetheless, the observed pattern of phylogenetic distributions has further validated the intimate phylogenetic connection of our examined ovine sequences with Chinese strains. Moreover, our results indicated that all incorporated breeds, includingAwassi, Karakul, and ovine other strains in the tree, descended exclusively from Chinese ancestors. This is not unusual since Chinse sheep have been characterized with high levels of biological diversity over the other breeds worldwide (Lv et al., 2022). However, The notable ADRB2 polymorphism identified in Karakul, followed by Awassi, may be associated with diverse characteristics beyond mere phenotypic distinctions, potentially implicating metabolic pathways governing these traits. Despite the lack of substantial empirical evidence to affirm or challenge these hypotheses, such conjectures must not be dismissed. The *ADRB2* variations existing in Awassi and Karakul breeds are likely to have a significant impact on the nuanced differences among ovine species, potentially introducing specific variations that go beyond *ADRB2* sequences to facilitate adaptations to varying environmental conditions.

The currently observed genetic variations can also be explained by the obvious and multi-aspect differences between both breeds. Due to its exceptional adaptation to various environmental conditions, including high temperatures, diseases, and parasites, the Awassi sheep breed is well-suited for both desert and temperate climates (Sharma et al., 2017). Additionally, genetic studies have identified a specific genetic role to impact heat tolerance in Awassi sheep, affecting their ability to cope with elevated temperatures (Al-Thuwaini et al., 2020). Meanwhile, the Karakul breed has been shown to exert a high level of performance due to its demonstrated adaptation to live in various conditions (Halil and Özbeyaz, 2020). Hence, further investigation is imperative to delve into the latent potential of the ADRB2 fragment under scrutiny in the diversity and distinctions of ovine species.

CONCLUSION

The *ADRB2* gene exhibited significant genetic diversity with the identification of twenty-seven variants, including insertions, silent SNPs, and missense SNPs. Genetic diversity analysis indicated that Karakul sheep displayed a higher number of haplotypes and variable sites compared to Awassi sheep, sug-

gesting greater genetic differentiation within the Karakul population. Analysis of molecular variance further supported this differentiation, with a majority of genetic diversity attributed to within-population variation. The median-joining network illustrated distinct clustering of haplotypes by breed, with a notable overlap of five haplotypes between Karakul and Awassi populations. Phylogenetic analysis traced the ancestry of these haplotypes back to Chinese origins, underscoring a shared genetic heritage despite distinct breed characteristics. Given the ability of the *ADRB2* gene to differentiate between these important Middle Eastern sheep breeds, its potential as a robust biological marker for tracing broader ovine genetic diversity is emphasized. Further research leveraging this marker could enhance breeding strategies and conservation efforts aimed at preserving and utilizing genetic diversity in sheep populations. Further research is needed to fully understand the functional implications of these *ADRB2* variations in both breeds, particularly regarding heat tolerance and adaptation to diverse environmental conditions.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Al-Atiyat RM, Aljumaah RS, Alshaikh MA, Abudabos AM (2018) Microsatellite-based genetic structure and diversity of local Arabian sheep breeds. Front Genet 9: 408.

Al-Atiyat RM, Tabbaa MJ, Barakeh FS, Awawdeh FT, Baghdadi SH (2021) Power of phenotypes in discriminating Awassi sheep to pure strains and from other breeds. Trop Anim Health Prod 53(1): 139.

Al-Barzinj YMS, Ali MK (2013) Genetic diversity among some sheep breeds in Sulaimani Governorate using RAPD-PCR Technique. J Life Sci 7(9): 971.

Aljubouri T, Hassan A, Al-Shuhaib MB (2022) Novel association between GnRHR gene and growth traits in Awassi and Karakul sheep. J Microbiol Biotechnol food Sci 11(5): e3241-e3241.

Al-Moman AQ, Ata M, Al-Najjar KA (2020) Evaluation of weight and growth rates of Awassi sheep lambs. Asian J Res Anim Vet Sci5(3): 26-32.

Al-Shuhaib MBS (2018) A minimum requirements method to isolate large quantities of highly purified DNA from one drop of poultry blood. J Genet 97(1): e87-e94.

Al-Shuhaib MBS, Hashim HO (2023) Mastering DNA chromatogram analysis in Sanger sequencing for reliable clinical analysis. J Genet Eng Biotechnol21(1): 115.

Al-Thuwaini TM, Al-Shuhaib MBS, Hussein ZM (2020) A novel T177P missense variant in the HSPA8 gene associated with the low tolerance of Awassi sheep to heat stress. Trop Anim Health Prod 52(5): 2405-2416.

Ali A, Javed K, Zahoor I, Anjum KM, Sharif N (2023) Identification of polymorphisms in the MSTN and ADRB3 genes associated with growth and ultrasound carcass traits in Kajli sheep. Anim Biotechnol 34(4): 847-862.

Ali W, Ceyhan A, Ali M, Dilawar S (2020) The merits of Awassi sheep and its milk along with major factors affecting its production. J Agric Food, Environ Anim Sci1(1): 50-69.

Aljubouri TRS, Hassan AF, Al-Shuhaib MBS, Mahyari SA (2020) Association of GnRH1 Gene with Growth Traits in Two Breeds of Sheep. Agric Res 10(2): 285-293.

Aljubouri TR, Al-Shuhaib MBS (2023) A missense SNP in the proopiomelanocortin (POMC) gene is associated with growth traits in Awassi and Karakul sheep. Anim Biotechnol 34(9): 4837-4850.

Aljubouri TRS, Hassan AF, Al-Shuhaib MBS (2021) Remarkable correlation of non-genetic factors with growth traits in two breeds of sheep. Iraqi J Agric Sci 52(2): 309-314.

Aljubouri TRS, Al-Shuhaib MBS (2021) Genotyping of mitochondrial D-loop sequences in three breeds of sheep. Biologia (Bratisl) 76(1): 203-211.

Alwan IH, Aljubouri TRS, Al-Shuhaib MBS (2023) A Novel Missense SNP in the Fatty Acid-Binding Protein 4 (FABP4) Gene is Associated with Growth Traits in Karakul and Awassi Sheep. Biochem Genet 1-23.

Burguete-García AI, Martínez-Nava GA, Valladares-Salgado A, Bermudez Morales VH, Estrada-Velasco B, Wacher N, Peralta-Romero J, Garcia-Mena J, Parra E, Cruz M (2014) Association of β1 and β3 adrenergic receptors gene polymorphisms with insulin resistance and high lipid profiles related to type 2 diabetes and metabolic syndrome. Nutr Hosp 29(6): 1327-1334.

Dahlman I, Arner P (2007) Obesity and polymorphisms in genes regulating human adipose tissue. Int J Obes 31(11): 1629-1641.

Diez-Tascón C, Littlejohn RP, Almeida PAR, Crawford AM (2000) Genetic variation within the Merino sheep breed: analysis of closely related populations using microsatellites. Anim Genet 31(4): 243-251.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. EvolBioinforma 1: 117693430500100000.

Fadhil IA, Al-Shuhaib MBS (2022) Phylogenetic differentiation between Awassi and Hamdani sheep using the mitochondrial 12S rRNA. Anim Biotechnol 33(5): 801-809.

French N, Yu S, Biggs P, Holland B, Fearnhead P, Binney B, Fox A, Grove-White D, Leigh JW, Miller W (2014) Evolution of campylobacter species in New Zealand. Campylobacter Ecology and Evolution 221-240.

Galal S, Gürsoy O, Shaat I (2008) Awassi sheep as a genetic resource and efforts for their genetic improvement – A review. Small Rum Res 79(2-3): 99-108.

Hadi YA, Mnati AA, Abdulfattah SY (2020) Study of genetic diversity using microsatellite markers in Iraqi sheep breeds. The Iraqi Journal of Agricultural Science 51(5): 1367-1374.

Halil E, Özbeyaz C (2020) Investigating various performance traits of Karakul sheep. Ankara Üniversitesi Vet Fakültesi Derg67(2): 113-120.

Huang Y-F, Zhao Y, He J-N (2017) Genetic diversity of three Chinese native sheep breeds. Russ J Genet 53(1): 118-127.

Khazaal NM, Alghetaa HF, Al-Shuhaib MBS, Al-Thuwaini TM, Alkhammas AH (2023) A novel deleterious oxytocin variant is associated with the lower twinning ratio in Awassi ewes. Anim Biotechnol 34(8): 3404-3415.

Khush GS (2023) Biodiversity is nature's gift for the survival of the human race: Some reflections. In Crop and Environment (Vol. 2, Issue 1, pp. 1-4). Elsevier.

Letunic I, Bork P (2019) Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res 47(W1): W256-W259.

Li R, Gong M, Zhang X, Wang F, Liu Z, Zhang L, Yang Q, Xu Y, Xu M, Zhang H (2023) A sheep pangenome reveals the spectrum of structural variations and their effects on tail phenotypes. Genome Res 33(3): 463-477.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11): 1451-1452.

Litonjua AA, Gong L, Duan QL, Shin J, Moore MJ, Weiss ST, Johnson JA, Klein TE, Altman RB (2010) Very important pharmacogene summary ADRB2. PharmacogenetGenom 20(1): 64-69.

Lucas T, Losert D, Allen M, Halaschek-Wiener J, Pratscher B, Friedrich C, Wolschek M, Fuchsjäger-Mayrl G, Schmetterer L, Pehamberger H (2004) Combination allele-specific real-time PCR for differentiation of β2-adrenergic receptor coding single-nucleotide polymorphisms. Clin Chem 50(4): 769-772.

Lv FH, Cao YH, Liu GJ, Luo LY, Lu R, et al. (2022) Whole-genome resequencing of worldwide wild and domestic sheep elucidates genetic diversity, introgression, and agronomically important loci. Mol Biol Evol 39(2):msab353.

Machová K, Marina H, Arranz JJ, Pelayo R, Rychtářová J, Milerski M, Vostrý L, Suárez-Vega A (2023) Genetic diversity of two native sheep breeds by genome-wide analysis of single nucleotide polymorphisms. Animal 17(1): 100690.

Mahram A, Herbordt MC (2015) NCBI BLASTP on high-performance reconfigurable computing systems. TRETS 7(4): 1-20.

Moradi MH, Nejati-Javaremi A, Moradi-Shahrbabak M, Dodds KG, Brauning R, McEwan JC (2022) Hitchhiking mapping of candidate regions associated with fat deposition in Iranian thin and fat tail sheep breeds suggests new insights into molecular aspects of fat tail selection. Animals 12(11): 1423.

Notter DR (1999) The importance of genetic diversity in livestock populations of the future. J Anim Sci 54(6): 61-69.

Obeidat M, Obeidat B (2022) Fattening performance and carcass traits of two fat-tailed sheep breeds.Trop Anim Health Prod 53(1): 375.

Ozerov MY, Tapio M, Kantanen J, Marzanova SN, Koreckaya EA, Lushnikov VP, Marzanov NS (2020) Environmental factors affecting genetic variation in coarse-wool sheep. Russ Agric Sci 46: 65-70.

Parsons YM, Cooper DW, Piper LR, Parsons YM, Cooper DW (1996) Genetic variation in Australian Merino sheep. Anim Genet 27(4): 223-228.

Sharma NK, Sharma MK, Gahlot GC (2017) Comparative performance of Marwari and Karakul sheep in Arid zone: growth. Vet Pract18(1): 111.

Sievers F, Higgins DG (2014) Clustal omega. Curr ProtocBioinforma48(1): 3-13.

Sponenberg DP (2020) Conserving the genetic diversity of domesticated livestock. In Diversity (Vol. 12, Issue 7, p. 282). MDPI.

Vaughn SE (2012) Review of the third edition of the guide for the care and use of agricultural animals in research and teaching. J Am Assoc Lab Anim Sci51(3): 298-300.

Wu J, Qiao L, Liu J, Yuan Y, Liu W (2012) SNP variation in ADRB 3 gene reflects the breed difference of sheep populations. Mol Biol Rep 39: 8395-8403.

Yang G, Hickford JGH, Zhou H, Fang Q, Forrest RH (2011) Extended haplotype analysis of ovine ADRB3 using polymerase chain reaction single strand conformational polymorphism on two regions of the gene. DNA Cell Biol30(7): 445-448.

Yang G, Zhou H, Hu J, Luo Y, Hickford JGH (2009) Extensive diversity of the ADRB3 gene in Chinese sheep identified by PCR-SSCP. Biochem Genet 47: 498-502.

Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL (2012) Primer-BLAST: a tool to design target-specific

primers for polymerase chain reaction. BMC Bioinformatics 13(1): 134.

Yilmaz O, Cemal I, Karaca O (2014) Genetic diversity in nine native Turkish sheep breeds based on microsatellite analysis. Anim Genet 45(4): 604-608.

Yousif AN, Abdullah SM, Shaker AS, Ameen QA, Mohammed MS, Muhammad SJ, Mustafa SM, Aziz CR (2023) Genetic diversity assessment of some Iraqi Sheep breeds using microsatellite DNA markers. TJAS 23(3): 41-50.

Zavala K, Vandewege MW, Hoffmann FG, Opazo JC (2017) Evolution of the β-adrenoreceptors in vertebrates. Gen Comp Endocrinol 240: 129-137.