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Research Article

Phenotypic and Genotypic Characterization of Sheep Breeds in Diverse Habitats of Baluchistan Province through the Analysis of the vertnin Gene

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Abstract: This study was conducted to identify the vertnin gene in four different sheep breeds of Balochistan by studying their genetic characteristics and analyzing molecular markers. For this purpose, four different sheep breeds, Balochi, Rakhshani, Harnai, and Bibrik, were selected. A total of 10 animals from each breed were chosen to study the impact of breed and the vertnin gene on carcass features. The selection of animals was based on their phenotypic characteristics such as age and teeth. Blood samples of 10 ml were collected and transported to the Laboratory of Molecular Genetics at the Faculty of Veterinary and Animal Sciences, LUAWMS Uthal, Balochistan. Genomic DNA (gDNA) extraction was carried out using a modified inorganic method. The results showed that Balochi had a change in amino acid cytosine (C) > into thymine (T) mutation, Rakhshani had a change in guanine (G) > into adenine (A) mutation, Bibrik had a change in adenine (A) > into cytosine (C) mutation, and Harnai had a change in thymine (T) > into cytosine (C) mutation. The maximum divergence was 0.15715 bp in relation to the comparison in all studied sheep breeds. The phylogenetic results showed that the Balochi breed has 95–97% similarities with the other breeds when sequences were compared. The results revealed that the gel-electrophoresis bands of the Balochi breed were slightly higher, respectively. It was concluded that the vertnin gene exists in different breeds of sheep and might show a broad range of differences, even with similar DNA sequences. However, the purity based on the vertnin gene is at high risk, as a notable difference was found between the Balochi and Harnai sheep.

Keywords: Vertnin Gene, Genotype, Phenotype Sheep Breeds, Phylogeny, Balochistan.

1. INTRODUCTION

Sheep (*Ovis aries*) were thought to be one of the earliest domesticated animals. Since the Neolithic agriculture period, they have provided a farmed source of milk, meat, wool, and hide. Archaeological data suggest that sheep were possibly first domesticated in the Fertile Crescent region of the Near East. Before present base pairs (bp) around 8000–9000 years ago, their domestication spread from the center in Asia, Africa, and Europe over the following few thousand years [1, 2]. For animal breeding and the protection of biodiversity, the application of molecular methods is very useful. It is of utmost importance to investigate the genetic potential using various biotechnological and molecular techniques to evaluate the genetic structure of sheep breeds [3]. The Balochi breed of fat-tailed sheep is able to adapt to extreme harsh environmental conditions in Baluchistan as well as the eastern part of the Islamic Republic of Iran [4, 5]. The Birbik and Harnai breeds are also fat-tailed and raised for mutton and wool purposes, commonly found in Dera Bugti district [6]. The Rakhshani breed is also famous for meat and milk, a fat-tailed breed habitat in the Rakshan valley as well as in Kalat, Janagal, and Makran cities of Balochistan. Rakhshani sheep are white in color but occasionally

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black and brown, spotted with a Roman-type nose and small ears [4]. With information regarding carcass traits and molecular genetic research on the *vertnin* gene; there is a vast variation in the number of thoracolumbar vertebrae bone among various species, including pigs (*Sus scrofa*) 21–23, [7, 8] sheep (*Ovis aries*) 19–21, and humans (*Homo sapiens*) 23–38 [9, 10]. It is reported that the *vertnin* gene has a highly influenced the lumbar and thoracic vertebral number, directly affecting carcass width, length, and height in ruminant carcasses [11]. Zhang *et al.* [2] has been reported that the *vertnin* gene is responsible for improving carcass quantity and quality. Various studies have been conducted on pigs and reported that vertebral variation in the thoracic region is considered a commercial choice in mammalian breeding. Similarly, confirmation has been done by observing the sheep vertebral variation increased in the potential of meat production positively [12]. However, no research has been seen on the influence of the *vertnin* gene on the breed carcass traits of sheep. This is one of the first studies conducted based on Pakistan sheep breeds to compare the effect of breed and *vertnin* gene on Balochi, Rakhshani, Harnai, and Birbik sheep breeds in Baluchistan, Pakistan.

2. MATERIAL AND METHODS

2.1. Experimental Animals

To observe the effect of breed on carcass traits of different sheep breeds (Balochi, Rakhshani, Harnai, and Bibrik), 10 male animals of 8 months of age were selected from each breed. In order to analyze and observe the characterization of the *vertnin* gene, the selection of animals followed phenotypic standards and a methodology similar to previous studies [13-15] conducted for morphological characterization.

2.2. Blood Collection and DNA Extraction

The 5 ml blood samples were collected from each animal's jugular vein. The collected blood samples were taken to the Laboratory of Molecular Genetics at the Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Baluchistan. Before processing, the samples were refrigerated and stored. DNA extraction was carried out using inorganic procedures recommended by [16] to study the genetic diversity and genotypic characterization of different sheep breeds in Baluchistan based on the *vertnin* gene. The PCR and DNA quality were assessed using standard markers in gel electrophoresis [16-2]. The details of primers of *vertnin* gene designed for this study given in (Table 1).

2.3. Genotype, Allele Frequency and Phylogenic Tree and Expression of Evolutionary Direction

The construction of the phylogenetic tree and the analysis of the protein sequences of the *vertnin* gene in four distinct sheep breeds from Balochistan were performed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean). A sample clustering procedure to determine an accurate percentage of evaluation (molecular clock hypothesis) requires a distance matrix to observe the taxa, which can be calculated from multiple alignments as described by Lori *et al*. [17]. After removing non-coding regions and editing the sequences, the phylogenetic tree was generated utilizing the NJ (neighbor-joining) procedure. This method employed the Q matrix, considering all branches, with a preference for selecting the most similar sequences with the lowest range values.

2.4. Sequencing

After preparation of the PCR products, prepared samples of the *vertnin* gene were sent for sequencing purposes to the Center of Applied and Molecular Biology (CAMB), University of Punjab, Lahore.

2.5. Statistical Analysis

The recorded data was typed to the computer in Microsoft Excel, and regression analysis was performed using the computer software (SAS-8.1).

3. RESULTS

3.1. Genotype and Allele Frequency

The analysis of genotype frequency for the *vertnin* gene in sheep breeds in Balochistan presented in (Table2). In this study, we analyzed the polymorphism distribution of the Balochi, Rakhsani, Harnai, and Bibrik sheep breeds. The results showed that Balochi had a change in amino acid cytosine (C) > into thymine (T) mutation, Rakhshani had a change

Table 1. Primer Sequences for *vertnin* gene used for current study.

Product	Primer sequence 5-3	Chromosomes	Position OAR 0.4	Size of product bp
1	Fwd AGTGTCATCCAGGTACCCGTTA	7	82533260	1,478
	Rvrs GCGGGACAATGGCACCTA	7	82534737	
2	Fwd AAAAGCTCTCCGAAGGAACCC	7	82534539	1,351
	Rvrs GCACCAAGCAGAACTTATGACC	7	82535889	

in guanine (G) > into adenine (A) mutation, Bibrik had a change in adenine (A) > into cytosine (C) mutation, and Harnai had a change in thymine (T) > into cytosine (C) mutation. However, based on the genotype and allele frequency, the loci of the *vertn* gene within the four sheep breeds of Baluchistan were in Hardy-Weinberg equilibrium (P 0.05) in the Balochi, Rakhshani, Bibrik, and Harnai breeds. The overall results for the Balochi breed showed that the CC genotype was more prevalent than the CT genotype at different levels. However, there was no significant difference in allele frequencies (Table 3).

3.2. Estimation of the Divergence

The divergence among breeds was observed with the help of the pair contrast procedure and base numbers. The data displayed in (Table 4) showed the number of base substitutions on each side of the sequences. The highest level of divergence was 0.15715 bp, which is similar in contrast to the different sheep breeds of Balochistan.

3.3. Phylogenetic Tree

Based on the molecular phylogenetic tree analysis, the *vertnin* gene sequences were divided into two major branches. The terminal nodes, representing present-day sequences, were used for classification, while internal nodes indicated ancestral sequences. The tree displayed a branching pattern with nodes bifurcating into two branches, reflecting distinct genetic patterns within the *vertnin* gene sequences. Comparative analysis of the sequences revealed significant taxonomic diversity, allowing for the exploration of phylogenetic relationships among different sheep breeds based on their *vertnin* gene sequences. Despite some genetic similarities, Balochi and Rakhshani sheep showed a distinct genetic distance, with divergence of 95% and 73%, respectively. The sequencing of the Balochi breed was aligned using MEGA6 software, known for its progressive algorithmic approach to sequence alignment and analysis are given in Figure 1.

Table 2. Genotype frequency analysis for vertnin gene in sheep breeds of Balochistan.

Breed	Genotype Frequency			Chi-Square Test (p-Value)
Balochi	TT	TG	GG	0.213
	0.565	0.35	0.28	
Rakhshani	AA	AG	GG	0.251
	0.45	0.35	0.27	
Bibrik	CC	CT	TT	0.236
	0.355	0.25	0.25	
Harnai	CC	CT	TT	0.291
	0.285	0.23	0.2	

Table 3. Allele frequency analysis for vertnin gene in sheep breeds of Baluchistan.

Breed	Allele Frequency		Chi-Square Test (p-Value)
Balochi	C	T	0.241
	0.775	0.75	
Rakhshani	A	G	0.233
	0.72	0.73	
Bibrik	A	G	0.254
	0.7	0.73	
Harnai	C	T	0.209
	0.65	0.695	

Table 4. The estimated divergence among the sequences of *VRTN* gene in four sheep breeds of Balochistan.

Breed	Balochi	Rakhshani	Bibrik	Harnai
Balochi	0.09555	-	-	-
Rakhshani	0.0982	-	-	-
Bibrik	0.11905	0.1065	-	-
Harnai	0.15715	0.1221	0.14415	-

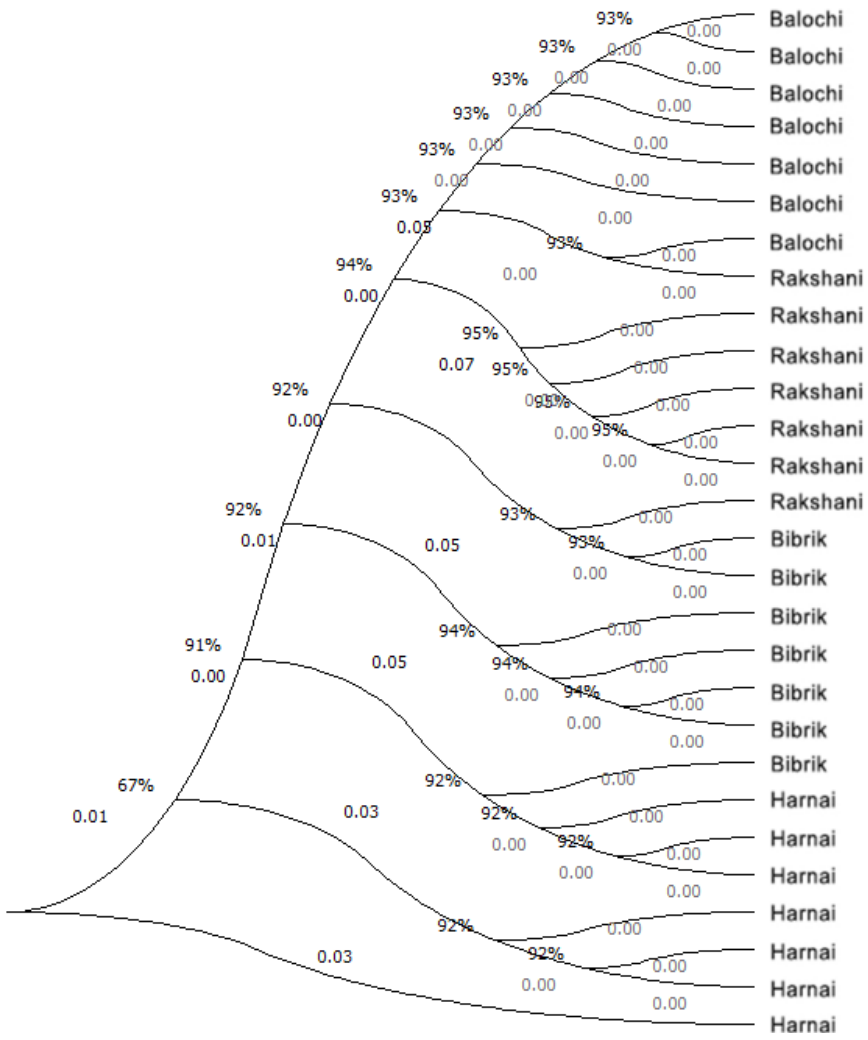

Fig. 1. Phylogenetic tree of estimated genotypic characterization of four sheep breed of VRTN gene sequencing.

3.4. PCR-Gel-Electrophoresis

PCR-gel electrophoresis was used to evaluate the bands of the *vertnin* gene in four different sheep breeds. The D-loop fragment of 778 bp (from position 1,478 to 1,351) was amplified with primers for *vertnin*. The results revealed that the bands of the Bibrik, Rakhshani, and Balochi sheep breeds were slightly higher than those of the Harnai breed (Figure 2).

3.5. Phenotypic Appearance

The results for thoracolumbar vertebral bone number, carcass height (cm), and carcass length (cm) in Balochi, Rakhshani, Harnai, and Bibrik sheep breeds were as follows. In the Balochi breed, the thoracolumbar vertebrae bone number was 20, with a carcass height of 76.12 ± 0.67 cm and a carcass length of 22.32 ± 0.18 cm. In the Rakhshani breed, the thoracolumbar vertebrae bone number was 18, with a carcass height of 71.61 ± 0.51 cm and a carcass length of 21.15 ± 0.19 cm. The Harnai breed had a thoracolumbar bone number of 18, a carcass height of 71.43 ± 0.93 cm, and a carcass length of 20.71 ± 0.31 cm. In the Bibrik breed, the thoracolumbar vertebrae bone number was 19, with a carcass height of 68.21 ± 0.7 cm and a carcass length of 18.13 ± 0.46 cm. The results showed a significant ($P < 0.05$) change in the region of thoracolumbar vertebrae has a positive influence on the carcass weight and carcass length of sheep meat details given in Table 5.

4. DISCUSSION

In our study, the genotype frequency analysis for the *vertnin* gene in different sheep breeds was

Table 5. Effect of thoracolumbar vertebral bone number on the carcass weight and carcass length of sheep breeds in Balochistan.

Breed	TVLN	Carcass height (cm)	Carcass length (cm)
Balochi	20a	76.12 ± 0.67a	22.32 ± 0.16a
Rakhshani	18c	71.61 ± 0.51b	21.15 ± 0.19b
Harani	18c	71.43 ± 0.93c	20.71 ± 0.31c
Bibrik	19b	68.21 ± 0.74	18.13 ± 0.46

*(P-Value is 0.003) The value with various superscript uppercase in same table showed significantly different ($P < 0.05$). The values with various superscript in lowercase in similar table showed highly significant different ($P < 0.05$).

conducted. In the Balochi breed, the genotype frequencies for the *vertnin* gene are TT: 0.211, TG: 0.565, and GG: 0.35. The Chi-Square test results indicate that these frequencies are significantly different from the expected frequencies ($P < 0.05$). Similar studies conducted previously and reported the least deviation among the expected frequencies

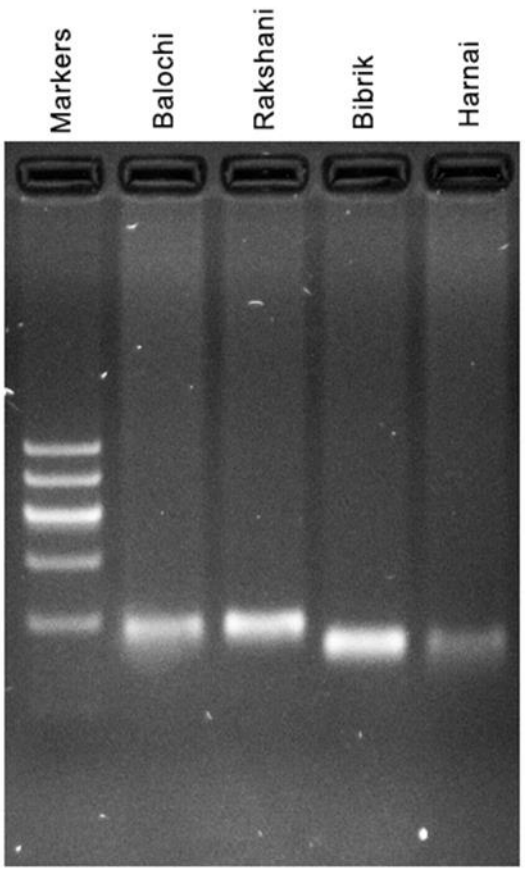

Fig. 2. PCR-amplified product of *vertnin* gene fragments from sheep breeds of Baluchistan (Marker: 1478bp to 1,351bp).

of breeds in the *vertnin* gene [18, 19]. Variations in the frequencies of different genotypes for the *vertnin* gene may indicate genetic diversity or potential breed-specific variations in the sheep populations of Baluchistan. [17-20] stated that to further understand these findings and put them into context, it would be beneficial to compare them with similar research conducted on other sheep breeds, both within the country and in other regions [21, 22]. By examining the genotype frequencies of the *vertnin* gene in other studies, we can gain a broader perspective on the genetic variations in sheep populations and potentially identify any patterns or trends. In conclusion, the genotype frequency analysis of the *vertnin* gene in sheep breeds of Baluchistan reveals significant variations in genotype frequencies across different breeds. Further research comparing these findings with other studies and exploring the functional implications of these genotypes can enhance our understanding of the genetic diversity and potential breed-specific characteristics of sheep populations. The result of allele frequency analysis for the *vertnin* gene in different sheep breeds of Baluchistan includes information on the gene, sampling breed, allele frequencies, and analysis of the Chi-Square test results. We observe that the p-values for all breeds are above 0.05, indicating that there is no significant association between the allele frequencies and the breeds at the specified level of significance. It has been reported that this means that the observed distribution of alleles in each breed is not significantly different from the expected distribution [12, 23, 24]. It's important to note that this analysis is specific to the sheep breeds of Baluchistan and the *vertnin* gene. Researchers and geneticists interested in this specific gene and breed combination can refer to this table to understand the allele frequencies and test results. The phylogenetic tree for the Balochi, Rakhshani, Bibrik, and Harnai sheep breeds was used in our study by narrow regions of axon with maximum association among the breed [25, 26]. The phylogenetic tree was formed on the base of the exon of the v*ertnin* gene, which was again confirmed through the classification of different mammalian species. The comparative study of sequences showed a large level of taxonomic analysis to explore phylogenetic linkage between various sheep breeds among the sequences of the *vertnin* gene. Although having similar characteristics among the genetic scattering rate, the phylogenetic tree and sequence percentage expressed the distance between Balochi

and Rakhshani sheep in the *vertnin* gene with percentages of 98 and 73, respectively (Figure 2). Furthermore, the sequencing of the Balochi breed was aligned using MEGA6 software, which is commonly used for layout. This is mainly utilized as the progressive algorithm layout for sequences. The results have shown that the Balochi breed has 98% similarities with the other three sheep breeds when sequences were compared (Figure 3). In the Balochi breed, the thoracolumbar vertebrae bone number was 20, with a carcass height of 76.12 ± 0.67 cm and a carcass length of 22.32 ± 0.18 cm. In the Rakhshani breed, the thoracolumbar vertebrae number was 18, with a carcass height of 71.61 ± 0.51 cm and a carcass length of 21.15 ± 0.19 cm. The Harnai breed had a thoracolumbar vertebrae number of 18, a carcass height of 71.43 ± 0.93 cm, and a carcass length of 20.71 ± 0.31 cm. In the Bibrik breed, the thoracolumbar vertebrae number was 19, with a carcass height of 68.21 ± 0.7 cm and a carcass length of 18.13 ± 0.46 cm. The results showed that a change in the thoracolumbar vertebrae region has a positive influence on carcass weight and length of sheep meat. Our study was supported by [1], who have reported that the number of thoracolumbar vertebrae is directly linked with body length, height, and carcass weight and length, which are economically important traits in farm animals' small ruminants. Another study was conducted by that carcass weight and carcass length is associated with body height and carcass traits in farm animals [10-26].

5. CONCLUSIONS

This study provides basic information on the genetic divergence between sheep breeds in Baluchistan based on the sequences of the *vertnin* gene. Researchers can use these estimates to study the genetic relationships, population structure, and evolutionary history of these sheep breeds, which can have implications for breeding programs, conservation efforts, and understanding the genetic diversity within Baluchistan's sheep populations.

6. FUNDING

Higher Education Commission of Pakistan provided funding for this study through PSDP project titled: Establishment of National Center for Livestock Breeding, Genetics and Genomics" at Lasbela University of Water Agriculture and Marine Sciences, Uthal, Balochistan.

7. ETHICAL STATEMENT

The Ethical Research Committee of the Directorate of Advanced Studies and Research Board of Sindh Agriculture University, Tandojam, Pakistan, approved all procedures of this study in its 131st meeting held on 13-11-2018. The approval was notified by the Directorate of Advanced Studies via letter No. DAS/5528 dated 28-11-2018.

8. CONFLICT OF INTEREST

All the Authors declared no conflict of interest.

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