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EXPLORING MUTATIONS IN THE GDF9 GENE FOR ENHANCED PROLIFICACY THROUGH BIOINFORMATICS ANALYSIS

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Abstract
This study comprehensively investigated sixteen mutations within the GDF9 gene in goats (*Capra hircus*), shedding light on their potential implications for goat reproduction. Utilizing bioinformatics tools, we analyzed the physicochemical properties, structural alterations, protein-protein interactions, and Gene Ontology (GO) associated with these mutations. The results revealed variations in molecular weight, instability index, and other physicochemical characteristics among the mutated GDF9 sequences. Structural modeling demonstrated subtle changes in functional domains induced by specific mutations. Protein-protein interaction network analysis unveiled close associations between GDF9 and key reproductive genes, emphasizing its central role in fertility. The study provides crucial insights for molecular breeding strategies aimed at enhancing goat prolificacy and reproductive performance. Understanding the genetic factors influencing goat reproduction is pivotal for developing targeted breeding approaches, ultimately contributing to sustainable and efficient livestock production. This research contributes to the growing body of knowledge surrounding the GDF9 gene in goats, offering a foundation for further exploration and practical applications in improving goat reproductive traits through molecular breeding.

Keywords: GDF9; Mutations; Reproductive Traits

Introduction
Goats (*Capra hircus*) are the first domesticated ruminants that have been serving humanity and are used as a source of milk and meat since 2500 B.C [1, 2]. Goats are referred as the "poor man's cow", as these support millions of landless and rural Pakistanis [3]. Because goats naturally adapt to their surroundings, they are a wonderful and valuable asset for farmers [4]. It is estimated that developing Asia and Africa account for 95% of the global goat population [5]. Pakistan is one of the Asian countries that produce goats and goat products. In terms of goat population, Pakistan ranks third in the world, behind China and India [6]. Based on the economic survey conducted in Pakistan, the estimated number of goats in the country is 72.2 million, representing 37.7% of all livestock [7].

There are 35 goat breeds in Pakistan, the majority of which are medium-sized, and their total population is 78.2 million [8]. With a few milch varieties like Nachi, the majority of goat breeds in Pakistan are grown primarily for meat [9]. The ability of these goat breeds to adapt to the specific dietary needs, diseases, and environmental stressors of a given geographic area has been made possible by evolutionary processes. Morphological characterization of goats is necessary to assess the degree of diversity among these breeds [10]. Hence, sustainable livestock management significantly boosts a nation's economy.

The transforming growth factor region β superfamily includes the Growth differentiation factor 9 (GDF9) gene, which is involved in the ovulation rate and follicular development mechanism in the ovaries. The GDF9 gene has three phenotypes: G1 (FecGI), Embrapa (FecGE), and high fertility (FecGH) [11] and is located on chromosome 5 [FecG] [12]. The GDF9 gene is involved in ovulation and reproduction. It can be used as a candidate gene to investigate reproductive activity in goats and sheep that is connected to litter size ability in relation to mutation and proliferation rates [13].

Individual genes, or genes with a major impact, have been identified through molecular genetics. One such gene is GDF9, which increases goat productivity by aiding in molecular reproduction [14]. Utilizing molecular genetics methods, it is possible to generate future generations of highly productive goats by leveraging the genes resistant to the local climate that are associated with their reproductive characteristics [15]. Furthermore, a sequence of well-organized cell-cell interactions between gametes and between sperm and somatic cells in the male and female reproductive systems constitute mammalian fertilization, and the autosomal GDF9 gene significantly influences follicular growth through altering the activity of granulosa cells [16, 17].

Goats' GDF9 single nucleotide polymorphism (SNP) has been investigated to identify the kind of mutation and amino acid alterations at SNP sites [18, 19]. One possible marker for economic features in goats, such growth and litter size, is the difference sequence surrounding the SNP location. Gene polymorphisms have so far been linked to higher ovulation rates and larger litter sizes. Another mutation in GDF9 was discovered, which results in arginine being substituted for serine at position 427 (S427R) (FecTT) [20]. A GDF9 mutation, which results in the substitution of cysteine for phenylalanine at position 345 (F345C) (FecGE), was reported by Silva et al in 2011 [21]. A phenotypic difference in the trait arises from the mutation, which may alter a portion of the gene controlling the trait or the gene entirely. The mutations are termed chromosomal mutations because they alter the number or shape of chromosomes. Alternatively, they can arise at the gene level, producing one or more nitrogenous bases [22]. It has been suggested that the primary factor influencing an animal's prolificacy may be the induction of amino acid alterations brought on by GDF9 gene mutations [23].

To the best of our knowledge, prior studies have solely recorded SNPs in the GDF9 gene among various goat breeds, with the functional implications of these observed SNPs remaining unexplored. Consequently, our research aims to identify previously reported GDF9 gene variants in goats and examine their potential impact on the reproductive traits of goats through the utilization of bioinformatics tools.

Materials and Methods

Literature search

We conducted a PubMed search using the keywords 'GDF9 SNPs' and 'Goats (*Capra hircus*)' to identify relevant studies. The search encompassed all original articles available until December 2023, resulting in the identification of a total of 177 articles.

Relevant data acquisition

Among the 177 studies, a total of 12 pertinent studies were selected based on the identification of SNPs in goats (*Capra hircus*) through a comprehensive review of titles, abstracts, and the full articles.

Gene sequence retrieval

Gene sequence of GDF9 in goat (*Capra hircus*) was extracted from the NCBI (https://www.ncbi.nlm.nih.gov/) GeneBank (NCBI Reference Sequence: NC_030814.1). GeneBank stores publicly available nucleotide sequences and associated metadata, serving as a crucial resource for researchers worldwide. Scientists access GenBank to retrieve, deposit, and analyze genetic information, fostering collaboration and advancing diverse fields like genomics, evolutionary biology, and biotechnology.

Characterization of physiochemical properties of wild type and mutated GDF9 proteins

Physiochemical properties, including molecular weight (MW), theoretical isoelectric point (PI), half-life, instability index (II), aliphatic index (AI), grand average of hydropathicity (GRAVY) of wild type and mutated GDF9 in goats was calculated using ProtParam tool (https://web.expasy.org/protparam/). ProtParam is a web-based tool that computes various physical and chemical parameters of a protein sequence. Developed by the ExPASy Bioinformatics Resource Portal, it aids researchers in analyzing protein characteristics like molecular weight, theoretical PI, amino acid composition, and more.

Homology modeling

The 3D models of wild type and mutated GDF9 in goats was generated using online tool Phyre^2 (http://www.sbg.bio.ic.ac.uk/phyre2), a completely web-based homology modeling tool for protein structure, functions and mutations.

Protein-protein interaction

Protein-protein interaction network of the GDF9 in goats was predicted using STRING (a database for known and predicted protein-protein interactions) available at; https://string-db.org/.

Gene enrichment analysis

DAVID (Database for Annotation, Visualization, and Integrated Discovery) is a bioinformatics resource that aids researchers in uncovering biological meaning from large-scale datasets [24]. It offers a comprehensive set of functional annotation tools for gene and protein analysis, enabling users to interpret the biological relevance of their data. With features like gene ontology analysis, pathway enrichment, and functional annotation clustering, DAVID facilitates the exploration of complex biological information, contributing to a deeper understanding of the functional implications within genomics research. In the present study, DAVID was employed to perform gene enrichment analysis of GDF9 interacting protein.

Results

Sequence retrieval

A total of sixteen previously reported mutations in the GDF9 gene in goats, comprising L61L (M1), P27R (M2), Q320P (M3), V397I (M4), A240V (M5), L50P (M6), A273V (M7), R358K (M8), A85G (M9), P27A (M10), E204Q (M11), L141L (M12), N112N (M13), D129D (M14), S165S (M15), and T217T (M16) (Table 1), were sourced from published studies. To assess the functional implications of these mutations, the wild-type sequence of the GDF9 gene in goats was obtained from NCBI GenBank, and mutated GDF9 sequences for each mutation were manually generated by incorporating these mutations into the wild-type sequence. Finally, one wild type and 16 mutated protein sequences of GDF9 were used for the downstream analyses.

Genomic characterization of GDF9

ProtParam was employed to evaluate the physiochemical attributes of both wild-type and mutated GDF9 protein sequences. The analysis revealed variations in molecular weight (MW), theoretical isoelectric point (PI), and instability index (II), aliphatic index (AI), and grand average of hydropathicity (GRAVY) among the reported mutated sequences. Interestingly, half-life remained

consistent across all reported mutations, while a specific mutation (M11) influenced the theoretical PI. Detailed results are presented in Table 1.

Table 1: Physicochemical characteristics of GDF9 mutations in goats predicted by ProtParam

Mutations	Molecular weight	Theoretical PI	Half Life (Hours)	Instability index (II)	Aliphatic Index (AI)	Grand average of hydropathicity (GRAVY)	References
Wild type	51902.61	9.13	30	54.78	77.73	-0.408	NCBI
M1 (L61L)	51902.61	9.13	30	54.78	77.73	-0.408	[25-28]
M2 (P27R)	51902.61	9.13	30	54.78	77.73	-0.408	[27]
M3 (Q320P)	51871.59	9.13	30	54.74	77.73	-0.404	[29, 30]
M4 (V397I)	51916.63	9.13	30	54.63	77.95	-0.407	[30-32]
M5 (A240V)	51930.66	9.13	30	54.55	78.15	-0.402	[33]
M6 (L50P)	51886.56	9.13	30	55.21	76.87	-0.420	[34]
M7 (A273V)	51930.66	9.13	30	54.78	78.15	-0.402	[35, 36]
M8 (R358K)	51874.59	9.13	30	54.26	77.73	-0.406	[37]
M9 (A85G)	51888.58	9.13	30	54.60	77.51	-0.413	[27, 36]
M10 (P27A)	51876.57	9.13	30	53.93	77.95	-0.400	[36]
M11 (E204Q)	51901.62	9.18	30	54.62	77.73	-0.408	[26]
M12 (L141L)	51902.61	9.13	30	54.78	77.73	-0.408	[38]
M13 (N112N)	51902.61	9.13	30	54.78	77.73	-0.408	[39, 40]
M14 (D129D)	51902.61	9.13	30	54.78	77.73	-0.408	[41]
M15 (S165S)	51902.61	9.13	30	54.78	77.73	-0.408	[41]

M16 (T217T)	51902.61	9.13	30	54.78	77.73	-0.408	[26]

Homology modeling

Utilizing the Phyre[2] web tool, we generated 3D structures for both wild-type and mutated GDF9 in goats. The outcomes revealed that mutations 1, 2, 7, 12, 13, 14, 15, and 16 induced subtle alterations in the functional domains of mutated GDF9 compared to the wild type, as illustrated in Figures 1. In contrast, other mutations exhibited no discernible impact on the functional domains of GDF9, as depicted in Figure 1. This analysis provides insights into the structural consequences of specific mutations on GDF9 in goats.

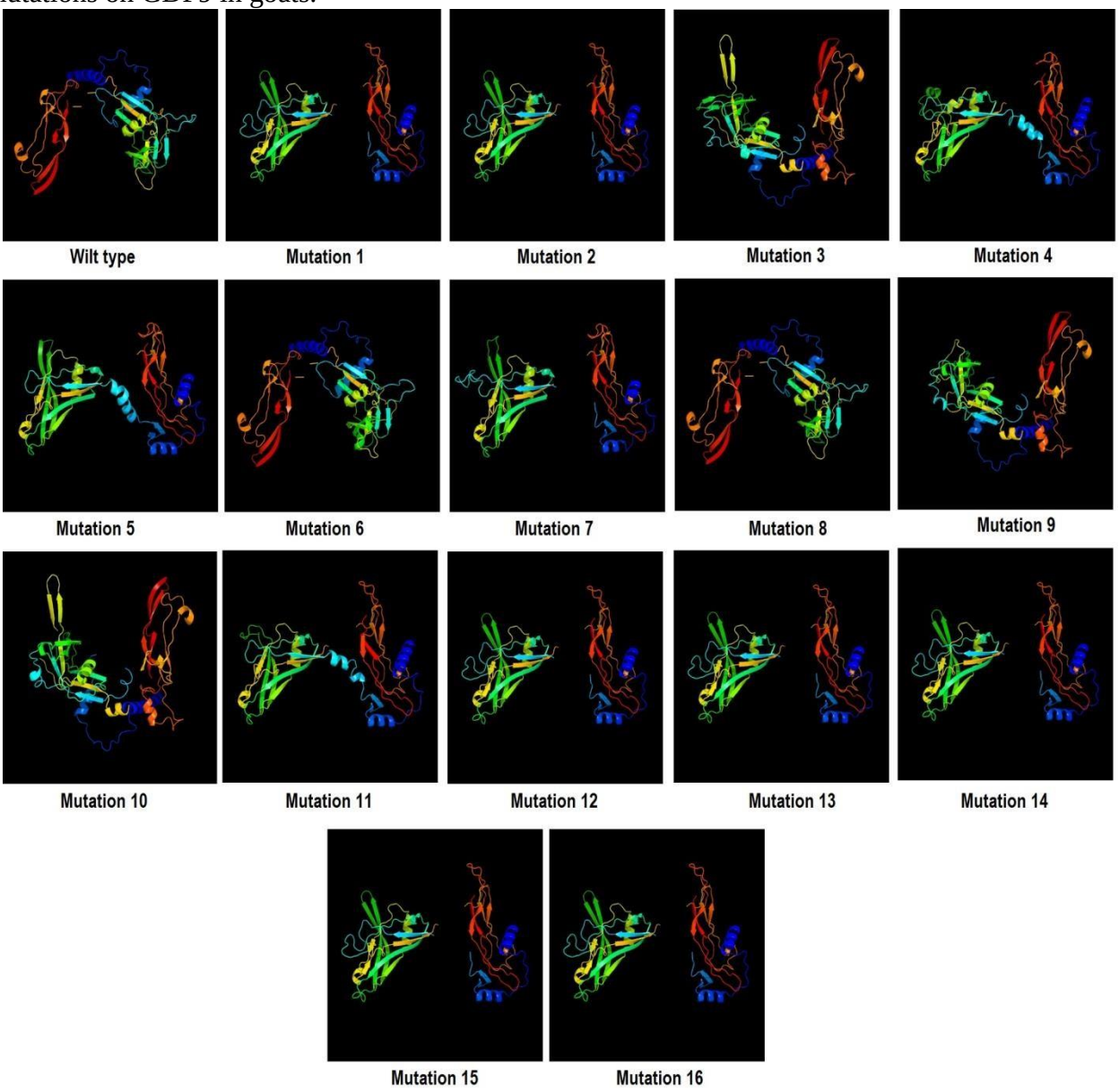

Figure 1: Schematic 3-D structures of mutated and wild type GDF9 proteins

Protein-protein interaction network and gene enrichment analyses

The construction of the protein-protein interaction (PPI) network aimed to delineate the specific molecular network of GDF9-associated proteins. STRING was employed for an in-depth exploration of protein-protein interactions. The findings revealed a close association between GDF9 and proteins such as BMP15, BMPR2, BMPR1B, ACVR1B, AMH, LHCGR, FSHR, ZP2, ZP3, and ZP4 as indicated in Figure 2. Additionally, Gene Enrichment analysis of GDF9-associated

proteins unveiled their involvement in diverse molecular functions (MF), biological processes (BP), and cellularcomponents (CC), as illustrated in Figure 3.

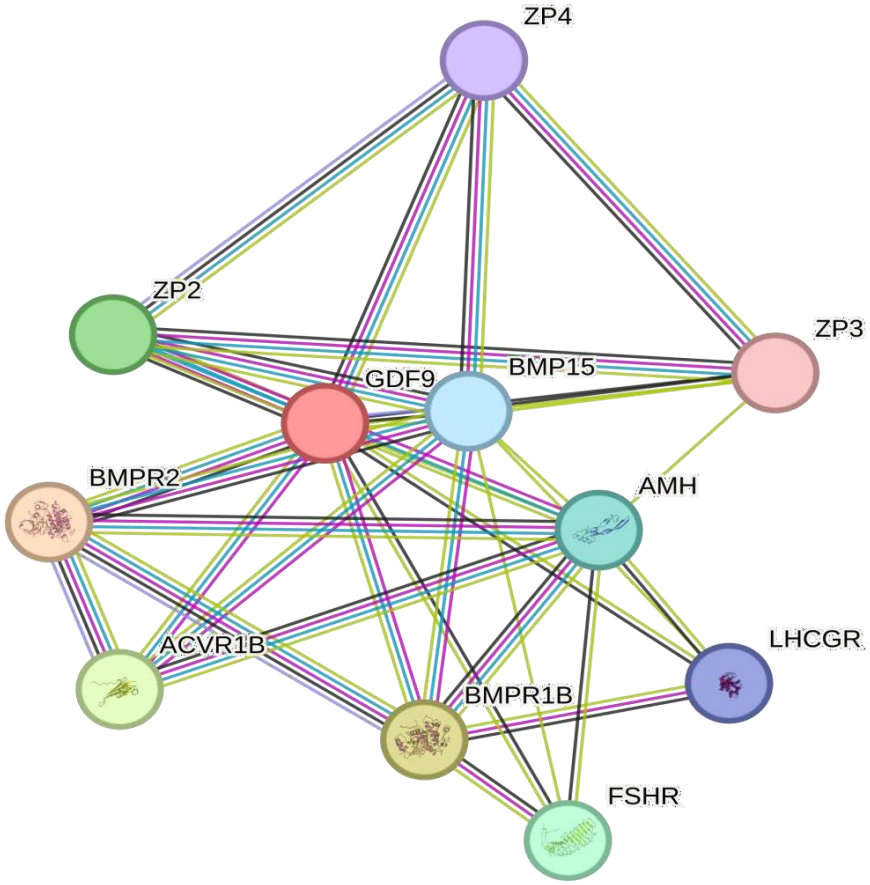

Figure 2: Protein-protein interaction of GDF9 in goats predicted using STRING.

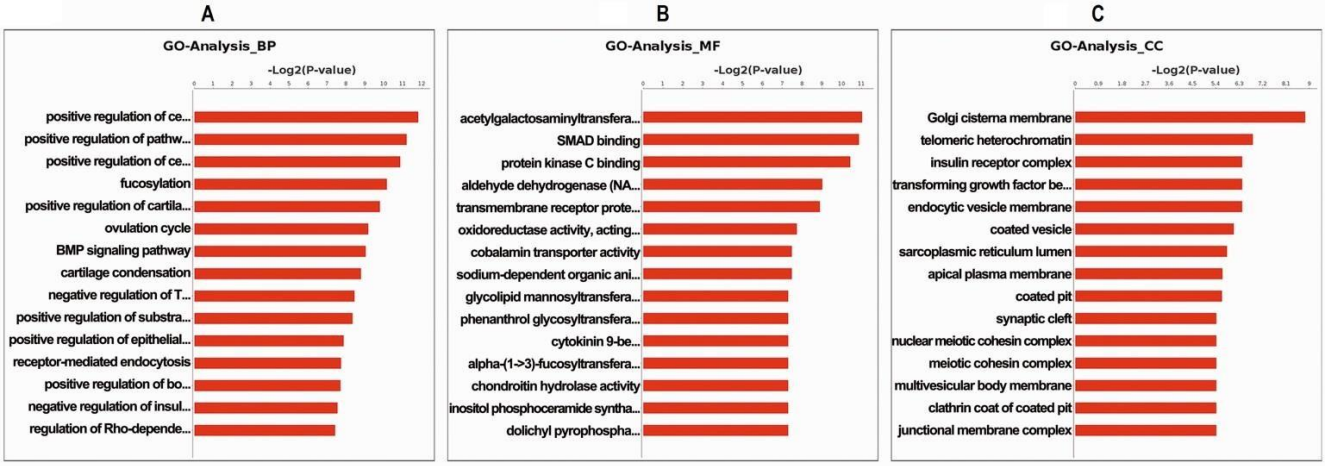

Figure 3: Gene Ontology outcomes of the GDF9-associated genes.

Discussion

The genes implicated in the biological regulation of a particular trait are considered "candidate genes" for association studies. When previous studies have identified the effects of the genes on the desired characteristics in the target species or in other species, the candidate gene approach is justified. Hence, the objectives of this research were to detect the existence of single nucleotide

polymorphisms (SNPs) in the GDF9 gene and to analyze their impact on goats using bioinformatics tools.

GDF9 is a crucial regulator that is released by female oocytes and is necessary for the development and maturation of ovarian follicles [42]. The GDF9 gene contains single nucleotide polymorphisms (SNPs) that have been linked to reproductive features in cattle. Some of these mutations have been successfully utilized in animal molecular breeding. The lack of a systematic sorting and analysis of the goat GDF9 gene SNPs from the published studies is noteworthy, nevertheless, since it prevents the identification of useful loci that may be used to increase goat prolificacy through molecular breeding.

In the present study, a total of sixteen GDF9 SNPs were identified in different goat breeds worldwide. Both synonymous (L61L, L141L, N112N, D129D, S165S, T217T) and non-synonymous (P27R, Q320P, V397I, A240V, L50P, A273, R358K, A85G, P27A, E204Q) mutations were analyzed using bioinformatics tools. Nonetheless, diverse research has demonstrated that distinct physiological impacts are associated with the same mutation location. For example, the relationship between a single SNP locus and reproductive traits differed throughout goat breeds. The effect of a mutation is determined by the variety of its impact on molecular function. Depending on the location, mutational consequences might be neutral, detrimental, or advantageous [43].

Physicochemical characteristics of GDF9 mutations in goats indicated that half-life remains constant while the molecular weight (MW), theoretical PI, instability index (II), aliphatic index (AI), grand average of hydropathicity (GRAVY) varies in all the reported mutations. In order to further investigate the modifications in the protein's structure following the mutation, the tertiary structures of the protein with and without the mutation were predicted, accordingly. It was discovered that both before and after the mutation, the area, including the mutation location, were consistently coiled erratically. The three-dimensional structure did differ in a few minor ways. According to earlier research, the minute alterations in the three-dimensional structure could influence the binding affinity of GDF9 with its receptors, as well as cumulus enlargement and ovulation. These factors could ultimately impact the rate of ovulation or the size of the litter as reported in previous studies [44]. Protein network analysis indicated that GDF9 has close association with BMP15, BMPR2, BMPR1B, ACVR1B, AMH, LHCGR, FSHR, ZP2, ZP3 and ZP4. A study indicated that these genes have an important role in increasing ovulation rate. GDF9 and BMP15 genes are produced by ovary and have adverse effects [45]. Nonetheless, data indicates that the fecundity is highly influenced by the genes GDF9 and BMP15 [46]. The dominant autosomal gene BMPR1B, or bone morphogenetic protein receptor-1B, is also responsible forfertility and twinning in sheep and goats [47]. Thus, these findings offer valuable information for creating molecular breeding methods for goats and enhancing their reproductive and productivity capabilities.

Conclusion

In conclusion, this study systematically examined sixteen mutations in the GDF9 gene in goats, providing insights into their potential impact on goat reproduction. Analysis of physicochemical properties revealed variations among the mutated sequences, emphasizing the functional relevance of specific mutations. Structural modeling showed subtle alterations induced by certain mutations in GDF9's functional domains. Protein-protein interaction network analysis highlighted close associations with key reproductive genes, indicating GDF9's pivotal role in fertility. These findings offer a foundation for molecular breeding strategies, showcasing the importance of understanding genetic factors to enhance goat prolificacy and reproductive performance.

Conflict of interest

None.

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None.

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