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Genetic variation analysis of the FecB gene in sheep

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Abstract
Single nucleotide polymorphisms (SNPs) play a pivotal role in genetic variation, particularly in missense variants that can significantly impact protein function. This study focused on the FecB gene, also known as BMPR1B, in sheep, which is critical for various physiological processes, particularly in reproduction. By analyzing SNP data retrieved from Ensembl-Biomart and Uniprot databases, we identified missense variants and utilized in silico tools to predict their deleterious effects and impact on protein stability. We found common deleterious mutations, P482L and R469K, which were predicted to decrease protein stability. Additionally, protein-protein interaction analysis revealed associations between FecB and proteins involved in important biological pathways. These findings contribute to our understanding of genetic variations in the FecB gene and their potential implications for sheep health and reproduction.

Keywords: Sheep, FecB gene, SNP analysis

Introduction
Single nucleotide polymorphisms (SNPs) represent a key form of genetic variation within the genome, arising from single base mutations in DNA. Constituting approximately 90% of all genetic variations, SNPs are highly prevalent and fundamental types of polymorphisms. Various publicly accessible databases such as dbSNP, GWAS Central, and SwissVar host extensive collections of SNP data. Of particular significance among SNPs are missense variants, also termed non-synonymous SNPs (nsSNPs), which induce alterations in the amino acid sequence during translation. Due to their widespread association with numerous disorders, nsSNPs are thought to significantly contribute to the functional diversity of coding proteins in human populations. These variations can impact protein solubility and disrupt protein structure, potentially influencing protein function and gene regulation mechanisms involving transcription and translation [1, 2].
The FecB gene, also known as BMPR1B (bone morphogenetic protein receptor type 1B), was first discovered in prolific Booroola Merino sheep. This gene harbors a mutation (A746G) within its coding region, resulting in the substitution of arginine with glutamine in the protein structure. The FecB gene assumes a crucial role in various physiological processes in sheep. Its proper functioning is vital for normal cellular activities and metabolic pathways. Research on the FecB gene in sheep is essential for understanding its impact on sheep physiology and reproduction. Genetic studies on this gene may provide valuable insights into inheritance patterns and potential mutations that could affect sheep health and reproductive traits. Investigating the FecB gene in sheep contributes to our understanding of genetic disorders or variations that influence sheep fertility and production [3].

2. Materials and Methods
2.1 Data set
The data of the gene FecB was retrieved from Ensembl-Biomart Databases (source: dbSNP; http://www.ensembl.org/ biomart / martview/) and Uniprot (https:// www.uniprot.org/uniprot/). We retrieved the information of SNPs (SNP ID, location, Gene stable ID, residue alteration, etc.).

2.2 Identification of deleterious nsSNPs

The substituted amino acids that alter protein function and phenotypic changes was predicated by SIFT (Sorting Intolerant from Tolerant) score from Ensembl database. SIFT predicted the deleteriousness of the SNP in the form of a tolerance index (TI) score ranging from 0.0 to 1.0, with a TI score of 0.05 or less as intolerant or deleterious [4, 5].
Further, to verify the identified deleterious SNPs from SIFT; POLYPHEN-2, PhDSNP, PredictSNP, and SNAP2 were used. Results of PhDSNP were obtained by the consensus classifier of PredictSNP.

2.3 Prediction of structural and functional effect on ANPEP gene

For prediction of protein stability change three different web servers I-Mutant 2.0, and MUPRO were used. These are Support Vector Machine (SVM)- based web server for the automatic prediction of protein stability changes upon single-site mutations [6, 7]. The input is a FASTA sequence of protein along with the residues change was provided. I-Mutant 2.0 predicts free energy change and RI value (reliability index). If the DDG value is negative, then the mutated protein will have less stability and vice versa for high stability.

2.4 Prediction of protein-protein interaction

For protein-protein interaction "Search Tool for the Retrieval of Interacting Proteins" (STRING; http://string-db.org/) was used [8].

3. Results and Discussion

3.1 Results and Discussion

A total of 14 missense variants and 24 synonymous variants were filtered out in the FecB gene during variant calling. The highest number of SNPs were present in the intronic region (2114), followed by the downstream region (122), upstream region (92).

3.2 Identification of deleterious nsSNPs

Based on SIFT score of variants obtained from the ensemble, 7 variants are found to be deleterious out of 14. Which were further analyzed using PolyPhen-2, PhDSNP, PredictSNP, and SNAP2. Two variants named P482L and R469K were found common deleterious (Table 1; Figure 1).

Table 1: In silico analysis of deleterious mutations

Variant ID	Variants name	Poly Phen-2	Score	SNAP2	SCORE	Predict SNP prediction	PhD-SNP prediction
rs1094003818	P482L	Probably damaging	1	Effect	38	Deleterious	Deleterious
rs418841713	Q305R	Probably damaging	0.996	Effect	26	Neutral	Neutral
rs591939617	A5V	Unknown		Neutral	-70	Neutral	Neutral
rs589500430	R469K	Probably Damaging	0.996	Effect	46	Deleterious	Deleterious
rs426338048	C45S	Benign	0.002	Neutral	-31	Neutral	Deleterious
rs412038619	R44C	Possibly Damaging	0.845	Effect	61	Deleterious	Neutral
rs603752979	R82H	Benign	0.083	Neutral	-86	Neutral	Neutral

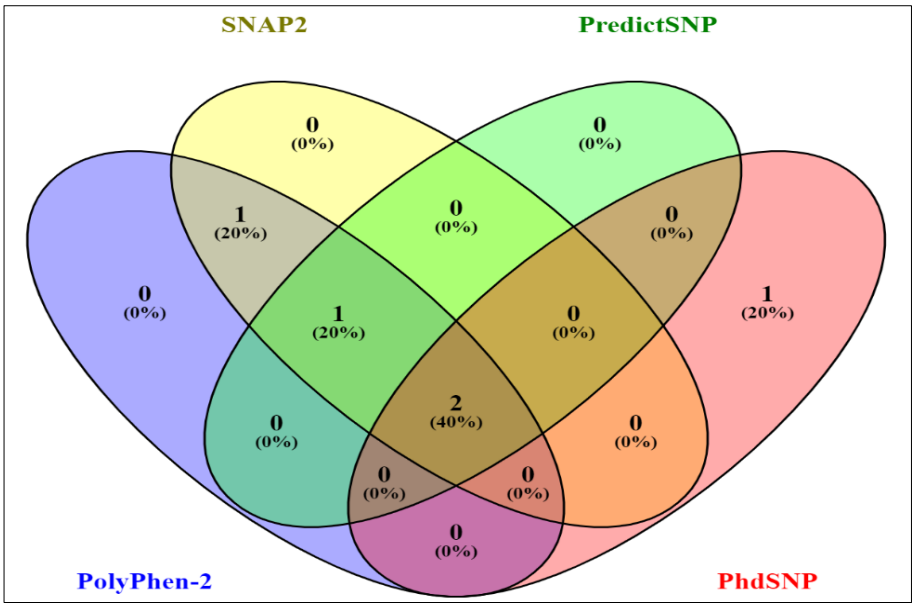

Fig 1: Common deleterious SNPs

Several similar studies done previously. In the bovine SLC11A2 gene, deleterious SNPs were predicted using SIFT, PolyPhen and Panther [9].

3.3 Prediction of change in protein stability due to mutation for the common mutation

For these two programs were used, I-Mutant 2.0, muPro. Through I-Mutatnt 2.0 and muPro predict decrease the protein stability of both variants (Table 2).

Table 2: Change in protein stability due to mutation

Deleterious variant	muPro	DDG	I-Mutant2.0	DDG
P482L	Decrease	-0.61366188	Decrease	-1.75
R469K	Decrease	-1.5694446	Decrease	-1.33

When considering a protein's structural and functional properties, stability is crucial. Any alteration in the stability of proteins may result in abnormal protein aggregation, misfolding, or destruction [10].

3.4 Protein-Protein interaction analysis

In the protein-protein interaction analysis by STRING, a total of ten proteins were found to be associated with functional FecB (BMPR1B) protein with a high confidence score (< 0.95) (Figure 2). Proteins like SMAD5, BMP15, GDF5, BMP4, BMP2, BMP6 were found to be associated with FecB protein.

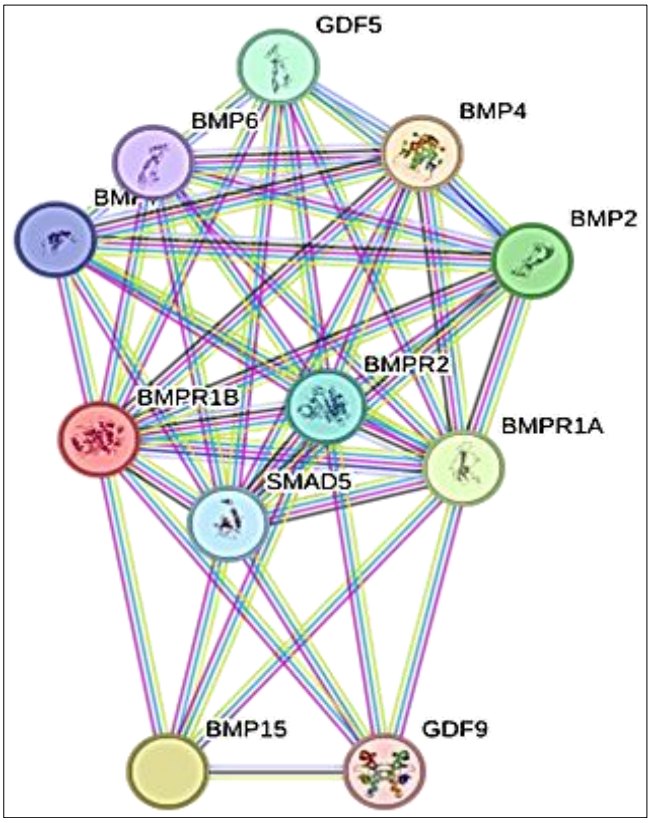

Fig 2: Protein-Protein interaction of FecB (BMPR1B) gene's protein

4. Conclusion

This study underscores the importance of investigating genetic variations in the FecB gene, particularly missense variants, which can have significant functional implications. The identification of deleterious mutations, such as P482L and R469K, highlights potential targets for further research into their effects on sheep physiology and reproduction. Additionally, the protein-protein interaction analysis provides insights into the functional network of FecB-associated proteins, shedding light on its role in key biological pathways. Ultimately, understanding genetic variations in the FecB gene contributes to advancements in sheep breeding and management practices, with implications for improving fertility and production traits in sheep populations.

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6. Conflict of interest

Authors have no conflict of interest in this study.

7. References

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