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Molecular Analysis of BMP4 Gene Polymorphisms in the Kamori Goat Breed of Khairpur, Sindh Pakistan

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ABSTRACT

Bone morphogenetic protein-4 (BMP-4) is a gene that plays a crucial role in the development and growth of various tissues in goat such as it is involved in the formation of the embryo particularly in the development of the mesoderm, which gives rise to muscles, bones and other connective tissues. BMP4 has been linked to reproductive traits in goats such as fertility and little size and development of hair, muscles and fat. BMP4 also involved in the development of various organogenesis such as heart, liver, lungs and kidney. It also play regulates the development of the nervous system. The motive of this research was to investigate the novality of SNPs in BMP-4 gene that have major effect on body growth development. The blood samples were taken from the Animal's Hospital of district Khairpur by using hygienic protocol and techniques. The blood tubes were transported into dry ice containing cooling box to Molecular Genetic laboratory of Department of Zoology for further process. In the first step DNA extraction from whole blood samples had been done by using of DNA extraction commercial kit and visualized on gel electrophoresis. Later on the PCR products were sent for DNA sequencing for findings of SNPs. The analysis shown that about 08 missense mutation were found in the Kamori goat breed. This mutations were found on the different base pairs (bp). While changes into genetic codon lead to changes into non-essential amino acids into essential amino acids which leads to enhancement of milk and meat qualities and quantities that was very good sign. The result suggested that Kamori goat breed can be implied for breed admixture and genetic marker assist for the selection of breeding.

INTRODUCTION

Goats are naturally curious. They have been considered as sensible as dogs by various surveys although goats are usually believed as hardy creatures. Goat's intestines are mostly used to make "catgut". It is still used in for anatomically human surgery sutures and strings used for musical instruments. The horn is mostly used to making spoons [1]. Most of individual used herds as a pet animal because of their ability to create a close bond with their human. They are live into community style [2]. The Kamori breed is mostly noticed in province of Sindh, Pakistan districts of Larkana, Dadu, Khairpur, and Shaheed Benazir Abad. They had different physical appearance along with prolonged ear lobes, collar and also long frame shape. Kamori breeds have healthier body weight. They have distinctive body color as whole pure shade of dark brown color with small patches of coffee bean-colored on its whole entire body. Genomic analysis verified that the appearance of Kamori breed with Pataire breed are hybrid because both of them have similar phenotypes as both of having long ears and brown skin coats. BMP-4 is a protein coding gene which concern connective tissues and soft tissues of the body structure. According to their construction human BMP4 is a 116-residue active carboxyl -terminal peptide that is produced as 40% residue preproprotein that cleaved by the post translationally. BMP4 contains 7 conserved and glycosylated residues [3]. A di-sulphide bridge and 3 pairs of cysteine amino acids hold the monomers together. A "cystine knot" is the name for this shape. BMPs are the member of TGF-beta as secreting signal molecule. About 20 members of BMPs found in mammals. Here are various family members such as BMP2, BMP4, BMP5, BMP7, BMP15 and many others. These all members express throughout limbs developmental. BMPs has been associated with initial limb modeling and development of skeletogenic. But BMP4 play main role in human as well as animal's body for maintaining many body activities such as cell propagation, diversity, and caspase-mediated cell death, stem cells including embryonic, hematopoietic, mesenchyme, neural stem cells. Target gene also performed main role in stem cell therapy [4].

Furthermore, BMP-4 gene promotes embryonic and extra embryonic mesoderm production in later stages of development. Target gene also play a critical role in accomplish of pregnancy in animals. But in some case, it also caused vasculogenic (dysfunction of the blood vessels) [5]. The most important gene is the considered as "BMP4 gene" which indicate to affect the fertility of organisms mostly human [6]. BMP-4 considered a growth factor (paracrine) and in aggregation with BMP-7 gene, it's function to regulates the initially development of ovarian follicles and primordial to primary follicle transition. It is dynamically important for availability of signs that specify the BMP4 gene has a critical part in the existence and inhibition of apoptosis in oocyte, although with the role of fertility BMP4 is significant source of oocytes conservation [6]. In Aves BMP-4 gene had expressed the effect on size of beak (nib) regarding to Darwin's finches. Lowering the amount of BMP-4 gene concern with beak size along with the depths and widths. On the other hand, high concentration of BMP-4 gene expressed phenotypically lofty rostrum. Genetically regulate the BMP-4 gene provide natural selection in bird's beak [7].

MATERIAL AND METHODS

Samples Collection

About thirty blood samples were collected from the indigenous Kamori goat breed at the Animals Hospital in district Khairpur Mir's employing careful sampling techniques. The selected animals were between 1.5 -2 years old. Using sterile disposable 5ml syringes, blood was drawn from the jugular vein of each animal. The blood was then transferred into 250ul of EDTA tubes to prevent coagulation. The EDTA tubes were subsequently transported in a cool box containing dry ice and stored at -4°C in the refrigerator for further DNA extraction at the Molecular Genetic Laboratory, Zoology Department Shah Abdul Latif Khairpur.

DNA Extraction

Extraction of DNA was performed by using of MQ Blood Genomic DNA Extraction Kit (MOLEQULE-ON®). Following blood sample collection, genomic DNA was extracted from the whole blood, adhering strictly to the manufacturer's guidelines. This extracted DNA served as the template for subsequent PCR amplification of the target gene.

Quantification of Target DNA

Nanodrop™ 1000 Spectrophotometer (Thermo Fisher Scientific) was used to quantify extracted DNA at the "Jamil-Ur-Rahman Center for Genome Research at Karachi University, Sindh Pakistan. Quantification of DNA had been done to ensure the presence of DNA into extracted sample from blood samples. Purity of DNA was measured by using the ratio of 260/280 nm absorbance (absorbance calculated by taken 260 nm divided by absorbance taken at 280nm). DNA is regarded as pure if it had reading between 1.7-1.8.

Primer Design and Synthesizing

Initially, BMP-4 gene sequences have been used to retrieve for the formation of primers designing on the website of National Center for Biotechnology Information (NCBI). Primers (Forward primers and Reverse primers) obtained from particular website and were blast again by using of NCBI website for checkered specificity.

Table 1

Sequences of forward and reverse primers of amplified gene BMP-4.

Gene	Forward Primers (5' ⟶ 3')	Reverse primer (3' ⟶ 5')	Base pair (bp)	Temperature™
BMP-4	CTACCGTACTCCCCAGACCC	GCACTACGGAATGGCTCCTAA	20	58

PCR Amplification

A reaction of mixture have been prepared for the PCR amplification of BMP-4 gene into PCR tube about (200ul). All reagents have optimization of concentration with total volume of 200 ul were transferred into PCR tubes. The reagents included into mixture were genomic DNA template (5ul), absolute red PCR Master Mix (7ul) from MOLEQULE-ON® Company, forward primer (2ul; 10pmol/ul), reverse primer (2ul; 10pmol/ul). The reagents included genomic DNA template (5ul), absolute Red PCR Master Mix (7ul) from MOLEQULE-ON® Company, PCR grade water or molecular DDH2O (Double Distal water) (4ul). Subsequently, PCR tubes were placed in Thermal Cycler Machine (Bio Rad T-100) for PCR amplification. PCR reactions were performed by following already published protocol described by Zhang [8].

Gel Electrophoresis

The amplified PCR products were visualized by electrophoresis on a 1.5% agarose gel containing ethidium bromide, mix well and put the gel into microwave oven for homogenized mixture, now pour the solution into gel tray and put the comb for formation of well, after solidification of gel. About 4ul of PCR product and 2ul of DNA loading dye were loaded into each well, alongside a 1kb DNA ladder. The gel was then submerged in 1xTBE buffer and subjected to electrophoresis at 70volt for 45 minutes, before being visualized under UV illumination using a Bio-Rad UV Trans-illuminator.

Sequencing, Purification and Data Analysis

For purification and sequencing of PCR product, amplified product of PCR samples was sent to Macrogen

Company, Korea for data analysis. The data of DNA sequences were analyzed by using of online genome browser ensemble.org, and blast the query of sequence of BMP-4 gene of selected goat breeds on sequence alignment tools. The software which compared the known sequence of DNA data with sequence data of already stored and provided about the BMP-4 gene.

RESULT

Quantification of extracted DNA

Quantification of DNA performed by using of Nanodrop Spectrophotometer measurement that revealed the DNA samples to identify the quantity ranged from 7.222 to 11.357 ng/ul. The quantity of DNA sufficient for performance of PCR amplification. Nanodrop also verified that isolated DNA was absolute pure since A260/A280 ratio was in the range of 1.09-1.81, also revealed one of the new results of nanodrop spectrophotometer exhibit purity and quantity of DNA samples.

Table 2

Shown the obtained results of DNA samples through nanodrop spectrophotometer measurement.

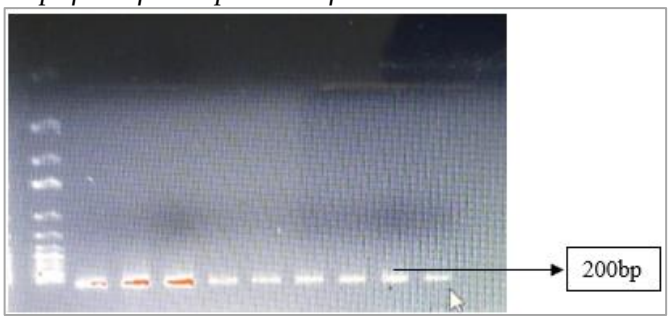
Sample ID	Goat Breeds	DNA Quantity (ug/ml)	DNA Purity (260/280nm)
S1	Kamori	7.221ug/ml	1.77
S2	Kamori	11.357ug/ml	1.14
S3	Kamori	7.866ug/ml	1.43
S4	Kamori	7.901ug/ml	1.81

PCR Amplification and Gel Electrophoresis

Gel electrophoresis procedure played a vital role to visualized amplified PCR product of BMP-4 gene from 9 samples of selected goat breeds and confirmed the size of amplified products as about 1000bp.DNA ladder ‘s bands produced into 1st well of gel and samples started from 2nd well to onwards.

Figure 1

Amplified of PCR product of BMP-4 Gene.



Identification and Sequencing of Single Nucleotide Polymorphism (SNPs)

The target gene’s nucleotide sequences from each sample of goat breed have been analyzed by using mutation detector online software (ensemble.org). This procedure was used alignment of sequence tool or blast tool. Consequently, alignment graphs had been displaying the mutations were found as shown in figure 2, 3.

Figure 2

Shown the Blast query of primary data on ensemble.org

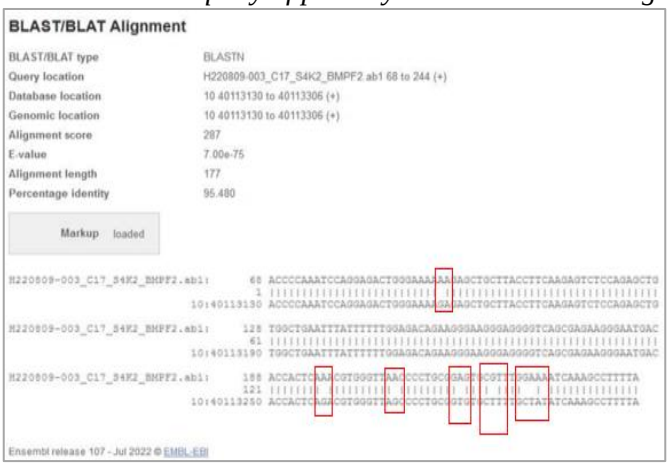
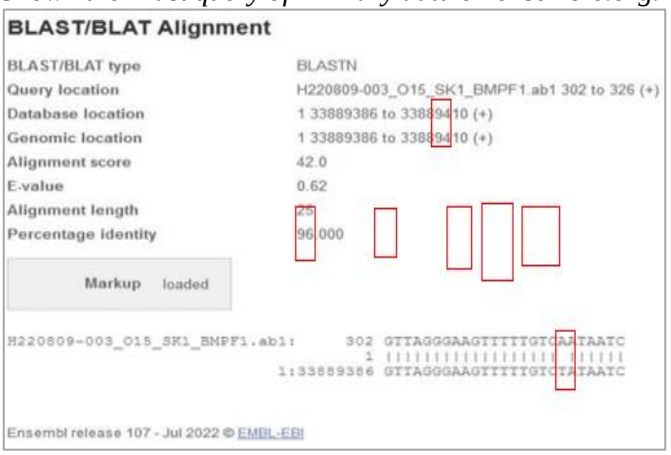


Figure 3

Shown the Blast query of Primary data on ensemble.org.



About 08 mutations were recognized in the Exon 1 region of BMP-4 gene in Kamori goat breed. While the mutations based on the variant into genetic codon. In the breed about 8 missense mutations were identified in which changes had been found from original amino acids to change in amino acids.

Table 3

Shown the Missense Mutation in the Kamori goat breed.

Sample Name (ID)	Position of Changing Nitrogenous Bases	Original Codon	Change Codon	Original Amino Acids	Change Amino Acids	Types of Point Mutations
SK1	321	AAT	TAT	Asparagine	Tyrosine	Missense mutation
	95	AAG	GAG	Lysine	Glutamic acids	Missense mutation
	197	AAC	GAC	Asparagine	Aspartic acid	Missense mutation
	108	ACC	GCC	Threonine	Alanine	Missense mutation

	118	AGT	TGT	Serine	Cysteine	Missense mutation
S4K2	131	AAT	TAT	Asparagine	Tyrosine	Missense mutation
	123	GTT	GTT	Valine	Phenylalanine	Missense mutation
	128	GAA	GAA	Glutamic Acids	Leucine	Missense mutation

DISCUSSION

DNA markers are poised to revolutionize animal breeding programs by providing unprecedented insights into genetic variation. At the forefront of this revolution are Single Nucleotide Polymorphism (SNPs), which represent the most common type of genetic variation in animals. These subtle variations often referred to as "snips" occur when a single nucleotide is altered in a DNA sequence. While frequently found in non-coding regions, SNPs can also exert a profound impact on gene function when located within or near genes. By serving as biological markers, SNPs enable researchers to pinpoint genes associated with desirable traits or disease susceptibility, ultimately informing strategies to enhance animal health, productivity and quality characteristics such as meat and milk production [9]. In this research study explored novel single nucleotide polymorphism (SNPs) within the BMP-4 gene, focusing on their potential and impact on meat traits, including quality and yield. A comprehensive genetic analysis of the BMP-4 gene's exon 1 region was conducted, yielding a comparative assessment of SNPs types and their frequencies within the breed. In this study PCR, gel electrophoresis and DNA sequencing techniques play important role to identify about 08 miss-sense mutations on the target region of the BMP-4 gene in Kamori goat breed [10].

There are different types of pint mutations such as silent mutation, deletion mutation, miss-sense mutation, and nonsense mutation. Each mutations have their own impact. There was no silent mutation found in the breed because silent mutation was very rare and had no effect on the meat quality and quantity traits because of changes in the genetic codon could not affect on the expression of genes on the amino acids coded due to this gene remain the same. There was no deletion mutation found in the Kamori breed because deletion mutation would be having negative impact on meat and milk quantity as well as quality. While in the deletion mutation changes into genetic codon leads to change into amino acids this can play a role to shorten the protein structure therefore this would lead to reduce in the quantity of meat [11].

In this study the missense mutation was noted in Kamori goat breed which is very effective symbol and could create a very good impact on meat quantity and milk quality as well. In this mutation modification occurred in the genetic codon led to change in non-essential amino acids (Glutamic acids) into essential amino acids (Leucine) [12]. If the variations occurred in DNA sequences are less than 1% then it considered as mutation and if it is greater than 1% than called SNPs. The result of this study described the Kamori goat breed shown the percentage was greater than 1. From the result the breed will might be have admixture to progress milk and meat quantities and qualities.

CONCLUSION

Present study revealed about eight missense mutation found BMP-4 Gene of Kamori goat breed. The mutations were identified by the help of PCR sequencing techniques based on genetic codon. Identification of missense mutations were concern with coding of various amino acids. Missense mutations are found useful for increased the body characteristics, traits, morphogenetic expression of animals. This change leads to enhance the meat and milk quantity of the breeds. This breed can be selected for breeding admixture and genetic marker assisted in selection of goat breed.

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