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GENETIC ANALYSIS OF BMP-4 GENE IN BADDI GOAT BREED OF DISTRICT KHAIRPUR, SINDH PAKISTAN.

Shaista Ghumro*
Javed Ahmed Ujan
Shahid Ali Jakhrani

Department of Zoology, Shah Abdul Latif University Khairpur Mir's, Sindh Pakistan
Corresponding Author: *shaistaghumro007@gmail.com*

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Abstract

Bone morphogenetic protein 4 is a protein that found in goat, cattle and humans are encoded by BMP4 gene. The candidate gene is found on chromosome 10q in goats. BMP4 gene is the subfamily of the superfamily named as transforming growth factor beta (TGF- β). It is an evolutionary conserved member of BMPs family. BMP-4 is a protein coding gene that concern with connective and soft tissues of the body structure. It plays main role in goats, cattle, human and other animal's bodies for maintaining the body activities such as cell proliferation, differentiation, apoptosis, and migrations. Present study was aimed to investigate the mutational variations found with same area of indigenous goat breeds. The Genomic DNA was extracted from the blood samples of Baddi goat breeds. The samples were transported and stored at 4°C for further processing of DNA extraction. The targeted region of BMP-4 gene that was amplified by the specific set of primers. The amplified products were sequenced by the ABI Genetic Analyzer 3500 and sequencing data was analyzed by Bio Edit v 7.2 and blasting was performed on ensemble.org. Results revealed that about 4 missense mutations found in Baddi goat breed with the help of PCR-Gel electrophoresis and DNA sequencing. The missense mutation was identified by the replacement of genetic codon from original codon such as first missense mutation found as replacement of Serine by Threonine at 157 Bp, second & third replacement from Glycine to isoleucine at 159 Bp and 173 Bp respectively, while fourth mutational replacement from Arginine to Serine at 177 Bp. However, the percentage of mutations in baddi breed was greater than 1% it means that there was polymorphism has been found with replacement of original amino acids with change amino acids that play a significant role in improvement of goats characteristic such body, muscles, milk and meat quantities. Obtained results revealed a great heterogeneity in genetic makeup of Baddi breed and hence could be used in genetic marker which assisted the selection of Baddi breed than others goat breeds.

Keywords:
DNA extraction, Electrophoresis, PCR, Polymorphism, Mutations.

Introduction

Goat (Capra hircus) is a domesticate animals found in both wild as well as domesticated areas. The goats are the member of Bovidae family, tribe Caprini, it is closely associated to the sheep species. More than 300 distinctive breeds of goat have been named (Hirst, 2008). It has been utilized for meat, milk (dairy products), fur and skin across many parts of the world. Goat's milk also turned into formation of cheese (Zeder *et al.*, 2000). Baddi goat breed is also called Pure Pateri goats. They are found in the Hyderabad, Khairpur, and Sanghar. In Sindh mostly of female and male breed had pure white body with brown color spots at skull, collar, and foot. The breeds are very heavier and possess elongated body figure. They have compact body shape, soft and straight lengthy hair (Mari, 2022).

BMP-4 means Bone Morphogenetic Protein. It is an evolutionary conserved associates of the BMP's family, it belong to superfamily of transforming growth factor beta (Mangino *et al.*, 1999). It is detected in the eyes, ventral peripheral zone, cardiac and circulatory fluid, and otic vesicles during the early embryonic development. Some other BMP members, performed a role in the formation of bones and cartilages, especially limb and tooth development. Target gene also involved in the development of adipose tissues (Winner *et al.*, 1995). BMP-4 is a protein (polypeptide) which encoded in human in the form of BMP4 gene. In human BMP-4 gene remains on chromosome number 14q22-q23. As it the associate with the superfamily that contain huge members of human and animal growth and development factors. The potentially demineralized extract bone involved in to stimulate endochondral osteogenesis in vivo extra skeletal site. BMP4 gene considered a Master gene that controls many aspects of development in all species, including palates production, sight development and various others. Many regulatory genes, such as BMP4 and hox genes that are conserved in this way. Orofacial cleft and Microphthalmia diseases have been occurred due the two mutations appeared that are concern with this gene protein in human and animals. The encoded protein also performed main part in pathophysiology with a numbers of cardiovascular illnesses and malignancies in humans (Oida, 1995).

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 expresses 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, mesenchymal, neural stem cells. Target gene also performed main role in stem cell therapy (Bandyopadhyay *et al.*, 2006). During the mammalian developmental process the dysregulation of the BMP signaling system can have serious implications. Many adult tissues rely on BMP4 for their preservation and performance (Bowers, 2007). By assuming a beige or brown phenotype during development, it promotes metabolically helpful in hyperplastic adipose tissue expansion and enhances intravenous adipose cell oxidative capacity in a paracrine way. In depth research on the human genome has been done to identify valuable features in Homo sapiens (Gustafson, 2015).

Material and Methods

Collection and transport of blood samples

Approximately, thirty samples of blood were collect from indigenous goat breed from the animal's hospital of district Khairpur Mir's by applying careful sample techniques. The breeds having an age of 1 to 2 years old. About (15ml) of sterilized disposable syringe had been used for taking the blood from goat's jugular vein of each animals. Subsequently, the blood from the syringe transferred into 250 ul(0.5M) of the

EDTA (ethylene diamine tetra acetic acid) tubes that protect the blood samples from anticoagulation, after that EDTA tubes transported into thermocol ice box which contain dry ice, then preserved the blood samples into the fridge at -4°C for further process of DNA extraction at Molecular Genetic lab in Zoology Department of Shah Abdul Latif University Khairpur, Sindh Pakistan.

DNA Extraction from Blood samples

DNA extraction has been done by using of MQ Blood Genomic DNA Extraction Kit. A volume of 100 ul of whole blood was add into 2.0 ml centrifuge tubes. Added the PBS solution to the tube to a final volume of 200ul. Vortex gently and let the tube was stand for 1 minute at the room temperature. 20ul of proteinase K Solution added then vortexing. About 200ul of Buffer CL mixed in the solution tubes. Incubated all tubes at the 56°C for 10 minutes in the water bath. A 200ul of 100% ethanol was add in the mixture and mix thoroughly by using pipetting. The contents were spin at 10,000 rpm for 2 minutes in the centrifuge machine. Discarded the flow through in the collection tube and column was again place back over same collection tube. 500ul of CW1 Solution was add in the tubes and spin at 10,000 rpm for 1 minute in the centrifuge machine. A volume of 500ul of CW2 Solution was added and spin at 10,000 rpm for 1 minute in the centrifuge machine. Discarded the flow through. A volume of 30-50ul of CE Buffer mixed to elute the genomic DNA and incubated for 2 to 3 minutes to increase the recovery yield while extracted DNA was stored 20°C for further process.

DNA Quantification and Gel electrophoresis

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 which could be used for further process of amplification. DNA is regarded as pure if the reading between 1.7-1.8 (Majida *et al.,* 2021). Agarose powder about 1.5 gram was add in 100 ml of TBE-buffer in a conical flask and mix well and heated in microwave oven to prepared the homogeneous solution. A volume of 110ul dilute solution of Ethidium bromide was add, poured over casting tray. After solidified gel placed into gel electrophoresis unit. However, 1st well of agarose gel was load by using of 1kb DNA ladder (2ul) as biomolecules. Rest of the other wells were loaded with 2uL of loading dye and 5uL of DNA template (PCR products) about 7uL in each wells. Afterwards, leave it at room temperature of about 60-70 Volts for 50-60 minutes (Zhang *et al.,* 2007).

PCR and DNA sequencing

A reaction of mixture have been prepared for the PCR amplification of BMP-4 gene into PCR tube about (200ul). All the reagents have optimization of concentration with total volume of 200ul were transferred into PCR tubes. 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 *et al.,* (2007). For purification and sequencing of PCR product, amplified product of PCR samples were sent to Macrogen Company, Korea for data analysis.

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

The present study was evaluate the Genetic Analysis of BMP-4 gene in Baddi goat breed of Khairpur district Sindh, Pakistan. According to

the result of DNA Quantification by Nano drop spectrophotometer isolated DNA was absolute pure since A260/A280 ratio and also revealed one of the new results of Nano drop spectrophotometer exhibit purity and quantity of DNA samples.

Table 1: Nano drop spectrophotometer of Baddi goat breed.

Breed	DNA quantity (ng/ul)	DNA purity (260/280)
Baddi goat	29.314 ng/ul	1.61
	11.063 ng/ul	1.09
	7.430 ng/ul	1.63
	7.411 ng/ul	1.60

Figure 1: Image has been taken during Nano drop performance at Karachi JURC center for genome and showed nice spectra 260/280 ratio.

Data analyzed on Ensemble.org genome browser in which blasting the query sequence of cast gene with selected breed's gene and found variations.

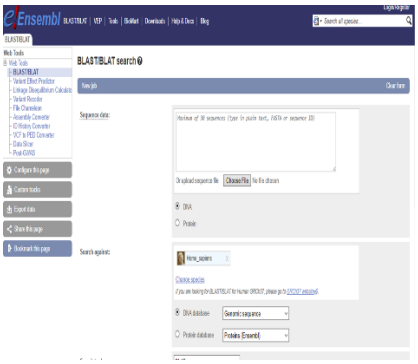

Figure 2. Blast on Ensemble.org of selected gene.

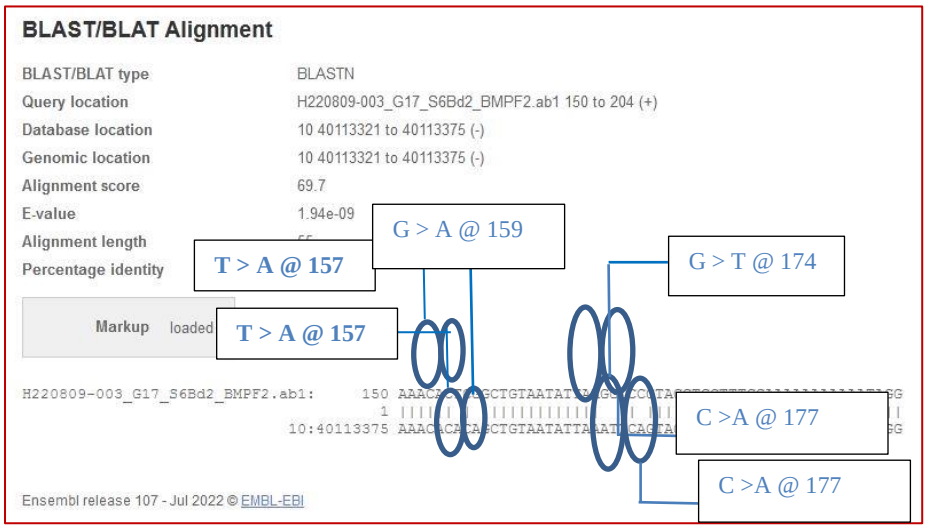

Figure 3. BLAST of Baddi goat breed from Ensemble.org.

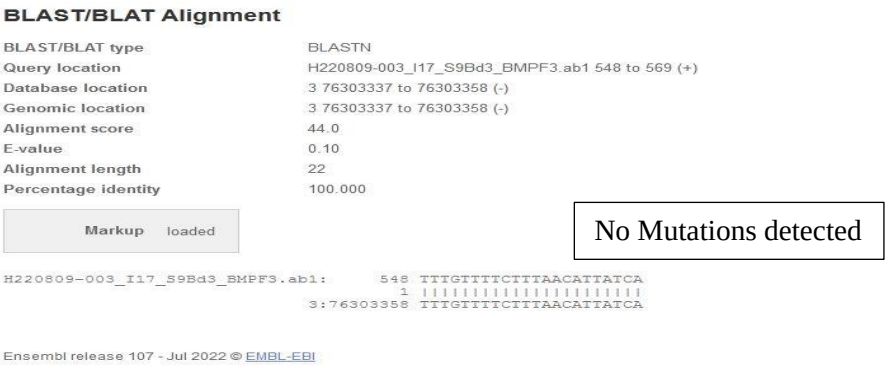

Figure 4. No mutation were detected in this sample.

Table 2. Showed the substitution of amino acids from original to variation (Muations).

Sample Names (ID)	Position of changing Nitrogenous base	Original Codon	Change Codon	Original Amino Acid	Change Amino Acids	Types of Point Mutation
S6Bd2	157	TCG	ACA	Serine	Threonine	Missense mutation
	159	GGT	ATT	Glycine	Isoleucine	Missense mutation
	173	GGT	ATT	Glycine	Isoleucine	Missense mutation
	177	CGT	AGT	Arginine	Serine	Missense mutation

Table 3: The Percentage of mutations in selected breed.

Name of Gene	Name of Breed	Found SNPs/ Mutations	Percentage method	Total Percentage %
BMP-4	Baddi Goat	4	4x100/200	2%

Discussion

High milk production is one of a dairy farmer's top concerns. In addition to milk composition features a new breeding goal has been established to meet the demands of a better diet (Krovvidi *et al.*, 2013). As a result, choosing farm animals with better dairy implementation is crucial for dual breeders and consumers. DNA markers are important in animal breeding programs. Genetic mapping and gene of all animal and plant generally has been transformed by DNA markers. SNPs also called "snips" are the extremely predominant variety of genetic variations found in mammals.

SNPs stands for single nucleotide polymorphism. The majority of the time, these modifications are found between genes. SNPs are recognized as biological markers that help researchers find genes associated with disease or genes involved in the expression of characteristics. SNPs that are located inside or close to a gene's regulatory domain may affect how genes function more directly. These mutations (SNPs) may cause sickness or occasionally have good effects such as increasing the output of milk or beef (Dodgson *et al.*, 1997). The work had been on title BMP4 gene and their relationship between these breed's marker traits. Maximum quantity of missense mutations have been found a very satisfactory symbol and could have a substantial impact on meat quantities and yielding of milk of that goat breed, meanwhile changed genomic codon produced alteration of dispensable amino acid into indispensable amino acid at numerous locations (Karuthadurai *et al.*, 2019). These result agreed with the research of (Bukhari *et al.*, 2013) and disagree with study conducted by (Singh *et al.*, 2015). In this study there were not any kind of silent mutations were found because it would not be effective on the qualities and quantities of milk, subsequently, the phenotypically gene expression would not be affected because of the amino acid code by gene endure similar.

Conclusion

If any kind of mutation occurred in DNA sequences are less than 1% considered mutation and if it is greater than 1% than called SNPs. In Baddi breed 4 types of missense mutations were found and their percentage is greater than 1% that assort in the genomic makeup that could be useful for breed admixture and possible better for meat and milk characteristics. This types of mutation could effect on skin texture of different animals, body trait and growth performance, genetic polymorphism and variant effect on gonadotropin hormones and fertility.

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Conflicts:

The authors declared that there is no conflict of interest regarding the publication of this research.

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