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Molecular Exploration of Zinc Finger BED-Type Containing 6 Gene for Growth Trait in Beetal Goat

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ABSTRACT

Zinc finger, BED-type containing 6 (ZBED6) is a newly discovered transcription factor. It functions as a repressor of IGF2 transcription in skeletal muscle myogenesis and development. It plays a role in organism development, signaling, cell-cell contact, hepatic fibrosis, clathrin-mediated endocytosis, and tight junction signaling cascades. Using C2C12 cells, chromatin immunoprecipitation (ChIP) sequencing revealed roughly 2,500 ZBED6 binding sites in the genome, with the inferred consensus motif matching the known binding site in Igf2. Igf2 expression, cell proliferation, wound healing, and myotube formation is all affected when Zbed6 is silenced in myoblast cells. Certain Gene Ontology categories, such as development and transcriptional control, were significantly enriched in genes related to ZBED6 binding sites. **Objective:** To explore the molecular expression of the ZBED6 gene that affect the growth trait in Beetal goat **Methods:** The BED6 gene's functional and signaling experiments revealed that it controls growth characteristics in goats. A total of 50 blood samples were taken. DNA was extracted using an inorganic technique. Primer3 software was used to build primers for PCR amplification. On the ABI 3130XL Genet Analyzer, PCR results were sequenced bi-directionally. **Results:** The CHROMAS program was used to examine the sequencing findings. SNPs were discovered using sequence alignment tools such as blast 2. The bovine ZBED6 gene was found on chromosome 16, has only one exon, and encodes 980 amino acids. The genomic DNA of both cow breeds was effectively amplified in this investigation by utilizing primer pairs for the ZBED6 gene. These animals had three SNPs, including one non-coding mutation in the promoter (SNP1: 826G > A) and two missense mutations in the CDS (SNP2: 680C > G and SNP3: 1043A > G). The missense mutations p. Ala 227 Gly and p. His 3" are found in SNP2 and 3. **Conclusions:** Novel variations have been discovered that might be used in the selection of superior goats with higher weight increase tendencies.

INTRODUCTION

Goats were among the chief domesticated animals and were modified from the wild form to *Capra aegagrus*. Starting around 10,000-11,000 years back, Neolithic agriculturists in the Near East started keeping little groups of goats for their milk and meat, for their excrement for fuel, and also for materials for apparel and building: hair, bone, skin, and ligament. Today there are more than 300 types of goats, and they live in atmospheres going from high elevation mountains to deserts. Late mitochondrial DNA research recommends that all goats today are slipped from a modest bunch of creatures and may have been trained in a modest bunch of better places [1]. Punjab is the most populated area that manages a substantial number of domesticated animals including goats and goats keeping a regular practice in country zones [2,3]. Beetal goats may be found across Punjab's watershed zones, including Jhelum, Gujrat, Mandi Bahauddin, Gujranwala, Lahore, Sheikupura, Faisalabad, Sargodha, Chakwal, Jhang, and Okara.

The body has a beautiful brown or reddish-brown color with white and black markings. The body is well-developed and reduced. The head is massive, the nose is Roman, and the ears are large, broad, and pendulous. Males have long spiraling horns, while females have short spiraled horns. They have powerful legs and a short tail, and the udder is all-around produced with long teats. In 120 days of lactation, the milk yield of an adult male and female weighing 46 and 36 kg is 200

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liters, and more than half of the births are twins or triplets. Beetal males are bred specifically to be conciliatory creatures. In eid ul azha, there is slaughter [4,5]. Animals' muscles can be divided into skeletal, cardiac, and smooth muscles. In the early developmental stages' muscles are produced by mesodermal cells. Somites are formed by the division of the paraxial mesoderm. Somites give rise to myotome, and further generate myoblast. Myoblasts proliferation starts with the activation of some growth factors such as fibroblast growth factors. Fibronectin secretes by myoblasts when enough secretions are produced by these factors, cause cell cycle arrest. Fibronectin directs to differentiation of muscles cells which is mediated by fusion and alignment of myoblasts. Calcium ions and some metalloproteinases are important for myoblast fusion. Myoblast fusion further leads to the formation of muscle fibers. Satellite cells are present in the skeletal muscles of healthy adults. These are mitotically undifferentiated cells in the quiescent phase. In case of any damage and injury to muscle cells leads to activation of proliferation and differentiation to heal injured fibers [6]. Some other studies form Southeast Asia were also reported on the breeding and other characteristics of beetal goat including the genetic studies [7-11].

Myogenic master regulators are a set of transcription factors that control myogenesis. MyoD, myogenic and transcriptional regulators are muscle-specific transcription factors, and their regulation, requisite myoblast differentiation which leads to skeletal muscles production. Myf5, MyoD, MRF4, and myogenic are members of myogenic regulatory factors (MRFs) containing basic helix loop helix domain, which are responsible for the activation of specific muscle genes during differentiation. Transcription of Myo-D expresses by skeletal muscles and its precursors while in non-muscle tissue and cells this phenomenon is silent due to methylation of distal enhancers at CpG sites [12,13]. SRF (serum response factor), MSTN, PAX3, PAX7, IGFI, and WWRTI are the transcription factors of skeletal precise MyoD, which controlled the transcription of it [16]. Insulin-like growth factors are mitogenic proteins which had a vital role in the maintenance of neuroendocrine regulation of development in all vertebrates. Most studies suggest that in a diversity of vertebrate species this growth factor complex, made of ligands, receptors, and highly-compatible binding proteins, evolved (evolution) [14]. The liver is the organ where IGF2 expression levels are very moreover it is also expressed in most tissues. Insulin-like growth factors I and II are the members of the IGF family and their function is complex in the skeletal muscles and myoblast cells. Both growth factors are proficient in utilizing insulin-like effects on intercessor metabolism. During myoblast proliferation, low levels of IGF 1 can be scrutinized. A study shows the 1.3-fold growth in body weight of mice due to the over the countenance of IGF. Mice (wild littermates) with knockout IGF1 or 2 have a birth weight of 60% while those that have null mutations lead to 30% of their wild-type littermates and mice dying shortly afterward [15]. IGFs arouse many cellular functions such as proliferation, migration, differentiation, and survival. IGFs bind to receptors known as IGF1 (also known as IGF1R) and IGF2 receptor and IGF1R show similarity in structure and sequence with Insulin receptor [16]. As a consequence of a mutation disrupting one of its binding sites in an intron of the IGF2 gene, ZBED6 was recently located within the first intron of ZgC3H11A, causing pigs to grow greater muscle [7]. ZBED6 (zinc finger, BED-type containing 6) is a recently discovered transcription factor that is a repressor of IGF2, a hormone that regulates growth, cell proliferation, and development in placental animals [17]. Chromatin Immunoprecipitation (ChIp) sequence data in murine C2C12 myoblast indicate that ZBED6 holds 2499 targeting sites while microarray data show 400 genes control differentially by ZBED6 in C2C12 myoblasts. Chromatin Immunoprecipitation (ChIp) also revealed the interaction between ZBED6 and QTN site in IGF2 [4]. To check the activity of ZBED6 during myogenesis, the expression of mRNA of IGF2 was measured in ZBED6 silenced and control C2C12 cells. In the early days, there were no significant effects but on day 6 there was an increase in IGF2 expression. Cells with inactive ZBED6 Showed increased proliferation and faster wound healing compared with control C2C12 cells [18].

METHODS

The research work was performed in the Molecular Biology and Genomics Laboratory of the Institute of Biochemistry and Biotechnology Department of Molecular Biology and Biotechnology, the University of Veterinary and Animal Sciences (UVAS), Lahore. Based on the weight of animals at the time of birth than at age of 3, 9, and 12 months only males were included in this study which was divided into two groups, 20 in each group**:** Beetal goat with a higher tendency towards weight and Beetal goat with a lower tendency towards weight. Phenotypic selection of low and high-weight animals was based on the record in which all animals' weight was mentioned. A total of 40 random animals (different families having no blood relation) having distinct phenotype features were collected in sterile vials from the University of Veterinary and Animal Sciences Pattoki campus Research Farm D Pattoki. Phenotypic selection of low and high-weight animals was based on the growth of the animals at 0 days, 3 months, 9 months, and 12 months. A blood sample (5 mL) was taken from each animal, primarily from the jugular vein, and placed in a 5 mL vacutainer tube containing an anti-coagulant (Ethylene diamine tetra-acetic acid) (ETDA). The samples were transported on ice to the Molecular Biology and Genomics Laboratory, IBBT,

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UVAS, Lahore, where they were kept at -20 °C.The organic method was used for DNA extraction from blood samples (Sambrook et al., 2006). The DNA was quantified using Nanodrop. ZBED6 gene in a goat is on chromosome number 16. It consists of only 1 exon (gene bank accession no. **NC_022308.1**). C2C12 cells were used for chromatin immunoprecipitation (ChIP) sequencing. C2C12 cells were used for chromatin immunoprecipitation (ChIP) sequencing. For amplification of exon of the ZBED6 gene from sequence available at NCBI (accession no. NC 022308.1), PCR primers were built using online Primer 3 program (www.primer3.com).

Primer Name	5'-3'Sequence
ZBED6 1F	AAATAACCTGGCTTGGAAGT
ZBED6 1R	GTATACTGCGGGTCCACAT
ZBED6 2F	TGCCCCTACTTTGTTAGCTT
ZBED6 2R	AATGGATGGGATGAGTGG
ZBED6 3F	TTCAACACTTCAACGACACC
ZBED6 3R	CTCACAGAAAAAGTCCTGAATG
ZBED6 4F	CATGCTTCCTGCGTTGTTTA
ZBED6 4R	GCCAATAAAACAAAGGGTCATC

Table-1: ZBED6 Gene Primers

To optimize primers for an effective polymerase chain reaction, a variety of PCR reaction mixes and PCR cycle settings were used, with the goal of achieving maximum amplification with the least number of reagents.

Step	Temperature	Time
Initial denaturation	95°C	4 min
1. Denaturation	94°C	30 sec
2. Annealing	63-53°C 60-50°C	45 sec
3. Extension	72 °C	45sec
	Repeat step 1 to 3 for 30 cycles	
Final extension	72 °C	10 In

Table2: Protocol for gene amplification

On 50ng DNA samples from the Beetal goat breed, all forward and reverse primers were amplified. Gel electrophoresis was used to validate PCR amplification. The gel was then photographed and studied under UV light in the Gel Documentation System (Bio-Rad). When a chosen region of DNA was amplified, the PCR products were eluted with the Favor Prep GEL/PCR purification kit and put on a 1% gel to check for quality. The PCR amplicons were sequenced after gel elution. Multiple amplifications from the original material were done to reduce the chance of detecting PCR-induced mutation. Sanger's chain termination approach was used to sequence the data. A single-standard DNA template, a DNA polymerase, a DNA primer, radioactively or fluorescently tagged nucleotides, and chain terminating nucleotides are all used in the traditional chain termination approach. Dideoxyribonucleoside triphosphate is a dideoxyribonucleoside triphosphate that stops DNA strand elongation. Four different processes using standard deoxynucleotides (dATP, dGTP, dCTP, and dTTP) and DNA polymerase were used to sequence the DNA. Only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, and ddTTP) was introduced to each process. These nucleotides lack the 3-OH group required to establish a phosphodiester link between two nucleotides, resulting in the end of DNA strand extension and the creation of DNA fragments of various lengths. For analysis of sequencing results, Chromas software was used. Results of sequencing were found in FASTA format. These FASTA format sequences were aligned by Blast software. From these aligned sequences Single Nucleotide Polymorphisms (SNPs) were identified. POPGENE 32 was used to determine allele frequencies, genotypic frequencies, Hardy- Weinberg equilibrium (HWE) at each locus, p-value, linkage disequilibrium, and Shanon Index.MEGA.5 software was used for phylogenetic analysis, and the rate of evolution ZBED6 gene in different species was determined. Thesis software (http://www.nhgg.org/analysis) was used for haplotype analysis and to determine the probability of each haplotype.

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RESULTS

The organic approach was used to recover DNA from goat blood samples. All forward and reverse primers were amplified on 50ng DNA samples of the Beetal goat breed using varied temperature ranges according to primer requirements. Gel electrophoresis (Figure 1) verified PCR amplification. Following the amplification of a desired region of DNA, PCR products were eluted using the Favor Prep GEL/PCR purification kit and loaded on a 1% gel to determine their quality (Figure 2). Polymorphic sites of the ZBED 6 gene were identified by using Multiple Sequence Alignment by CLUSTALW in Betal goat (Figure 3).

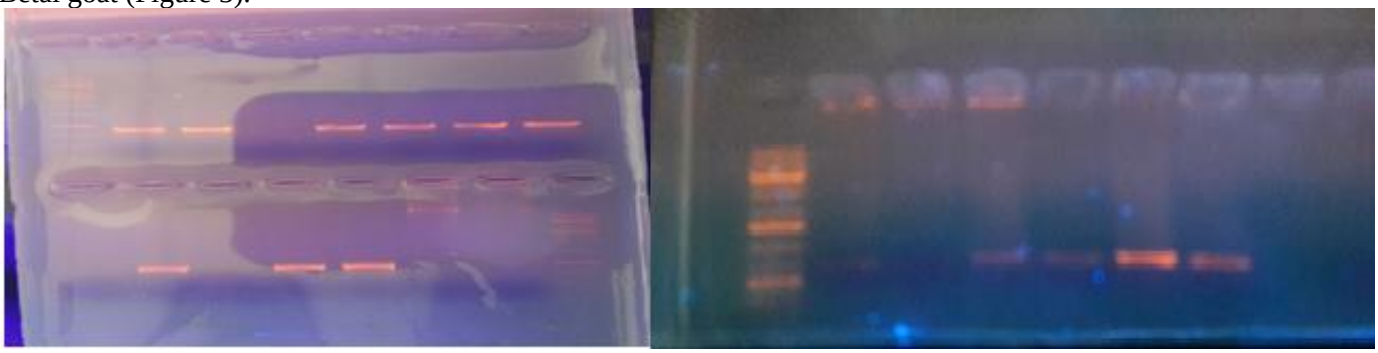


Figure 1: Gel-Electrophoresis picture of PCR products

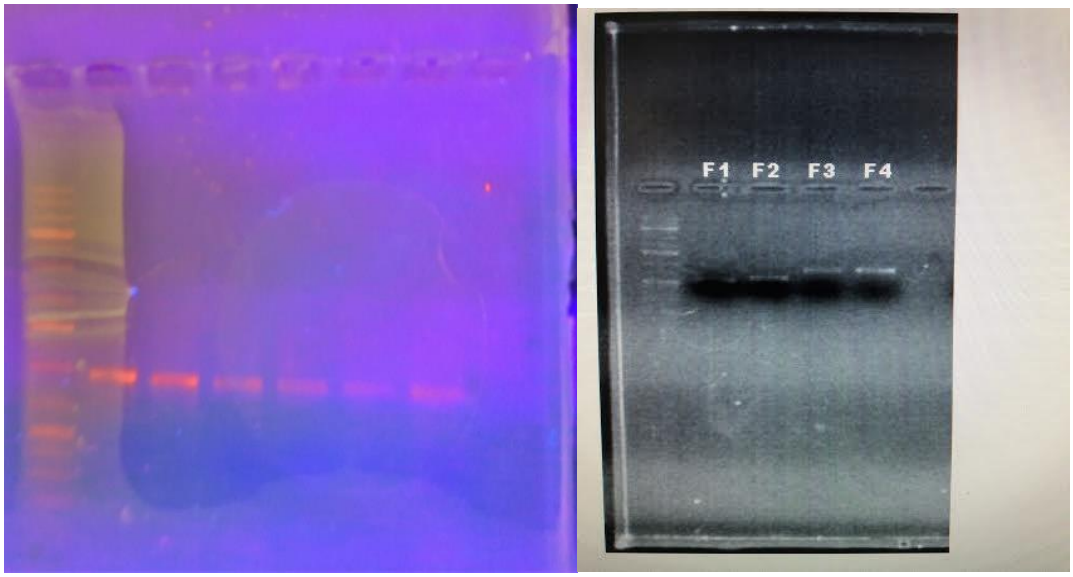
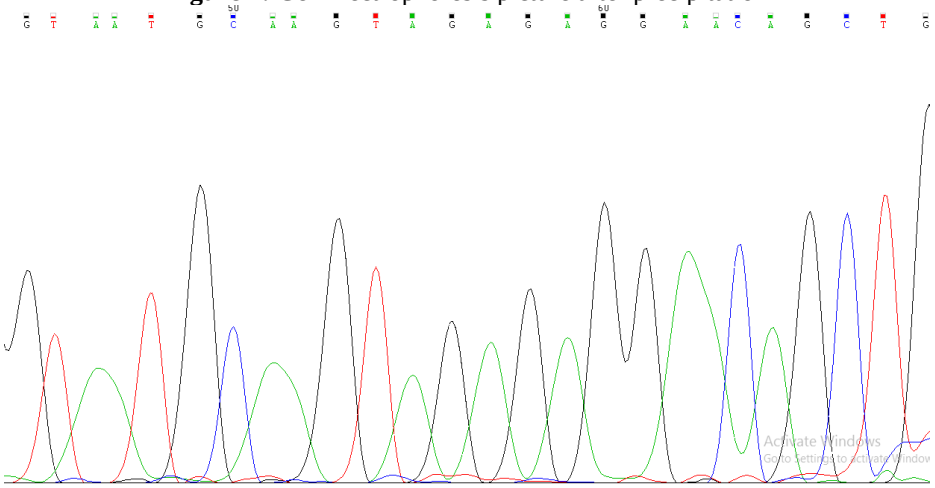


Figure 2: Gel-Electrophoresis picture after precipitation



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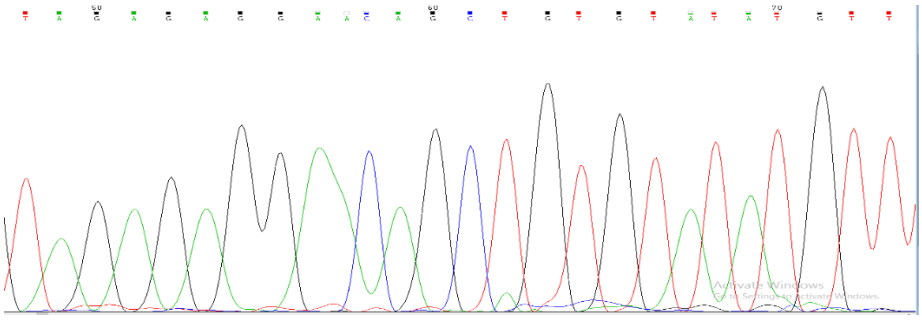

Figure 3: Identification of SNPs

DISCUSSION

ZBED6 recently discovered within the first intron of ZgC3H11A in a result of mutation distracting one of its binding sites in the IGF2 gene's intron [7]. ZBED6 (zinc finger, BED-type containing 6) is a recently discovered transcription factor that acts as a repressor of IGF2, a hormone that regulates growth, cell proliferation, and development in placental animals [8]. Chromatin Immunoprecipitation (ChIp) sequence data in murine C2C12 myoblast indicate that ZBED6 holds 2499 targeting sites while microarray data show 400 genes control differentially by ZBED6 in C2C12 myoblasts. Chromatin Immunoprecipitation (ChIp) also revealed the interaction between ZBED6 and QTN site in IGF2 [4]. To check the activity of ZBED6 during myogenesis, expression of mRNA of IGF2 was measured in ZBED6 silenced and control C2C12 cells. In early days there were no significant effects but at day 6 there was an increase in IGF2 expression. Cells with inactive ZBED6 Showed increased proliferation and faster wound healing compared with control C2C12 cells [10]. ZBED6 is expressed in all nine tissues studied in cattle and has a wide tissue distribution (3 individuals per stage). The heart, intestines, and LDMs showed the highest relative expression levels of bovine ZBED6 mRNA, which subsequently reduced in abundance over the three developmental phases of myogenesis and muscle maturation. The expression patterns of ZBED6 were found to be high in the heart and muscle tissue and low in the rest of the body. The bovine ZBED6 gene is found on chromosome 16, has just one exon, and encodes 980 amino acids, according to SNPs. The genomic DNA of both cow breeds was effectively amplified in this investigation utilizing primer pairs for the ZBED6 gene. These animals had three SNPs, including one non-coding mutation in the promoter (SNP1: 826G > A) and two missense mutations in the CDS (SNP2: 680C > G and SNP3: 1043A > G). The missense mutations p. Ala 227 Gly and p. His 3 are found in SNP2 and 3.

Conclusions: Novel variations have been discovered that might be used in the selection of superior goats with higher weight increase tendencies.

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