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Genetic Polymorphism of GH, IGF1 AND BMP15 Genes in Maraz Goat

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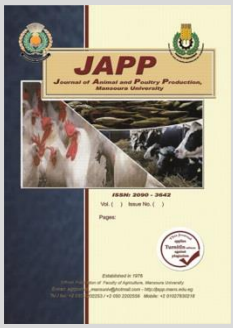

ABSTRACT

The Studies of genetic polymorphism in local animals aim at evaluating the genetic diversity of animal breeds chiefly for conservation purposes. In this research, single nucleotide polymorphism (SNP) was used to detect point mutation of GH, IGF1, BMP15 genes in Maraz goat. Forty blood samples were collected from Maraz goat, DNA was isolated and primer was used to amplify GH, IGF1, and BMP15 genes. Finally, sequencing methodology was applied for PCR products. Results of GH gene showed only one SNPs (A□G) at nucleotide number 58pb. The sequence results of IGF1 illustrated two point mutations at nucleotide number 5502 and 5675 bp. While according to data sequences no point mutation was detected of BMP15 gene. These outcomes revealed that the SNP marker of the GH, IGF-I, and BMP15 genes will be a possible molecular marker for growth traits and cashmere fiber in Maraz goat. This research displays that the genetic similarities between Maraz goat and other breeds match their geographical distribution which indicates that the Maraz goat is robustly separated from the other breeds.

Keywords: GH, IGF1, BMP15, SNPs, diversity, Maraz goat.

INTRODUCTION

Polymerase chain reaction (PCR) was imagined by Mullis in 1983 and licensed in 1985. Its standard depends on the utilization of DNA polymerase which is an in vitro replication of explicit DNA groupings. This technique can create a huge number of duplicates of a specific DNA section "the succession of interest, the DNA of interest, or the objective DNA" from a DNA remove (DNA format). Truth be told, if the grouping of interest is available in the DNA extricate, it is conceivable to specifically recreate it in exceptionally huge numbers. The intensity of PCR depends on the way that the measure of DNA in the cluster isn't, in principle, a restricting variable. Accordingly, we can enhance nucleotide arrangements from minute measures of DNA extricate. Consequently, PCR is a decontamination or cloning strategy. DNA extricated from a life form or test containing DNA of different beginnings can't be straightforwardly breaking down. It contains numerous masses of nucleotide successions. In this manner, it is important to separate and purge the arrangement or successions that are of revenue, either the grouping of a quality or non-coding successions "introns, transposons, smaller than expected or microsatellites". From quite a mass of groupings that establishes the DNA network, PCR can thusly choose at least one arrangements and intensify them by replication to a huge number of duplicates. When the response is finished, the measure of DNA in the exhibit that isn't in the territory of interest won't have changed. Or maybe, the measure of intensified succession (s) "the DNA of interest" will be exceptionally huge. PCR makes it conceivable to enhance a sign from a foundation clamor, making it an atomic cloning technique and the clone is gotten back to virtue (Rheinberger and Müller-Wille, 2018).

Maraz goat is local variety in Kurdistan area and reared basically for their fine hair and meat. They are more modest in size than the nearby black goats, Khoshanow (2002) and Aziz (2009) reported that the live body weights of Maraz goat reared in private farms is from 27.6 to 31.2 kg for males and from 24.9 to 25.4 kg for females. According to hair, four distinct colors (white, red, brown and some are a mixture of these colors) of Maraz goats have been known, and adapted to survive under harsh feed and environment conditions (Alkass and Juma, 2005).

In our region studies to identify the molecular markers and gene polymorphisms in sheep were made by Hama Khan *et al.* (2019) who studied the genetic polymorphism of some genes which affect production performances related to milk traits in Karadi sheep.

Investigation the hereditary make-up of native breeds at the DNA level and candidate genes has given researchers the tools for open doors for genetic improvement and the initial phase in the overall methodology of marker assisted selection. The application of molecular genetics approaches is of great interest in the identification of genetic variations in genetic markers which are associated with the most economically important production traits in animals (Jiang *et al*., 2002; Arora and Bhatia, 2006; Missohou *et al*., 2006).

The physiological processes of metabolic pathways in animals are heavily influenced by different genes. Polymorphisms in these genes, which show relationships with particular production performance, are valuable markers for marker assisted selection. Single nucleotide polymorphisms (SNP) are the most common forms of variation in the genome and they can be used to investigate the combinations between them and the quantitative and qualitative traits of animals (Wang *et al*., 2010).

Growth hormone quality gives directions to making the development hormone protein. Development hormone is created in the development invigorating somatotropic cells of the pituitary organ, which is situated at the base of the mind. Development hormone is vital for the typical development of the body's bones and tissues. Insulin growth factor1 (IGF1) ties its receptor, IGF1R, enacting a few flagging pathways. Associated with multiplication, separation, endurance, development, apoptosis and recovery.

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IGF1 "Insulin Like Growth Factor 1" is a Protein Coding quality. Illnesses related with IGF1 incorporate Insulin-Like Growth Factor I and Pituitary Gland Disease. Among its connected pathways are Apoptosis Modulation and Signaling and Transcriptional misregulation in disease. Bone morphogenetic protein 15 (BMP-15) is a protein that in people is encoded by the BMP15 quality. It is engaged with folliculogenesis, the cycle where early stage follicles form into pre-ovulatory follicles. Growth hormone (GH), insulin growth factor1 (IGF1), and bone morphogenetic protein 15(BMP15) genes are candidate genes and play a vital role in performance production in goats (Supakorn, 2009). The purpose of this study is to find the genetic polymorphism of "GH, IGF1 and BMP15" genes in Maraz goat using SNPs.

MATERIALS AND METHODS

DNA samples and Isolation

Blood samples were taken from an aggregate of 40 Maraz goats. Five milliliter blood was gathered from every creature from jugular vein into 10 ml Vacutainer tubes containing the anticoagulant, ethylenediaminetetra-acidic corrosive (EDTA) and blood tests were put away at 20°C until DNA extractions. DNA was extricated from every one of the blood test utilizing Quick-DNATM Miniprep Kit-Catalog Nos. D3024 and D3025 (Zymo Research Corp.).

Polymerase chain reaction (PCR) runs with specific primer

Polymerase chain reaction (PCR), a strategy used to make different copies of a specific piece of DNA quickly and absolutely. The polymerase anchor reaction enables analysts to get the tremendous measures of DNA that are required for various assessments and frameworks in sub-nuclear science, logical examination, formative science, and clinical diagnostics.

Enhancements were finished utilizing a warm cycler with the last response volume of 50µL. An expert blend for tests was readied and an aliquot of 40µL filled in each PCR tube. Ten µL of test DNA was added to each cylinder to make the last volume 50µL, control response was set up without DNA. A GoTaq® Green Master Mix (ADM7122 00000311719, Promega-USA) fuses with 25µL Taq DNA polymerase (25Units/mL, dNTPs 200µM, and MgCl2 1.5mM), 4µL groundwork (0.1-1µM, forward and adores), 10µL (50ng) of DNA layout and 11µL DNase free water. In this examination the accompanying convention was utilized: initial denaturation at 94°C for 5 min to thoroughly denature the DNA design, followed by 35 examples of denaturing at 94°C for 45s, reinforcing for min at temperatures express for the marker and extension at 72°C for 1 min, with a last growth adventure at 72°C for 5min followed by limit at 4°C forever. The PCR things are electrophoreses on 1.5% agarose gel recolored with ethidium bromide to test the upgrade accomplishment. In the current assessment, an amount of three express loci primers which were portrayed in (Table 1).

Table 1. The primers sequence and annealing temperature used in this study

Locus	Sequence (5′–3′)	Annealing temperature	Amplicon size (bp)	Reference
GH	CTCTGCCTGCCCTGGACT GGAGAAGCAGAAGGCAACC	55°C	388	Hua *et al*., (2009)
IGF1	CACAGCGTATTATCCCAC GACACTATGAGCCAGAAG	56°C	322	Liu *et al*., (2010)
BMP15	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC GGATGCAATACTGCCTGCTTG	63°C	120	Hamid *et al*., (2009)

RESULTS AND DISCUSSION

Growth hormones quality is actually situated on goat chromosome 19q22 (Schibler *et al*., 1998; Pinton *et al*., 2000). It is made of 1,800 base sets (bp), which comprises of 5 exons and 4 interon (Kioka *et al*., 1989). In the current investigation exon 2 and 3 intensification of GH quality delivered 383 bp, which the DNA grouping of GH quality of Maraz goats coordinate with various successions in NCBI the first one with (KC789517.1) by 99% character score which (A→G) at position 58 (Figure 1).

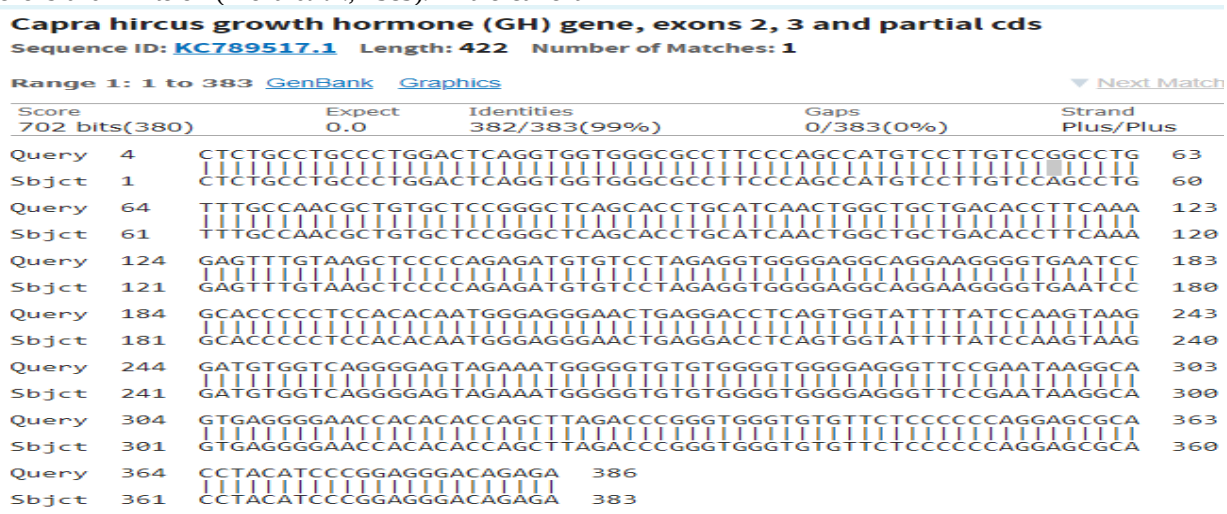

Figure 1. Sequence alignment of Maraz goat growth hormone GH gene with KC789517.1 sequence.

Othman *et al*. (2015) detailed a SNP (G→A) at position 55 of GH1 locus (422 bp) in the Egyptian Baladi, Barki, and Zaraibi goat breeds utilizing HaeIII/PCR-RFLP and quality sequencing. Some previous works have been discovered polymorphism of GH quality in the administrative district, untranslated locales and exons. Ge *et al*., (2003) and Yu *et al*., (2004) outlined polymorphic locales have been accurately described for nucleotide and amino corrosive changes. Likewise, two polymorphic destinations were situated in every one of exon 1 and exon 2; 4 locales in exon 3, 7 destinations in exon 4 and 5 destinations in exon 5 (Missohou *et al*., 2006).

The IGFs compound framework, which is made out of IGF-I, IGF-II, IGF-I receptor, IGF-II receptor and six restricting proteins (IGFBP-1 IGFBP-6), envision a critical division being created, improvement and duplication similarly as developing (Miller and Gore, 2001; Li *et al*., 2009; Lan *et al*., 2007c). IGF-I quality is encoded by a solitary quality on chromosome 5 (Schibler *et al*., 1998), make of three pioneer exons (1 w, 1 and 1a) and three exons (3, 4 and 6), in which exon 3 and exon 4 encode the develop IGF-I peptide (Mikawa *et al*., 1995). IGF-I assumes a critical function in digestion, mammogenesis, lactation (Zhang *et al*., 2008), by animating anabolic cycles, for example, cell expansion, skeleton and hair development and protein union. Arrangement investigation in this examination uncovered two point changes, which the first was replacement (C→G) at position 5675 and the subsequent one was a nucleotide transversion from Guanine (G) to Cytosine (C) at position 5502 of the succession with increase number D26119.2 (Figure 2). A few polymorphisms of IGF-I quality and their relationship with creation characteristics were accounted for in goats. Zhang *et al*. (2008) detailed the polymorphism in intron 4 of the IGF-I and its relationship with development qualities in the Nanjiang Huang goat. The relationship of g.5752 G>C and g.1617 G>A changes with milk yield and body size in Chinese dairy goats were accounted for with a huge impact of these minor departure from the analyzed gainful characteristics (Deng *et al*., 2010). Qiong *et al*., (2011) delineated the connection between IGF-1 variety and cashmere creation characteristics just as body weight in three Chinese goat breeds. An epic SNP was distinguished in exon 4 and it is fundamentally connected with cashmere creation attributes. As indicated by study which led Naicy *et al*., (2017) of India from Sequencing the PCR results of IGF1from two Indian goats uncovered a point change (C→T).

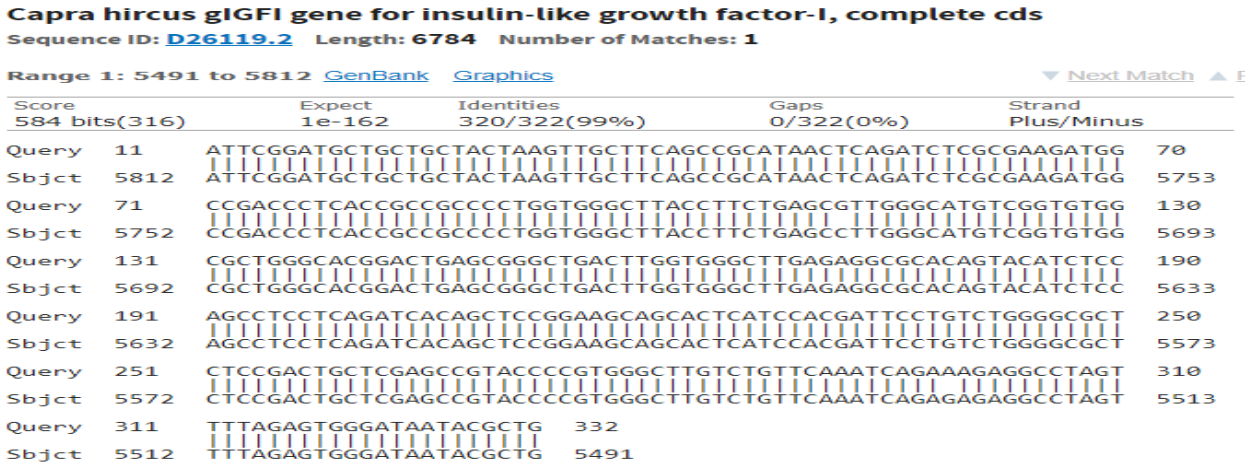

Figure 2. Sequence alignment of Maraz goat IGF1 gene with D26119.2 sequence.

The sequence alignment of 97 bp out of 122 bp of Maraz goats BMP15 gene, exon three with published sequence (GU732196.1, Markhoz goat) was carried out using BLAST and showed 100% identity (Figure 3). This results was the same as compare with published sequences accession number: KY780297.1 Tibet Cashmere goat, and EU743938.1, Jining Grey goat). As per research evidence all these breeds are bred for production cashmere fiber, and also they have some common characteristics such as body weight. This information on the genetic analysis can be used with physical and phenotypic characteristics and management practices for registration of Maraz goat as a cashmere breed. These results indicate Maraz goat remains pure to produce cashmere, in addition it will help us to declaration this breed as an important breed in the world to produce cashmere fiber. According to Hua *et al*., (2007) in Chinese goats none of the polymorphism of the BMP-15 gene was tested in goats from six breeds (or flocks). The same result was observed by Jamshidi *et al*., (2009) in Sangsari Sheep Breed of Iran. The absent of mutation in BMP-15 of six breeds of Chinese goats was reported by He *et al*., (2006). Zare *et al*., (2007) also detected no point mutation of BMP-15 gene from 240 blood samples of Shal ewes by using of PCR- RFLP.

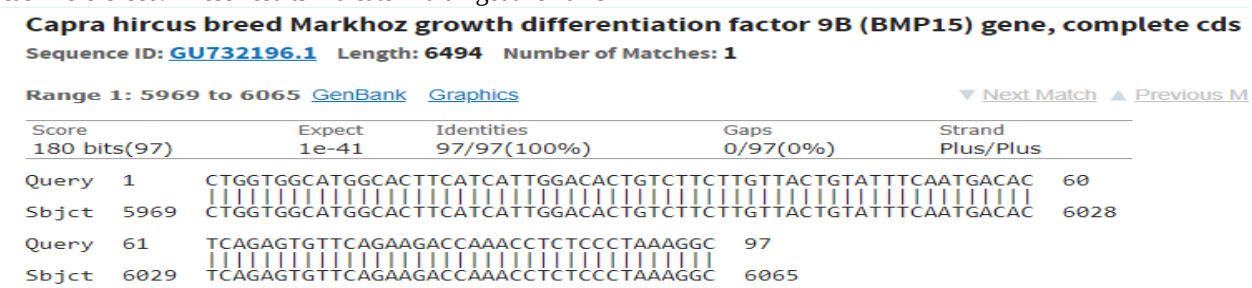

Figure 3. Sequence alignment of Maraz goat BMP15 gene with GU732196.1 sequence.

The Neis genetic distances for GH, IGF1 and BMP15 of Maraz goat with some *Capra hircus* recorded in GenBank are present in Table 2. Higher identity of GH sequence was presented between Maraz goat, Capra_hircus (KU288603.1), Capra_hircus (KU288612.1) and Barki with percentage of 100% while lower one was found between Maraz goat and Capra_hircus (KC789517.1), Capra_hircus (KU288609.1) and Capra_hircus (KU288610.1) with an average (0.02%). In IGF1, the distances of Maraz goat and other goat breeds were much closer which the highest was (1.27%) with Malabari (KP256000.1), while the lowest was (1.17%) with Capra_hircus (HQ731040.1). As per the findings, BMP15 locus of Maraz goat show very high levels of genetic similarity with an average 100% within

population with Markhoz goat (GU732196.1), Tibet Cashmere (KY780297.1), Nandidurga (MK421566.1), Telangana (MK421559.1), Jamnapari (MN629925.1) and Capra hircus (MN629926.1), and low similarity (0.010481) with Nandidurga (MK421565.1). This similarity between breeds of the different country may suggest increasing crossbreeding as a consequence of increase in transhumance and farmers' preference for larger animals.

According to a study was conducted Al-Barzinji and Hamad (2017) to find out the genetic variation among different hair Maraz goats (Brown, Black and White) and local (Black, white, Koor and Pnok) goats, as it turned out the genetic distance among several goat breeds ranged from 9.11 to 43.33%, in addition the highest genetic distance 43.33% recorded between Maraz goat and other goat breeds and between local

Koor and other goat (except Maraz goats) breed (37.79%). However, the lowest genetic distance recorded between local white and Pnok. Also, some previous researchers studied the genetic diversity in different goat breeds (Oliveira *et al*., 2005; Moradi *et al.*, 2014; Kumari *et al*., 2013). Genetic variation is the raw material for the animal breeders, who utilize domestic animal species to people's needs. Furthermore, the increasing genetic data on goat breeds using different genetic markers will help to understand the evolutionary history of goat. In addition, it will help to refine the definition of breed (Henderson, 1984; Bearden and Fuquay, 2000). Mahfouz *et al*.(2008) reported the genetic similarity ranged from 0.8332 to 0.9750 in five Egyptian sheep breeds. In addition Al-Barzinj1 and Ali (2013) reported the mean of heterozygosity between three Kurdish sheep (the Hamdani, Karadi, and Jaff) with the Awassi sheep breed was 0.3678.

Table 2. Pairwise distance for GH, IGF and BMP15 of Maraz goat with some *Capra Hircus* recorded in GenBank

Capra Hircus	**GH**	***Capra Hircus***	**IGF1**	***Capra Hircus***	**BMP15**
Maraz Goat		**Maraz Goat**		**Maraz Goat**	
Guizhou_Xiang (JN012229.1)	0.01	Malabari (KP256000.1)	1.27	Bidri (MK421561.1)	0.010485
Capra_hircus (KC789517.1)	0.002	Tappady_Black (KP256001.1)	1.25	Nandidurga (MK421565.1)	0.010481
*Capra_hircus (*KU288609.1)	0.002	Capra_hircus (KX432967.1)	1.23	Markhoz (GU732196.1)	0.00
Capra_hircus (KU288610.1)	0.002	Capra_hircus (KX432968.1)	1.26	Tibet Cashmere (KY780297.1)	0.00
Capra_hircus (KU288603.1)	0.00	Capra_hircus (FJ957939.1)	1.19	Nandidurga (MK421566.1)	0.00
Capra_hircus (KU288612.1)	0.00	Capra_hircus (D26119.2)	1.20	Telangana (MK421559.1)	0.00
Barki (KU976149.1)	0.00	Capra_hircus (HQ731040.1)	1.17	Jamnapari (MN629925.1)	0.00
Baladi (KX032517.1)	0.00			*Capra hircus* (MN629926.1)	0.00
Capra_hircus (EU048226.1)	0.00				

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تعدد الأشكال الجينية لجينات GH و IGF1 و BMP15 في الماعز المرعز

كاروا ن محمد حمة خان

قسم علم الحيوان ، كلية علوم الهندسة الزراعية ، جامعة السليمانية ، السليمانية ، إقليم كوردستان ، العراق

تهدف دراسات تعدد الأشكال الوراثية في حيوانات المزرعة إلى تقييم الاختلافات الجينية لسلالات الحيوانات لأغراض الحفظ بشكل أساسي. في هذه الدراسة ، تم استخدام تقنية تعدد أشكال النوكليوتيدات المنفردة (SNP) لاكتشاف الطفرة الوراثية لجينات GH و IGF1 و BMP15 في ماعز مرعز. تم جمع أربعون عينة دم من ماعز مرعز ، وعزلت DNA واستخدمت بادأ لتضخيم جينات GH و IGF1 و BMP15. أخيرًا، تم تطبيق طريقة تفاعل البلمرة المتسلسل PCR. أظهرت نتائج جين GH بوجود فقط واحد SNPs بتغير A الى G عند نيوكليوتيد رقم pb58. أوضحت نتائج تسلسل IGF1 طفرتين وراثيتين عند النوكليوتيدات رقم 5502 و 5675 bp. ولم يتم الكشف اي طفرة في جين BMP15. أشارت هذه النتائج إلى أن علامة SNP لجينات GH و IGF-I و BMP15 قد تكون علامة جزيئية محتملة لصفات النمو وألياف الكشمير في ماعز مرعز. توضح دراستنا أن العلاقات الوراثية بين ماعز المرعز والسلالات الأخرى تتوافق مع توزيعها الجغرافي بالإضافة إلى أن ماعز المرعز منفصل بشدة عن السلالات الأخرى.