Advances in Environmental Biology, 5(7): 1850-1853, 2011
ISSN 1995-0756

This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLE

Sequence Assigning of the Bone Morphogenetic Protein 15 (BMP15) Genes in Markhoz Goats

[1]Hourad Ghoreishi, [2]Jalal Shayegh, [3]Abolfazl Barzegari, [4]Naser Maheri-Sis, [4]Abolfazl Gorbani, [1]Ahmad Babazadeh Bedoustani, [4]Sadegh Fathi Yosefabad

[1]Tabriz branch, Islamic Azad University, Tabriz, Iran.
[2]Department of Veterinary Medicine, Shabestar branch, Islamic Azad University, Shabestar, Iran.
[3]Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
[4]Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran.

Hourad Ghoreishi, Jalal Shayegh, Abolfazl Barzegari, Naser Maheri-Sis, Abolfazl Gorbani, Ahmad Babazadeh Bedoustani, 2Sadegh Fathi Yosefabad: Sequence Assigning of the Bone Morphogenetic Protein 15 (BMP15) Genes in Markhoz Goats

ABSTRACT

This research was performed to sequence the bone morphogenetic protein 15 (BMP15) from Transforming Growth Factor beta (*TGFβ*) family in Markhoz breed of goat. This gene controls the ovarian follicle development, ovulation rate and fertility. Blood samples were collected from Sanandaj Markhoz goat breeding station and DNA was extracted using the phenol chloroform method. The primers for exon 1 and 2 were designed and used for amplification of the gene fragments through the simple PCR procedure. The length of exon 1 and 2 are 328bp and 857bp, respectively. Sequence detection was performed after amplification of the gene parts. A mutation at exon 1 in base No.200 with C→T type and two mutations at exon 2 in base No.573 with G→A type and No.755 with T→G type were identified. These mutations were reported and registered in the NCBI gene bank with number of GU732196.

Key words: BMP15, *TGFβ*, Markhoz goat, ovulation rate, PCR, sequencing.

Introduction

TGFβ super family consists of more than 40 members, most of them have regulating roles in fertility [2]. One of the latest members of this super family is Bone Morphogenetic protein 15 (BMP15) or GDF9B [3,4,10]. Some polymorphisms have been identified in the BMP15 gene that is one of the main causes of follicular growth alterations and infertility in Inverdale, Hanna, Cambridge and Belclair sheep [5]. BMP15 is necessary for normal follicular development in sheep. These findings show the essential role of ovaries in normal follicular growth regulation and ovulation rate [1]. Juengel *et al*. [13] have performed an experiment to find the relation of GDF9 and BMP15 with ovulation rate and progesterone concentration. They represented that the presence of GDF9 and BMP15 is vital for normal follicular function and growth, before and after follicular development [9]. Hanrahan *et al* [11] examined polymorphisms of GDF9 and BMP15 genes and their relation with increased ovulation rate in Belclare and Cambridge sheep breeds. Homozygote ewes for both mutant genes were infertile and heterozygote ones had increased ovulation rate [7].

BMP15 protein has defined to encode with two exons in humans and mice: The exon one encodes a signaling peptide that consists 17 amino acids and the first section of propeptide region.

The exon two encodes the rest of the propeptide

Corresponding Author

Hourad Ghoreishi, Teacher of Tabriz branch, Islamic Azad University, Tabriz, Iran.

and complete 125 amino acids region [5] BMP15 gene is located on X chromosome and its protein coding region length is 1182bp that encodes a protein with 393 amino acids in sheep. This gene with approximately 6.6kb length has two exons that are 328bp and 829bp for exon 1 and 2 respectively. A 5.3kb interon separates these exons [8]. The aim of this study was assigning the BMP15 gene in Markhoz goats.

Materials and methods

Approximately 10cc blood was collected aseptically from the jugular vein of five does. EDTA used as anticoagulant. Blood samples collected from Markhoz breeding station in Sanandaj, northwest of Iran. Samples stored at -20°C. DNA extraction performed using standard Phenol-Chloroform method. The extracted DNA was tested via the 0.8% Agarose gel electrophoresis and spectrophotometery procedure. Primers for exon 1 and 2 were designed using Oligo5 software [table 1].

These primers can amplify the 328bp and 857bp fragments for exon 1 and 2 respectively. PCR products each of them has 50µl volume containing 26µl PCR master kit, 1µl DNA, 3µl from each of the forward and reverse primers (with 10pmol density, each) and 17µl DNAase free water, were prepared and tested with the 1.2% Agarose gel electrophoresis method, and then sequenced by Macrogen Inc. South Korea.

Table 1: Primers used for exon 1 and 2 of BMP15 gene amplification.

Gene	Primer	Primer sequence (5´-3´)	Annealing temperature (Oc)
BMP15 (exon1) 328bp	F1	TTTTCAAGATGGTCCTCCTG	59
	R1	TCTGAGAGGCCTTGCTACAC	
BMP15 (exon2) 857bp	F2	CAGTTTGTACTGAGCAGGTC	59
	R2	TTTGCCGTCACCTGCATGTG	

Results:

-Exon 1 and 2 Amplification:

PCR products were tested using the 1.2% Agarose gel electrophoresis procedure and the results showed that desired fragments amplified properly and no unspecific fragments were seen (Fig.1).

- BMP15 Sequence:

Markhoz breed BMP15 gene exons were sequenced for the first time. The length of exon 1 and 2 assigned as 328bp and 857bp respectively. Sequence results showed some mutations: one in exon 1 at nucleotide No. 200 with C?T type and two mutations in exon 2, which are at nucleotide No. 573 with Gβ A type and at nucleotide No. 755 with T?G type respectively [Fig. 2]. In exon 1, the mutation is in the second base of (TCA) codon that codes Serine, where C was replaced with T and the codon changes to (TTA), which codes Leucine so the mutation is missense. The first mutation of exon 2 that happened in nucleotide No. 573, changes the first base of (GGC) codon from G to A and coded amino acid changes from Glycine to Serine, so this is a missense mutation too.

In the second mutation of exon 2, the third base in (CCT), codon changes from T to G, but there is no change in coded amino acid that is Proline, and the mutation is silent.

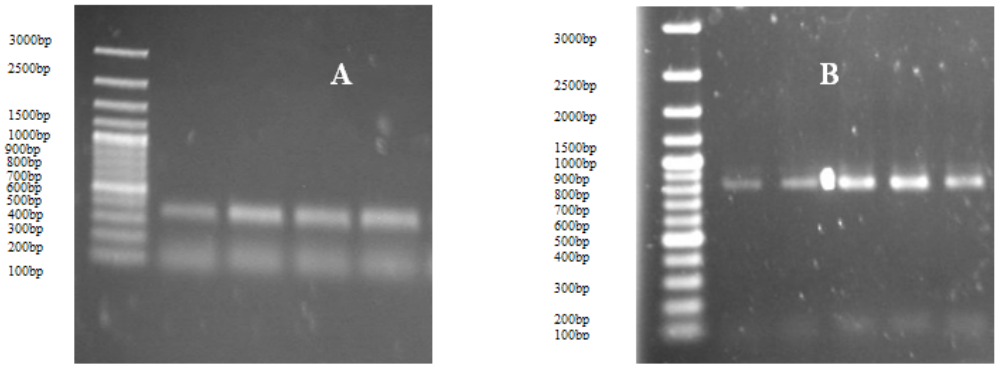

Fig. 1: Electrophoresis images of amplified exon1 (A) and exon2 (B) of BMP15 gene.

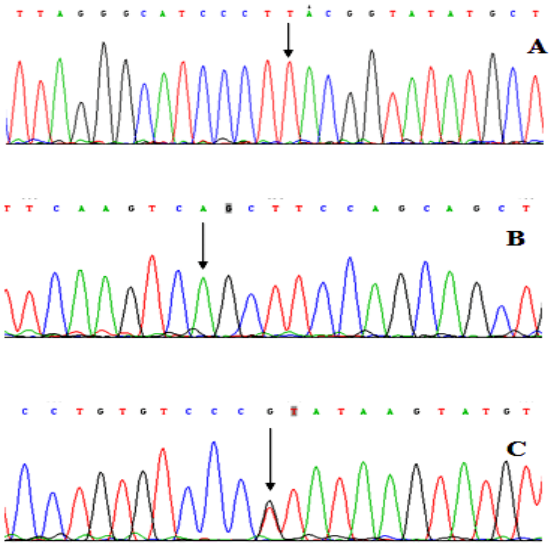

Fig. 2: Mutations in exon1 (A) and 2 (B and C) of BMP15 gene.

Discussion:

Several genes are defined to have associations with fecundity in some animals. It is verified that some loci in BMP15, GDF9, BMPR-1B, other genes, and their mutations have major effects in sheep prolificacy [11, 12]. Some of mentioned loci and genes were studied in goats and it is determined that their influence in prolificacy of goats were not as significant as of sheep [13, 14]. In this research we sequenced Markhoz BMP15 gene exons and found three single nucleotide mutations. These mutations need further studies to confirm their relevance with goat fecundity.

With considering the importance of this gene in fertility and ovulation rate, continuous study and research of this gene and other candidate genes, the use of techniques such as PCR-RFLP, SSR-PCR or PCR-SSCP look essential. It is recommended using larger flocks with pedigree. Thus, minute comparisons between gene sequences, and mentioned mutations in prolific and non-prolific does by appropriate methods suggest. In addition, it is advisable to inspect other parts of this gene (for example introns) and to use infertile animals.

Acknolowdgment

The authors appreciate Dr. Ali Rezapour (head of Agriculture and natural resources faculty of Islamic Azad University Tabriz branch), Mr. Shahram Rashidi (Markhoz breeding station manager) and his assistant Mr. Jamal Kakekhani, Mr. Mahmoodi (domestic animal improvement assistant of Jahade Keshavarzi organization of Kurdistan) and Islamic Azad University Shabestar branch for their support and cooperation. Authors are grateful to Mr. Alireza Lotfi for his co-works in formatting and submitting.

References

1. Bodensteiner, K.J., C.M. Clay., C.L. Moeller and H.R. Sawyer., 1999. Molecular cloning of the ovine Growth/Differentiation factor-9 gene and expression of growth/differentiation factor-9 in ovine and bovine ovaries. Biol. Reprod., 60: 381-386.
2. Chang, H.C., W. Brown and M.M. Matzuk., 2002. Genetic analysis of the mammalian transforming growth factor-b superfamily. Endoc Rev., 23: 787-823.
3. Dai, R., 2004. Analyzed relationship between CLPG, BMP15 gene polymorphic and production performance of seven sheep populations in north of Xinjiang. Nanjing Agric. Univ. Master's Thesis.
4. Davis, G.H., 2004. Fecundity genes in sheep. Anim. Reprod. Sci., 82-83: 247-253.
5. Davis, G.H., 2005. Major genes affecting ovulation rate in sheep. Genet. Sel. Evol., 37(Suppl.1): S11-S23.
6. Davis, G.H., L. Balakrishnan., I. K. Ross., T. Wilson., S. M. Galloway., B. M. Lumsden., J. P. Hanrahan., M. Mullen., X. Z. Mao., G. L. Wang., Z.S. Zhao., Y.Q. Zeng., J.J. Robinson., A.P. Mavrogenis., C. Papachristoforou., C. Peter., R. Baumung., P. Cardyn., I. Boujenane., N.E. Cockett., E. Eythorsdottir., J.J. Arranz and D.R. Notter, 2006. Investigation of the Booroola (FecB) and Inverdale (FecX(I)) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Anim. Reprod. Sci., 92: 87-96.

7. Deldar-Tajangookeh, H., Zare A. Shahneh, Javad M. Zamiri, M. Daliri, H. Kohram and A. Nejati-Javaremi. 2009. Study of BMP-15 gene polymorphism in Iranian goats, African J. Biotechnol., 8: 2929-2932.
8. Galloway, S.M., K.P. McNatty., L.M. Cambridge., M. P. Laitinen., J. L. Juengel, T.S. Jokiranta., R.J. McLaren., K. Luiro., K.G. Dodds., G.W. Montgomery., A.E. Beattie., G. H. Davis and O.Ritvos., 2000. Mutations in an oocytederived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat. Genet., 25: 279-283.
9. Guan, F., S.R. Liu., G.Q. Shi and L.G. Yang, 2007. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Anim. Reprod. Sci., 99: 44-52.
10. Guo-Hua, H., C. Shi-Lin, A. Jun-Tao, Y. Li-Guo, 2008. None of polymorphism of ovine fecundity major genes FecB and FecX was tested in goat. Anim. Reprod. Sci., 108: 279-286.
11. Hanrahan, J.P., S.M. Gregan., P. Mulsant., M. Mullen., G.H. Davis., R. Powell and S.M. Galloway., 2004. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovis aries). Biol. Reprod., 70: 900-909.
12. He, Y.Q., M.X. Chu., J.Y. Wang., L. Fang and S.C. Ye. 2006. Polymorphism on BMP15 as a candidate gene for prolificacy in six goat breeds. J. Anhui Agric. Univ., 33: 61-64.
13. Juengel, J.L., N.L. Hudson., D.A. Heath., P. Smith., K. Reader., S.B. Lawrence., A.R. O'connell., M. Laitinen., M. Cranfield., N.P. Groome., O. Ritvos and K.P. McNatty., 2002. Growth differentiation factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. Biol. Reprod., 67: 1777-1789.
14. Knight, P.G. and C.Glister., 2003. Local roles of TGF-beta superfamily members in the control of ovarian follicle development. Anim. Reprod. Sci., 78: 165-183.