Bioinformatics analysis of growth differentiation factor-9 and bone morphogenetic protein-15 similarity in sheep, goat and cattle

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Abstract

Litter size is one of the important economic traits in farm mamales. Many genetic and management factors affect on litter size. Growth differentiation factor-9 (GDF-9) and Bone morphogenetic protein-15 (BMP-15) are two genetic factors affecting this trait in sheep, goat, and cattle. In this study, 6 protein sequences of GDF-9 and BMP-15 selected from UniProt database and then some bioinformatics analysis were investigated such as multiple alignment, motifs identify, tertiary structure, and homology search. The results were indicated that GDF-9 and BMP-15 protein sequences in sheep, goats and cattle are very similar in their about 112 COOH-terminal amino acids and there are two motifs in both proteins. In other words, COOH-terminal of both genes have similar amino acids and function which support multiple alignment results. This similarity have been conserved and is common in TGF-beta family.

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Introduction

In recent decades, improvement of reproductive traits in farm animals especially in sheep, has been considered by breeders and researchers, because a small increase in litter size (the number of offspring produced at one birth by an animal) can make a lot of return (Ghaffari et al., 2009). Increasing litter size by genetic selection and management methods, with the purpose of improve the production efficiency, is aim of the animals industry in many countries. This will happen by increasing the number of follicles released in each ovarian cycle that require special genetic, nutrition and administration conditions (Lawloret al., 2007). Many mammals including primates, sheeps, cattles and goats normally have an ovulation rate (i.e. the number of mature oocytes released during one reproductive cycle) of one or sometimes two, where as other mammals such as rats, mice, hamsters, cats, dogs and pigs have ovulation rates that vary between 4 and 15 (McNatty et al., 2005).

There are several hormones and biochemical compounds that is involves in control of reproductive activity in animals. In sheep, three prolificacy loci have been discovered, namely bone morphogenetic protein receptor type-1B (BMPR-1B; or activin-like kinase 6, ALK6), known as FecB on chromosome 6 (Souza et al., 2001); growth differentiation factor-9 GDF-9, known as FecG on chromosome 5 (Hanrahan et al., 2004); and bone morphogenetic protein-15 BMP-15 known as FecX on chromosome X (Hanrahan et al., 2004; Galloway et al., 2000). In a study conducted by Hanrahan and coworkers (2004) on Cambridge and Belclare sheep, a substitution of serine with phenylalanine in position 395 (S395F) (FecGH) of the GDF-9 gene was found to be associated with increased ovulation rate. The oocyte-secreted polypeptide growth factors, GDF-9 and BMP-15 (also known as GDF-9B) have both been revealed to be essential for ovarian follicular growth and function. (Galloway et al., 2000; Juengel et al., 2002; Hanrahan et al., 2004; Juengel et al., 2004). Both factors are the members of the transforming growth factor-β (TGF-β) superfamily. The TGF-β superfamily including a group of structurally related proteins affecting a wide range of differentiation processes during embryonic development (McPherron and Lee, 1993). GDF-9 and BMP-15 regulate the number of follicles ovulated in sheep, a mammal that normally has a low ovulation rate (Dong et al., 1996). The GDF-9 gene can be considered as a strong candidate gene for increased ovulation rate and litter size in sheep (Vage, 2013). Like the human and mouse genes, ovine GDF-9 spans approximately 2.5 kb and contains two exons and one intron. Exon 1 spans 397 bp and encodes for amino acids 1–134, while exon 2 spans 968 bp and encodes for amino acids 135-453. The single intron spans 1,126 bp. The putative 453-amino acids (aa) prepropeptide is likely to be proteolytically cleaved to release a 135-aa mature COOH-terminal polypeptide, which should be a constituent of the biologically active GDF-9 ligand (McPherron and Lee, 1993).

GDF-9 has an unusual cysteine patterning having only 6 cysteines in its mature region, compared to the seven conserved cysteines found in most TGF-β family members (Laitynien et al., 1998).

Due to the importance and similarity of functions of GDF-9 and BMP-15 proteins, study on protein structures sequences of them in different species; in order to identify similarities and differences of them; could be useful. In the present study, the bioinformatics analysis was used to study GDF-9 and BMP-15 proteins diversity and structure in several sheep, goat and cattle using biological data in stored bioinformatics databases.

Material and methods

In the present study, the 6 protein sequences of GDF9 and BMP15 selected from UniProt database; URL: (http://www.uniprot.org). All studied protein sequences were reviewed (swiss-prot proteins).

Multiple alignment of GDF-9 and BMP-15 executed using ClustalW2 with default setting and then colored based on conservation percent using Jalview program; both available at http://www.ebi.ac.uk/Tools/msa/clustalw2. MEME program (http://meme.nbcr.net) was used to identify...
protein sequence motifs. Finally, protein 3D structure of GDF-9 was calculated using SWISS-MODEL program and was observed using Pymol program. Then, the secondary structure and homology of and BMP-15 genes were examined by using the Jalview program.

**Result and discussion**

Alignment Results of GDF-9 and BMP-15 protein sequences in sheep, goats and cattle showed that these two proteins are very similar in their about 112 COOH-terminal amino acids, but in the other parts of their sequences similarity is low (Fig. 1). Similarity of some parts both GDF-9 and BMP-15 protein sequences shows that these two gene have similar function which support Galloway et al., 2000; Juengel et al., 2002; Hanrahan et al., 2004; Juengel et al., 2004.

![Fig. 1. Alignment of GDF-9 and BMP-15 proteins in sheep, goat, and cattle. Yellow color shows match amino acids between two genes sequences.](image)

Protein sequence analysis of GDF-9 and BMP-15 in sheep using MEME showed that there are two motifs in both proteins (Fig. 2). The first motif, with 43 amino acids length, is between amino acids 402-452 in the GDF-9 gene and the region between amino acids 350-392 in BMP-15 gene.

(D[QS]NS]VP[QR]PSCVP[AY][SV][P][IL]S[IV][L][A][E][AP][DN][GS][IL][YE][DG][MIA][QT][KS][CTC]

The second motif, with 43 amino acids length, is between amino acids 350-387 in the GDF-9 gene and the region between amino acids 290-327 in BMP-15 gene.

(N)[EQ][C][ES][LH][DP][F][QR][LV][SF][QS][QL][GK]
Motif analysis shows that COOH-terminal of both genes have been conserved and also are similar. In other words, COOH-terminal of both genes have similar amino acids and function which support multiple alignment results.

**Conclusion**

GDF-9 and BMP-15 proteins, has similar motif at the COOH-terminal of their protein sequences which is multifunctional peptide that controls proliferation, differentiation and other functions in many cell types. In fact, this domain is common of TGF-beta family. Any protein which has this domain had function like other TGF-beta family proteins. Regarding conservation of these part of GDF-9 and other members of TGF-beta, any change in residues of this domain, make probably make the proteins to lose their function.

**Reference**


Dong JW, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. 1996. Growth differentiation factor 9 is required during early ovarian folliculogenesis. Nature 383, 531-535. [http://dx.doi.org/10.1038/3835.31a0](http://dx.doi.org/10.1038/3835.31a0)


Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, Galloway SM. 2004. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge
http://dx.doi.org/10.1095/biolreprod.103.023.093

http://dx.doi.org/10.1095/biolreprod.102.00.7146

http://dx.doi.org/10.1095/biolreprod.103.023.333

http://dx.doi.org/10.1016/S0925-4773(98)00.161-0

http://dx.doi.org/10.1186/2046-0481-60-6-359

http://dx.doi.org/10.1016/j.mce.2004.08.01.3


http://dx.doi.org/10.1046/j.1365-2052.2002.t01-11-00.876.x


Souza CJ, MacDougall C, Campbell BK, McNeilly AS, Baird DT. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. Journal of Endocrinology 169, 1-6. 
http://dx.doi.org/10.1677/joe.0.169R0.01

http://dx.doi.org/10.1186/1471-2156-14-1