

MOLECULAR ANALYSIS OF PROTEINS OF BMP15 GENE IN RUMINANTS AND NON-RUMINANTS

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Abstract

Bone morphogenetic protein 15 (BMP15) is a member of the transforming growth factor β (TGF β) superfamily that is expressed by oocytes and play key roles in granulosa cell development and fertility in animal. It is a gene that has major effect on ovulation rate and fecundity leading to its classification as a fecundity gene with the name *FecX*. This study investigated the molecular genetic variation of proteins of BMP15 gene of cattle, sheep, goats, swine, and chicken with a view to providing relevant genetic information for breeding and selection programmes in the studied species using computational method. A total of thirty seven (37) BMP15 nucleotide sequences comprising goats (18), sheep (6), cattle (6), swine (4) and chicken (3) were retrieved from the GenBank (NCBI) (www.ncbi.nlm.nih.gov). Sequence alignment, translation and comparison of the BMP15 gene of the various species were done with ClustalW using IUB substitution matrix, gap open penalty of 15 and gap extension penalty of 6.66. The amino acid composition revealed the abundance of leucine amino acid in all the studied species, and the predicted 3D BMP15 protein structure aligned well with the template. The information emanating from this study may be important for chemist, drug developers, breeders and molecular biologists in the development of functional hypotheses about hypothetical proteins, improving phasing signals in crystallography, selecting sites for mutagenesis and thus help in marker-assisted selection (MAS) for high litter size productivity in various livestock species.

Keywords: BMP15, protein, variation, in-silico, livestock

Introduction

Application of biotechnology in the production of livestock has proven to be the most effective panacea to the problem of inadequate protein supply in developing countries like Nigeria. Hence, the need to embrace this technique which offers enhanced possibilities for manipulating biological systems for the benefit of mankind such as improved growth rate, better reproductive performance amongst other benefits (Hosoe, 2011). Identifying genes of major effect offers the opportunity to improve production efficiency, product quality and product diversity in livestock industry, through utilizing them in breeding programs (Moradband *et al.*, 2011).

Improved reproductive performance requires in-depth knowledge of the genes associated with reproduction especially those related to ovulation and litter size like Bone morphogenetic protein 15 (BMP15). BMP15 is a member of the transforming growth factor β (TGF β) superfamily that is expressed by oocytes and play key roles in granulosa cell development and fertility in animal models along with growth differentiation factor 9 (GDF9). It is one of the major causal mechanisms underlying either the highly prolific or infertile phenotypes of several sheep breeds (Demars *et*

al., 2013) and other mammalian species including man (Palmer *et al.*, 2006). Since BMP15 is a major reproductive gene, and gene codes for protein which regulates biological processes, this study was undertaken to investigate in-silico the various physicochemical properties of BMP15 gene and thus, predict the secondary and tertiary structures of the gene with a view to providing relevant genetic information for breeding and selection programmes.

Materials and Methods

A total of thirty seven (37) BMP15 nucleotide sequences comprising goats (18), sheep (6), cattle (6), swine (4) and chicken (3) were retrieved from the GenBank (NCBI) (www.ncbi.nlm.nih.gov). The Genbank accession numbers of the sequences are JF824149.1, JF82414 8.1, NM001285588.1, JQ350892.1, JQ350891.1, JQ350090.1, HM462258.1, HM462255.1, HM462254.1, FJ429281.1, GU732196.1, EU743938.1, JX860305, JX860304, JN655670.1, JN655669.1, EU095935.1, EU888137.1 (Goat); KF644561.1, NM001114767.1, EU402923.1, FJ600405.1, JN655672.1, JN655671.1, (Sheep); DQ463368.1, NM001031752.1, EU712722.1, AY572412.1, DQ489533.1, AY304484.1 (cattle); NM.001005155.1, AY295074.1, AH012830.1,

AY295073.1 (swine); AY725199.1, NM001006589.2 and AY729025 (chicken).

Sequence alignment, translation and comparison were done with ClustalW as described by Larkin *et al.* (2007). ProtParam Tool was used for the computation of various physical and chemical properties of the BMP15 gene using amino acid sequences. The computational parameters included molecular weight, theoretical pI (isoelectric point), amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) (Gasteiger, 2005). The amino acid sequences of BMP15 gene was further subjected to secondary structure prediction using Expasy's SOPMA tool. The Phyre and Phyre2 servers were used to predict the 3D structure of BMP15 gene of the species of interest (goat, sheep, cattle, swine and chicken) in the present study. These servers predict the three-dimensional structure of a protein sequence using the principles and techniques of homology modeling (Kelley and Sternberg, 2009).

Results

The amino acid composition of the BMP15 proteins of goats, sheep, cattle, swine and chicken is shown in Table 1. Goat, sheep, cattle and swine shared one or more amino acid between them while chicken was the only specie that did not share the same amino acid composition with the rest of the studied species, and its BMP15 protein appeared richer in alanine, arginine, aspartate, glycine, leucine, proline, serine, threonine and tyrosine, but it has the lowest content of asparagine, cysteine, glutamate, histidine, isoleucine, lysine and methionine. However, Swine BMP15 proteins appeared richer in cysteine, glutamine, methionine and phenylalanine but had the least values for tryptophan, threonine, proline and serine.

The physico-chemical characteristics of proteins of goats, sheep, cattle, swine and chicken predicted by ProtParam are shown in Table 2. Half-life and net charge were the only parameters that were the same across the species. Apart from the number of amino acid (constant between goat, sheep, cattle and swine), theoretical pI (same for goat and sheep, and cattle and swine, respectively), extinction coefficient (same for goat and sheep), all other parameters varied across the studied species.

Prediction of secondary structures of proteins revealed that the percentage of alpha helix was

highest in swine (36.80%) and lowest (25.15%) in chicken (Table 3).

A total of 329 residues (84% of goat and sheep BMP15 amino acid sequence each) were modelled with 100.0% confidence by the single highest scoring template (Figures not shown). For cattle, 307 residues (78% amino acid sequence) were modelled with 100.0% confidence by the single highest scoring template (Figure 3). Also, 312 residues (79% of swine BMP15 amino acid sequence) and 291 residues (87% of chicken BMP15 amino acid sequence) were modelled with 100.0% confidence by the single highest scoring template (Figures not shown). The percentage identity with the template BMP15 protein was 79 and 87%, respectively.

Discussion

BMP15 is a polymorphic gene whose polymorphism has been proven to be associated with increased ovulation rate, sterility and litter size of farm animals (Wang *et al.*, 2011; Hanrahan *et al.*, 2004). Physico-chemical and structural characteristics of an amino acid can be used to explain mutation through correlation (Taylor, 1986). The BMP15 proteins of the various species in this present study often bind to the positively charged DNA, which may regulate gene expression. The positioning and properties of amino acids are key to understanding many biological processes. The leucine side chain is very non-reactive and is thus rarely directly involved in protein functions like catalysis, although it can play a role in substrate recognition. In particular, hydrophobic amino acids can be involved in binding/recognition of hydrophobic ligands such as lipids (Betts and Russel, 2003). The information on amino acid composition of BMP15 proteins may be important for chemist, drug developers, breeders and molecular biologists.

The prediction of protein structure from amino acid sequences is a fundamental scientific problem and is regarded as a grand challenge in computational biology and chemistry. The accuracy of protein structure prediction depends critically on sequence similarity between the query and template and if a template is detected with >30% sequence identity to the query, then usually most or all of the alignment will be accurate and the resulting relative positions of structural elements in the model will be reliable (Kelley and Sternberg, 2009). Findings from this study were reliable as all the query sequences of various species were detected with more than 30% identity.

Conclusion

The amino acid composition reveals the abundance of leucine amino acid in all the studied species, and the predicted 3D BMP15 protein structure aligns well with the template. The information emanating from this study would be relevant in performing further genotype-phenotype research and the SNPs selected as markers of fecundity will in turn help in improving livestock production in Nigeria.

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Table 1. Amino Acid Composition of the BMP15 Proteins of Goat, Sheep, Cattle, Swine and Chicken

Amino acid %	Goat	Sheep	Cattle	Swine	Chicken
Alanine	4.6	4.3	4.6	5.3	11.0
Arginine	7.1	7.1	7.4	8.1	9.6
Asparagine	3.3	3.3	3.0	3.3	2.1
Aspartate	2.3	2.3	2.3	2.5	3.9
Cysteine	2.0	2.0	2.0	2.3	1.8
Glutamine	6.3	6.3	6.1	6.6	4.5
Glutamate	5.3	5.3	5.3	5.1	2.4
Glycine	5.8	6.1	5.6	5.8	7.2
Histidine	4.8	4.8	5.1	4.6	3.6
Isoleucine	4.3	4.3	4.6	4.6	2.1
Leucine	12.9	12.7	12.9	13.5	14.4
Lysine	3.8	3.8	3.8	3.0	0.9
Methionine	2.0	2.0	2.0	2.3	0.9
Phenylalanine	2.5	2.5	2.5	3.0	1.5
Proline	8.6	8.6	8.4	7.9	10.2
Serine	7.4	7.4	7.9	6.6	8.1
Threonine	4.3	4.3	4.1	3.8	4.5
Tryptophan	2.3	2.3	2.3	1.8	2.1
Tyrosine	3.3	3.3	3.0	3.0	3.6
Valine	6.9	7.1	7.1	6.9	5.4

Table 2. Physico-Chemical Characteristics of Protein of Goat, Sheep, Cattle, Swine and Chicken Predicted By PROTPARAM

Specie	No of AA	Mol. Wt	pI	Q	EC	Half life	II	AI	GRAVY
Goat	394	45006.0	9.44	+ve	69370	30	47.24	91.75	-0.324
Sheep	394	44978.0	9.44	+ve	69370	30	47.52	91.24	-0.329
Cattle	394	45025.1	9.52	+ve	67880	30	49.84	93.48	-0.298
Swine	394	45014.2	9.52	+ve	56880	30	46.36	95.48	-0.241
Chicken	334	36553.9	9.97	+ve	56755	30	59.07	91.23	-0.210

AA=amino acid, Mol. Wt= molecular weight, pI=isoelectric point, Q=net charge, II=instability index, AI= aliphatic index, GRAVY=grand average of hydropathicity.

Table 3. Prediction of Secondary Structures of BMP15 Protein of Goat, Sheep, Cattle, Swine and Chicken

Specie	Alpha helix (%)	Extended strand (%)	Beta turn (%)	Random coil (%)
Goat	30.46	18.27	8.12	43.15
Sheep	30.46	18.27	7.61	43.65
Cattle	32.99	17.26	8.12	41.62
Swine	36.80	16.50	9.90	36.80
Chicken	25.15	13.77	10.78	50.30

Parameters :

Window width : 17

Similarity threshold : 8

Number of states : 4