Polymorphism study of BMP15 gene in Indonesian Goats

D Maharani1, S Elieser2, I G S Budisatria1, A Baturabara2, A P Z N L Sari1, D N H Hariyono1

1 Faculty of Animal Science, Universitas Gadjah Mada, 55281, Indonesia
2 Indonesia Goat Research Center Sei Putih, North Sumatera, 20585, Indonesia

Corresponding author: d.maharani@ugm.ac.id

Abstract. Bone Morphogenetic Protein 15 (BMP15) is the family of Transforming Growth Factor β (TGFβ) superfamily which essential for early ovarian folliculogenesis. The aim of this study was to detect the genetic variation within BMP15 gene in four Indonesian goat breeds. A total of 27 blood samples of Gembrong, Kosta, Samosir, and Kacang goats reared in Indonesia Goat Research Center Sei Putih, North Sumatera were collected. Sequence alignment using of 4 samples represent each breed has revealed one synonymous mutation in position g.735A>G (position number refer to GenBank JQ320890), which did not induce the change of lysine in position 135. Genotyping based on SNP g.735A>G was accomplished using BbsI restriction enzyme with PCR-RFLP method. All three genotype (AA, GG, and AG) showed in Gembrong, while in Samosir and Kacang goat the GG genotype was absent. Interestingly, Kosta goat only has AA genotype. The A allele (83%) was higher than G allele (17%), followed with AA (70%, n=19), AG (26%, n=7), and GG (4%, n=1) genotypes in all sample breeds. The hardy-weinberg equilibrium analysis resulted the sample population was not deviated (X2<5.59). It can be suggested the SNP g.735A>G might be used for further study in association the gene with reproductive traits in goat.

1. Introduction

Previous studies on the genetics of prolification trait in sheep have three recommended major fecundity genes, namely Bone Morphogenetic Protein Receptor type IB (BMPRIB); Growth Differentiation Factor 9 (GDF9) and Bone Morphogenetic Protein 15 (BMP15) [1]. BMP15 gene is the family of Transforming Growth Factor β (TGFβ) superfamily located in the X chromosome contains two exons length 1179 nucleotides, separated by 5.4 kilobases intron in sheep. BMP15 encodes a prepropeptide of 393 amino acid, which has 125 amino acid as an active mature peptide [2]. BMP15, also known as FecX gene, have an important role to oocyte development in the ovary, cellular growth, and differentiation [3,4]. Six mutations within BMP15 gene have been detected so far in sheep. There are FecXH and FecXI in Hanna and Inverdale sheeps [5], FecXG and FecXB in Galway and Beclare sheeps [6], FecX in Lacaune sheep [7], and FecX in Rasa Aragonesa sheep [8]. He et al. [9] tested the six mutations in BMP15 gene which associated to high fecundity in sheep to Chinese Hainan goat. However, the result showed none of the six mutations were detected. The same result also reported by Chu et al. [2] in Jining Grey goat, Hua et al. [10] in Boer and Haimen goats, and Ahlawat et al. [1] in Indian goats. Those, the previous study suggested that higher prolificacy in goat may be associate with different mutation than sheep. To prove the study of BMP15 gene, we elaborated the preliminary study of polymorphism the gene in Indonesian goats.
2. Material and methods

A total of 27 blood samples were used to explore the genetic variations within BMP15 gene in Indonesia goat breeds (Gembrong = 5, Kosta = 4, Samosir = 8, Kacang = 10). The blood samples were collected from the jugular vein into EDTA vacutainer tubes and transferred to the laboratory. Blood samples were kept in -20 °C before further procedure.

The blood samples was extracted using gSYNC DNA Extraction kit (Geneaid, New Taipei City, Taiwan). The quality of isolated DNA was determined using agarose gel electrophoresis (0.8%). Amplification of 141 bp DNA was performed in SEDI G Thermal Cycler. A total of 25 μL mix reaction consist of 2 μL genome DNA, 9.5 μL Double-Distilled Water (DDW), 12.5 μL MyTaq™ HS Red Mix (Bioline, UK), and 0.5 μL each primer as describe by Javanmard et al. [11] in Table 1. The PCR reaction was accomplished by initial denaturation at 94 °C for 5 min, followed by 36 cycles of denaturation at 94 °C for 1 min, annealing at 61 °C for 1 min, extension at 72 °C for 30 min, with a final extension at 72 °C for 4 min. The PCR products were visualized through 1.5% agarose gel stained with ethidium bromide.

Table 1. Primer sequence, PCR product size and restriction enzyme for PCR amplification and PCR-RFLP of BMP15 gene

| Primer   | Primer sequence (5’ – 3’) | Product size (bp) | Restriction enzyme |
|----------|---------------------------|-------------------|-------------------|
| BMP15-F  | CACTGTCTTCTTGTTACTGTAATTCAATGAC | 141 bp            | BbsI              |
| BMP15-R  | GATGCAATACTGCTGTCTGTT     |                   |                   |

Four samples (one sample for each breed) of PCR product have been sequenced and confirmed the gene target and single nucleotide polymorphism by 1st BASE DNA Sequencing Services (Selangor, Malaysia) using automated DNA Sequencer. Raw sequence data were edited using BioEdit software. Sequence and amino acid alignments were performed with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) to identify single nucleotide polymorphism (SNPs) within the BMP15 gene in Indonesia goat breeds. Manual examination of electrophoregrams was used to confirm the polymorphic site. The DNA sequences were translated into amino acid using the ExPASy translate tool (https://web.expasy.org/translate/).

Genotyping of the sample was performed using Restriction Fragment Length Polymorphism (RFLP) with the BbsI restriction enzyme. Digestion performed in approximately 20 μL total volume consists of 15 μL PCR product, 2.8 μL DDW, 2 μL buffer and 0.2 μL restriction enzyme. The digested product was run on 2% agarose gel.

The allele and genotypes frequencies were calculated using standard procedure given by Falconer and Mackay [12].

Genotype frequency = \( \frac{\text{Number of animals of a particular genotype}}{\text{Total number of animals}} \)

Allele frequency = \( \frac{2D + H}{2N} \)

where, D is the number of homozygote animals, H is the number of heterozygote animals, and N is the total number of animals.

A Chi-square test was performed to test the allelic and genotypic frequencies for Hardy-Weinberg equilibrium. The following mathematical model was:

\[ X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i} \]
Where, $X^2$ is Chi-square value, $O_i$ is observed frequency, $E_i$ is expected frequency, n is the number of possible outcomes of each event.

3. Results and discussion

Litter size is one of major reproduction trait which has an important value in goat breeding. However, due to the low heritability of litter size and other majors of reproduction traits, it is hard to undergo traditional selection to improve goat breeding speed. DNA tests are the key to utilization of genes to be a useful tool or marker to be a rapid and economical method to increase the reproductive capability including litter size [9].

One single polymorphism site (SNP g.735A>G) of the BMP15 gene has been recognized by sequence alignment of four Indonesia goat breeds with the sequence of Capra hircus (Genbank acc no. EU847289) (Figure 1). Manual inspection of the electrophoregram showed a clear peak of the AA, GG, and AG genotypes (Figure 2). However, no amino acid variant was found in position 135 from 284 amino acid within the BMP15 gene exon 2.

The 141 bp of BMP15 PCR product was digested using BbsI restriction enzyme (5’...GAAGAC(N)2...3’) to genotype the sample based on SNP g.735A>G. Agarose electrophoresis revealed two types of restriction pattern; the homozygous AA animals have only one fragment-sized (141 bp) and the heterozygous AG animals have three fragments-sized (54 bp, 87 bp, and 141 bp), as shown in Figure 3.
The result of allele and genotype frequencies was shown in Table 2. Gembrong goat have both three genotypes. However, the GG genotype was absent in Kosta, Samosir, and Kacang goats. Overall, the frequency of AA genotype (70%, n=19) higher than AG (26%, n=7) and GG (4%, n=1) genotypes. The highest homozygous AA animals found in this study was Kosta goat (100%) followed by Samosir (88%) and Kacang (60%), Gembrong (40%) goats. The A allele (83%) was higher than G allele (17%). Kosta, Samosir, and Kacang goats have 80 – 100% of A allele frequencies followed by Gembrong (60%). Allendorf et al. [13] stated that a polymorphic site could be named as SNP if it has <0.99 allele frequency in a large population or <0.95 allele frequency in a smaller population.

The allele and genotype distribution were analyzed by Pearson’s Chi-square method and the result indicated that the sample population have not deviated from Hardy-Weinberg equilibrium (P>0.05). The allele and genotype frequencies will be constant as long as there were no selection, mutation, migration, non-random mating and genetic drift in population [13].

Table 2. The allele and genotype frequencies, and HWE analysis of BMP15 gene in four Indonesia goat breeds

| Breed  | Genotype frequency | Allele frequency | X² |
|--------|--------------------|------------------|----|
|        | AA     | GG   | AG | A   | G   | |
| All breed | 0.70  | 0.04 | 0.26 | 0.83 | 0.17 | 0.12 |
| Gembrong | 0.40  | 0.20 | 0.40 | 0.60 | 0.40 | 0.14 |
| Kosta   | 1.00  | 0.00 | 0.00 | 1.00 | 0.00 | 0   |
| Samosir | 0.88  | 0.00 | 0.12 | 0.94 | 0.06 | 0.04 |
| Kacang  | 0.60  | 0.00 | 0.40 | 0.80 | 0.20 | 0.62 |

4. Conclusion
One synonymous mutation SNP g.735A>G in BMP15 gene has been indicated in Indonesian goats. The allele and genotype distribution of SNP g.735A>G in BMP15 gene were not deviated from the Hardy-Weinberg equilibrium. The SNP g.735A>G might be used for further study in association the gene with reproductive traits in goat.

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