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Polymorphism of Bone Morphogenetic Protein Receptor-IB (BMPR- IB) Gene with Litter Size and Kids Growth of Some Goat Breeds in Egypt.

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Key words
Goat,

BMPR- IB gene

polymorphism

ABSTRACT:

Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR–RFLP) and Single Nucleotide polymorphism (SNP) techniques were used to study the association between bone morphogenetic protein receptor IB (BMPR IB) gene polymorphism with litter size trait and kids growth. Forty four Female goats were precisely selected according to their litter size and kids growth. PCR amplification of 190 bp of the BMPR-IB gene was genotyped in all goats and sequenced only in those produced the highest and lowest litter size and kids growth. Restriction analysis of PCR-RFLP using Ava II and Hind III of the BMPR-IB gene (190-bp) do not produce restriction fragments. By DNA sequencing, eight single nucleotide polymorphisms (SNP's) at seven different positions were obtained. Furthermore, with translation of SNPs to corresponding amino acids, change of six amino acids in three female goats were obtained as the following, Baladi goat with high litter size, glutamic acid (E) changed to aspartic acid (P) and isoleucine (I) changed to valine (V). In high litter size, Zaraibi goat, valine (V) changed to leucine acid (L) and glutamine (Q) changed to histidine (H) and threonine (T) changed to proline (P). These findings can be used in a marker-assisted selection (MAS) for selection for high litter size trait in goats. There are negative relationships in most goats between SNPs in BMPR IB gene and relative growth gain (RGG).

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1. INTRODUCTION

Recently, goats become an important aspect of animal production in Egypt. Therefore, increasing productivity of goats will contribute to improve the living standard of the rural people. The possible improvement in productivity has been reported due to changes in management practices (Adu et al., 1988; Van Vlaenderen, 1989; Odubote et al., 1992). Improvement of productivity can also be done by selection of superior genotypes by using genetic markers. Soller and Beckman (1982) were the first authors reported the potential benefit of selection for genetic markers (marker-assisted selection) for genetic response in dairy cattle breeding program. The Booroola fecundity gene (FecB) increased ovulation rate and litter size in sheep and was inherited as a single autosomal locus (Wilson et al. 2001). Last discoveries have revealed that the high prolificacy in Booroola sheep was the result of a mutation (FecB) in the bone morphogenetic protein receptor 1B (BMPR1B) gene (Souza et al. 2001). This discovery led to the development of the DNA test which enabled researchers to screen the mutation in other prolific breeds. Besides Booroola, the gene has been reported to be present in the Garole, Javanese, Hu and Small Tail Han sheep breeds.

The aim of the present study was to investigate associations between litter size and kids relative growth gain (RGG) in Baladi, Zaraibi, Damascus and Alpine goat breeds with BMPR-IB gene polymorphisms based on molecular genetic level using PCR-RFLP and SNPs markers.

2. MATERIALS AND METHODS

2.1. Animals:

Forty four female goats from four breeds (Baladi, Zaraibi, Damascus and Alpine) reared under Egyption conditions were precisely selected according to litter size (high and low) and kids relative growth gain (RGG) from Sakha Animal Production Research Station, Animal Production Research Institute, Ministry of Agriculture, Kafr El-Sheikh Governorate, Egypt. RGG was determined according to Brody (1945) with the following equation:

Relative Growth gain (RGG) =

$$\frac{\text{Body weight (g)-body weight in the previous week (g)}}{0.5\{\text{Body weight (g) +body weight in the previous week (g)}\}} \times 100$$

Animals were noticed apparently healthy and free from any clinical disorders or diseases.

2.2. Blood sampling and DNA extraction:

Blood samples were collected by jugular vein puncture into tubes containing anticoagulant disodium EDTA, stored at – 20 °C until used for DNA extraction.

DNA was extracted from blood samples using QIA amp DNA Blood Mini Kit (Qiagen).

2.3. PCR Amplification of BMPR IB gene:

A segment of 190 bp of BMPR IB gene of 44 female goats was amplified with the use of A segment of 190 bp of BMPR IB gene of 44 female goats was amplified with the use of primer sequence CCAGAGGACAATAGCAA AGCAAA- (Forward) and CAAGATGTTTTCA TGCCTCATCA ACAGGTC (Reverse) (Ghaffari et al. 2009). PCR was performed in reaction mixture of 25 µl consisting of 5 µl DNA template, 5 µl GoTaq® Flexi Buffer 5X Green or Colorless, 0.5 µl dNTP (10mM) (promega), 1.0 µl forward primer (25 pmol), 1.0 µl reverse primer (25 pmol), 0. 25 µl GoTaq® Hot Start Polymerase (5u/µl) and compete the volume to 25 µl H2O. Thermal cycling was carried out by initial denaturation at 95 C for 4 minutes followed by 34 cycles each at 95 °C for 30 sec for DNA denaturations, 60 °C for annealing, extension at 72 for 30 sec. and final extension at 72 °C for 5 min. then the samples were held at 4°C. The amplified DNA fragment were separated on 3٪ agarose gel, stained with eithedium bromide, visualized on a UV transilliuminator and photographed by gel documentation system.

2.4. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP):

The 190 bp amplified DNA fragments were digested with restriction endonucleases (RE) Hind III and Ava II. The PCR-RFLP was carried out in reaction volume of 20 µl consisted of 7 µl H_2O, 2 µl buffer, 10 µl PCR product and 1 µl enzyme (Zhou et al., 2005) .

2.5. DNA sequencing:

DNA sequencing for 190 bp amplified fragment was performed from 18 female goats (9 high and 9 low litter size) of four breeds and have different RGG ranged from 0.6 to 1.2. The sequence was done in Automated DNA sequencing using 3130 X DNA Sequencer (Genetic Analyzer, Applied Biosystems, Hitachi, Japan). The fragment submitted to NCBI Gene Bank data base for getting the accession number.

Results were analyzed by Chromas 1.45 (http://www.technelysium.com.au) and Blast 2.0 software (Altschul et al. 1990). By using Clustalw (1.8), sequence alignment was compared with Capra hircus breed boar BMPR-IB genes that are available in the Gene Bank database sequence ID gene (accession number gb|EU847290.1|). Differences between individual sequence and reference sequences available in the Gene Bank were classified as single-nucleotide polymorphisms (SNPs)

3. RESULTS

3.1. PCR amplification using specific primer for bone morphogenetic protein receptor IB (BMPR IB) gene.

PCR amplification of BMPR IB gene yielded a fragment of 190 bp as shown in Figure (1)

3.2. Genotyping of bone morphogenetic protein receptor IB (BMPR IB) gene (190-bp) using RFLP technique

Restriction analysis of PCR-RFLP-of BMPR IB gene (190-bp) using restriction endonuclease (Hind III and Ava II) produced one fragment of 190 bp. The two restriction enzymes do not digest the amplified fragments in all goat breeds under study.

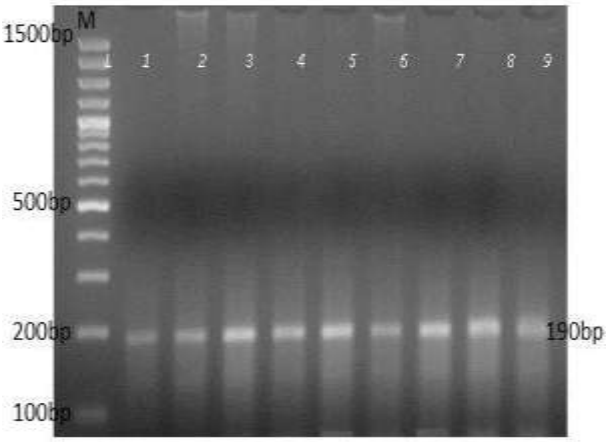

Figure (1): Lane 1-9 are PCR product of BMPR IB gene (190-bp) of Zaraibi goat breed and Lane M is a DNA marker

3.3. DNA sequencing

The results of direct sequencing of 190 pb fragment of BMPR-IB gene in 18 female from four goat breeds (nine highest and nine lowest litter size) and their blast was shown in Figure (2).

3.4. Direct sequencing methods for screening of SNPs and changed amino acids:

Sequencing of 190 bp fragment of BMPR-IB gene revealed nucleotide varaitions among high and low litter sized goats as observed in figure 3 and Table 1. The changed amino acids were presented in figure (4) and table (1)

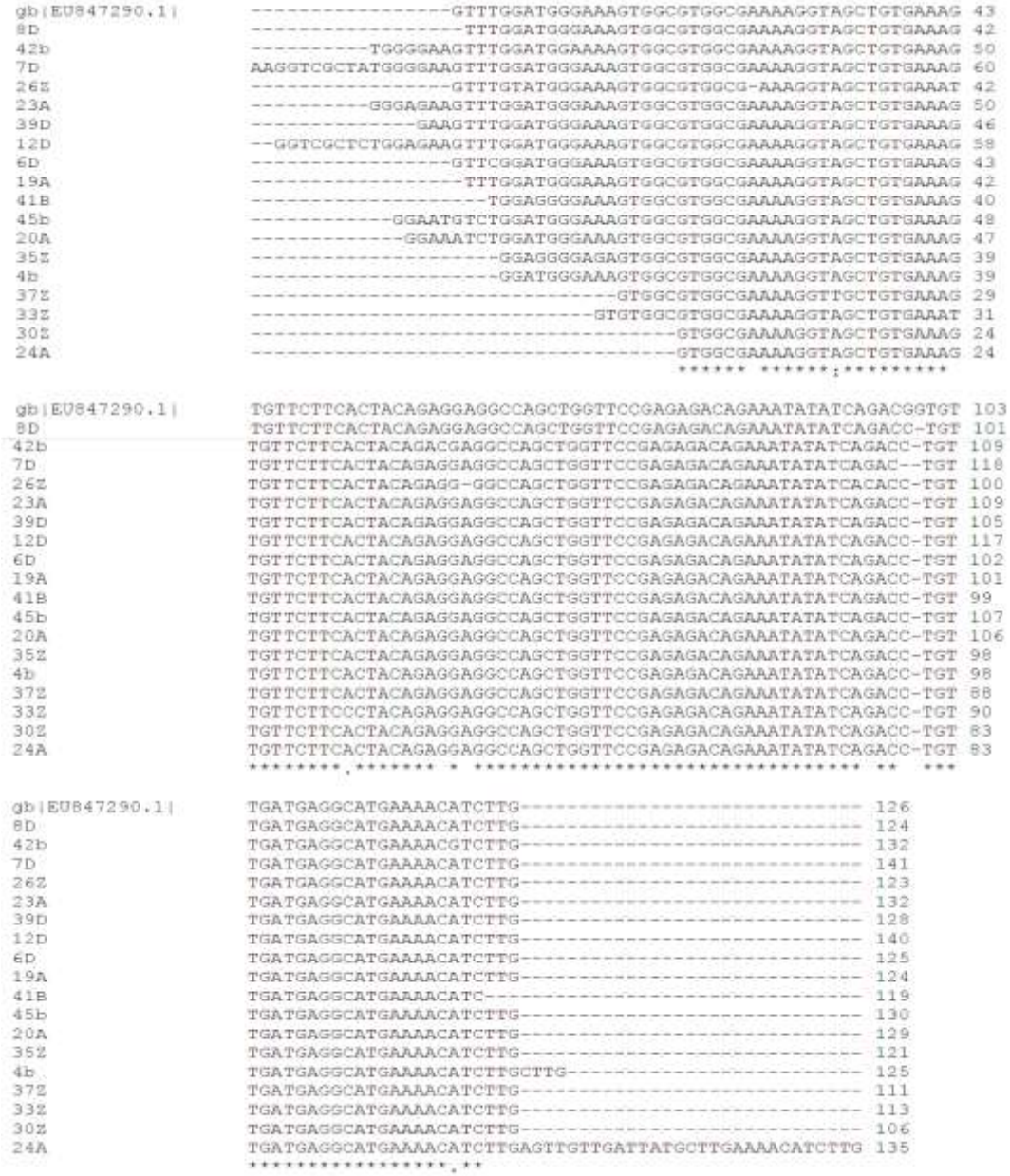

Figure 2. Summary of changed nucleotide to corresponding amino acids for four goats breeds under study.* astrickis means sharing (common) nucleotides
Goats No 26Z, 33Z, 42b, 23A, 12D, 19A, 39D, 37Z and 24A are high litter size.
goats 6D, 7D, 8D, 4b, 20A, 35b, 45b, 30Z and 41b are lowest litter size.
Z for Zaraibi, B for baladi, A for alpine and D for Damascus.

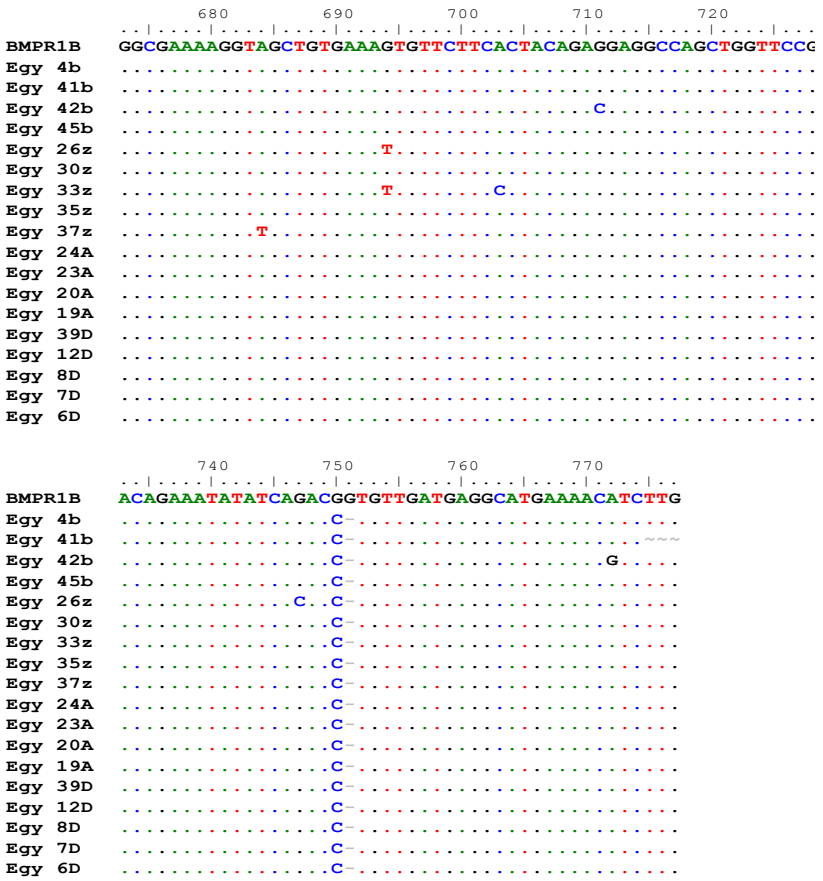

Figure 3. Summary of changed nucleotide in four goats breed under study

Goats No 26Z, 33Z, 42b, 23 A, 12 D, 19 A, 39 D, 37Z and 24A are high litter size.
goats 6D, 7D, 8D, 4b, 20A, 35 b, 45 b, 30 Z and 41b are lowest litter size.
Z for Zaraibi, b for Baladi, A for Alpine and D for Damascus.

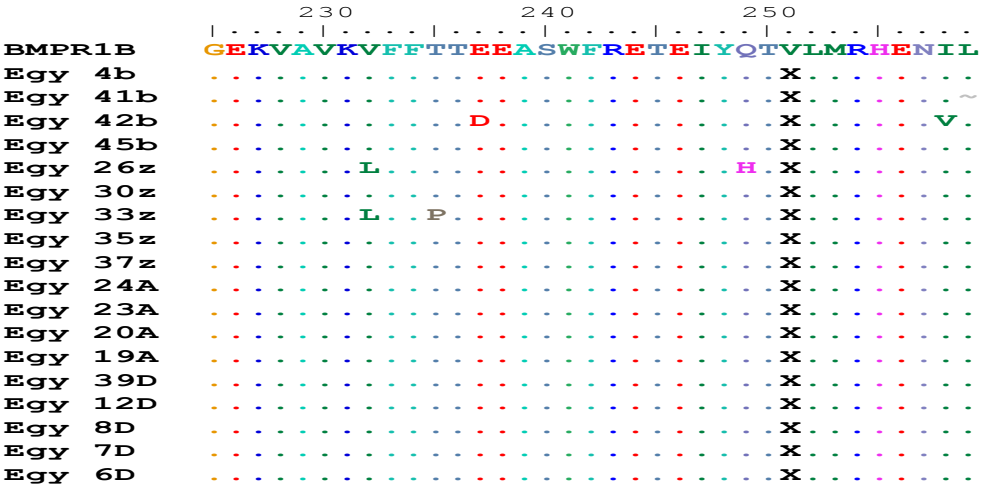

Figure 4. Summary of changed nucleotide to corresponding amino acids for four goats breeds under study.

Goats No 26Z,33Z, 42b, 23A, 12D, 19A, 39D, 37Z and 24A are high litter size.
goats 6D,7D, 8D, 4b, 20A, 35b, 45b, 30Z and 41b are lowest litter size.
Z for Zaraibi, B for baladi, A for alpine and D for Damascus.

From these results, it was found that, there were many SNPs which can be used as a marker for selection for high litter size, in animal number 42b of high litter size (4 kids) there were two SNPs at nucleotide number 711 and 772. The two corresponding amino acids changed were the amino acids number 237 (glutamic to aspartic acid) and number 258 (isoleucine to valine). These two SNPs can be used as a marker-assisted selection for improvement of litter size in this breed. Moreover, in Zaraibi breed there were many SNPs can be used as a marker- assisted selection for high litter size. The goat number 26z has two SNPs at nucleotide number 694 (G→T) and 747(G→C), the two corresponding amino acids were changed, amino acid number 232 from valine to leucine and number 249 glutamine to histidine. The animal number 33Z also had two SNPs, at nucleotide number 694 (G→T) and 703 (A→C) and the corresponding amino acid changed were from valine to leucine also at 232 position and threonine to proline at 235 position. The goat number 37z (high litter size) has one SNPs at nucleotide number 684 (A→T).

3.5. Single nucleotide polymorphisms in different goat breeds have different relative growth gain.

Relative growth gain, SNPs and corresponding changed amino acids in BMPR IB were shown in table 4. Within Baladi goat breed, goat number 42b has two SNPs at 711 (G→C) and 772 (A→G) and RGG equal to 0.8. In Zaraibi goat breeds the goat number 26z has two SNPs at 694 (G→T) and at 747 (G→C) with RGG equal to 0.6. The goat number 33z has also two SNPs at 694 (G→T) and at 703 (A→C) with 0.6 RGG. The goat number 37z has one SNPs at 684 (A→T) with 0.85 RGG. Alpine goat breeds showed RGG = 1.2 % where that goats didn't show any SNPs between them also in Damascus goat breeds, goats numbers 39D, 6D and 8D have RRG = 0.9 while goat number 7D has RGG = 0.6 and goat number 12D has RGG = 0.8.

There was a negative relationship in most goats between SNPs in BMPR- IB gene litter size and RGG. Only within Baladi breed, the SNPs at 711 (G→C), goat number 42b give high litter size and 0.8 RGG, also within Zaraibi goat breeds there was one SNPs at nucleotide 684 (goat number 37Z) lead to high litter size and 0.85 RGG . These two SNPs can be used as a genetic marker for improvement of these two economic traits.

Table 1. Single nucleotide polymorphism, amino acid variations litter size and relative growth gain of goats under study.

Goat number	SNPs number and type	Amino acid number and type	Litter size	RGG
4b	-	-	Low	0.86
41b	-	-	Low	1.0
42b	711(G→C) 772 (A→G)	237(glutamic acid→aspartic acid) 258 (isoleucine→valine)	High	0.8
45b	-	-	Low	1.0
26z	694 (G→T) 747 (G→C)	232 (valine→leucine) 249 (glutamine →histidin)	High	0.6
30z	-	-	Low	0.9
33z	694 (G→T) 703 (A→C)	232 (valine to leucine) 235 (threonine →proline)	High	0.6
35Z	-	-	low	0.87
37Z	684 (A→T)	No change amino acid	High	0.85
6D	654 T→C)	-	Low	0.9
7D	-	-	Low	0.6
8D	-	-	Low	0.9
12D	-	-	High	0.8
39D	-	-	High	0.9
19A	-	-	High	1.2
20A	-	-	Low	1.2
23A	-	-	High	1.2
24A	-	-	high	1.2

4. DISCUSSION

The polymorphism of genetic markers gave some useful information in studying the relationships among breeds and their evolution. It can also be used for indirect selection if there were some relationships between these markers and some economically important quantitative traits. Many researchers employed the random amplified polymorphic DNA markers technique to characterize and estimate genetic distances between goat breed (Williams et al. 1990; Welsh and Mecldhmd 1990; Nyamsamba et al. 2002; Ouafi et al. 2002)

Litter size and lamb growth are important economic values in goat breeding and reproduction. Many aspects of the *FecB* gene, including reproductive endocrinology (Smith et al., 1993), ovary development (Cognie et al., 1998), litter size, organ development and body mass (Smith et al., 1996) have been studied.

Mulsant et al. (2001), Souza et al. (2001) and Wilson et al. (2001) reported that bone morphogenetic protein receptor IB (BMPR-IB) gene mutation was responsible for the high prolificacy associated with the *FecB* gene in Booroola Merino sheep. This mutation is located in the kinase highly conserved domain of the bone morphogenetic protein receptor IB, and is characterized by 'precocious' differentiation of ovarian follicles, leading to the production of large numbers of ovulatory follicles that are smaller in diameter than wild-type follicles (Souza et al., 2003).

Restriction endonuclease (RE) *(Hind III- Ava II)* were used to digested the amplified fragment showing no differences between goats under the study, the restriction enzymes do not digest this fragment .

The obtained results agree with the results obtained by El Hanafy and El-Saadani (2009) where their study revealing absence of restriction site of AvaII restriction enzyme in five studied sheep breeds.

SNPs detected in BMPR –IB gene can be used as marker assisted selection (MAS) to select for high litter size. Consequently, these eight SNPs markers in goat BMPR –IB gene may be useful in genetic improvement of litter size in goats under study. In this study nucleotide number 750 changed from G to C in all four goat breeds. This considered MAS selection to the four goat breeds in Egypt. Furthermore, these SNPs affected on amino acids translation. This mutation considered sense mutations which change amino acids. Moreover there are two SNPS can be used as genetic marker for selection of high litter size in Baladi at nucleotide number 711 and 772 and the two corresponding changed amino acids where 237 and 258. In Zaraibi breed also there are two SNPS can be used as a MAS for high litter size. These two SNPs are the nucleotide number 694 and 747; the two corresponding amino acids are amino acid number 232 and 249.

It was reported that the SNPs in BMPR IB gene has negative effects on fetal body weight, body size and development during pregnancy (Smith et al., 1993). The differences probably result from the effects of different breeds and different phases of development, environmental conditions, different lambing and nutrition or effects altogether and the mechanism requires advanced research.

However, these results indicate that molecular genetic markers (SNPs) can be used for marker assisted selection (MAS) for high litter size goats and accelerate the rate of genetic improvement on litter size.

5. ACKNOWLEDGEMENTS

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