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Original article

GROWTH HORMONE GENE POLYMORPHISM IN DOMESTIC AND WILD GOAT BREEDS IN KURDISTAN REGION OF IRAQ USING PCR-RFLP AND SNP MARKERS

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Summary

Abbas, A. M., J. M. S. Jubrael & A. B. Mohammed, 2022. Growth hormone gene polymorphism in domestic and wild goat breeds in Kurdistan region of Iraq using PCR-RFLP and SNP markers. *Bulg. J. Vet. Med.* (online first).

In Iraq generally and Kurdistan region especially, goats are an important resource for meat and milk production. It is well known that growth hormone (*GH*) is involved in a variety of biological activities in livestock animals, including reproduction, growth, lactation, metabolism etc. The goal of this research was to reveal the polymorphism of *GH* gene in different *Capra hircus* breeds (native, Shami, Meriz and Kamori goat) and in wild mountain goat (*Capra aegagrus)* via PCR-RFLP technique and direct seqencing. *GH1* (exon 2 and 3) and *GH2* (exon 4) polymorphisms on the *GH* gene were investigated. In all breeds, *GH1-Hae* III/RFLP revealed only two genotypes (homozygous AA and heterozygous AB), with absent genotype BB. The genotype frequency was 0.886 for the AB genotype and 0.114 for the AA genotype; the allelic frequency was 0.558 for the A allele and 0.442 for the B allele. The average of observed heterozygosity was 0.882 and observed homozygosity was 0.118, indicating that the *GH1* was polymorphic. The sequence data of *GH1* gene of Meriz and wild goats revealed a SNP at the position 58 (C to T) of the wild goat that has led to a change in amino acid proline to serine. *GH2* digestion by *Hae* III, on the other hand, was monomorphic with the CC homozygous genotype. The results of PCR-RFLP and SNP experiments from this investigation were evaluated as very useful in genotype analysis of local goat breeds/populations. Thus, DNA polymorphisms in the growth hormone gene could be used as reliable genetic markers in breeding programmes in this region.

Key words: *Capra aegagrus, Capra hircus*, *GH* gene, *Hea* III restriction enzyme, PCR-RFLP, sequencing, SNP

INTRODUCTION

In Iraq, including Kurdistan region, the domestic goat (*Capra hircus*) is a popular livestock species primarily raised for meat and milk (Scherf, 2000). Therefore, combining trials with emphasis on management and genetic progress such as employing reliable markers may be of great importance to improve animal production (Masoudzadeh *et al*., 2020b). Animal growth hormone *(GH)* genes are frequently utilised to assess genetic variation in goats and cattle (Gooki *et al.,* 2019). In

goats, the *GH* gene is a 2.5-kilobyte peptide with five exons and four introns encoded by a single gene located on chromosome 19 (19q22) (Schibler *et al*., 1998; Wickramaratne *et al*., 2010). The pattern of this gene is important for protein, lipid, and carbohydrate metabolism, as well as postnatal longitudinal growth and development, tissue growth, lactation, and reproduction among other things (Akers, 2006; ThidarMyint *et al*., 2008).

Various approaches have been developed to evaluate polymorphism or genetic variation within and between populations (Okumus & Mercan, 2007; Hussain *et al*., 2013; Mahrous *et al*., 2018). Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) is one of most widely utilised procedures. The direct sequence is an effective approach for detecting nucleotide differences in amplified DNA fragments (Akamine *et al*., 2009). For many plants and animals, single nucleotide polymorphism (SNP) screening has also been the method of choice for identifying and correlating characteristics with sections of the genome (Rafalski, 2002).

The main research studies reported in Iraq were related to the two domestic goat breeds (native and Meriz), which were morphologically characterised (Taha, 1990; Alkass & Merkhan, 2013). At molecular level, Mohammed (2013) reported the use of SSR marker for characterisation of the Meriz and native goat breeds in Duhok province.

To the best of our knowledge PCR-RFLP and SNPs on growth genes in goat breeds in Kurdistan region of Iraq have not been reported. Thus, as a first step towards goat genetic improvement in Kurdistan Region – Iraq, based on the growth hormone gene, the goal of this study was to look for genetic polymorphisms in the caprine *GH* gene in some domestic goat (*Capra hircus*) and wild goat (*Capra hircus*) breeds in Duhok province.

MATERIALS AND METHODS

DNA extraction

Blood was collected from 71 female goats from different herds (26 native, 10 Shami, 27 Meriz, 3 Kamori, and 5 wild mountain goats) in 10 mL tubes containing 2.7% EDTA as an anticoagulant and kept at 4 °C until used. Blood genomic DNA was extracted using the phenol-chloroform method (Powell & Gannon, 2002). A Nanodrop spectrophotometer was used to determine the purity and concentration of genomic DNA.

Polymerase chain reaction

For amplifying the *GH1* and *GH2* loci of goat *GH* gene primers given by Amie

Table 1. PCR primers used in this study

Polymorphic locus	Primer sequence 5'–3'	PCR product size (bp)
Exon 2 and 3 (GH1)	F. TCAGCAGAGTCTTCACCAAC R. CAACAACGCCATCCTCAC	422 bp
Exon 4 (GH2)	F. CTCTGCCTGCCCTGGACT R. GGAGAAGCAGAAGGCAACC	116 bp

According to Amie Marini *et al*. (2012).

Marini *et al*., (2012), two sets of primers were utilised (Table 1).

Amplification reaction contained 1 µL (100 ng) genomic DNA, 1 µL of each forward and reverse primer (both concentration 10 pmol/µL), 10 µL of 2×PCR master mix (ADDBIO INC) and 7 µL of deionised distilled water for 20 µL volume. The PCR programme included a preliminary step of denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, annealing at 56 °C for *GH1* and 62°C for *GH2* for 45 s, extension at 72 °C for 1 min, followed by a final extension at 72°C for 5 min and storagee at 4 °C. The PCR products were analysed using 1.5% agarose gel electrophoresis. Red safe stain was used to stain the gels, which were then viewed using a UV transilluminator.

Restriction fragment length polymorphism (RFLP)

The reaction mixture was performed in a total volume of 25 µL of each sample, which contained 10 µL of PCR amplicons and that digested with 10 units of the *Hae*III restriction enzyme (Gena Bioscience) then incubated at 37 °C for 6 h. The digested amplicon fragments were separated by 2.5% agarose gel electrophoresis; 100 bp ladder DNA marker was run with digested PCR products for measuring of the bands. Gels were stained with Red safe stain, then visualised with a UV trans-illuminator and photographed. Data for each locus were analysed by PopGene programme v. 1.31 (Yeh *et al*., 1999).

GH1 gene sequencing

The PCR result products, for each identified genotype of *GH1* gene in Meriz and wild goat breeds were sequenced by Macrogen (Seoul, Korea). To find each single nucleotide substitution between distinct genotypes, the NCBI/BLAST/ blastn suite (https://blast.ncbi.nlm.nih.gov /Blast. cgi? PAGE_TYPE=BlastSearch) was used to perform sequence analysis and alignment.

RESULTS

The results of repeated PCR experiments of the two growth hormone genes *GH1* and *GH2* revealed successful and reproducible amplifications in all tested breeds. As *GH1* was regarded (exon 2 and 3), an amplicon of 422 bp was found across all tested goat breeds (Fig. 1) and for *GH2* (exon 4) an amplicon of 116 bp was detected (Fig. 2).

The PCR-RFLP results of the *GH1* gene digested with restriction enzyme *Hae* III produced two different alleles (A and B). The allele B revealed an uncut fragment of 422 bp whereas allele A was found to be cleaved into two fragments,

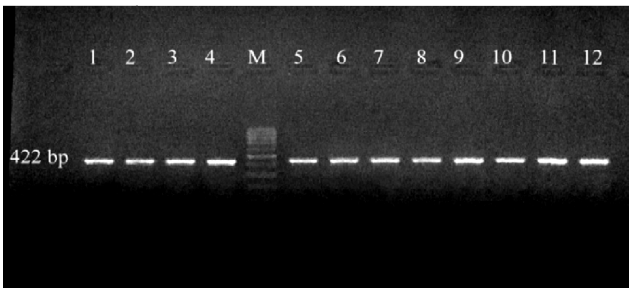

Fig. 1. Agarose gel (1.5%) electrophoresis. Lane M: 100 bp ladder, lanes 1–12: the 422 bp PCR product of *GH1* gene.

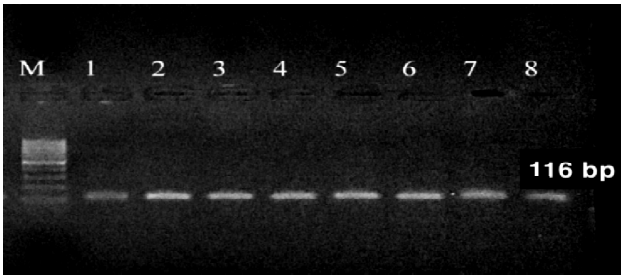

Fig. 2. Agarose gel (1.5%) electrophoresis. Lane M: 100 bp ladder, lanes 1–8: the 116 bp PCR product of *GH2* gene.

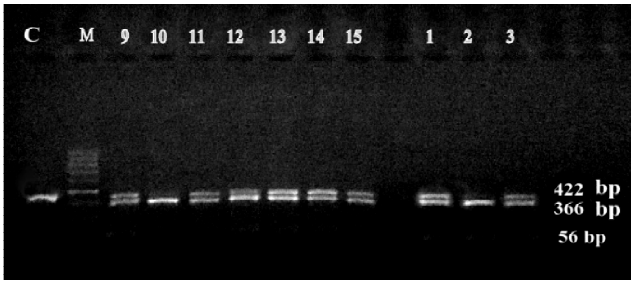

Fig. 3. Agarose gel (2%) electrophoresis of PCR-RFLP patterns of *GH1* using *Hae* III. C: undigested amplified PCR product as control, L: 100 bp marker. All samples represent the AB genotype except for lanes 2 and 9 (AA genotype).

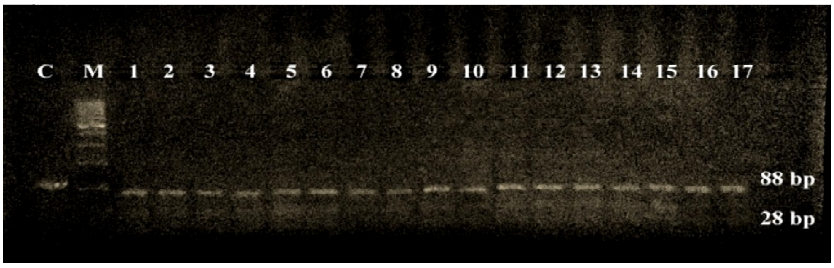

Fig. 4. Agarose gel (2%) of PCR-RFLP patterns of *GH2* using *Hae* III. C: undigested amplified PCR product as control, M: 100 bp marker. All samples represent the CC genotype.

one fragment of 366 bp and another one of 56 bp (Fig. 3). The restriction analysis of 422 bp *GH1* fragment further revealed polymorphisms through distinguishing two genotypes, a homozygous genotype AA (366 and 56 bp) and a heterozygous genotype AB (366 , 56 and 422 bp). The results also indicated the absence of homozygous genotype BB.

The results of *GH2* gene digestion (116 bp) with *Hae* III produced two fragments of 88 bp and 28 bp representing homozygous genotype CC across all individuals (Fig. 4). However, the results did not reveal genetic makeup of DC and DD.

The detailed data analysis of allele and genotype frequencies of *GH1* is given in Table 2. The genotype frequencies of AB

Table 2. Genotype and allele frequency of *GH1* locus in five goat breads

Population	Number	Observed AA genotype	Observed AB genotype	Genotype frequency			Allele frequency	
				AA	AB	BB	A	B
Shami	10	0	10	0	1.0	0	0.5	0.5
Kamori	3	1	2	0.33	0.67	0	0.67	0.33
Wild goat	5	1	4	0.2	0.8	0	0.6	0.4
Meriz	27	0	27	0	1.0	0	0.5	0.5
Native goat	26	1	25	0.04	0.96	0	0.52	0.48
Average		0.042	0.958	0.114	0.886	0	0.558	0.442

Table 3. Availability (A), observed and expected homozygosity and heterozygosity in *GH1* locus of 5 goat breeds

Population	A	Observed homozygosity	Observed heterozygosity	Expected homozygosity	Expected heterozygosity
Shami	1.000	0.000	1.000	0.4737	0.5263
Kamori	1.000	0.333	0.667	0.4667	0.5333
Wild goat	1.000	0.200	0.800	0.4667	0.5333
Meriz	1.000	0.000	1.000	0.4865	0.5135
Native goat	1.000	0.0588	0.9412	0.4866	0.5134
Average	1.000	0.118	0.882	0.476	0.524

in Shami and Meriz goats were 1.0 which may be attributed to the fact that all individuals carried AB genotype whereas in the Kamori, native and wild genotypes, the frequencies were 0.67, 0.96 and 0.8 respectively (Table 2). The average allelic frequencies of *GH1* in this study revealed that the allelic frequency of the allele A (0.558) was higher than that of allele B (0.442) (Table 2). The highest allelic frequency was recorded for allele A (0.67) in Kamori goat breed and the lowest was in the Meriz and Shami type with a value of 0.5. In the case of allele B, the Meriz and Shami type had the highest frequency (0.5) whereas, the lowest one was in Kamori with 0.33 (Table 2).

Several genetic diversity parameters based on allelic frequencies analysed in this study for *GH1* are shown in Table 3. The average observed homozygosity (observed Ho) value was 0.118, while for expected homozygosity (expected Ho) was 0.476. The observed He value was 0.882, while that of expected He was 0.524. The highest observed heterozygosity was found in Shami and Meriz (1.000) and the lowest: in Kamuri (0.667). According to *GH2,* the average observed heterozygosity in this region was 0.000 and the observed homozygosity was 1.000.

The sequence data obtained from the PCR products of Meriz and wild goats further confirmed the two distinctive genotypes AA and AB characterised earlier by PCR-RFLP results. This was evident by the fact that the *Hae* III restriction site (GGCC) at the position 56 of allele A was not changed, thus it was cleaved into two fragments 366 and 56 bp as shown on Fig. 5. However, the nucleotide sequence of genotype AB revealed a point mutation (SNP) at position 58 of allele B where the nucleotide C is changed to T (Fig. 6). This mutation has led to removal of *Hae* III

CTCTGCCTGCCCTGGACTCAGGTGGTGGGCGCCTTCCCAGCCATGTCCTTGTCC**GGCC**CATTTGCCA
ACGCTGTGCTCCGGGCTCAGCACCTGCATCAACTGGCTGCTGACACCTTCAAAGAGTTTGTAAGCTC
CCCAGAGATGTGTCCTAGAGGTGGGGAGGCAGGAAGGGGTGAATCCGCACCCCCTCCACACAATGG
GAGGGAACTGAGGACCTCAGTGGTATTTTATCCAAGTAAGTCTCTGCCTGCCCTGGACAGCCTTCTT
CCTCACCTACTCAAGTGTATGTCGAGTGCGCAGCTGGTCAGTCGTGTCTGACTCTTTGAGACTCTCC
ACGGACGGTATCCCCCCAGGAGCGCACCTACATCCCGGAGGGACAGAGATACTCCATCCAGAACAC
CCAGGTTGCCTTCTGCTTCTCC

Fig. 5. The *GH1* nucleotide sequence of genotype AA of Meriz goat showing the intact *Hae* III restriction site in allele A.

CTCTGCCTGCCCTGGACTCAGGTGGTGGGCGCCTTCCCAGCCATGTCCTTGTCC**GGCT**CATTGCCAA
CGCTGTGCTCCGGGCTCAGCACCTGCATCAACTGGCTGCTGACACCTTCAAAGAGTTTGTAAGCTCC
CCAGAGATGTGTCCTAGAGGTGGGGAGGCAGGAAGGGGTGAATCCGCACCCCCTCCACACAATGG
GAGGGAACTGAGGACCTCAGTGGTATTTTATCCAAGTAAGTCTCGGCCTGCCCTGGACAGCCTTCTT
CCTCACCTACTCAAGTGTATGTCGAGTGCGCAGCTGCTCAGTCGTGTCTGACTCTTTGAGACTCTCC
ACGGACGGTATCCCCCCAGGAGCGCACTCCTATCCCGGAGGGACAGAGATACTCCATCCAGAACAC
CCAGGTTGCCTTCTGCTTCTCC

Fig. 6. The *GH1* nucleotide sequence and chromatogram of genotype AB of wild goat showing the point mutation of *Hae* III restriction site in allele B.

Allele A

Leu, Cys, Leu, Pro, Trp, Thr, Gln, Val, Val, Gly, Ala, Phe, Pro, Ala, Met, Ser, Leu, Ser, Gly, **Pro**, Phe, Ala, Asn, Ala, Val, Leu, Arg, Ala, Gln, His, Leu, His, Gln, Leu, Ala, Ala, Asp, Thr, Phe, Lys, Glu, Phe , Glu, Arg, Thr, Pro, Ile, Pro, Glu, Gly, Gln, Arg, Tyr, Ser, Ile, Gln, Asn, Thr, Gln, Val, Ala, Phe, Cys, Phe, Ser

Allele B

Leu, Cys, Leu, Pro, Trp, Thr, Gln, Val, Val, Gly, Ala, Phe, Pro, Ala, Met, Ser, Leu, Ser, Gly, **Ser**, Phe, Ala, Asn, Ala, Val, Leu, Arg, Ala, Gln, His, Leu, His, Gln, Leu, Ala, Ala, Asp, Thr, Phe, Lys, Glu, Phe , Glu, Arg, Thr, Leu, Ile, Pro, Glu, Gly, Gln, Arg, Tyr, Ser, Ile, Gln, Asn, Thr, Gln, Val, Ala, Phe, Cys, Phe, Ser

Fig. 7. The amino acid sequences of allele A and B.

restriction site which in turn led to change in the amino acid proline to serine (Fig. 7).

DISCUSSION

These results of *GH1* and *GH2* were found to be in line with research reported by Hua *et al* (2009) in Boer goat bucks in China. The digestion of *GH* fragments with the restriction enzyme *Hae III* showed that *GH1* locus was polymorphic (Fig. 3) and *GH2* gene was monomorphic in all tested goat breeds (Fig. 4). Similar results were reported in several studies. Hua *et al*. (2009) used gene sequencing and PCR-RFLP methods to investigate the variability of the *GH1* locus in Boer goats, as well as the relationship between growth traits and polymorphism. When they compared the AB to the AA genotype, it was found that the AA genotype resulted in a considerable reduction in weaning weight and birth chest girth. These findings were similarly consistent with those of Seevagan *et al*. (2015), who found no BB genotype in the *GH* gene locus *GH1*. He also noted that the mutation was recessive lethal in this locus. The death of gene carriers prior to or shortly after birth was defined by lethal gene expression. This work differ from that of Amie Marini *et al*. (2010), who revealed polymorphisms with AA, BB and AB genotypes after digestion of a 422 bp *GH1* fragment.

In addition, comparable findings were reported by Amie Marini *et al*. (2012), using PCR-RFLP to investigate the genetic diversity of *GH2* in Kalahari and Savanna goats and discovering that the *GH2* was monomorphic and both breeds were homozygous for the CC genotype (88 and 28 bp). Using the same method, Zhang *et al*. (2011) investigated the *GH2* gene variance in both Boer and Matou Chinese goat breeds and discovered that the genotype CC was much more common than the CD genotype. They also found that the CC genotype's litter size was substantially greater than the genotype CD. In addition, Hua *et al*. (2009) investigated the polymorphism of the *GH2* region in Boer goat breeds and its relationship to several growth parameters using gene sequencing and PCR-RFLP. They discovered genotype CC (88 and 28 bp) and also CD genotype (116, 88, and 28 bp). The authors found that weaning weight and weaning height were observed to be higher in the CC genotype than in the CD genotype.

In all breeds, genotype AB in *GH1* locus was found to be more common than genotype AA. These results were in agreement with data from several other reports such as that of Amie Marini *et al*. (2012), studying Savanna and Kalahari goats, that of Zhang *et al.* (2011) studying Matou goat in China, and the study of Othman *et al* (2015) who studied the Egyptian goat breeds Baladi, Barki and Zaraibi. However, these findings contrasted with those published in India by Singh *et al* (2015) in both Barbari and Sirohi goat breeds reporting that allelic frequency of the allele A was lower than that of the allele B. However these frequencies were similar to those reported by Hua *et al*. (2009) and Kumari *et al* (2014), who discovered that the Boer goat population, as well as the Indian native goat population, had a higher frequency of allele A.

The concept that various breeds/populations have been kept under a variety of environmental factors and hence are susceptible to diverse evolutionary processes to variable degrees could explain these disparities in allelic frequencies. Furthermore, discrepancies in the frequency of allelic variants in different breeds and populations may have been influenced by sample fluctuations (Singh *et al*., 2015).

In this study the observed He value of *GH1* was higher than the expected He, indicating that the high genetic diversity observed in these goat breeds may be due to crossbreeding performed by local farmers between goats. It is also reported that heterozygosity value is influenced by the number of samples, number of alleles and their frequency (Nei & Kumar, 2000). In contrast the *GH2* locus was homozygous in all sampled breeds this may also indicate the presence of inbreeding within the populations (Coulson *et al.,*1998).

The sequence data obtained from the PCR products of *GH1* in Meriz and Wild goat which detected AA and AB genotype respectively showed the point mutation (cytosine to thiamine) in *Hae* III restriction site, position 58 in wild goat (AB genotype) that led to change in amino acid sequence (proline to serine). Several other studies have documented many SNPs in *Hae* III restriction site in different goat breeds. Wickramaratne *et al.* (2010) reported five point mutation of growth hormone gene (G200T, A815G, A1753, C1763T, and A1789G) in the Osmanabadi and Sangamneri goat breeds. Hua *et al.* (2009) and Zhang *et al.* (2011) also discovered a SNP (A781G) in the *GH1* gene in Boer goat bucks and Boer and Matou breeds respectively.

Most of these researchers reported that the presence of heterozygous goats with AB genotype led to the increase in birth chest girth, weaning weight, body weight,

length and height, that may be attributed to glycine and serine in the amino acid sequence of AB genotype where serine is involved in metabolic processes that burn glucose and fatty acids for energy and glycine, on the other hand, is essential for the body to produce protein (Nelson & Cox, 2005).

CONCLUSION

The outcome of PCR-RFLP and SNP in this investigation revealed that digestion of amplified fragments with the restriction enzyme *Hae* III was very useful in genotype analysis of the local breeds/populations demonstrating that only *GH1* revealed polymorphism and *GH2* was detected as monomorphic. The sequence data obtained in this study also confirmed the genotype analysis. As a result, growth hormone genes could be employed as a reliable genetic marker in genetic diversity assessment, assisting in selection and formulating strategies for Kurdistan region/ Iraq goat breeding programmes such as increasing the number of heterozygote *GH* gene goat breeds.

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