INTER AND INTRASPECIFIC COMPARATIVE ANALYSIS OF GROWTH HORMONE GENE FOR SOME FARM RUMINANT SPECIES

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Molecular genetics techniques help in discovery of the genes or candidate genes which affect the important economic traits, the use of this techniques for genetic improvement depend on the ability to genotype individuals with better potentialities for specific economic traits. The information from candidate genes helps in planning breeding programs through increasing the accuracy of selection, selection differential and consequently the response to selection.

Growth hormone gene (GH) plays a fundamental role in the regulation of growth and metabolism in vertebrates (Davidson, 1987; Sami, 2007). This gene is a about 1800 bp in length with four intervening sequences and consists of five exons of about 648 nucleotides (Gordon *et al*., 1983) with chromosome region 19q26 in cattle, 11q25 in sheep (Hediger *et al*., 1990), 19q22 in goat (Schibler *et al*., 1998; Pinton *et al*., 2000). The gene produces growth hormone from the anterior pituitary, is necessary for prenatal and postnatal growth and metabolism in animals (Yamano *et al*., 1988; Supakorn, 2009). Furthermore, Malveiro *et al.* (2001), Hattori, (2009) and Hua *et al.* (2009) reported that this hormone is influencing animal growth, milk yield traits and immune stimulant properties.

Polymorphism at DNA level might be responsible for the alteration of gene functions leading to changes in a disease situation or impair/enhance a production trait. Furthermore, GH gene polymorphism was known to give possible selection criterion for fertility, weight and milk production traits (Mullen *et al*., 2011; Ishida *et al*., 2010; Ibeagha-Awemu *et al*., 2008).

MATERIALS AND METHODS

DNA samples

Blood samples were collected from three species (cattle, sheep and goat). The samples were randomly taken from three breeds of cattle viz. Simmental, Brown Swiss and Baladi, and three breeds of sheep viz. Abodlik, Awasi and Rahmani and two breeds of goat viz. Damascus and Baladi. Blood samples of Damascus and Awassi were obtained from farms of National Research Centre (NRC), while Baladi cattle samples were obtained from

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Animal Production Research Institute (APRI) and Brown Swiss and Simmental samples were obtained from Sanad farm, Abodlik and Baladi goat samples were obtained from local herds in the south of Egypt (Halaib and Shalateen) and Rahmani samples were obtained from those collected by Kayali (2013) from Animal Production Research Institute (APRI), Agricultural Research Center.

Approximately 5 ml blood per animal were obtained by jugular venipuncture in a K3-EDTA tube containing anticoagulant (Tripotassium-ethylene diamine tetracetic acid (K3-EDTA). The genomic DNA was extracted from whole blood using a commercial kit (ISOLATE II Genomic DNA Kit, Bioline, Cat No. BIO-52066).

PCR conditions

According to the conserved regions of available sequences of bovine, ovine and caprine GH genes that obtained from GenBank, NCBI, one sets of universal primers were designed to amplify intron 2, exons 3 and intron 3 of the GH gene. The primer sequence was: Forward F: 5´-TGCTG ACACC TTCAA AGAGT- 3´; Reverse R: 5´-CAGTG AGATG TGAAG CAGCT- 3´.

PCR products were electrophoretically separated on 1.5% agarose gel, stained with ethidium bromide to test the amplification success. PCR reaction was performed in a final volume of 25 µl, containing 1 µl genomic DNA, 1 µl each primer, 12.5 µl GoTaq® Green Master Mix (Promega, Madison USA) and nuclease free water up to 25 µl. The PCR program consisted of a Denaturing step at 94°C for 3 min, followed by 30 cycles [Denaturation 92°C (30 s), Annealing 50°C (30 s), Extension 72°C (30 s)] and a final Extension step at 72°C for 3 min. This program is similar for all species except annealing temperature, which was 45°C in sheep. Successful PCR products were purified using Gene JET PCR Purification Kit Cat. No.# K0701 (Thermoscientific, Lithuania).

DNA sequencing and analysis

Purified fragments were sequenced by sequencing service (Macrogen, Netherlands). Analysis of the sequencing data was performed using the Geneious program v8.1.

RESULTS AND DISCUSSION

The sequence lengths for GH gene of cattle breeds were 599, 573 and 403 bp for Simmental, Brown Swiss and Baladi, respectively. While sequence lengths of sheep breeds were 490, 486 and 484 bp for Abodlik, Awassi and Rahmani, respectively. Whereas sequence lengths of goat breeds were 535 and 598 bp for Damascus and Baladi, respectively. The variations in sequence lengths were due to cutting out of unsure bases.

Variations among species

Variation between the consensus sequences of the three species was detected and then compared with the available

sequences of bovine, ovine and caprine GH genes that obtained from GenBank, NCBI to exclude the polymorphic variation within species. There are 20 interspecific genetic variations (monomorphic within every species, polymorphic between species). All of these variations located in intronic region, whereas exon 3 didn't have any species-specific genetic variations. It is observed that intron 2 has a large number of this genetic variation, thirteen, while intron 3 have only seven. This means that the variations between species in this region centralize at the regulatory level.

All of inter-specific genetic variations can be used to distinguish two groups of animals, large ruminant (cattle) and small ruminant (sheep and goat) except for the variations in positions 202 and 454, these distinguishing goats from cattle and sheep.

The Forced PCR-RFLP method can be used to discriminate cattle, goat and sheep depending on the presence or absence of the restriction site. *BfaI* (recognition sequence C↓TAG) can be used to distinguish between cattle and small ruminant (sheep and goat), it cleaves intron 3 in sheep and goat, but not in cattle due to the absence of the nucleotide A in recognition site at position 373. To distinguish the two species of small ruminant (sheep and goat) we can use *AvaI* (recognition sequence C↓YCGRG) which cleaves intron 3 in sheep, but not in goat because of absence of the nucleotide C in the recognition site at position 454. Application of the Forced PCR-RFLP method can be used to uncover meat adulteration. Some of interspecific genetic variations caused variations in transcription factors-binding site prediction (Fig. 1).

Although intronic variation cannot change the amino acids sequence of the protein, there is augment evidence that variants in intronic region also play significant roles in amendment gene expression patterns (Le Hir *et al*., 2003; Pagani and Baralle, 2004). The influence of non-coding regulatory variants on complex traits may be more than the influence of coding-region variants (Clop *et al*., 2006; Pagani and Baralle, 2004). Intronic mutations may affect splice sites and consequently mRNA stability and may lead to truncated protein products or to the lack of them (Ibeagha-Awemu *et al*., 2008). Generally, Introns function in a number of different ways, such as sources of non-coding RNA, carriers of transcriptional regulatory elements, contributors to alternative splicing, enhancers of meiotic crossing over within coding sequences and signals for mRNA export from the nucleus as reported by Fedorova and Fedorov (2003) and Dario *et al*. (2008).

Variations among cattle breeds

There is no single nucleotide polymorphisms (SNPs) identified between the three breeds of cattle.

Variations among goat breeds

Five SNPs were identified between the two breeds; all of them are transition

(Table 1). Two SNPs were detected in exon 3, one SNPs led to an amino acid change, G353A which alters the amino acid codon GGC to AGC. This substitution led to an amino acid mutation serine to glycine (39ser>gly).

Variations in the exons may lead to changes in amino acids sequence of the expressed protein and may affect positively or negatively its function and consequently traits that are influenced by this protein. The effects of this substitution may lead to eliminating, inactivating, unstable product or amendment protein functions to suit different environments (King *et al*., 2006; Ibeagha-Awemu *et al*., 2008).

The other variations in exons did not change in amino acid sequences. Some researcher stated association between synonymous variation and production trait (Yao *et al*., 1996; Silveira *et al*., 2008; Chung *et al*., 1996; Dybus, 2002; Fan *et al*., 2010). Synonymous mutations can alter mRNA folding, led to a decrease or an increase in the mRNA stability and translation, and also can alter the methylation pattern which leads to alterations in chromatin structure and either to a decrease or an increase in the rate of transcription. (Duan *et al.*, 2003; Capon *et al*., 2004; Ramser *et al*., 2008).

As shown in Table (2), the T>C Transition changed a common codon AAC to a less used codon AAT (both coding asparagine). This may be having important consequences for cellular processes but cannot be deduced from simple analysis of DNA sequence, it needs further analysis before drawn any conclusion.

Variations among sheep breeds

There are two SNPs identified between the three breeds, all of them are Transition (Table 3). G353A alter the amino acid codon GGC to AGC. This substitution led to an amino acid mutation Serine to Glycine (39Ser>Gly).

CONCLUSION

Inter-specific genetic variations can be considered as species differentiating marker, and requires further studies to know their effects on the gene expression.

Based on the newly discovered finding with regard to the synonymous mutation, we need to look at them thoroughly and requires further studies to know their effects on the production traits to be used for genetic improvement of livestock animals. Baladi goat has a large variation and could be used in genetic improvement.

SUMMARY

Growth hormone affects a lot of physiological processes and traits, such as metabolism, milk and meat production. Polymorphism at DNA level might affect gene function and consequently the trait. The aim of this study was to identify the variation in the growth hormone gene between and within species (cattle, sheep and goat). The results showed that all variations between species located at intronic

region, whereas exon 3 didn't have any species-specific genetic variations. There is no SNPs identified between the breeds of cattle, whereas the variation within breeds of sheep and goat located at an intronic and exonic region.

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Table (1): Variations between goat breeds.

Nt. no	Breeds		Type	Location	Flanking region	Restriction enzyme
	Baladi	Damascus				
73	C	T	Transition	Intron 2	CCCC/TTCC	*(-)*
329	R[1]	A	Transition	Exon 3	ACGG/AGCA	*(-)*
337	Y[2]	Y	Transition	Exon 3	GAAT/CGAG	*(-)*
400	T	C	Transition	Intron 3	ATCT/CTAA	*(AflII, BfrI* and *MseI)*
435	G	A	Transition	Intron 3	CCTG/AGGG	*(AvrII, BfaI* and *BlnI)*

[1.] is a Purine (Adenine or Guanine), 2 is a Pyrimidine (Cytosine or Thymine), Nt. no is nucleotide number

Table (2): Goat synonymous SNPs and codon usage (codon usage database).

SNP	*Type*	*Codon*	*Amino acid*	*Codon usage*
337T>C	Transition	AAT>AAC	Asparagine	15.7>25.3

Table (3): Variation among sheep breeds, type, location, and the sequence of flanking region for the site of variation.

Nt. no	Breed		Rahmani	Type	Location	Flanking region
	Abodlik	Awassi				
83	A	R[1]	A	Transition	Intron 2	GAAA/GTGG
265	R	R	R	Transition	Exon 3	ACGG/AGCA

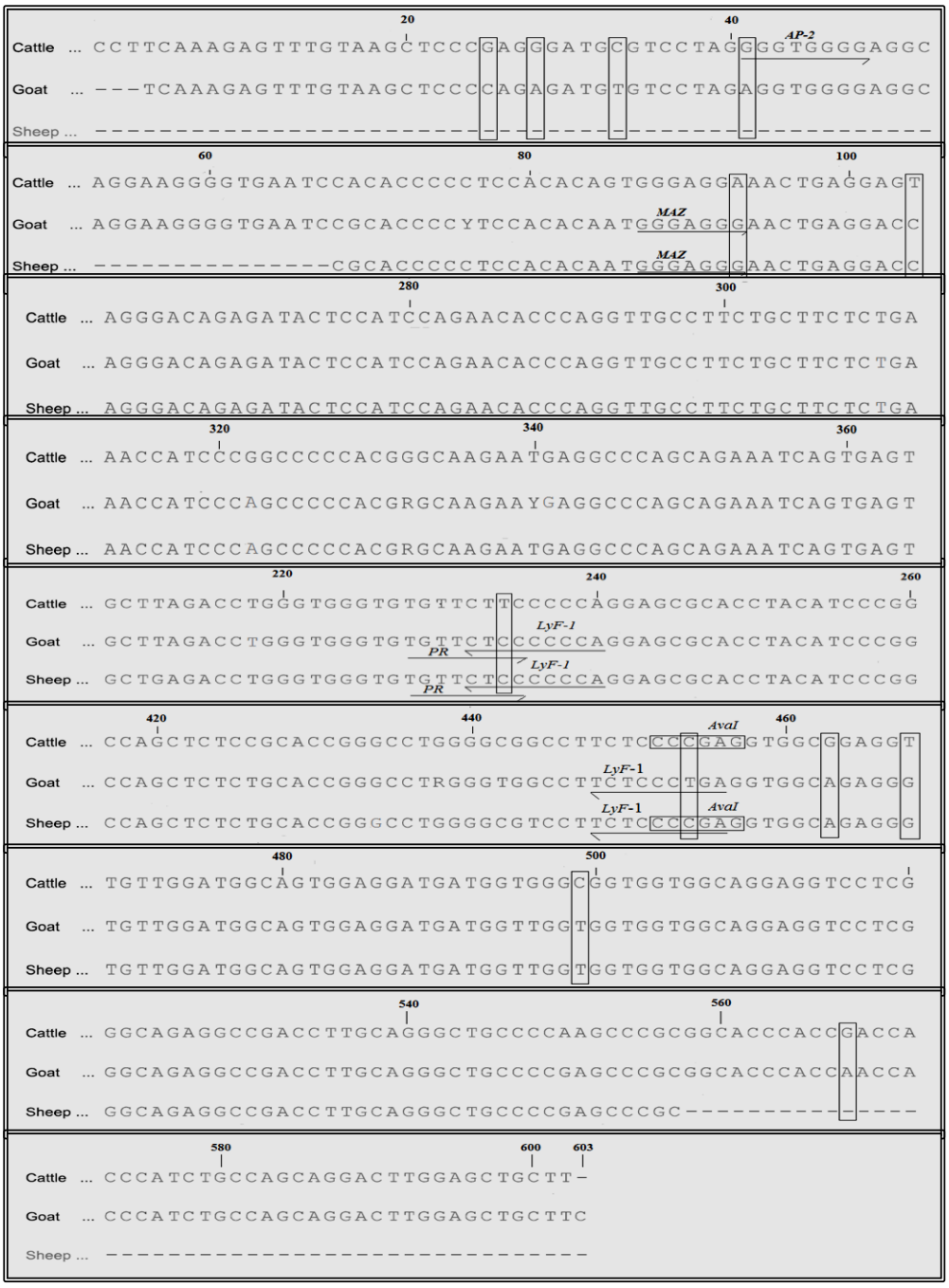

Fig. (1): Alignment of GH consensus sequence of cattle, goat and sheep: Exon 3 span from 242 to 358, the interspecific genetic variation represented by vertical boxes, restriction site represented by horizontal boxes, ——→ representing TFBSs in sense strand, ←—— senting TFBSs in antisense strand.