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 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...
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 varia-
tion within species. There are 20 interspe-
cific genetic variations (monomorphic 
within every species, polymorphic be-
tween 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 reg-
ulatory level.

All of inter-specific genetic varia-
tions can be used to distinguish two 
groups of animals, large ruminant (cattle) 
and small ruminant (sheep and goat) ex-
cept 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. BfαI (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 distin-
guish the two species of small ruminant 
(sheep and goat) we can use AvaI (recog-
nition 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. Appli-
cation 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 signif-
icient 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 muta-
tions may affect splice sites and conse-
quently mRNA stability and may lead to 
truncated protein products or to the lack of 
them (Ibeagha-Awemu et al., 2008). Gen-
erally, Introns function in a number of 
different ways, such as sources of non-
coding RNA, carriers of transcriptional 
regulatory elements, contributors to alter-
native 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 pol-
ymorphisms (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
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 |
|--------|----------|--------|----------|-----------------|-------------------|
| 73     | Baladi   | C      | Transition| Intron 2        | CCCC/TTCC         |
| 329    | Damascus | T      | Transition| Exon 3          | ACGG/AGCA         |
| 337    |          | R\(^1\) | Y        | Exon 3          | GAAT/CGAG         |
| 400    |          | T      | C        | Intron 3        | ATCT/CTAA         |
| 435    |          | G      | A        | Intron 3        | CCTG/AGGG         |

\(^1\) 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 |
|--------|-------|---------|--------|----------|-----------------|
| 83     | A     | R\(^1\) | A      | Transition| Intron 2        |
| 265    | R     | R       | R      | Transition| Exon 3          |

\(^1\) is a Purine (Adenine or Guanine), \(^2\) is a Pyrimidine (Cytosine or Thymine), Nt. no is nucleotide number
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, representing TFBSs in antisense strand.
