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RESEARCH ARTICLE

Molecular comparative study of growth hormone receptor (*GHR*) gene in Egyptian and Saudi breeds of sheep (*Ovis aries*)

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Abstract

In the present study, the amplified fragment including part of sheep *GHR* gene exon 10 spanned bases from 590 to 783 based on nucleotide positions of *Ovis aries* isolate Ovis 1 (DQ062717) in GenBank. One band was detected by PCR analysis of exon 10 of sheep GHR gene in individual sheep. In order to explore the genetic variation, forty two samples including three Egyptian breeds (Rahmani, Osseimi and Barki) and two Saudi breeds (Najdi and Harri). The sequencing pattern revealed that the most common transitions were A to C and G to A, followed by T to G, G to T and A to G. The most rare transitions were A to T, C to T and T to A. there were some deletions such as A deletion was found in Barki H, Rahmani H and Harri; T deletion was found in Barki H and Barki L; C deletion was found in Osseimi H and G deletion was found in Barki H. There was only one insertion "A insertion" in Rahmani L. The relative similarities between different breeds revealed that Barki L and Najdi similar to O. aries with 92%, followed by Osseimi H (91%), Rahmani H (90%) and Osseimi L (87%). The highest similarities between Egyptian and Saudi breeds under study was found between Osseimi H and Najdi (96%), Rahmani H and Najdi (91%), and between Osseimi H and Harri (91%), then Rahmani H and Harri (91%). The lowest similarities between Egyptian and Saudi breeds were found between Harri and Barki L (69%) and between Najdi and Barki L (75%). The phylogenetic tree of sheep GHR gene in the different breeds showed that the most related breeds were Osseimi H (Egyptian) and Najdi (Saudi), Ossiemi H and Harri (Saudi), Najdi and Rahmani H, and Harri and Rahmani H..

Introduction

The domestic sheep is a multi-purpose animal, and the more than 200 breeds now in existence were created to serve these diverse purposes. However, several hundred breeds of sheep have been identified by the FAO, with the estimated number varying somewhat from time to time: e.g. 863 breeds as of 1993 **(Maijala, 1997)**, 1314 breeds as of 1995 **(Scherf, 2000)** and 1229 breeds as of 2006 **(FAO, 2007)**.

Almost all sheep are classified as being best suited to furnishing a certain product: wool, meat, milk, hides, or a combination in a dual-purpose breed. Other features used when classifying sheep include face color (generally white or black), tail length, presence or lack of horns, and the topography for which the breed has been developed. This last point is especially stressed in the UK, where breeds are described as either upland (hill or mountain) or lowland breeds **(Brown and Meadowcroft, 1996)**. A sheep may also be of a fat-tailed type, which is a dual-purpose sheep common in Africa and Asia with larger deposits of fat within and around its tail.

The growth hormone receptor (*GHR*) is a member of the cytokine/hematoprotein superfamily of receptors **(Maj and Zwierzchowski, 2005)**, and consists of three functional domains: an extracellular (ligand-binding) domain, a transmembrane domain and a cytoplasmic (signal-transducing) domain. It is required for growth hormone (*GH*) to carry out its effects on target tissues **(Moody et al., 1995)**. The binding of *GH* to *GHR* causes receptor dimerization, and thus initiates signaling cascades through the cytoplasmic domain **(Frank, 2001)**. Also, the *GHR* mediates the biological actions of *GH* on the target cells by the regulation of the transcription of other genes, including insulin-like growth factor-I, metabolic enzymes and transcription factors **(Argetsinger and Carter-Su, 1996; Herrington and Carter-Su, 2001)**. The *GHR* gene of most mammalian species consists of 9 coding exons (exons 2–10) and several alternative untranslated exons in the 50-noncoding region **(Jiang and Lucy, 2001)**.

Several investigations have been carried out on genetic polymorphism in *GHR* gene of human **(Buzi et al., 2007; Millar et al., 2008)**, chicken **(Feng et al., 1998)**, bovine **(Ge et al., 2000, 2003; Di Stasio et al., 2005; Varvio et al., 2008)** and ovine **(O'Mahoney et al., 1994; Viitala et al., 2006; Ma et al., 2007)**. Also, some of *GHR* gene polymorphisms affecting the growth and production traits have been reported in recent years. Several examples exist in bovine a wide variety of mutations of the *GHR* gene has been suggested as genetic markers for the traits related to the milk yield **(Blott et al. 2003; Viitala et al., 2006)**, meat production and growth **(Di Stasio et al., 2005; Ge et al., 2003; Sherman et al., 2008)**.

Materials and Methods

Animals

Whole blood samples were collected from sheep animals belonging to three main sheep breeds reared in Egypt and two Saudi sheep breeds. The blood samples were collected from different farms belonging to Animals Production Institute. The three breeds used in this study are, Rahmani (from Animal Breeding Research Station in Sero), Domiata), Barki (from Animal Breeding Research Station in Borg El-Arab, Alex) and Osseimi (Animal Breeding Research Station in Seds, Bani Swif) while the two Saudi sheep breeds are, Najdi and Harri (from slaughter house) in Jeddah.

DNA extraction

Genomic DNA was extracted from the whole blood according to the method described by **Miller et al. (1988)** with minor modifications. Briefly, 10ml of blood taken on EDTA were mixed with 25 ml of cold 2X Sucrose-Triton and 15 ml double distilled water. The tubes were placed on ice for 10 min and mixed by inversion several times. After centrifugation, at 5000 rpm for 15min at 4ºC, the pellet was re-suspended by 3ml of nucleic lysis buffer. The content was mixed with 108 µl of 20% SDS and 150 µl of Proteinase K. The tubes were placed in a water bath at 37ºC overnight. After the incubation, the tube contents were transferred to a 15-ml polypropylene tube and 2 ml of saturated NaCl was added and shaken vigorously for 15s. After centrifuging at 3500 rpm for 15 min at 4ºC, the supernatant was transferred to a clean 15-ml polypropylene tube and mixed with absolute ethanol. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The precipitated DNA was picked up using the heat sealed pasture pipette, then washed twice in 70% ethanol and exposed to air to dry completely. The DNA was dissolved in 200 µl TE buffer in 1.5-ml Microfuge tube and kept overnight in an incubator at 37ºC. DNA concentration was determined and diluted to the working concentration of 50 ng/µl, which is suitable for polymerase chain reaction using NanoDrop1000 Thermo Scientific spectrophotometer.

Polymerase chain reaction (PCR)

The primer was synthesized at Macrogen (Korea) for the amplification of fragment including part of sheep *GHR* gene exon 10, upper primer 5′- GCCAAAACAATAAGACTGGGAACC-3′ and lower primer 5′-GGCTGTAGTGGTAAGGCTTTCTG-3′ to amplified a fragment 218 bp with annealing temperature 62°C (Hickford et al. 2010). The DNA fragments of the studied genes were amplified through polymerase chain reaction technique developed by **Mullis et al. (1986)**. A PCR cocktail consists of 1.0 mM upper and lower primers, 0.2 mM dNTPs and 1.25 units of Taq polymerase. The cocktail was aliquot into PCR tubes with 100 ng of sheep DNA. The reaction was cycled for 35 cycles according to the specific protocol suitable for each primer. The amplification will be verified by electrophoresis on 2% agarose gel (w/v) in 1x TBE buffer using 100-bp ladder as a molecular weight marker for confirmation of the length of the PCR products. The gel will be stained with ethidium bromide (1 µg/µl) and visualized on UV trans-illuminator.

Sequence analysis

The PCR products represent *GHR* gene detected in this study was purified and sequenced by Macrogen Incorporation (Seoul, Korea) to identify the SNPs. Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite.

Results and Discussion

The conservation of animal genetic resources has been a topic of discussion since the 1950s **(Simon, 1984)** from biological, economic, cultural and emotional standpoints. It is essential to avoid the loss of genetic variability, in part because these resources may be valuable for future breeding requirements **(Hodges, 1984)**. Genetic variability in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, particularly in light of increasing demands for animal products around the world. Conservation should be based on a deep knowledge of the genetic resources of each specific breed. It is therefore important to make efforts to characterize genetically indigenous breeds.

In the present study, the amplified fragment including part of sheep *GHR* gene exon 10 spanned bases from 590 to 783 based on nucleotide positions of *Ovis aries* isolate Ovis 1 (DQ062717) in GenBank. One band was detected by PCR analysis of exon 10 of sheep *GHR* gene in individual sheep. In order to explore the genetic variation in exon 10 of sheep *GHR* gene in analyzed populations, forty two samples including three Egyptian breeds (Rahmani, Osseimi and Barki) (Figure 1) and two Saudi breeds (Najdi and Harri) (Figure 1). Rahmani breeds including six samples high meat yield and five low meat yield breeds, Osseimi breeds including six samples high meat yield and five low meat yield breeds, Barki breeds including five high meat yield and four low meat yield breeds, six samples from Najdi breed and five samples of Harri breed. All forty two PCR products were sequenced in both directions. The more common pattern corresponded to the sequencing results of the GenBank accession number DQ062717 (Figure 2).

Investigation of *GHR* gene polymorphism is of great interest, since mutation of *GHR* gene might affect its binding capacity and signaling pathway, thereby alter the *GH* activity in the target tissues **(Di Stasio et al., 2005)**, and ultimately affect the milk and meat production traits. Several investigations have been carried out on genetic polymorphism in *GHR* gene of ovine **(O'Mahoney et al., 1994; Viitala et al., 2006; Ma et al., 2007)**.

Growth hormone (*GH*) is one of the major regulators of postnatal growth and metabolism in animals, and thus *GH* affects growth rate, body compositions, health, milk production and aging by direct actions and indirect effects including the secretion of insulin-like growth factor I (*IGF-I*) **(Ho and Hoffman 1993; Lincoln et al., 1995)**. Bovine *GH* gene is a single-copy gene located in the band region of q26-qter in cattle chromosome 19. It is composed of four introns and five exons with approx. 1800 bp of length **(Vukasinovic et al., 1999)**, encoding 191 (or 190) amino acid residues with a four-helix structure **(Secchi and Borromeo, 1997)**. The ovine *GH* and bovine *GH* genes are 97.5% homologous in the coding regions **(Jacqueline et al., 1988)**.

The sequencing pattern revealed that the most common transitions were A to C and G to A, followed by T to G, G to T and A to G. The most rare transitions were A to T, C to T and T to A. there were some deletions such as A deletion was found in Barki H, Rahmani H and Harri; T deletion was found in Barki H and Barki L; C deletion was found in Osseimi H and G deletion was found in Barki H. There was only one insertion "A insertion" in Rahmani L (Figure 2 and Table 1).

The relative similarities between different breeds and *O. aries* 1 (DQ062717) were reported in Table 2, which Barki L and Najdi similar to *O. aries* with 92%, followed by Osseimi H (91%), Rahmani H (90%) and Osseimi L (87%). The highest similarities between Egyptian and Saudi breeds under study was found between Osseimi H and Najdi (96%), Rahmani H and Najdi (91%), and between Osseimi H and Harri (91%), then Rahmani H and Harri (91%). The lowest similarities between Egyptian and Saudi breeds were found between Harri and Barki L (69%) and between Najdi and Barki L (75%).

The phylogenetic tree of sheep *GHR* gene in the different breeds showed that the most related breeds were Osseimi H (Egyptian) and Najdi (Saudi), Ossiemi H and Harri (Saudi), Najdi and Rahmani H, and Harri and Rahmani H. the most related breed to the *O. aries* isolate Ovis 1 was Barki L. (Figure 3).

It is currently accepted that genetic variability is high in sheep landraces. The maintenance of a great number of local sheep breeds with diversified production conditions offers resistance to the tendency toward the reduction of genetic variability **(Flamant, 1991)**. Therefore, it is necessary to evaluate the variability within each breed.

The SNP found in the exon 10 for sheep and cattle in which 3 and 14 SNPs have been identified, respectively **(Ge, 2000; Blott et al., 2003; Varvio et al., 2008)**. Previous studies reporting association analyses of variants of *IGF-1* and milk production and growth traits in cattle include *IGF-1* SNPs **(Islam et al., 2009). Yilmaz et al. (2005)** found the same patterns that corresponded to three genotypes of AA, AB, and BB in mixed breed sheep.

This clustering based on the nucleotide sequences of *GHR* gene exon 10 clearly shows the phylogenetic inter-relationship among these domesticated species, and also is generally in agreement with the known species relationships.

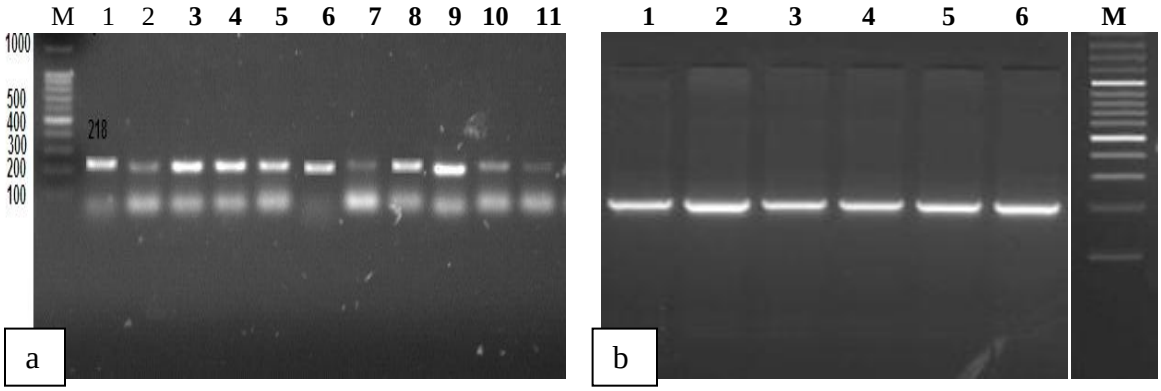

Figure 1: a) Ethidium bromide-stained gel of PCR products representing amplification of *GHR* gene in sheep. Lane 1. 100-bp ladder marker, Lanes 2-11: 218-bp PCR products amplified from sheep DNA. b) PCR amplification bands after gel purification

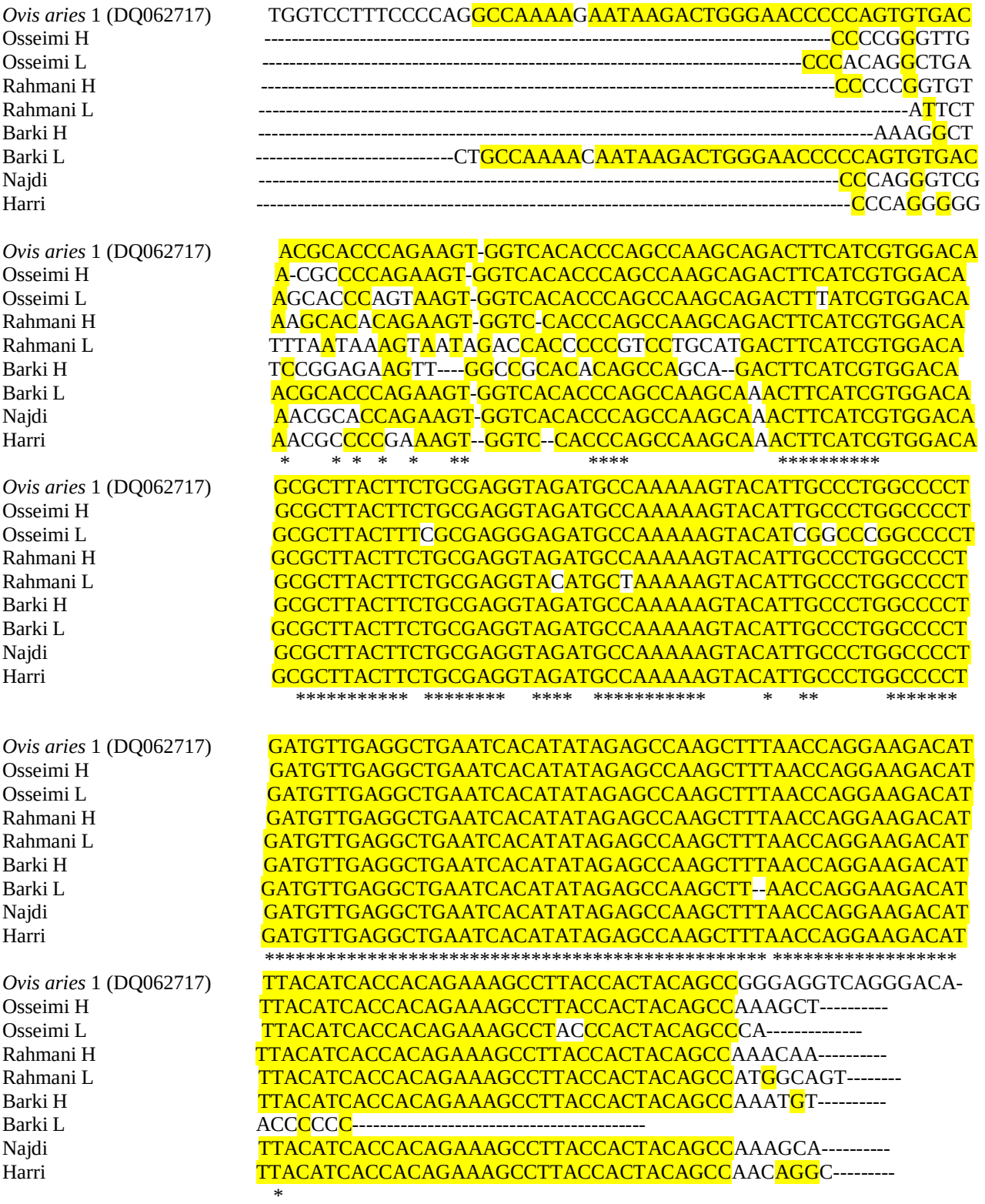

Figure 2: Multiple sequence alignment of nucleotide sequences of growth hormone receptor (*GHR*) gene in the eight different Saudi and Egyptian sheep breeds. The *Ovis aries* isolate Ovis 1 was downloaded from the GenBank with accession number DQ062717. Alignment was done with ClustalW 2.0 software.

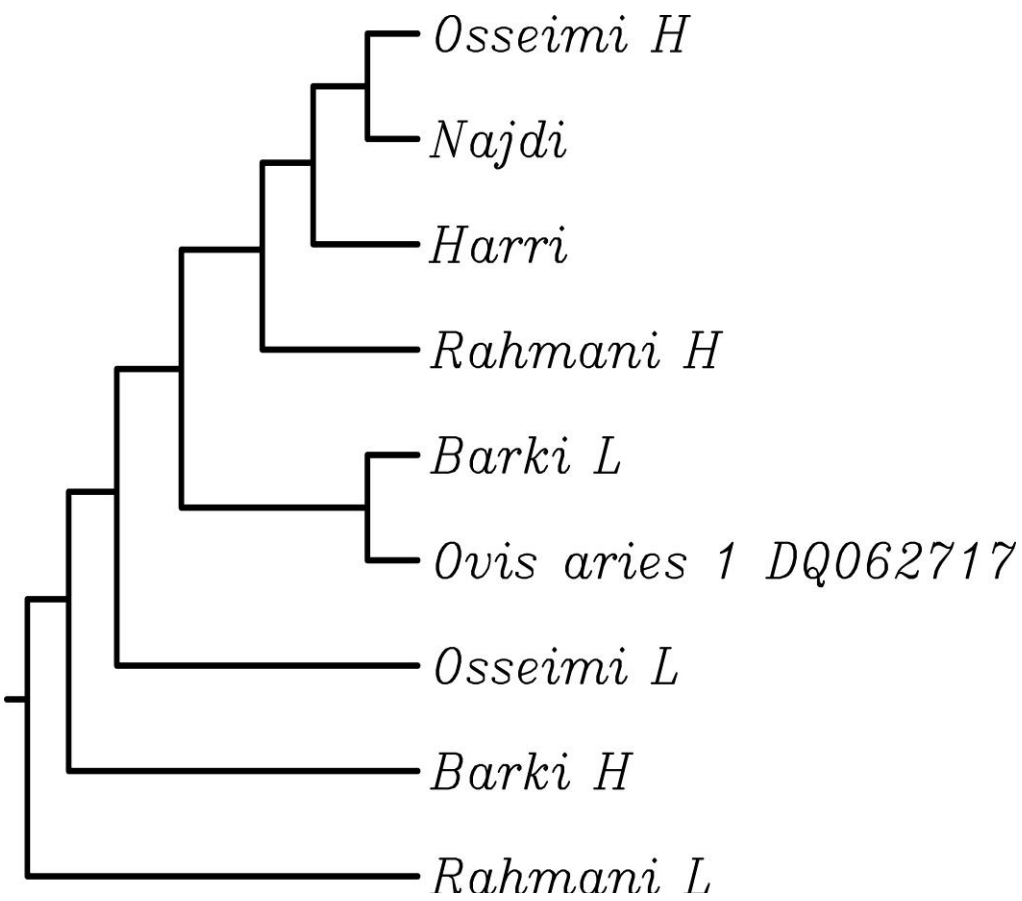

Figure 3: Phylogenetic tree of sequence of growth hormone receptor (*GHR*) gene showing the relationship of *Ovis aries* (Saudi and Egyptian) breeds to the *Ovis aries* isolate Ovis 1 (DQ062717).

Table (1): *List of the transitions, deletions and insertion in the GHR gene sequence in different Saudi and Egyptian breeds related to the Ovis aries isolate Ovis 1 (DQ062717)*

Transitions	Osseimi (H)	Osseimi (L)	Barki (H)	Barki (L)	Rahmani (H)	Rahmani (L)	Najdi	Harri
A to C	2	3	1	3	2	2	3	2
G to C	3	1	2	1	1		2	3
T to G	2	1	1		1	1	2	1
G to T	2	1	1	1	1	3	1	
A to T	1		4			3		
C to G	2	1	2				2	2
G to A	3	2	6	1	5	5	6	4
A to G	1	1	3		1	3	1	2
T to C		4	1	2	1	1		1
C to A		2	3		1	3	2	1
C to T		1	1		1	4		
T to A		1	1	1				1
A deletion			1		1			1
T deletion			1	1				
C deletion	1							
G deletion			1					
A insertion						1		

Table (2): *Relative similarity of sequence of growth hormone receptor (GHR) gene of Ovis aries (Saudi and Egyptian) breeds with Ovis aries isolate Ovis 1 sequences obtained from gene data bank*

Breeds	Osseimi (H)	Osseimi (L)	Barki (H)	Barki (L)	Rahmani (H)	Rahmani (L)	Najdi	Harri	*O. aries* 1 (DQ062717)
Osseimi (H)	100								
Osseimi (L)	85	100							
Barki (H)	86	79	100						
Barki (L)	73	70	64	100					
Rahmani (H)	90	81	84	71	100				
Rahmani (L)	81	73	79	63	79	100			
Najdi	96	84	85	75	91	80	100		
Harri	91	81	85	69	91	78	91	100	
*O. aries*1 (DQ062717)	91	87	85	92	90	82	92	88	100

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