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RESEARCH ARTICLE

Association between Growth Hormone Single Nucleotide polymorphism and Body Weight in Four Saudi Camel (*Camelus dromedarius*) Breeds

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ABSTRACT

The aim of this study was to evaluate the relationship between growth hormone gene (GH) polymorphism and estimated body weight in Arabian camels. This was achieved by sequencing and alignment of the growth hormone (GH) gene in four Saudi Arabian camel breeds (Majaheem, Saheli, Waddah and Homor); searching for single nucleotide polymorphisms (SNPs) and correlating them with estimated body weight. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect SNPs in the genotype of 200 animals from each breed. Thirteen SNPs (two insertion and eleven substitution) were detected in the Majahem breed, and one was detected in the Waddah and Homor breeds each at position 419 (C419T). Two SNPs (C419T and T450C) were detected in the Saheli breed. Of these the T450C SNP was associated with increased estimated body weight. Both male and female Saheli camels with the CC genotype had higher body weights than the CT and TT genotypes ($P \leq 0.05$).The SNP T450C, which was detected only in camels of the Saheli breed, was correlated with greater body weight. Consequently, this SNP may be a useful marker in the selection of camels for higher growth rate and meat production.

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INTRODUCTION

Camels are present in large numbers in the arid parts of Africa and Asia. In the Kingdom of Saudi Arabia (KSA), the camel population is estimated 240,000 heads (FAO, 2011), representing 51% of the total tropical livestock units (TLU) in the country (Abdallah and Bernard, 2012).

The development of molecular-genetic techniques has made it possible to identify differences between individuals at the DNA level. Recently, genetic polymorphisms, at candidate genes affecting economic traits, have stimulated considerable research interest because of their potential utilization as an aid to genetic selection and to demarcate evolutionary relationships in different livestock breeds (Sodhi *et al.,* 2007). Association of several polymorphic sites (SNPs) in different candidate genes with economic traits has been widely used and accepted as a selection tool in different commercially important species like cattle (Ge *et al.,* 2003), sheep (Bastos *et al.,* 2001), and goats (Neelam *et al.,* 2007).

In farm animals, promising candidate genes for many traits are in the growth hormone axis. Growth hormone (GH) is a polypeptide hormone with diverse biological activities including somatogenic, lactogenic, insulin-like and diabetogenic effects (Ishag *et al.,* 2010). The biological effects of GH are coordinated through changes in tissue metabolism, including nutrient partitioning and thus can play a key role in increasing growth performance or milk yield (Etherton and Bauman, 1998). In ruminants, GH is known to be responsible for galactopoesis and for persistency of lactation. It coordinates physiological processes so that nutrients are partitioned for milk synthesis (Svennersten-Sjaunja and Olsson, 2005), and results in more efficient milk production in dairy cows. In other commercial livestock, increased concentrations of GH are of economic importance because they are often associated with faster growth and reduced fat stores (McMahon *et al.,* 2001). Moreover, other researchers have considered the gene encoding GH to be a promising candidate as a marker for selection purposes (Parmentier

et al., 1999). Genetic variation at molecular level is pervasive in all breeding programs and these variants can be a potential marker-gene resource. The use of modern genetic selection programs and the development of new reproductive technologies enable concentration of desirable genes within a breeding population (Croquet *et al.,* 2006). The camel growth hormone gene extends over about 1900bp, and like other mammalian GH genes, it splits into 5 exons and 4 introns (Maniou *et al.,* 2001).

For camels to contribute more to the agricultural economy, it is necessary to select genotypes with high productivity for meat and/or milk. Therefore, the aim of the study reported here was to survey the body weight of four different Saudi Arabian camel breeds and to attempt to determine the genetic relationship between them using molecular genetic markers. The study attempted to determine genetic diversity in the current camel population and to establish, through sequencing the growth hormone gene and detection of SNPs, whether these are genetic markers that could be used for 'marker assisted selection' in future camel breeding programs.

MATERIALS AND METHODS

This study was approved by the Ethical Committee of the King Fahed Centre for Medical Science, King Abdulaziz University, KSA.

Survey and sample collection: Ten camel farms in the East Region of KSA were visited over a period of 10 days. At each farm, body measurements were taken on 10 adult female and 10 adult male camels from each of four camel breeds (Majaheem, Saheli, Homor and Wodoh) to estimate body weight using the Schwartz and Dioli (1992) equation, as given below:

$$Y = SH\,(m) * TG\,(m) * AG\,(m) * 50$$

Where: Y = The weight in kg, SH (m) = The height of the shoulder in meters, TG (m) = The chest girth behind the chest pad in meters, AG (m) = The abdominal girth over the highest part of the hump in meters.

Blood samples were taken from 200 individuals of each breed, collected in BD Vacutainer™ plastic tubes (Fisher Scientific, Cat. # 02-689-7, USA) for DNA extraction.

DNA isolation and PCR amplification: Genomic DNA isolation from 200 samples from each breed was performed using a blood DNA extraction kit (Qiagen, Cat no. 51104, Germany). The first 781bp of GH gene that resembled exon1 and 2 and intron 1 and 2 from10 samples from each camel breed were amplified using primers that were designed by PRIMER3 software (http://www.-genomewi.mit.edu) as per the published camel GH gene sequence (AJ575419) of NCBI database (Maniou *et al.,* 2004). PCR was conducted using PCR master mix (Qiagen, Cat no 201443, Germany). The size and purity of the PCR products were estimated by subjecting samples to 1% agarose gel electrophoresis.

GH gene sequencing and sequence analysis: The PCR product was extracted from agarose gel and purified using QIA quick PCR Purification Kit (Qiagen, Cat no 28104, Germany). The purified product was sequenced (10 samples from each breed) in forward direction using sequencing kit (Applied Biosystems, Cat. no 4337455, USA). Results were analyzed with Chromas Pro 1.49 (http:/www.technelysium.com.au) and Blast 2.0 (Altschul *et al.,* 1990) software. The sequences were aligned and the position of the identified SNPs was numbered as per the complete camel reference sequence of accession AJ575419 (Maniou *et al.,* 2004), using Clustal W software (Thompson *et al.,* 1977).

GH genotype detection: GH genotyping was performed using PCR-RFLP in 200 samples from each breed. A 508bp fragment encompassing the mutations containing sequence was amplified using specific primers (Ishag *et al.,* 2010). One part of the obtained product was digested with *MspI* restriction endonuclease (Thermo Scientific, Cat. no, ER0541, Wehlistrasse, Austria). The digested fragments were C allele, unrestricted fragment with 508 pb length and T allele that was restricted to 312 and 196 pb length fragments. The other part was digested with *HinP1I* restriction endonuclease (Thermo Scientific, Cat. no, FD0484, Wehlistrasse, Austria). The digested fragments were T allele, unrestricted fragment with 508 bp length and C allele that was restricted to 343 and 165 bp length fragment. The alleles were resolved by electrophoresis then visualized and photographed using Gel Decomentation System (Pharmacia, Biotech, USA).

Statistical analysis: Genotype and allele frequencies were determined by gene counting. However the t-test was used to determine differences in gene frequencies between populations. Estimated body weights of the different genotypes were subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS, 2000).

RESULTS

GH sequencing: Sequencing of GH in the four different camel breeds revealed the presence of 13 SNPs in Majaheem breed; two insertions and 11 substitution SNPs. The insertion SNPs (G and A) were detected between nucleotide 179 and 180 in exon1. The substitution SNPs included five SNPs occurred in exon 1, four of them transversion (C140T, C141T, C177A, T178A) and one transition mutation (G179A), five transversion mutations in interon 1 (C211G, C217A, T218G, G377C, C419T) and one transition mutation in interon 2 (G615A). In the Saheli breed, there were two transversion SNPs, one of which was present only in this breed at exon 2 at position 450 (T450C) and the other present in all breeds in intron 1 at position 419 (C419T). In the Waddah and Homorbreeds, only one SNP was detected in intron one at position 419 (C419T; Table 1 and Fig. 1).

GH genotyping and alleles frequencies: The four selected camel breeds were successfully genotyped using the C419T and T450C SNP. In the first SNP, all camel breeds were found to be carriers of the T allele with an allelic frequency ranging between 0.44 for the Waddah breed and 0.47 for the Majaheem and Homor breeds,

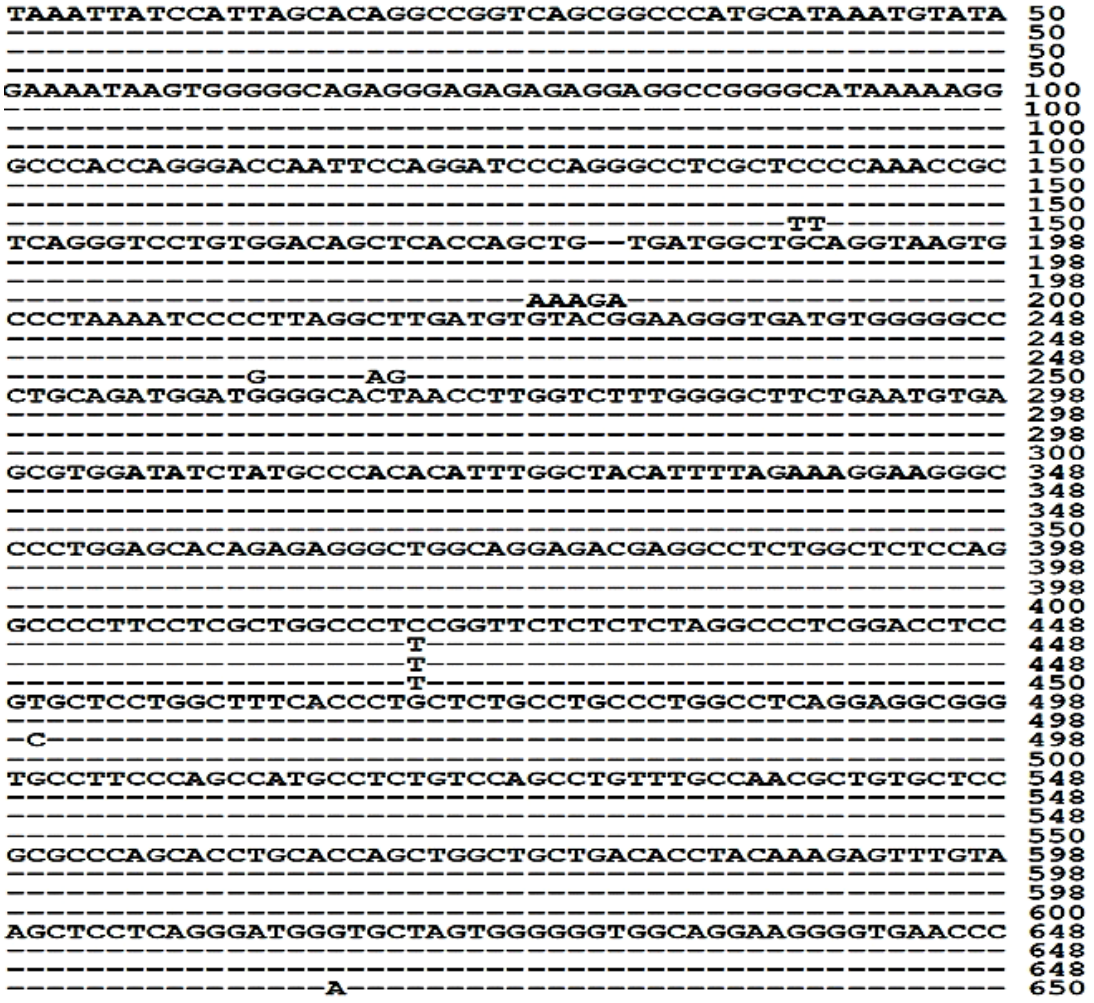

Fig. 1: Sequences alignment of Waddah, Homor, Saheli and Majaheem camel growth hormone gene with online *Camelus dromedaries* gene having accession number AJ57541.9

Table 1: Single nucleotide polymorphisms (SNPs) detected by sequencing of some Saudi Arabian camel breeds' growth hormone gene and aligned with *Camelus dromedarius* gene having accession number AJ575419.1

Location	Sequence change	Nucleotide position	Name of SNPs	Deduced AA change	Camel Breed			
				-	S	M	W	H
5′UTR	C >T	140	C140T	-	nd	D	nd	nd
5′UTR	C >T	141	C141T	-	nd	D	nd	nd
5′UTR	C >A	177	C177A	-	nd	D	nd	nd
5′UTR	T >A	178	T178A	-	nd	D	nd	nd
5′UTR	G >A	179	G179A	-	nd	D	nd	nd
5′UTR	-/G	179-180	179G180	-	nd	D	nd	nd
5′UTR	-/A	179-180	179A180	-	nd	D	nd	nd
Intron 1	C >G	211	C211G		nd	D	nd	nd
Intron 1	C >A	217	C217A		nd	D	nd	nd
Intron 1	T >G	218	T218G		nd	D	nd	nd
Intron 1	G >C	377	G377C		nd	D	nd	nd
Intron 1	C >T	419	C419T		D	D	D	D
Exon 2	T >C	450	T450C	V9A	D	nd	nd	nd
Intron 2	G >A	615	G615A		nd	D	nd	nd

UTR=untranslated region; nd=not detected; D=detected; S=Saheli; M=Majaheem; W=Waddah; H=Homor.

while the highest frequency of the C allele was observed in the Waddah (0.56) and the lowest (0.53) in Majaheem and Homor breeds. The genotype frequency revealed three genotypes, heterozygous (CT) was the same in the Saheli, Majaheem and Homor breeds (0.50) and least frequent (0.49) in the Waddah breed. The homozygote (TT) had the highest genotype frequency (0.22) in the Majaheem and Homor breeds, while the homozygous genotype (CC) was most frequent (0.32) in the Wahhah breed. No significant differences were observed in the genotype and alleles frequencies among camels of four breeds (Table 2).

The other SNP that has been studied is T450C, in which T450 is substituted by C. The T450C SNP was present only in the Saheli breed. All the camel breeds except the Saheli were found to carry only the T allele,

Table 2: Genotype and allele frequencies of the SNP C419T in growth hormone gene in some Saudi Arabian camel breeds (n = 200 each)

Breeds	Genotypes			Alleles	
	TT	CT	CC	T	C
Saheli	0.21	0.50	0.29	0.46	0.54
Majaheem	0.22	0.50	0.28	0.47	0.53
Waddah	0.19	0.49	0.32	0.44	0.56
Homor	0.22	0.50	0.28	0.47	0.53

Table 3: Genotype and allele frequencies of the SNP T450C in the growth hormone gene in some Saudi Arabian camel breeds (n = 200 each)

	Genotypes			Alleles	
	TT	CT	CC	C	T
Saheli	0.04*	0.32*	0.64*	0.80*	0.20*
Majaheem	I	0	0	0	I
Waddah	I	0	0	0	I
Homor	I	0	0	0	I

* Within row differ significantly (P<0.05).

with an allelic frequency 1, and one genotype (TT) that was manifested by an undigested fragment (508bp) by RFLP. However, the Saheli breed carried both the T and C alleles in an allelic frequency of 0.2 and 0.8 respectively, and three genotypes, the homozygote for the mutant allele (C), heterozygote (CT) and the homozygote (T) allele in frequencies of 0.64, 0.32 and 0.04 respectively (Table 3). The chi-square ($\chi 2$) test showed that each breed was in Hardy-Weinberg equilibrium (HWE). Significant difference ($P \leq 0.05$) was observed among the genotype and alleles frequencies (Table 3).

Association analysis: Associations of different SNPs with the adult camel body weight in different breeds are given in Tables 4 and 5. With respect to the C419T SNP, no significant difference in body weight was found between different genotype variants in any breed (Table 4). With respect to the T450C SNP, males and females of the Saheli breed with the homozygote CC (mutant allele) had the heaviest body weights (842±16 and 644±34 kg; $P \leq 0.05$), 19-22% greater than the TT genotype. Individuals with the heterozygote CT had body weights intermediate between the two monozygotes. The body weight for CT and CC genotypes of the other three camel breeds was not recorded because these breeds did not show mutation in T450C, and they do not have CT or CC genotypes (Table 5).

DISCUSSION

Growth hormone is the major regulator of growth and metabolism in mammals, controlling growth rate, health, milk production, body composition, and aging through modulation of the expression of many genes (Sumantra *et al.,* 1992; Ho and Hoffmann, 1993; Lincoln *et al.,* 1995). The GH gene in mammals has 5 exons and 4 introns (Maniou *et al.,* 2004), of about 1.7 kb in sheep (Byrne *et al.,*1987) and porcine (Vize and Wells, 1987) and 1.9 kb in the camel (Maniou, 2003) and horse (Zhang *et al.,* 2004).

In previous studies on camels, only one SNP was detected at position 419 (C419T) in Sudanese (Ishag *et al.,* 2010), and Pakistani dromedary camels (Shah, 2006) compared with 13 SNPs in the current study. These additional SNPs have not been detected previously in camel GH and are novel to the current study. However, other SNPs in GH have been documented in other species. For example, more than 10 SNPs have been recorded in cattle (Chikuni *et al.,* 1994; Ge *et al.,* 2003*;* Musa, 2007), and seven SNPs in sheep (Bastos *et al.,* 2001). Twenty four and 19 SNPs were detected in *Osmanabadi* and *Sangamneri* goats respectively (Wickramaratne *et al.,* 2010), and 11 SNPs were identified in Iranian Fars native fowl GH (Aminafshar and Fathi, 2012).

The C419T SNP with C to T variation, where a restriction site for *Msp*I provoked by the "C" variant, was used as a special restriction fragment length polymorphism (PCR-RFLP) for genotype screening of the camel breeds studied. In the current study, the frequency of the T allele ranged from (0.44 to 0.47), and the frequency of C allele from 0.53 to 0.56. The genotype frequency revealed three genotypes, heterozygous (CT) was the same in Saheli, Majaheem and Homor breeds (0.50) and least frequent (0.49) in the Waddah breed. The homozygous (TT) had the highest genotype frequency (0.22) in the Majaheem and Homor breeds, while the homozygous genotype (CC) was most frequent (0.32) in the Wahhah breed. This is in line with the findings for Pakistani dromedary camels (Shah, 2006), but in Sudanese camel breeds, the frequency of the T allele in the Bishari and Anafi breeds (0.57 and 0.48) was significantly ($P<0.05$) higher than in those of Kenani, Rashaidi, Lahwee and Kabbashi breeds (0.32, 0.33, 0.30 and 0.33, respectively (Ishag *et al.,* 2010).

The other SNP that has been studied is T450C, in which T450 is substituted by C. That mutation was detected only in the Saheli breed. It is located in exon2 changing the code GTG of amino acid valine number 9 to GCG the code of amino acid alanine, this mutation resulted in a restriction site for *HinP1I* provoked by the "C" variant, and was used as special restriction fragment

Table 4: Association between genotypes of SNP (C419T) and body weight of adult male and female camels in some Saudi Arabian camel breeds (P>0.05).

Genotype	Estimated body weight (kg)							
	Saheli		Majaheem		Waddah		Homor	
	M	F	M	F	M	F	M	F
TT	790±34	605±25	670±23	571±09	611±10	554±34	633±18	517±10
TC	770±25	590±32	650±30	553±18	600±34	540±26	610±41	500±12
CC	783±36	612±26	658±24	560±15	590±26	530±31	600±35	511±14

Table 5: Association between genotypes of SNP (T450C) and body weight of adult male and females camel in some Saudi Arabian camel breeds

Genotype	Estimated body weight (kg)							
	Saheli		Majaheem		Waddah		Homor	
	M	F	M	F	M	F	M	F
TT	690±27[c]	540±34[c]	659±4	561±16	600±25	541±2	614±26	509±22
CT	780±20[b]	600±32[b]	nd	nd	nd	nd	nd	nd
CC	842±16[a]	644±34[a]	nd	nd	nd	nd	nd	nd

Values with different letters in the same column are significantly different at P≤0.05. nd=not detected.

length polymorphism (PCR-RFLP) for genotype screening of the camel breeds studied.

The C419T SNP was not associated with any difference in body weight in any breed. This strongly suggests that this mutation does not affect camel body weight. However, with respect to the T450C SNP, the three variants TT, TC and CC were observed only in the Saheli breed. In this breed, the individuals with the homozygote CC (mutant allele) had a body weight 19-20% greater than the TT homozygote, with the heterozygote CT being approximately intermediate; 7-8% greater than the TT homozygote respectively. This strongly suggests that this SNP may be the cause of the greater body weight that is apparent in the Saheli breed. This may result from the change in the single peptide of the GH, where the amino acid V9 changed to A. Further studies are needed to confirm this hypothesis. The other SNPs that appeared in Majaheem breed also warrant further investigation.

Conclusion: This study detected 13 SNPs (two insertion and eleven substitution) in the Majaheem camel breed, while only one SNP was detected in the Waddah and Homor breeds and two in the Saheli breed. The SNP T450C detected only in Saheli individuals was correlated with greater body weight so it may be a useful marker in the selection of camels for higher growth and meat production.

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