Phenotypic and molecular characterization of six Sudanese camel breeds

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Abstract

The objective of this study was to sequence the growth hormone (*GH*) gene in Sudanese camel breeds (Kenani, Lahwee, Rashaidi, Anafi, Bishari and Kabbashi) searching for single nucleotide polymorphisms (SNPs) and contribute to the phenotypic characterization of the multitude of camel ecotypes in Sudan. This will also afford the chance of investigating the possibility of the presence of correlations between body measurements and SNPs of *GH* gene. A length of 1732 bp, spanning the region between -44 bp upstream of the first exon and +37 bp downstream of the last exon was sequenced in two animals from each breed. The sequence comparison of Sudanese camel *GH* sequences with the GenBank sequence identified one single nucleotide polymorphism (SNP). The SNP was detected in the non coding region (intron 1) in position AJ575419:g.419C>T. A PCR-RFLP method was used to genotype 181 animals representing the six tested Sudanese breeds for detected SNP. The Bishari and Anafi breeds that are classified as riding camels had slightly higher T allele frequencies (0.57 and 0.48, respectively) than those of the other four breeds which are classified as pack camels. The effect of genotype with regard to the SNP g.419C>T on those traits was not significant.

Keywords: Camels, Sudan, growth hormone, polymorphisms, body measurements
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Introduction

Camels provide mankind with a range of products and services, e.g. wool, meat, milk and draught power. They have been domesticated about 3000 years ago and are present in high numbers in the arid parts of Africa (*ca*. 11.5 million animals in this region in 1992), particularly in the arid lowlands of eastern Africa (Somalia, Sudan, Ethiopia, Kenya and Djibouti) (Schwartz & Dioli, 1992). Sudan is rated second in numbers of camel population in the world after Somalia with an estimation of 4078 thousand head (Ministry of Animal Resources, 2006), concentrated in two main regions; the Eastern states (Butana plain and Red Sea mountains) and Western regions (Darfour and Kordofan) (Agab, 1993). The camel ecotypes in Sudan serve numerous functions in their respective production systems (e.g. milk, meat, racing, riding, packing) and are bred and selected for sustainable performance.

Phenotypic and genetic characterization to assess the existing biodiversity and differences among the Sudanese camel ecotypes is an essential prerequisite to facilitate the conservation and utilization programme in an effective and meaningful way. However, the Sudanese camel ecotypes are not well classified or defined, with very limited information available. The development in 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 substantial research interest because of their impending 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 much investigated in different animal species. Work based on characterization of candidate genes and their association with animal performance in camels is meagre compared with cattle (Lucy *et al*., 1991; Schlee *et al*., 1994; Ge *et al*., 2003), sheep (Wallis *et al*., 1998; Bastos *et al*., 2001) or goats (Wallis *et al*., 1998; Neelam Gupta *et al*., 2007).

In farm animals, promising candidate genes for many traits are in the growth hormone axis. The growth hormone is a polypeptide hormone with diverse biological activities including somatogenic (growth promoting), lactogenic, insulin-like and diabetogenic effects. The camel growth hormone (*GH*) gene extends over about 1900 bp, and like other mammalian *GH* genes; it splits into 5 exons and 4 introns (Maniou *et al.*, 2001).

There is no direct evidence of a correlation between body measurements and growth hormone in camels. However, Liu, *et al*. (2010) studied the correlation between GDF5 gene and body measurement in cattle, Ardiyanti, *et al*. (2009) investigated the association of *GH* gene and carcass traits in cattle, while Hua, *et al*. (2009) estimated the correlation between *GH* gene and growth traits in goats.

The aim of this study was to sequence the growth hormone (*GH*) gene in Sudanese camel breeds searching for single nucleotide polymorphisms (SNPs) and contribute to the phenotypic characterization of the multitude of camel ecotypes in Sudan. This will also afford the chance of investigating the possibility of the presence of correlations between body measurements and SNPs of the *GH* gene.

Materials and Methods

Hair samples were obtained from 181 unrelated individuals of Sudanese camels. Thirty one hair samples were collected from the Kenani breed and 30 hair samples from each of the Rashaidi, Lahwee, Anafi, Bishari and Kabbashi breeds. Genomic DNA was extracted from hair roots by using the Nucleospin® tissue kit (Macherey-Nagel), following manufacturer instructions. DNA concentration was measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc).

Based on the published nucleotide sequence information of the camel *GH* gene (GenBank accession no. AJ575419, Maniou *et al*., 2004) primer pairs were designed to amplify four *GH* fragments (Kgh1b, Kgh1, Kgh2 and Kgh3) by using primer 3 programme (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). The gene from -44 bp upstream of the first exon to +37 bp downstream of the last exon was analyzed. The primer sequences, location and size of the amplified fragments are shown in Table (1). PCR was performed in a reaction volume of 25 µL using 100 ng of DNA, 0.2 mM of each primer, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.5 units of GoTaq flexi-DNA polymerase (Promega).

The amplification programmes consisted of 37 cycles. The first one was characterised by denaturation at 94 °C for 2 min, annealing with the special primer temperature (Table 1) for 30 s and an extension step at 72 °C for 40 s. The next 36 cycles involved a denaturation step at 94 °C for 1 min, annealing at 51 to 57 °C for 30 s and extension at 72 °C for 40 s with the exception that in the last cycle the extension time was 10 min. The PCR products were visualized by ethidium bromide staining following electrophoresis on 2% agarose gel (Biorad) in TAE buffer and photographed under UV light.

Two animals of each breed were sequenced. The PCR products amplified by using the standard methods were cut from agarose gel (2%) and purified using JustSpin Gel Extraction columns (Genaxon). Nucleotide sequencing was carried out according to the dideoxynucleotide chain-termination technique (Sanger *et al*., 1977) by using a BigDye™ Terminator v1.1 Ready Reaction cycle sequencing kit and an ABI PRISM 310 nucleotide sequencer (Applied Biosystems).

The 181 animals of the six tested Sudanese camel breeds were genotyped for SNP AJ575419:g.419 C>T (intron 1) using the PCR-RFLP method. A 613 bp fragment (primer pair KGH1) covering the sequence containing the mutation site was amplified. The amplicon was digested with *MspI* restriction endonuclease (Promega) at 37 °C for four hours to distinguish between the two alleles. For each reaction, 15 µL of PCR product, 2 µL buffer, 2.5 µL H_2O and 0.05 µL enzyme containing 5 units of *MspI* were used. The digested fragments (C allele, unrestricted: 613 bp; T allele, restricted: 349 bp and 264 bp) were analyzed by electrophoresis in 2% agarose gels, stained with ethidium bromide and photographed under UV light (Figure 1).

A detailed questionnaire survey was designed to obtain information on general characteristics and descriptions of the camel breeds such as body colour, hair length and distribution, hump and udder size.

Chest girth was measured by metric tape immediately behind the breast pad; abdominal girth was determined over the highest part of the hump and the shoulder height was measured for each animal. Weights of animals were then estimated using the Boue formula (1949) as follows:

$$P = 53\ TAH$$

Where P is body weight (kg); T chest or heart girth (cm) which was taken immediately behind the chest; An abdominal girth (cm) which taken over the highest part of the hump; and H is shoulder height (cm).

Genotype and allele frequencies were determined by gene counting. The Chi-square test was employed to evaluate whether the populations were in Hardy-Weinberg equilibrium. However, the t-test was used to determine differences in gene frequencies between populations. The data on the estimation of body weights and phenotypic measurements of the different genotypes were subjected to analysis of variance (ANOVA) using the general linear model (GLM) from the Statistical Analysis Software (SAS Institute Inc., 2000). The statistical model used was:

$$Y_{ijkl} = \mu + S_i + A_j + G_k + e_{ijkl}$$

Where Y_{ijkl} is the observation on each trait of the *ijkl*th animal, μ is the general mean of each trait, S_i is the fixed effect of *i*th sex, A_j is the covariance of *j*th age, G_k is the fixed effect of the *k*th genotype and *eijkl* is the random error effect associated to the *ijkl*th observation.

Table 1 The primer sequences, location and size of the amplified fragments

Name	Annealing temperature (°C)	Product size (bp)	Sequence (5'-3')
KGH1B up	56	508	Cagggaccaattccaggat
KGH1B low			Ccatccctgaggagcttaca
KGH1 up	51	613	Gtcctgtggacagctcac
KGH1 low			Tgtcctcctcactgcttta
KGH2 up	57	671	Tcaggatgggtgctagtg
KGH2 low			Tggtgaagaccctgctgag
KGH3 up	57	687	Cttctcgctgctgctcatc
KGH3 low			Gcactggagtggcactttc

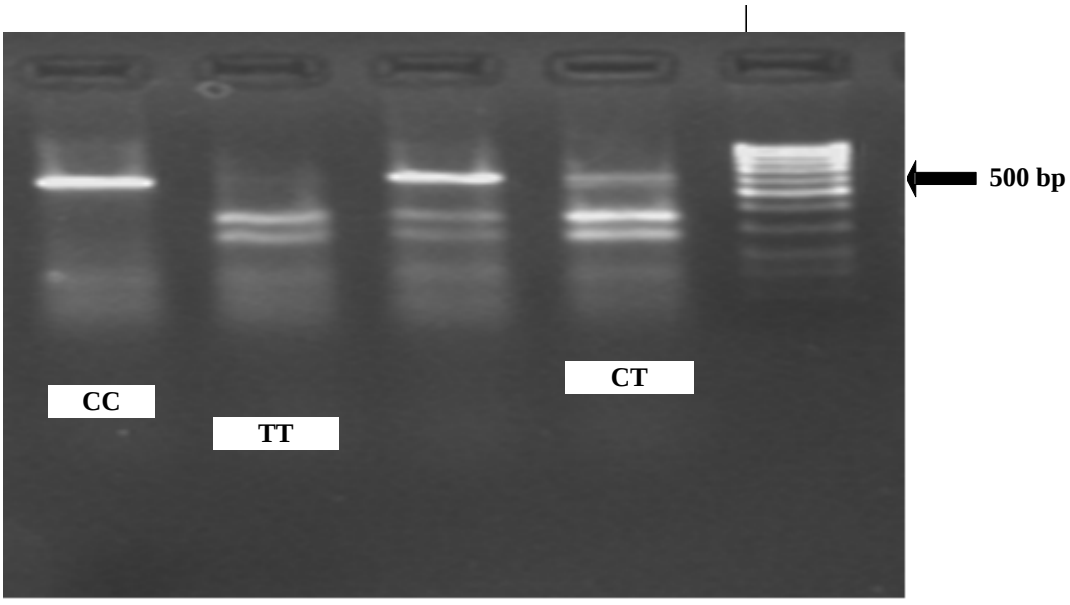

Figure 1 Different genotypes of *MspI* restriction for SNP g.419 C>T for all studied camel breeds.

Results

The nucleotide sequence of the *GH* gene of Sudanese camels resulted in 1732 bp, spanning the region between -44 bp upstream of the first exon and +37 bp downstream of the last exon. The sequence comparison of the tested six Sudanese camel breeds *GH* sequences with the references of GenBank sequence (AJ575419) descending from dromedary camels identified one single nucleotide polymorphism (SNP). The SNP was detected in a non coding region (intron 1) in position g.419C>T relative to the GenBank sequence.

The genotype frequencies of the SNP g.419C>T in intron 1 in Sudanese camels are listed in Table 2. All camel breeds were found to be carriers of the T allele with an allelic frequency ranging between 0.30 for the Lahwee breed and 0.57 for the Bishari. The heterozygous (CT) was most frequent among the Rashaidi breed and least frequent among the Kenani breed. The homozygous (TT) had the highest genotype frequency in the Bishari and Anafi breeds, while the homozygous genotype (CC) was most frequent among the Lahwee and Kenani breeds. The chi-square (χ^2) test showed that each breed was in Hardy-Weinberg equilibrium (HWE) ($P < 0.01$). Differences in genotypes and alleles frequencies between breeds were tested for significance using the t-test (Table 2). The frequency of the T allele in the Bishari breed was 0.57, which was significantly ($P < 0.05$) higher than in all those of other breeds except the Anafi breed.

Table 2 Genotype and allele frequencies of the SNP g.419 C >T in *GH* gene in Sudanese camel breeds

Breed	Genotypes			Alleles	
	TT	TC	CC	T	C
Kenani	0.19	0.26	0.55	0.32[a]	0.68
Rashaidi	0.10	0.47	0.43	0.33[a]	0.67
Lahwee	0.17	0.27	0.57	0.30[a]	0.70
Anafi	0.30	0.37	0.33	0.48[ab]	0.52
Bishari	0.37	0.40	0.23	0.57[b]	0.43
Kabbashi	0.13	0.40	0.47	0.33[a]	0.67
Overall	0.21	0.36	0.43	0.39	0.61

[a,b] Within columns allele frequencies with different superscripts differ significantly ($P > 0.05$).

Table 3 points out the phenotypic description of the six camel breeds studied. The results revealed that most ecotypes generally have more or less similar morphological features (grey, brown, yellow colour, large size, heavily built animals with a developed hump) except for the Rashaidi, Anafi and Bishari, which are classified as pack (heavy) camels and called Arabi camels. The Rashaidi camel is also classified as a pack camel but it has different phenotypic characteristics (dark grey, pinkish red colour, light weight and short at shoulders) compared to the other Sudanese pack camels. Moreover, the Rashaidi breed has large size udders and well developed milk veins which may qualify it to be classified as a dairy camel. The results of these phenotypic descriptions also showed that the Anafi and Bishari breeds have similar features (white, yellowish colour and light weight) and are classified as riding camels in Sudan.

The data in Table 4 present the least squares means and standard errors of the abdominal girth, chest girth, shoulder height and body weight. The estimated least squares means of the abdominal girth, chest girth, height at shoulder and body weight were 2.42 ± 0.02 m, 1.97 ± 0.01 m, 1.86 ± 0.01 m and 439.05 ± 4.75 kg, respectively. These results (Table 4) indicate that breed had a significant ($P < 0.01$) influence on all studied traits, while the SNP g.419C>T genotypes had no significant effects ($P > 0.05$) on those traits. The results also revealed that age of animal significantly ($P < 0.01$) influenced abdominal girth, chest girth and body weight. Sex had a significant ($P < 0.01$) effect on chest girth, height at shoulders and body weight. The results also showed that the KEN breed had significantly higher values for chest girth (2.08 ± 0.02 m) and body weight (501.65 ± 11.79 kg) compared to the other breeds. Male camels had a significantly ($P < 0.05$)

greater chest girth, shoulder height and heavier body weight than the corresponding traits of female camels. In addition, the homozygous genotype of the SNP g.419 (TT) had the highest, but not significantly different (P >0.05) abdominal girth, chest girth, height at shoulders and body weight; followed by those of the heterozygous (TC), while the homozygous (CC) had the lowest values.

Table 3 Phenotypic descriptions of the Kenana, Kabbashi, Rashaidi, Lahawee, Anafi and Bishari camel breeds in the Sudan

Characteristics	Breed					
	Kenani	Kabbashi	Rashaidi	Lahawee	Anafi	Bishari
Body colour	Dark brown, grey	Red, grey, yellow	Reddish, dark grey	Red, brown, yellowish	White, yellowish	White
Colour pattern	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform
Hair length	Medium, long	Medium, long	Short, medium	Medium	Short	Short
Wool distribution	Whole body	Whole body	Whole body	Whole body	Whole body	Whole body
Face profile	Flat	Flat	Flat	Convex	Flat	Concave
Rump profile	Roomy	Flat	Flat	Sloping	Flat	Flat
Hump size	Large, medium	Small	Small, medium	Large	Small	Small, medium
Hump orientation	Erect	Erect	Erect	Erect, bent sideways	Erect	Erect
Hump location	Middle, to the back	Middle	Middle	Middle	Middle, to the back	Middle
Ears size	Large	Medium	Large	Large	Large	Medium
Ears orientation	Erect	Erect	Backward	Erect	Forward	Backward, erect
Tail base	Thick	Thin	Thin	Thick	Thin	Thin
Tail length	Long	Short, medium	Medium	Medium	Long	Long
Udder size	Large, medium	Medium	Large	Medium, large	Rudimentary	Rudimentary
Teat size	Large, medium	Medium	Large	Medium, large	Rudimentary	Rudimentary

Discussion

The *GH* sequences of the six tested Sudanese camel breeds were aligned and compared with the GenBank camel *GH* sequence AJ575419. Only one SNP was identified in a non coding region (intron 1) in position AJ575419:g.419C>T. It is noteworthy to state that numerous mutations in this gene were documented in other species, *viz*. in cattle more than 10 SNPs have been recorded (Chikuni *et al*., 1994; Ge *et al*., 2003; Musa, 2007). Also, many SNPs have been reported in sheep (Bastos *et al*., 2001). Neelam Gupta *et al*. (2007) found several SNPs in the growth hormone gene of Black Bengal goats. The lack of SNPs in *GH* of Sudanese camels may be due to the probability that all these ecotypes may have originated from the same stock and not enough time has passed for segregation and generation of new mutants. The detected SNP in the *GH* gene of Sudanese camel was previously reported in Pakistani dromedary camels (Shah, 2006).

The Anafi and Bishari breeds tended to have a higher T allele frequency compared to those of the other four breeds. However, the difference in the T allele frequency was significant only between Bishari and the other four breeds. The Anafi and Bishari breeds have the same morphological appearance (white coat and relatively light weight), and both are classified as riding camels. This SNP is only one of a large probable number of mutations in the whole genome but it is possible that the higher T allele frequency in Anafi and Bishari is the result of a probable similar ancestral origin. However, these suggestions require extensive

studies to verify them. Other breeds (Kenani, Lahwee, Rashaidi and Kabbashi) have higher body weights and are classified as pack camels (draught animals). Generally they have almost similar T allele frequencies (0.30 to 0.33).

The results of phenotypic descriptions showed that the Rashaidi breed has large size udders and well developed milk vein which may qualify it to be classified as a dairy camel. These findings are similar to those reported by Wardeh (2004). Regarding the udder and teats feature, Kenani, Kabashi and Lahwee camels have well developed udders (medium to large size). This probably explains their capacity in milk production and may be classified as dual purpose (beef and dairy) camels.

Table 4 Least square means and standard errors of abdominal girth (AG), chest girth (CG), shoulder height (SH) and body weight (BW)

Parameter	No.	AG (m) Mean ± s.e.	CG (m) Mean ± s.e.	SH (m) Mean ± s.e.	BW (kg) Mean ± s.e.
Age group		**	**	NS	**
4 - 6 years	56	2.31 ± 0.03	1.92 ± 0.01	1.85 ± 0.01	404.36 ± 6.86
7 - 9 years	61	2.49 ^{b} ± 0.03	2.02 ^{b} ± 0.01	1.89 ^{a} ± 0.01	470.98 ^{b} ± 7.23
10 - 12 years	43	2.49 ^{b} ± 0.04	2.02 ^{b} ± 0.01	1.87 ^{a} ± 0.01	465.14 ^{b} ± 8.97
≥13 years	21	2.47 ^{b} ± 0.05	2.01 ^{b} ± 0.02	1.89 ^{a} ± 0.02	464.22 ^{b} ± 12.82
Breed		**	**	**	**
Kenani	31	2.51 ^{b} ± 0.04	2.08 ^{b} ± 0.02	1.95 ^{c} ± 0.01	501.65 ^{b} ±11.79
Rashaidi	30	2.58 ^{b} ± 0.04	1.96 ^{a} ± 0.02	1.78 ^{a} ± 0.01	439.10 ^{a} ± 12.76
Lahwee	30	2.50 ^{b} ± 0.04	1.99 ^{a} ± 0.02	1.86 ^{bc} ± 0.01	452.48 ^{a} ± 12.62
Anafi	30	2.40 ^{b} ± 0.04	1.96 ^{a} ± 0.02	1.86 ^{b} ± 0.01	424.83 ^{a} ± 11.94
Bishari	30	2.38 ^{ab} ± 0.04	1.97 ^{a} ± 0.02	1.86 ^{b} ± 0.01	424.37 ^{a} ± 12.34
Kabbashi	30	2.23 ^{a} ± 0.04	1.97 ^{a} ± 0.02	1.92 ^{c} ± 0.01	450.67 ^{a} ± 11.95
Sex		NS	**	**	**
Female	131	2.43 ± 0.02	1.96 ± 0.01	1.84 ± 0.01	432.02 ± 6.00
Male	50	2.44 ^{a} ± 0.03	2.02 ^{b} ± 0.01	1.91 ^{b} ± 0.01	465.68 ^{b} ± 9.60
SNPg.419C>T		NS	NS	NS	NS
TT	38	2.48 ± 0.03	2.01 ± 0.02	1.88 ± 0.01	463.21 ± 10.78
TC	65	2.43 ± 0.03	1.97 ± 0.01	1.87 ± 0.01	442.87 ± 8.43
CC	78	2.40 ± 0.03	1.99 ± 0.01	1.87 ± 0.01	440.48 ± 7.86
Overall mean		2.42 ± 0.02	1.97 ± 0.01	1.86 ± 0.01	439.05 ± 4.75

** Significant at P <0.01; NS not significant at P >0.05.
^{a,b,c} Within rows means with different superscripts differ significantly at P <0.05.

The body weights obtained in this study for the Bishari and Anafi breeds are in agreement with the findings of Wardeh (1989), Khouri (2000) and Wardeh (2004). Male camels had significantly higher body measurements than the females, which was similar to that reported by Dioli *et al.* (1992) and Mehari *et al.* (2007) who stated that there is quite distinctive sexual dimorphism in camels, i.e. the male camels is usually taller and of heavier weight than the females. These differences in tested traits between male and female camels may reflect differences in the hormonal secretions and their activities in the two sexes.

The age group, 7 to 9 years, had significantly higher values of the studied traits, followed by those of the age group 10 to 12 years, then those of the age group ≥13 years. However, the age group 4 to 6 years had significantly lower values of tested traits than those of the other age groups. This means that camels reach maturity (growth peak) within 7 to 9 years of age; after which the different measurements decline. This trend is reflected in the growth curve of the Sudanese camels.

An association was indicated between *GH* polymorphism and carcass characteristics in cattle (Ardiyanti *et al*., 2009). However, the evidence obtained from this study does not support the presence of any association between body measurements and *GH* polymorphism.

Conclusion

The Dromedary camel contributes significantly to family food security in semi dry and dry climates, and is a major component of the agro-pastoral systems in vast pastoral areas in Asia and Africa. This study demonstrated that only one single nucleotide polymorphism was detected in the growth hormone gene of the studied Sudanese camel breeds after sequencing. Though it is located in a non coding region (intron 1), it can be used as a genetic marker in different genetic studies, e.g. to determine genetic relationships and to establish the phylogenic tree for different camel breeds. This study showed that there were no associations between growth hormone genotypes and body measurements. Further research and more studies with larger numbers of animals are required to investigate these associations between growth hormone genotypes and camel body measurements.

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