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ORIGINAL ARTICLE

Genetic Characterization of Growth Hormone Gene in Dromedary Camels

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Abstract

INTRODUCTION: Advances in molecular genetic techniques have simplified the process of identifying individual differences at the DNA level. Genetic polymorphisms at candidate genes controlling economic features have recently drawn a lot of scholarly attention due to their potential as a tool for genetic selection and the identification of evolutionary linkages in livestock.
OBJECTIVE: This work aimed to explore the genetic characteristics of the *GH* gene in dromedary camels.
METHODS: Thirty dromedary she-camels were used to provide blood samples using disodium EDTA as an anticoagulant for DNA extraction. Using the polymerase chain reaction (PCR), the 613-bp *GH* gene was amplified. The dromedary she-camels that were enrolled were examined for polymorphisms in the *GH* gene (613 bp) using the PCR-DNA sequencing method.
RESULTS: The *GH* gene sequence comparison among the camels under investigation revealed nucleotide sequence variation in the form of the A430C single nucleotide polymorphism (SNP), which has been submitted to GenBank under the accession numbers gb|PP265532| and gb|PP265533.
CONCLUSION: Selecting camels may involve breeding techniques and marker-assisted selection (MAS), as the indicated SNP in the *GH* gene may be considered a genetic marker that may be used to predict productive performance.

Keywords: Dromedary camels, Genetic polymorphism, Growth hormone gene

1. Introduction

In semi-arid and desert parts of Asia and Africa, camels are essential to millions of people's livelihoods and play important socioeconomic roles. During severe droughts, camels proved to be the most resilient domestic animals [1]. The camel has survived several droughts and is still breeding and producing. The camel, with its versatility in a wide range of temperatures, is the best species to change genetically in reaction to global warming. The science of genomics has raised consumer awareness, and as a result, camel breeding companies are increasingly emphasizing meat quality and considering quality attributes to be essential components of selection programs.

Over the past 10 years, camel meat quality has improved dramatically through genetic research, which is essential to maintaining meat consumption. Studies have indicated the possible advantages of using camel meat to cure stickiness, sciatica, cancer, and infections [2]. Egypt has four main types of camels: the Maghrabi, which is used for milk and meat; the Somali, Sudani, Falahi or Baladi, which is used for agriculture and transportation; and the Mowallad, a hybrid of the Maghrabi and

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Falahi [3]. Therefore, the secret to enhancing animals may lie in both environmental factors and genetic abilities.

Molecular genetic markers are a powerful tool for genome research because they allow one to connect heritable traits to underlying chromosomal variance. Advances in molecular genetic techniques have simplified the process of identifying individual differences at the DNA level. Genetic polymorphisms at candidate genes controlling economic features have recently drawn a lot of scholarly attention due to their potential as a tool for genetic selection and the identification of evolutionary linkages across different cattle breeds [4]. Because it is widely distributed and abundant throughout the genome, this type of polymorphism accounts for the majority of genetic diversity. Development of single nucleotide polymorphism (SNP) markers including the coding region of the genome is necessary to understand the relationship between genetic and phenotypic variations in camels or other animals. In the mammalian genome, single base pair mutations, or SNPs, happen about every 1000 base pairs (bp) [5].

In ruminants, galactopoesis and lactation persistence are known to be caused by *GH*. It promotes the production of more protein, DNA, RNA, and glycogen and accelerates the breakdown of glucose, higher fatty acids, and depot fat in the tissues. It balances physiological processes to distribute nutrients for the production of milk [6]. Biological effects of growth hormone (*GH*), a polypeptide hormone, include those that are insulin-like, diabetogenic, lactogenic, and somatogenic (growth-promoting). The polypeptide chain of *GH* contains 191 amino acid residues, and its molecular weight exceeds 22 000. Similar to other *GH* genes found in mammals, the camel *GH* gene is divided into 5 exons and 4 introns. About 1900 bp make up its length [7]. *GH* can have a markedly positive effect on milk yield or growing performance because it alters tissue metabolism, which includes how food is partitioned. In agricultural animals, breastfeeding, reproduction, and metabolism are all regulated by the *GH* axis [8]. The *GH* gene can be used as a candidate gene for marker-assisted selective (MAS) breeding in native camels after possible correlations with the development and production attributes. Numerous studies have examined the *GH* gene polymorphism and its effects on growth rate and milk production-related factors in a variety of livestock species [9–11].

This study's goal was to investigate the genetic characterization of the *GH* gene in dromedary camels through the use of PCR-DNA sequencing.

2. Materials and methods

2.1. Ethics statement

The research ethics committee at Mansoura University's Faculty of Veterinary Medicine set rules for the care of experimental animals, which were adhered to in the collection of samples and handling of the study's animals. (code M/179).

2.2. Animals

A private farm close to El-Amria in Alexandria, Egypt, donated thirty dromedary camels that appeared to be in good health. A 10 ml of blood were extracted from each camel's jugular vein using a vacutainer tube that contains EDTA as an anticoagulant in order to prepare the blood for DNA extraction.

2.3. DNA extraction and polymerase chain reaction (PCR) amplification

Genomic DNA was extracted from whole blood (QIAGEN, Germany, Lot No. 163040813) using a QIAGEN whole blood genomic DNA extraction kit and the manufacturer's instructions. The DNA's quality, purity, and concentration were assessed by Nanodrop in preparation for more investigation. PCR technique was performed to amplify fragments of 5′ UTR of *GH* (613-bp). The primer sequences were designed according to the PubMed published sequence of *Camelus dromedarius* (>MT478654.1 *C. dromedarius* isolate B490 *GH* precursor gene, *GH*-T allele, exons 1 and 2 and partial cds) is:

F: 5′- GTCCTGTGGACAGCTCAC -3′.

R: 5′- TGTCCTCCTCACTGCTTTA -3′.

A 100 μl total volume PCR blend was created using a thermal cycler. Included in each reaction volume were 1.5 μl of each primer, 50 μl of PCR master mix (Jena Bioscience, Germany), 41 μl H_2O (distilled water), and 6 μl DNA. Four minutes were spent subjecting the reaction mixture to a denaturation temperature of 94 °C. 35 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 45 s, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min comprised the cycling procedure. Samples were kept in storage at 4 °C. Representative PCR analysis findings were identified using agarose gel electrophoresis, and fragment patterns were observed using a UV visualization equipment (USA: Gel Document, Alpha-chem. Imager).

2.4. DNA sequencing

PCR products containing target bands were utilized to sequence DNA in both forward and reverse orientations using an ABI 3730XL DNA sequencer (Applied Biosystem, USA) employing the enzymatic chain terminator technique developed by Sanger *et al*. [12]. Utilizing BLAST 2.0 and Chromas 1.45, the DNA sequencing data was analyzed [13]. To ensure adequate concentrations and purity, as well as a high yield, the PCR product was measured using a Nanodrop (Uv–Vis spectrophotometer Q5000/USA) [14]. SNPs were identified as differences between the PCR findings of the gene under study and the reference sequences stored in GenBank. Based on the data alignment from DNA sequencing, the MEGA6 software program was used to assess variance in the amino acid sequence of the studied genes across the tested animals [15].

3. Results

3.1. Genetic characterization of GH gene in dromedary camels

PCR-DNA sequencing for 613-bp fragment of *GH* gene elaborated nucleotide sequence variants in the form of A430C SNP among thirty she-camel. Representative amplified DNA nucleotides submitted to GenBank with accession codes gb|PP265532| and gb|PP265533.

Out of the thirty dromedary she-camels; eight of them possessed the A430C SNP. The detected SNP was verified by utilizing typical DNA sequence differences between the nucleotide sequence obtained from GenBank and the *GH* gene examined in the investigated dromedary camels (Fig. 1). Fisher's exact test revealed a significant difference ($P < 0.01$) in the frequency of the identified SNP among the enrolled dromedary she-camel.

As illustrated in Fig. 2, the neighbor-joining phylogenetic tree of the representative sequenced samples, when compared with the GenBank reference accession number gb|MT478654.1|, revealed that animals exhibiting the discovered SNP were located in one cluster, and the remaining animals were in a different cluster.

4. Discussion

In this study, PCR-DNA sequencing was used to molecularly characterize a 613-bp fragment of the camel *GH* gene in dromedary she-camels. Our findings showed that the nucleotide sequences of the enrolled she-camels varied (submitted to GenBank with accession codes gb|PP265532| and gb|PP265533|). It is interesting to note that the specified SNP was considered unique compared with the GenBank sequence.

To maximize camel potential, camel genetic variety must be preserved while camel genetic progress takes place. Many studies have examined the genetic variety of the camel GH gene, however, because camels have not undergone extensive selection, it has been suggested that camels have a high genetic variability [16,17]. These variations have been found using several genome sequencing programs referred to as expressed sequence tag programs [18]. After they are gathered, these markers are quite helpful because they are simple to use and repeatable. Though their application in dromedaries is still relatively new, they have been employed in several studies concerning genetic diversity and relevant phenotypic traits for other animals. In recent studies, they are starting to be applied to evaluate gene diversity at the individual gene level [19]. The main driving force for gene mapping in domestic animals is the potential to use gene maps to locate and map the genetic loci responsible for genetic variation in traits of economic value. The ultimate objective is to apply this knowledge to MAS using DNA level polymorphisms [20]. The purpose of this study was to highlight the genetic characteristics of the *GH* gene and explore potential applications for marker-assisted selection.

The genotypes of the 419 C greater than T SNP were found in six Sudanese camel breeds: Kenani, Lahwee, Rashaidi, Anafi, Bishari, and Kabbashi. While the Bishari and Anafi breeds are classified as riding camels, the T allele frequencies of the four breeds of camels that fall within the pack camel category were slightly lower [21].

Mohamed *et al*. 2013 [22] discovered SNPs in the coding region of the *GH* gene using PCR product restriction employing MspU (419 C→T) and HinPII (450 T→C) endonucleases after the gene region was amplified. Ali *et al*. 2014 [23] found that the 450 T > C SNP in the *GH* gene was significantly correlated with the higher estimated body weight. Also Shawki *et al*. 2015 [24] discovered an SNP (419 C > T) in the *GH* intron1 after genotyping 23 Maghrabi camels bred in Egypt. While Hedayat-Evrigh *et al*. 2015 [25] used the sequencing and aligning method to identify seven mutations in the *GH* gene. Two of these involved substitution mutations that changed the amino acid sequence of the *GH* protein.

Abdel-Aziem *et al*. 2015 [26] amplified a 613-bp segment of camel *GH* in five breeds of camels farmed in Egypt: Somali, Mowaled, Maghrabi, Falahy, and

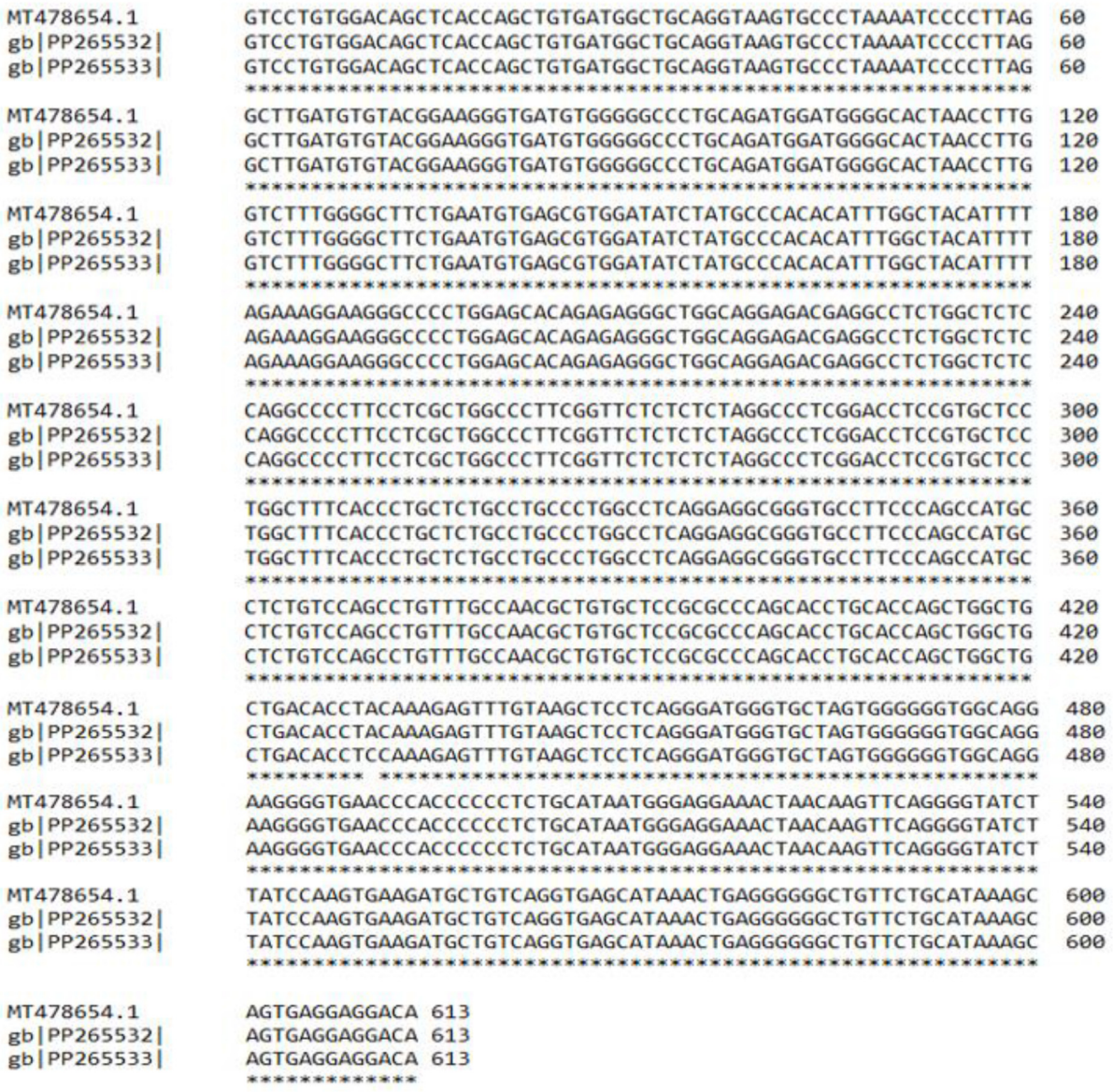

Fig. 1. The identified growth hormone gene single nucleotide polymorphism using representative DNA sequence discrepancies between the dromedary camels studied in this investigation and the nucleotide sequence acquired from GenBank.

Sudani. The results show that compared with the other four investigated camel breeds, the Maghrabi breed—which is classified as a dual-purpose camel breed—had a higher frequency for allele C (0.75). Sabahat *et al*. 2020 [27] obtained Marecha camel DNA samples from the Camel Breeding and Research Station located in Rakhmani Bhakar, Pakistan. Whereas the *GHR* gene has three significant polymorphism sites, the *GH* gene only has two. Among them, the *GH* gene's T1720A polymorphism changed leucine to histidine, while the *GHR* gene's A211927G polymorphism changed methionine to valine. In 93 Indian camels, Jyotsana *et al*. 2021 [28] amplified a 613-bp region of the camel *GH* gene (38 Jaisalmeri and 55 Sindhi camels). The PCR-RFLP investigation revealed three genotypes in both breeds: CC, CT, and TT, using the restriction enzyme *MspI*. It was found that the CT genotype predominated in Sindhi breeds, whereas the CC genotype predominated in Jaisalmeri breeds. The C allele was more prevalent than the T allele in both breeds.

The results of this study are new SNPs in the dromedary camel breed's *GH* gene. There may be other candidates for this gene to be investigated in the hunt for markers connected to camel production attributes. It can be used in camel breeding initiatives that aim to improve the growth characteristics of Egyptian-bred camel breeds through MAS. The

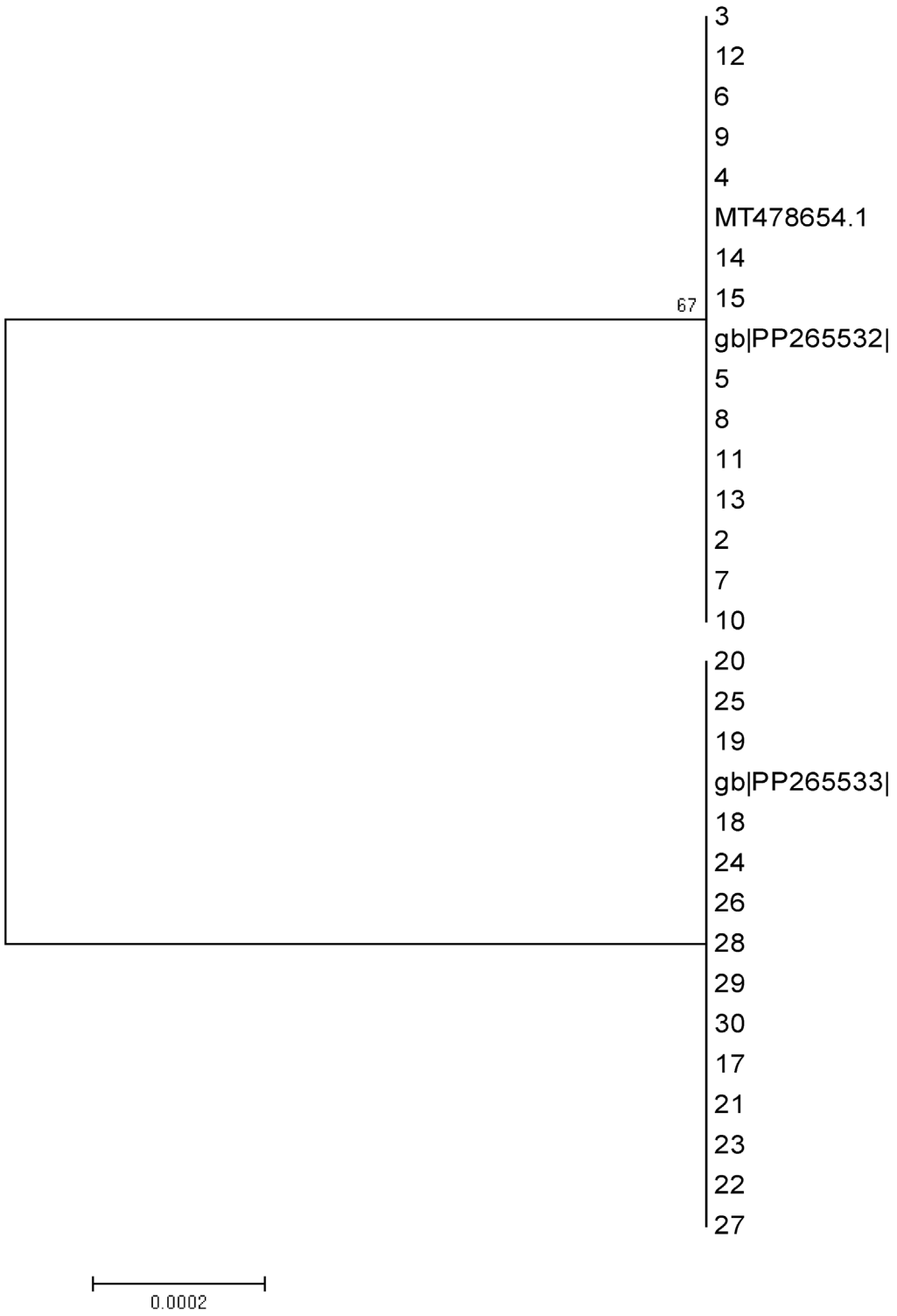

Fig. 2. Neighbor joining phylogenetic tree of growth hormone gene between investigated dromedary camels compared with the reference accession number gbjMT478654.1j.

body weight and daily increase can be predicted using these discovered SNPs as Al-Sharif *et al*. 2022 [29] collected blood samples from seventy camels using vacutainer tube containing EDTA as an anticoagulant for DNA extraction. The 5′ UTR of the *GH* gene (286 bp) was examined for polymorphisms using PCR-DNA sequencing. SNPs were found in the *GH* gene among the enrolled camels. Also Afifi

et al. 2014 [30] used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique to genotype 200 animals from each breed to identify SNPs. The Waddah and Homor breeds each have one SNP at position 419 (C419T), whereas the Majahem breed had thirteen SNPs (two insertions and eleven substitutions). There are two SNPs (C419T and T450C) in the Saheli breed. Among them, the T450C SNP was associated with an increased estimated body weight. El-Kholy *et al*. 2016 [31] revealed that one SNP alteration in the 377 A→T position of the *MYF5* gene coding region resulted in the substitution of lysine for the amino acid residue methionine. Three SNP polymorphisms were discovered in the *GH* gene 5′-flanking region that are associated with the meat productivity indices, namely in the 111 (G→A or G→C) and 380 (G→A) locations.

In genome-wide association studies and large-scale candidate gene studies, researchers often scan a large number of SNP markers one by one to detect SNP-trait association signals. Single SNP analysis has been favored as a simple and effective method, assuming that the size and design of the studies are sufficient to capture the marginal direct or indirect link of an SNP with complex economic variables [32,33].

5. Conclusion

Nucleotide sequence variations were found in the *GH* gene using PCR-DNA sequencing in the thirty dromedary she-camels that were recruited. One potential genetic marker for predicting daily weight gain and body weight is a SNP in the *GH* gene. In the future, when more camels are being studied, breeding techniques and MAS may be employed in camel selection.

Ethical approval

The authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page, with approval number (code M/179) obtained from the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University, Egypt.

Availability of data and materials

The Data that support the findings of this study are available from the corresponding author upon reasonable request.

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Authors' contributions

A.A. writing review and editing, writing original draft, methodology, data curation, conceptualization. A.K. writing original draft, methodology. A. AE. and H.E. writing original draft, data curation.

Conflict of interest

The authors declare no conflicts of interest.

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