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Detection of *Msp*I polymorphism and the single nucleotide polymorphism (SNP) of *GH* gene in camel breeds reared in Egypt

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Growth hormone (GH) is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary gland in a circadian and pulsatile manner, the pattern of which plays an important role in postnatal longitudinal growth and development, tissue growth, lactation, reproduction as well as protein, lipid and carbohydrate metabolism. The aim of this study was to detect the genetic polymorphism of *GH* gene in five camel breeds reared in Egypt which are Sudany, Somali, Mowaled, Maghrabi and Falahy, using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) technique. Also, this work aimed to identify the single nucleotide polymorphism (SNP) between different genotypes detected in these camel breeds. The amplified fragment of camel *GH* at 613-bp was digested with the restriction enzyme *Msp*I and the result reveals the presence of three different genotypes; CC, CT and TT in tested breeds. Significant differences were recorded in the genotype frequencies between these camel breeds. The result shows that the Maghrabi breed that is classified as a dual purpose camels had higher frequency for allele C (0.75) than those in the other tested four breeds. The sequence analysis declared the presence of a SNP (C264T) in the amplified fragment which is responsible for the elimination of the restriction site C^CGG and consequently the appearance of two different alleles C and T. The nucleotide sequences of camel *GH* alleles T and C were submitted to nucleotide sequences database NCBI/Bankit/GenBank and have accession numbers: KP143517 and KP143518, respectively. It is concluded that only one SNP C→T was detected in *GH* gene among the five tested camel breeds reared in Egypt and this nucleotide substitution can be used as a marker for the genetic biodiversity between these camel breeds. Also, due to the possible association between allele C and higher growth rate, we can used it in marker assisted selection (MAS) for camels in breeding program as a way for enhancement of growth trait in camel breeds reared in Egypt.

Key words: Camel breeds in Egypt, *GH*, PCR, RFLP, SNPs.

INTRODUCTION

The population of old world camelidae in the world is estimated to be 18.5 million heads. Dromedary camels comprise 95% of them while the remaining 5% are Bactrian camel. Bactrian camels are found mainly in the cold high altitudes of Asia. The Near East, North Africa and the Sahel region have about 70% (12.6 million) of the world's Dromedary camels. Somalia and Sudan together own about half of this number (Kesseba et al., 1991). In Egypt, the camel population was about 120 thousand head, (SADS, 2009). It was reported that many camel

breeds are reared in Egypt but the main camel breeds are Maghrabi (a dual purpose animal; (meat and milk) and Falahy, Sudany, Somali and Mowaled (meat type animal) (Mahrous et al., 2005). Camels are economically important animals in Egypt where they are dual purpose animals. In the Nile Valley and Delta, they are mainly raised for meat production and for some agricultural labors. In the desert, they are raised equally for meat and milk production, while some for labors and transport, and some especially for camel racing. The main biological role of growth hormone is to control postnatal growth, although it also has effects on metabolic regulation, lactation and reproduction (Louveau and Gondret, 2004; Daverio et al., 2012). Growth hormone gene, with its functional and positional potential role in the regulation of growth and metabolism in animals, has been widely used for marker in several livestock species, including the cattle (Dybus, 2002; Ge et al., 2003; Beauchemin et al., 2006; Katoh et al., 2008), sheep (Marques et al., 2006) and goat (Malveiro et al., 2001; Boutinaud et al., 2003). The camel growth hormone gene extends over about 1900 bp, and as other mammalian *GH* genes, it is organized in five exons separated by four introns (Maniou et al., 2004). It is a 22 KDa single chain polypeptide primarily produced and secreted by the somatotrophs of the anterior pituitary gland in circadian and pulsatile manner (Dybus, 2002).

Genetic polymorphism can be identified by several techniques; one of the most commonly used methods is PCR-RFLP. It is a powerful method for identifying nucleotide sequence variation in amplified DNA and can detect single base substitutions in enzymatic restriction sites (Amie Marini et al., 2012). The present study aimed to detect the genetic polymorphism of *GH* gene in some camel breeds reared in Egypt using PCR-RFLP technique and identify SNPs between different genotypes detected in these breeds.

MATERIALS AND METHODS

Blood samples and DNA extraction

Blood samples were collected from jugular vein of 15 individual camels from each tested breed; Fallahi, Maghrabi, Mowalled, Sudany and Somali. Genomic DNA was extracted from the whole blood according to the method described by Miller et al. (1988) with minor modifications. Briefly, blood samples were mixed with cold 2x sucrose-triton x-100 and centrifuged at 5000 rpm for 15 min at 4°C. The nuclear pellet was suspended in lysis buffer, sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water bath at 37°C. Nucleic acids were extracted with saturated NaCl solution and then washed with 70% ethanol. The DNA pellet was dissolved in 1x TE buffer and its concentration was determined by using NanoDrop1000 (Thermo Scientific) Spectrophotometer. Thereafter, it was diluted to the working concentration of 50 ng/µl to be used for polymerase chain reaction.

Polymerase chain reaction (PCR)

A PCR cocktail containing 1.0 mM forward and reverse primers specific for growth hormone gene, 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin (w/v), 0.1% Triton X-100 and 1.25 units of Taq polymerase was used. The cocktail was aliquot into PCR tubes with 100 ng of camel DNA. The reaction was run at 94°C for 5 min, 35 cycles of 94°C for 1 min, 55°C for 30 s, 72°C for 45 s and a final extension at 72°C for 5 min. The PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide to check amplification product. The primers used in this study were designed on the basis of DNA sequence of the camel *GH* gene (Ishag et al., 2010). The primer sequences are: F: 5′-GTCCTGTGGACAGCTCAC-3′ and R: 5′-TGTCCTCCTCACTGCTTTA-3′

RFLP Genotyping

In order to detect variants of *GH* gene in different camel breeds, the PCR products were digested using restriction enzyme; *Msp*I (Fermentas). 10 µl of PCR product was digested with 1 µl of FastDigest restriction enzymes for 5 min at 37°C. The restriction fragments were subjected to electrophoresis in 2% agarose/ ethidium bromide gel (GIBCO, BRL, England) in 1x TBE buffer (0.09 M Tris-boric acid and 0.002 M EDTA). Gels were visualized under UV light and documented in FX Molecular Imager apparatus (BIO-RAD).

DNA sequencing

The PCR products representing each detected genotype of *GH* gene in different camel breeds, were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite to detect each single nucleotide substitution between different detected genotypes. Results of endonuclease restriction were carried out using FastPCR. The nucleotide sequences of different alleles for camel *GH* gene were submitted to GenBank (NCBI, BankIt).

RESULTS AND DISCUSSION

Growth hormone has proven to be the major regulator of postnatal growth and metabolism in mammals and thus affects growth rate, body composition, health, milk production and aging by modulating the expression of many genes (Carnicella et al., 2003; Ge et al., 2003). Growth hormone has also been suggested as responsible for more important and prolonged cell proliferation in hypertrophied animals (Schoenfeld, 2010). The growth hormone gene in mammals was comprised of five exons and four introns (Maniou et al., 2004). The coding region of *GH* consisted of five exons,

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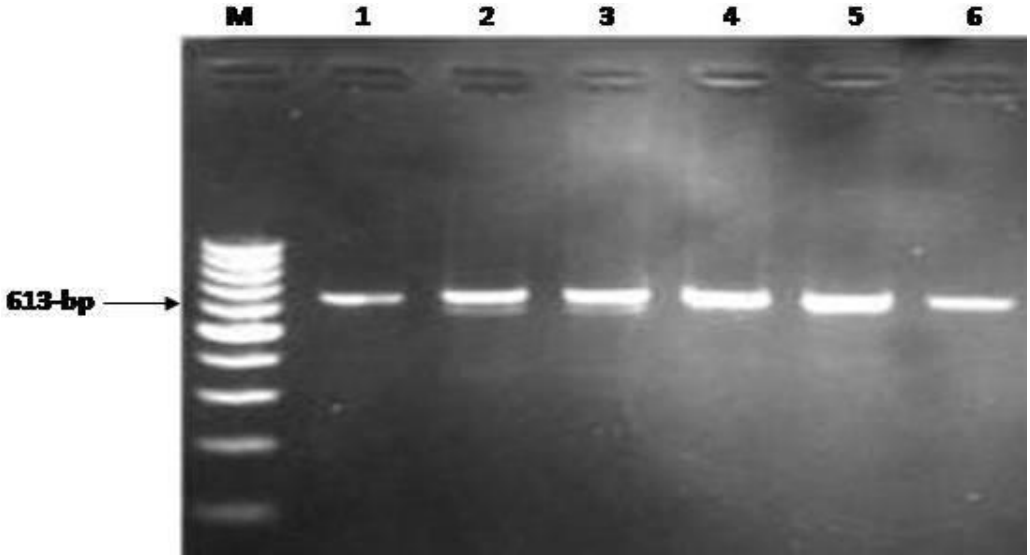

Figure 1. Electrophoretic pattern of PCR-amplified fragment from *GH* gene in camels. M: 100-bp ladder marker. Lanes 1-6: 613-bp PCR product amplified from camel DNA.

covering a total transcribed area of about 1.7 kb in sheep and humans (Byrne el al., 1987; Vize and Wells,1987), 2.7 kb in cattle (Yao et al.,1996) and 1.9 kb in camel (Maniou, 2003) and horse (Zhang et al., 2004). Camels provide mankind with a range of products and services; like 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, particularly in the arid lowlands of Eastern Africa (Somalia, Sudan, Ethiopia, Kenya and Djibouti) (Schwartz and Dioli, 1992). 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 (Ishag et al., 2010). Camels are economically important animals in Egypt where they are dual purpose animals (meat and milk). Improvement of camel productivity and detection of biodiversity between different camel breeds has necessitated the genotyping of the productivity trait genes in these breeds. The development in molecular genetics 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). We aimed in this work to detect the genetic polymorphism of *GH* gene in some camel breeds reared in Egypt using PCR-RFLP technique and identify SNPs between different genotypes detected in these breeds. The primers used in this study flanked 613-bp fragments consist of 36 base pairs from exon 1, 243 base pairs from intron 1, 161 base pair from exon 2 and 173 base pairs

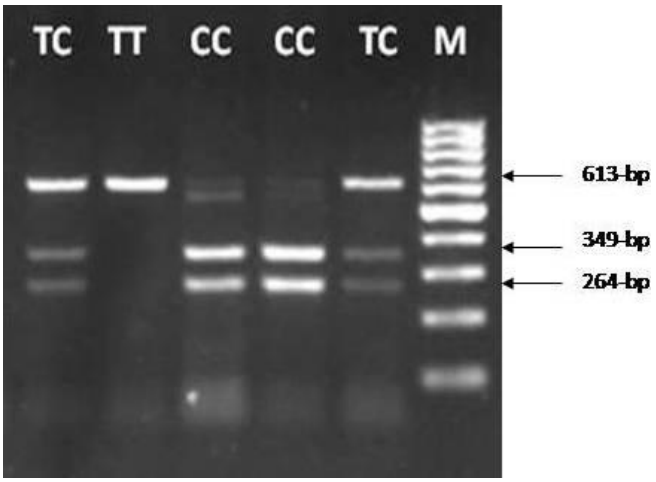

Figure 2. Different genotypes obtained after digestion of PCR products of camel *GH* gene with *Msp*I; TT: homozygous genotype with one undigested fragment at 613-bp, TC: heterozygous genotype with three fragments at 613-, 349- and 264-bp and CC: homozygous genotype with two digested fragments at 349- and 264-bp. M: 100-bp ladder marker.

from intron 2 of camel *GH* gene. The amplified fragments obtained from all tested camels were of 613-bp (Figure 1). These PCR amplified fragments (613-bp) were digested with *Msp*I endonuclease. Depending on the presence or absence of the restriction site (C^CGG) at position 264^265, we can easily differentiate between three different genotypes: CC with two digested fragments at 349-and 264-bp, TT with undigested one fragment at 613-bp and CT with three fragments at 613-, 349- and 264-bp (Figure 2).

Table 1. Genotype and allele frequencies of the *GH* gene in different camel breeds.

Camel breed	Genotype frequencies			Allele frequencies	
	CC	CT	TT	C	T
Falahy	0.20	0.40	0.40	0.40	0.60
Maghrabi	0.50	0.50	0.00	0.75	0.25
Mowaled	0.10	0.40	0.50	0.30	0.70
Somali	0.09	0.18	0.73	0.18	0.82
Sudany	0.23	0.62	0.15	0.54	0.46
Total	0.22	0.42	0.36	0.43	0.57

GTCCTGTGGACAGCTCACCAGCTGTGATGGCTGCAGGTAAGTGCCCTAAAATCCCCTTAGGCTTGATGTGTACG
GAAGGGTGATGTGGGGGCCCTGCAGATGGATGGGGCACTAACCTTGGTCTTTGGGGCTTCTGAATGTGAGCGT
GGATATCTATGCCCACACATTTGGCTACATTTTAGAAAGGAAGGGCCCCTGGAGCACAGAGAGGGCTGGCAGG
AGACGAGGCCTCTGGCTCTCCAGGCCCCTTCCTCGCTGGCCCTCCGGTTCTCTCTCTAGGCCCTCGGACCTCC
GTGCTCCTGGCTTTCACCCTGCTCTGCCTGCCCTGGCCTCAGGAGGCGGGTGCCTTCCCAGCCATGCCTCTGT
CCAGCCTGTTTGCCAACGCTGTGCTCCGCGCCCAGCACCTGCACCAGCTGGCTGCTGACACCTACAAAGAGTTT
GTAAGCTCCTCAGGGATGGGTGCTAGTGGGGGGTGGCAGTAAGGGGTGAACCCACCCCCCTCTGCATAATGGG
AGGAAACTAACAAGTTCAGGGGTATCTTATCCAAGTGAAGATGCTGTCAGGTGAGCATAAACTGAGGGGGGCTG
TTCTGCATAAAGCAGTGAGGAGGACA

Figure 3. Nucleotide sequence of allele C with nucleotide **C** at position 264 in the amplified fragment.

GTCCTGTGGACAGCTCACCAGCTGTGATGGCTGCAGGTAAGTGCCCTAAAATCCCCTTAGGCTTGATGTGTACG
GAAGGGTGATGTGGGGGCCCTGCAGATGGATGGGGCACTAACCTTGGTCTTTGGGGCTTCTGAATGTGAGCGT
GGATATCTATGCCCACACATTTGGCTACATTTTAGAAAGGAAGGGCCCCTGGAGCACAGAGAGGGCTGGCAGG
AGACGAGGCCTCTGGCTCTCCAGGCCCCTTCCTCGCTGGCCCTTCGGTTCTCTCTCTAGGCCCTCGGACCTCC
GTGCTCCTGGCTTTCACCCTGCTCTGCCTGCCCTGGCCTCAGGAGGCGGGTGCCTTCCCAGCCATGCCTCTGT
CCAGCCTGTTTGCCAACGCTGTGCTCCGCGCCCAGCACCTGCACCAGCTGGCTGCTGACACCTACAAAGAGTTT
GTAAGCTCCTCAGGGATGGGTGCTAGTGGGGGGTGGCAGTAAGGGGTGAACCCACCCCCCTCTGCATAATGGG
AGGAAACTAACAAGTTCAGGGGTATCTTATCCAAGTGAAGATGCTGTCAGGTGAGCATAAACTGAGGGGGGCTG
TTCTGCATAAAGCAGTGAGGAGGACA

Figure 4. Nucleotide sequence of allele T with nucleotide **T** at position 264 in the amplified fragment.

The result shows that all tested camel breeds have the three genotypes with different frequencies with the exception of Maghrabi breed which carries only CC and CT genotypes with absence of TT genotype (Table 1). The highest frequencies of three genotypes were present in Maghrabi breed for CC homozygous genotypes (0.50), Sudany breed for CT heterozygous genotype (0.62) and Somali breed for TT homozygous genotype (0.73). The frequencies for alleles C and T ranged from 0.18 to 0.75 and from 0.25 to 0.82, respectively, in tested camel breeds. These three detected genotypes are resulted from the presence of two different alleles; C (Figure 3) and T (Figure 4) in tested camel animals. The sequence analysis of the purified PCR products representing these three detecting genotypes CC, CT and TT declared the presence of a single nucleotide polymorphism (C→T) at position 264 which is responsible for the elimination of restriction site C^CGG and consequently the differentiation between these three different genotypes C/C (Figure 5), C/T (Figure 6) and T/T (Figure 7). The nucleotide sequences of camel *GH* alleles T and C were submitted to nucleotide sequences database NCBI/ Bankit/GenBank and have accession numbers: KP143517 and KP143518, respectively. In the present study, sequence analysis revealed a single nucleotide polymorphism C→T, where nucleotide "C" furnished cutting site for *Msp*I restriction enzyme. Restriction reaction resulted three different genotypes; homozygous without restriction (TT), homozygous with restriction (CC) and heterozygous animals (T/C). The results show significant differences in the allele frequencies between the breeds. This finding agrees with the results of Shah (2006) which explained the significant differences in allele frequencies among Pakistani camel breeds and also the similar results were reported by Ishag et al. (2010) in Sudanese camel breeds where they

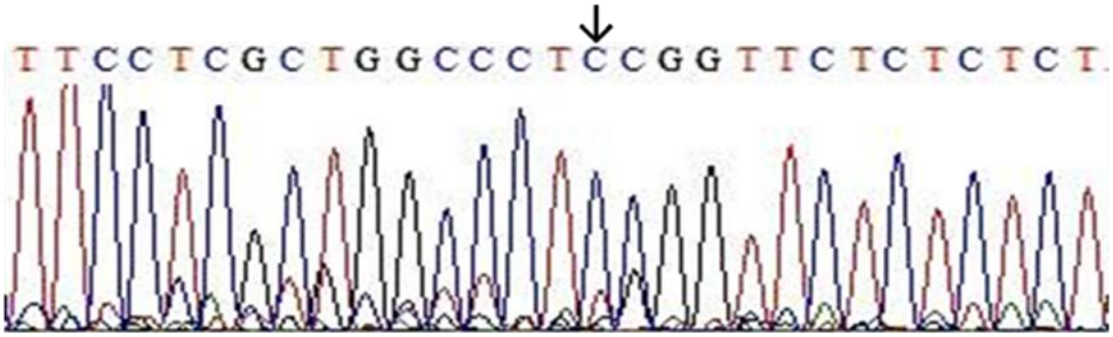

Figure 5. Genotype C/C.

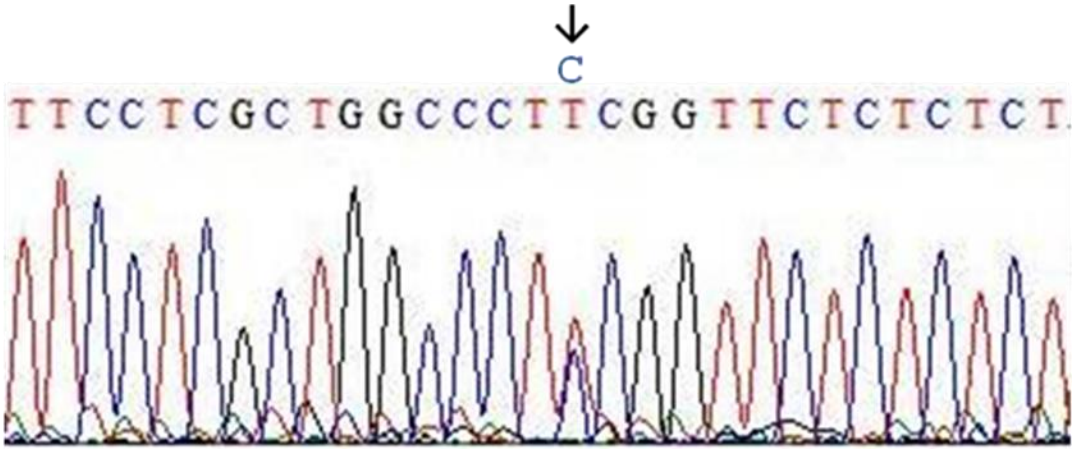

Figure 6. Genotype C/T.

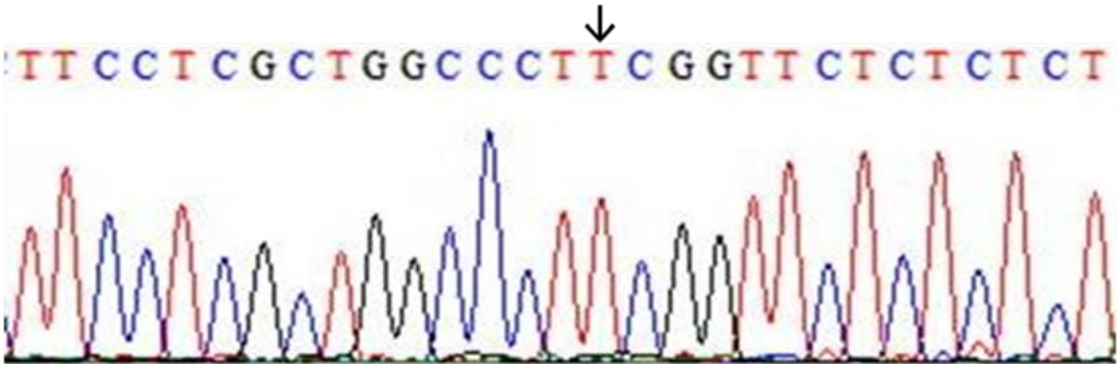

Figure 7. Genotype T/T.

studied the RFLP and SNP of *GH* gene in six Sudanese camel breeds; Kenani, Lahwee, Rashaidi, Anafi, Bishari and Kabbashi. The sequence comparison of Sudanese camel *GH* sequences with the GenBank sequence identified one SNP 419C>T in intron 1. The Bishari and Anafi breeds that are classified as riding camels with relatively light weight 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. On the other hand, other breeds (Kenani, Lahwee, Rashaidi and Kabbashi) have higher body weights with low T allele frequencies (0.30 to 0.33) and

high C allele frequencies (0.67 to 0.70). The relationship between *GH* polymorphism and body weight was evaluated in four Arabian camel breeds (Majaheem, Saheli, Waddah and Homor) by Afifi et al. (2014). Thirteen (13) SNPs (two insertion and 11 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 and 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.

In our study, the frequency of allele C in the Maghrabi breed that is classified as a dual purpose camels was higher (0.75) than those in the other tested four breeds. This allele may be associated with body weight as reported by Shah (2006), Ishag et al. (2010) and Afifi et al. (2014). Consequently, this allele may be considered as a useful marker in the selection of camels for higher growth rate and meat production. It is concluded that only one SNP C→T was detected in *GH* gene among the five tested camel breeds reared in Egypt and this nucleotide substitution can be used as a marker in different genetic studies including the detection of genetic biodiversity and establishment of phylogenic tree for different camel breeds reared in Egypt. Also, due to the possible association between allele C and higher growth rate, this can be used in marker assisted selection (MAS) for camels and enter the camels possess this allele in breeding program as a way for enhancement of growth trait in camel breeds reared in Egypt.

Conflict of interests

The authors did not declare any conflict of interest.

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