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Novel polymorphisms of the *IGF1R* gene and their association with average daily gain in Egyptian buffalo (*Bubalus bubalis*)

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ABSTRACT

The objective of this study was to detect insulin-like growth factor 1 receptor (*IGF1R*) polymorphisms, their allele, and genotype frequencies and to determine associations between these polymorphisms and growth traits in Egyptian water buffalo. Three loci of the *IGF1R* coding region were amplified by RT-PCR and, subsequently, subjected to sequence analysis, followed by single-strand conformation polymorphism to identify different allelic patterns. A total of 11 novel polymorphisms were detected; 6 SNPs among Egyptian water buffaloes and 5 polymorphisms compared with Indian buffalo (Y12700). Three of those polymorphisms; GAG Indel polymorphism, C261G, and G263C SNPs, were nonsynonymous mutations. The GAG Indel polymorphism led to deletion of E (glutamic) amino acid (aa) in the IGF1R of Egyptian water buffaloes compared with Indian buffalo. However, C261G SNP, which replaced A (alanine) by G (glycine) aa, and G263C SNP, which changed A (alanine) to P (proline) aa, were detected among Egyptian water buffaloes. Three different single-strand conformation polymorphism patterns were observed in exon 21: CC/CC, GG/GG, and CG/GC with frequencies of 0.291, 0.253, and 0.556, respectively. The heterozygous animals (CG/GC) had a higher ADG than homozygous animals (CC/CC and GG/GG) from birth to 6 mo of age. We conclude that the heterozygous haplotype, C261G/G263C, in exon 21 of the *IGF1R* gene is associated with the ADG during the early stages of life (from birth to 6 mo of age) and could be used as a genetic marker for selection of growth traits in Egyptian buffalo.

1. Introduction

The water buffalo (*Bubalus bubalis*) holds great promise and potential for animal production. This species shows a great capacity of environment adaptation and has leaner meat that contains less fat and cholesterol than beef while having a comparable taste. Although there are many advantages to raising water buffalo, these animals remain underused and show suboptimal meat production potential. Therefore, genetic improvement of these animals is of economic importance.

The IGF1 receptor (IGF1R) is a heterotetrameric plasma membrane receptor that is composed of two α-subunits (130 kDa each) and two β-subunits (90 kDa each) linked by disulfide bonds [1]. The human *IGF1R* gene consists of 21 exons spanning >310 kb of genomic DNA [2]. The exon/intron organization of the bovine *IGFIR* gene is similar to its human equivalent, with 21 exons spanning >301.2 kb [3,4].

As a main receptor of IGFs (can bind both IGF1 and IGF2), IGF1R mediates the transduction of metabolic signal of cell proliferation, bone growth, and protein synthesis in the GH/IGF pathways [5]. The expression of *IGF1R* in almost all embryonic tissues suggests its importance during embryogenesis [6]. The postnatal increase of muscle mass

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results from increasing size of existing myofiber (hypertrophy) because of activation of satellite cells, processes that depend on the interaction between IGF1 and its receptor, IGF1R [7,8].

As a result of its physiological importance, there are many published studies on the association between *IGF1R* polymorphisms and growth traits. A G3148A SNP in exon 11 of the human *IGF1R* gene led to substitution of glutamic acid by lysine [9]. This nonsynonymous SNP caused dysfunctions of the *IGF1R* gene and led to growth retardation [10,11]. Six novel SNPs in the chick *IGF1R* gene were associated with early growth traits [12]. In addition, an association study between *IGF1R* SNPs and body weight in small line and large line quails showed the presence of an SNP at position c.2293G>A, which led to the replacement of a valine with an isoleucine (V765I) [13]. Moreover, a nonsynonymous SNP that changes a highly conserved arginine at amino acid 204 to histidine in the IGF1R contributed to tiny size in dogs [14]. Furthermore, TaqI SNP was determined in intron 16 of the bovine *IGFIR* gene [3]. This SNP is not useful because of the absence of the B allele in *Bos taurus* and its low frequency in *Bos indicus* [3,15]. In addition, no association between the genotypes of this polymorphism and growth or carcass traits was detected in cattle [16,17]. Recently, 220 SNPs were identified in the *Bos taurus IGF1R* gene, but the gene and genotype frequencies and association between the genotypes of these polymorphisms and growth traits of beef cattle have not been investigated yet (reviewed by Szewczuk et al [18]).

To date, no study on associations of the *IGF1R* gene with growth traits has been reported in buffalo. The objective of this study was to detect *IGF1R* polymorphisms, their allele, and genotype frequencies and to determine associations between these polymorphisms and growth traits in Egyptian water buffalo.

2. Materials and methods

This work was reviewed and approved by the Animal Care and Welfare Committee of Kafrelsheikh University, Egypt.

2.1. Animal source

Two hundred Egyptian water buffaloes were used in this study. They were maintained at El-Nataff El-Gidid Experimental Stations, Mahalet Mousa, Kafrelsheikh Governorate. All animals were pure Egyptian water buffaloes, based on farm records and animal appearance. Moreover, these animals were artificially inseminated by fresh semen collected from buffalo studs on the farm. Animals used in this study were chosen at random. All records of growth traits (body weight, ADG, body height, body length, and chest girth) for different growth periods (at birth and at 3, 6, 9, 12, 18, and 24 mo of age) were collected from the farm records.

2.2. Muscle biopsies

Skeletal muscle biopsies were collected from the middle gluteal muscle under local anesthesia (2% lidocaine). The collection site was first shaved and washed thoroughly with antiseptic solution, and then a sterilized large (5 mm) Bergstrom biopsy needle was inserted at a constant depth by using the percutaneous needle biopsy technique. The muscle sample (~75–110 mg) obtained from each biopsy was snap-frozen in liquid nitrogen and then stored at −80°C until used for RNA extraction.

2.3. Total RNA isolation

Each muscle sample was homogenized, and total RNA was extracted with the use of total RNA purification kit according to the manufacturer protocol (Fermentas, K0731; Thermo Fisher Scientific, Waltham, MA). The extracted RNA was dissolved in nuclease-free water, and the concentration and purity were determined with Nanodrop (UV–visible spectrophotometer Q5000; Quawell) by absorption at 260 and 280 nm. The quality and integrity of total RNA were assessed by inspection of the ribosomal RNA bands (18S and 28S) in ethidium bromide-stained 1% agarose gels under UV light, and electrophoresis of the RNA confirmed that it was intact. The RNA samples were stored at −80°C.

2.4. Reverse transcription

Total RNA (5 μg per sample) was reverse transcribed into cDNA with the use of Revert Aid H minus Reverse Transcriptase (Fermentas, EP0451; Thermo Fisher Scientific) and a mix of oligo(dT) (0.5 μg/reaction), 4 μL of 5× Reaction Buffer, 0.5 μg of RNase Inhibitor, 2 μL of dNTP Mix in a 12.5 μL total reaction volume at 42°C for 60 min. To terminate the reaction, the tubes were heated at 70°C for 10 min and then were stored at −80°C.

2.5. PCR

To amplify 3 portions of the coding region of the *Bubalus bubalis IGF1R* gene, 3 pairs of PCR primers (Table 1) were designed with Primer 5.0 software according to the published nucleotide sequence information of the *Bos taurus* IGFIR (GenBank accession NM_001244612). The PCR was

Table 1
Forward and reverse primer sequences for 3 coding regions of the *IGF1R* gene, annealing temperatures, size of PCR products, and localization of the representative parts of these regions.

Locus	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Ta (°C)	Size (bp)	Coding region
IGF1R.1	GGGGACTCCCTGTTTTTCTCC	GCTGTGTGAGAAGACGACCA	55	549	Exon 1–2
IGF1R.2	AGATCCTAGGGGAGGAGCAG	CACGATCAGCTGAGAAGAGG	55	668	Exon 5–9
IGF1R.3	ATGCTGTTTGAACTGATGCGCATGTGCTGG	CCGCTCGTTCTTGCGGCCCCCGTTCAT	55	354	Exon 20–21

Abbreviation: Ta, annealing temperature.