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# Detection of SNPs in growth hormone and insulin like growth factor -1 genes in two divergently selected lines of Japanese quail

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## Abstract

**The Japanese quail (Coturnix japonica) is used for producing both meat and egg in many countries and as a model for animal research purposes. Two lines of Japanese quail that were differentiated by high body weight (HBW) and low body weight (LBW) had been improved by selection for body weight at four weeks of age. The objective of this study was to detect single nucleotide polymorphism (SNP) in Growth hormone (*GH*) and Insulin like Growth Factor -1 (*IGF-1*) genes in the two Japanese quails selected lines which based on the weight of the body at four weeks of age. DNA has been extracted from fifty blood samples by commercial kits and amplified by polymerase chain reaction (PCR). DNA sequencing revealed nucleotide polymorphisms between the two Japanese quails selected lines. The results of this investigation revealed that, one nucleotide change (T/C) in the intron 2 of *GH* gene. However, there were no nucleotide differences in *IGF-1*gene between the two selected lines. It concluded that, the SNP discovered in *the GH gene* may provide appropriate markers for associating researches of candidate genes with imperative economic measurements in Japanese quail. However, further studies are necessitating detecting mutation in another region of IGF-*1*.**

Key words: Growth; Japanese quails; Growth hormone; IGF-1; SNP

## Introduction

Japanese quail belongs to order Galiformes, genus Coturnix, and species japonica. Coturnix japonica is the scientific nomination of Japanese quail, which vary from Coturnix coturnix (common quail)[18)]. It has been used worldwide as an experimental animal with remarkable characteristics such as short generation interval, rapid growth rate with less feed, water and housing requirements and low maintenance costs[8)]. Although, Japanese quail has various benefits as a laboratory bird, but its genome sequence is not accessible now. The genome sequence of Japanese quail will deliver important genomic resources to accelerate different studies and to authenticate divergent lines of Japanese quail[14)].

Growth is a complex physiological pathway that occurs from fertilization until maturity in birds; consequently precise measurement of entire

growth phase cannot be conducted easily. Thus, practical and simplified measures used to assess growth of chickens such as body weight and weight gain[1]. The advent of molecular genetics techniques potentially offered an approach for selecting animals for breeding at an early age and to select from a broad variety of traits as well as to improve the reliability in expecting the mature phenotype of the animal[9]. The use of molecular marker-assisted selection (MAS) has established to be efficient and led to improvement in the production performance[16].

Single nucleotide polymorphisms (SNPs), including deletion, insertion, and substitution, has a crucial role in the transcription and translation of genes and extensively distributed along the chicken genome[2]. DNA sequencing is a technique that has been used to identify great numbers of SNP positions in the genome. *GH* has many physiological roles in animals, such as enhancing muscle growth, bone formation and regulating fat content, all of which affected the animals' growth and development[19].

In birds, *GH* has an imperative role in growth, but was also involved in a variety of secondary functions such as egg production, aging and reproduction[13]. In all mammals the *GH* gene extends over 2-3 kb and comprised five exons split by four introns[11].The *GH* gene polymorphism has been reported in chicken, but not in quails[12]. Insulin-like growth factor gene (*IGF1*) considered as a candidate gene in chicken related to growth, metabolism, body composition, skeletal traits, adipose tissue development and deposition of fat. However, the majority of the *GH* functions in chickens were governed by *IGF1*[15]. Therefore, the aim of the current study was to detect SNPs in *GH* and *IGF-1* genes in two divergently selected body weight lines of Japanese quails.

## Materials and Methods

Birds were collected from the research unit in Faculty of Agriculture, Zagazig University, Egypt. The experiment was performed in agreement with the Zagazig University Animal Ethics Committee instructions (ANWD-206).

*Birds and Management:*

Three hundred (300) Japanese quails, divergently selected over three generations for body weight at four weeks of age, were used in this trial to obtain two distinct lines (n=150/line; high body mass (183.37±4.60g HBM), low body mass (100.15±1.64g LBM). Birds were kept in cages (30x30x25cm LxWxH) throughout the study at the poultry research farm of the Fac. of Vet. Med., Zagazig Uni., Egypt. All observations and collected samples were conducted with the third generation. A standard diet was provided *ad libitum* during the rearing period (0-6 weeks age), with 24% crude protein and 12451 KJ/kg ME.

*Blood Sampling and DNA extraction:*

Fifty birds (50) from each selected line (HBM and LBM) were assigned randomly to collect blood samples at 6$^{th}$ week age by a braquial vein puncture. Blood samples were withdrawn into vacutainer EDTA tubes, and placed immediately inside an ice box and transferred to the laboratory. The samples were stored at -20 until DNA has been extracted. The genomic DNA has been extracted from blood by DNeasy Blood & Tissue Kit (QIAGEN, Germany) following manufactures protocol. The quality of the extracted DNA was assessed by agarose gel electrophoresis and the quantity was evaluated by UV spectrophotometer.

*PCR amplifications:*

The PCR primers for the *GH* and *IGF-1* genes were designed based on GenBank accession numbers EF521468.1 and GQ896357.1, respectively, using the software Primer3 Program (v.0.4.0). The *IGF-I* and *GH* primer sequences were F: 5-TTTGCCAGAAGAGGGAGAGA-3; R:5-GCAGAAGCAGACAACACACA-3 and F:5-TCCAGTAGGGGTTGAGATGC-3; R:5-CTGTCTGAGGTGCCGAAAAC-3, with size product 418bp and 466bp, respectively. PCR was performed using the T-professional thermal cycler

(Biometra, Germany) and DreamTaq Green PCR Master Mix (Thermo Scientific, fermentas). Each reaction mixture comprised of 25$\mu$l of the master mix, 2$\mu$l of the DNA template, 1.5$\mu$l of each primer (10pmol/$\mu$l) and some deionized water to have a final volume of 50$\mu$l. Cycles applied were: 94°C for 3min; followed by 37 cycles of 20sec at 94°C, 20 Sec at 54.5°C for *GH gene* and, 53°C for *IGF-1*, 30sec at 72°C; and a final extension of 7min at 72°C. The PCR products were screened by 1.5% agarose gel electrophoresis in 1×TAE buffer and were visualized in a gel documentation system with transilluminator.

*DNA sequencing:*

The Gene JET PCR purification kit (Fermentas) has been used to purify the band of interest based on the manufacturer's directions. The purified DNA fragments were immediately sequenced using both the forward and reverse primers of PCR amplification. The sequencing technique has been carried out by European Custom Sequencing Centre (GATC Biotech AG, Germany). The acquired sequences were entered manually using Chromas Lite Ver. 2.01,(http://www.technelysium.com.au/chromas.html) and aligned with Clustal Omega software to detect nucleotide replacements.

## Results and Discussion

The investigation of candidate genes is one of the foremost techniques to reveal whether definite genes are associated with the economic traits in farm animals. SNPs in the candidate genes that might be evidence for association with the specific economically related trait are constructive for enhancing marker-assisted selection[5]. The amplification product of *GH* gene was 466bp which, distinguished by 1.5% agarose gel electrophoresis.(Fig. 1A. The PCR product of multiple individuals has been sequenced and submitted to the GenBank database under Accession number: KM262764.1. In this study, one nucleotide has been changed in intron 2 of *GH* gene and the mutation was T→C transitions when compared the DNA sequences between HBW and LBW (Fig. 1B).

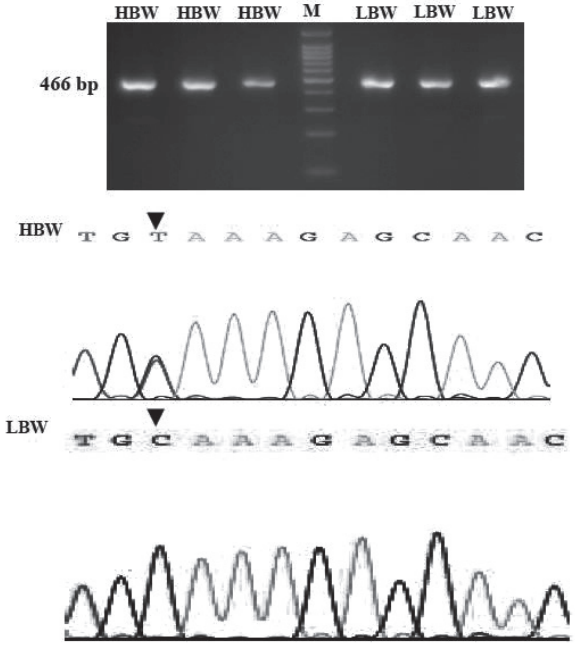


**Fig. 1(A)** PCR amplification of *GH* gene in two selected lines of Japanese quail (high body weight (HBW) and low body weight (LBW). M: 100 bp plus ladder. **(B)** Nucleotide sequence alignment between HBW and LBW lines of Japanese quails. Arrow refers to site of nucleotide change.

Quantitative traits controlled the poultry productive performance, which could be influenced by various environmental aspects and genes. One of these genes was the *GH*. *GH* gene polymorphisms had been studied in several poultry species; in chicken[22], goose and ducks[21]. In the present investigation, the polymorphism of intron 2 of the quail *GH* gene was examined. Previous investigations had revealed that the polymorphisms of the avian *GH* gene could be detected not only at intron 2, but also at exon in different regions of this gene as in ducks and geese[4]. Meanwhile, polymorphisms in intronic regions of the avian *GH* gene were detected at

intron 1, 3 and 4 in chickens[22], intron 2 in ducks and intron 3 in geese[21]. Chang et al. [4] detected 19 SNPs in a region of 2087 bp in a duck GH gene, while Mehdi and Reza[17] declared a significant effect on body weight traits with SNP at G662A in the chicken.

The amplification of *IGF-1* revealed a DNA fragment with 418bp spanning over exon 1 regions (Fig. 2A). The PCR product of multiple individuals has been sequenced and submitted to the GenBank database under Accession number: KM278222.1. The nucleotide did not reveal any difference in *IGF-1* coding region when compared the sequence between HBW and LBW selected birds (Fig. 2B).

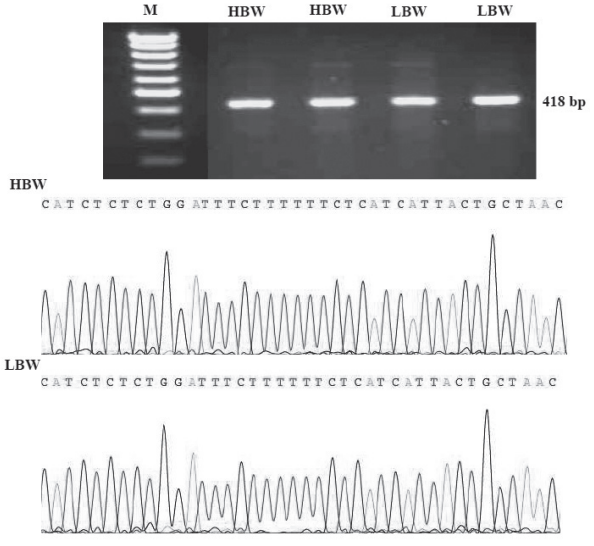


**Fig.2. (A)** PCR amplification of *IGF-1* gene in two selected lines of Japanese quail (high body weight (HBW) and low body weight (LBW). M: 100 bp plus ladder. **(B)** Nucleotide sequence alignment between HBW and LBW lines of Japanese quails.

The current results were comparable with other[7]. On the contrary, several studies had been performed in chicken *IGF-1* genes which noticed mutations in the promoter region[20], introns 2 and 5[10] and 5'-flanking, exon 3 and 3'-flanking regions of the *IGF*[3]. *IGF-1* has a crucial responsibility in the muscle tissue development and positively influenced the muscle growth[6]. In birds, *IGF1R* is notorious to control two IGFs with different affinities. Thus, the functional variation associated with the *IGF1R* SNP, might influence the growth traits only in these selected Japanese quail lines.

In conclusion, single nucleotide polymorphism (T/C) was identified in *GH* gene, which showed disparities between high and low selected lines and presented appropriate markers for associating studies of candidate genes with imperative economic traits in Japanese quail. While, there was no difference in *IGF-1* gene between the two selected lines (high and low). Therefore, further studies are necessitating for sequencing other regions of *IGF-1* genes.

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