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Growth hormone 1 and insulin 2 gene polymorphism in Egyptian chickens

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ABSTRACT

The genetic polymorphism of two genes related to growth production in four chicken breeds (Dokki 4, Inshas, El-Salam and Mandarah) were studied. PCR-RFLP and single nucleotide polymorphism (SNPs) analysis were studied in the two genes namely *GH1* and *insulin* 2. Blood samples were collected randomly from 84 chickens, DNA was extracted and genotypes for two genes were detected using PCR-RFLP analysis. The locus *GH1* (467 bp) showed two genotypes (GG, AG) in four chicken breeds due to the presence of two *Msp1* restriction sites (C^CGG) in intron1. The sequence analysis revealed two substitutions (C/T) in Dokki 4 and Inshas breeds, G/A substitution in Dokki 4 and Mandarah breeds and insertion of T nucleotide in four chicken breeds. In *insulin* 2 gene (371 bp), two genotypes (TT, TC) were recorded in four chicken breeds due to presence of one restriction site in intron1. The nucleotide substitutions (A/G) and (T/C) were observed in all Egyptian chicken breeds except Inshas breed and A/T transversion was observed in all breeds. The sequences of detected SNPs were submitted to GenBank database with the accession numbers MG906782–MG906789 in *GH1* gene and the accession numbers MG906790–MG906791 in *insulin* 2 gene. In conclusion, the presence of certain polymorphisms could increase the phenotypic variety and will be supportive in selection and breeding programs.

Key words: Chicken, Egypt, GH1, Insulin 2, SNPs

Poultry production is considered as one of the most important and assorted component of agriculture all over the world. Growth achievement and carcass merit are very important economic factors in broiler production which are controlled by various complex genes (Anh *et al.* 2015). Previous studies reported that somatotropic axis genes plays critical role in chicken growth and development as it consists of main components like growth hormone (*GH*), insulin-like growth factors (*IGF-1* and *2*) and their corresponding receptors and carrier proteins as well as other hormones such as leptin, insulin and thyroid hormones (Kadlec *et al.* 2011). The growth hormone (191 amino acid) plays an essential role as a growth regulator for several physiological processes such as carbohydrate, lipid and protein metabolism as well as immune system maintenance which indirectly affected by the insulin-like growth factor I (*IGF-1*), that produced in the liver and other tissues through *GH* stimulation (Zhao *et al.* 2011).

Great progress has been made in the genetic improvement of chicken growth using Marker-assisted selection (MAS) that based on molecular markers, which playing crucial role in shorten the breeding process and save a lot of time and money (Abdlhag *et al.* 2015). Single nucleotide polymorphism (SNPs) has gained interest as it is a type of DNA polymorphism (deletion, substitution and insertion of single nucleotide) which is bi-allelic and spread along the genome (Khoa *et al.* 2013) where in chicken genome over 2.8 million SNPs were detected (Bassam and Dihya 2016). Single nucleotide polymorphisms (SNPs) of somatotropic axis genes influence physiological and growth traits (Lei *et al.* 2007). PCR-RFLP was used to detect two SNP locations in Egyptian chicken *GH* gene at intron 4 beside other new SNPs which is correlated to growth traits (Heba *et al.* (2017).

Insulin gene is considered as elect gene in the genetic analysis for animal complex traits such as body composition, growth rate and fat deposition, it also plays an important role in potassium homeostasis (Wilcox 2005). Studies on variations of the *INS* gene in chickens revealed 24 SNP using 4 divergent breeds (Nie *et al.* 2005). The data from chickens and human suggest that the *INS* gene is highly polymorphic and could be a candidate in mapping quantitative trait loci in domestic animals (Qui *et al.* 2006).

In this study, PCR-RFLP and sequencing analysis were used to investigate the growth hormone (*GH1*) and *insulin* 2 genes genotypes in four Egyptian chicken breeds (Dokki4, Inshas, El-salam, Mandarah) and identify SNPs of *GH1* and *insulin 2* genes.

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MATERIALS AND METHODS

Sampling and DNA processing: Blood samples were collected randomly from 84 chickens of four local Egyptian chicken strains (Dokki4, Inshas, El salam, Mandarah) as 21 chickens from Mandarah, 24 chickens from Inshas, 20 chickens from El-Salamand and 19 chickens from Dokki4.

Blood samples were collected in tubes containing EDTA as anticoagulant and transported to the laboratory and kept at –20°C. DNA was extracted and purified from blood samples using the whole blood salting out technique described by Miller *et al*. (1988). The DNA quality and quantity was determined using UV spectrophotometer at optical density of 260 and 280 nm and also by loading genomic DNA on 1% agarose gel electrophoresis.

GH1 and insulin 2 *genotypes defined by PCR-RFLP:* The *GH1* and insulin 2 genes were typed in four Egyptian chicken breeds. The primer (5'-AACATCCTCCCCAA-CCTTTC-3', 5'-CCCTGTCAAGGTTAGGCTCA-3') was designed to amplify the fragment (467 bp) of the chicken *GH1* gene with accession number AY461843 and primer (5'-CTCCATGTGGCTTCCCTGTA-3',5'-GGCTTCTTGG-CTAGTTGCAGT-3') was designed to amplify the fragment (371 bp) of the chicken *Insulin* 2 gene with accession number AY438372, according to Khoa *et al*. (2013).

Restriction fragment length polymorphism (RFLP): The PCR products were restricted using restriction enzyme *Msp1* (Fermentas, Germany). PCR products (10 µl) were digested with 5 units of the fast restriction enzyme including specific buffer (Fermentas, Germany) in a final reaction volume of 15 µl. The reaction mixture was incubated at 37°C in water bath for 30 min. The obtained restricted fragments were visualized on 2% agarose gel electrophoresis stained with ethidium bromide. The bands were photographed using digital gel documentation system (BioRad, USA). Genotype and allele frequencies were determined using free Lab. Image V2.7 software. It is available free from Proland company (Germany) (http://www.labimaging.com/servlet/engine/home/start.html).

Genotype and sequence analysis: The genotypic and allelic frequencies for *GH1* and insulin 2 genes were analyzed in four chicken breeds. The PCR products representatives for each detected genotype of *GH1* and insulin 2 genes were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment of sequence products were carried out using NCBI/BLAST/ blastn and BioEdit software to identify each single nucleotide substitution between different detected genotypes.

RESULTS AND DISCUSSION

Improvements in growth rate and related characteristics using modified selection programs as molecular genetics selection or by a transgenic approach on individual genes is a talented method to genetically develop economically important traits in chickens and understanding the biology of the regulatory factors, genes encoding these factors and the regulation of their expression (Shahnaz *et al.* 2008). *GH1* and insulin genes considered as one of the most important genes which affect chicken performance traits, and plays a vital role in both growth and metabolism rates (Anh *et al.* 2015).

GH1 gene

For *GH1* gene, PCR amplification inspects a region of 467 bp (Supplementary Fig. 1). Two genotypes were created due to the presence of two *Msp1* restriction sites (C^CGG) in intron1 at positions 225–226 and 351–352. GG genotype in which one *Msp1* restriction site was cut lead to generation of two fragments, viz. 225 and 242 bp. AG genotype in which two *Msp1 restriction* sites were cut where the 2nd restriction site was present in 242 bp fragment lead to generation of four fragments 225, 242, 126 and 116 bp (Supplementary Fig. 2).The two genotypes (GG, AG) were found in four chicken breeds studied (Dokki4, Inshas, El-salam, Mandarah) while AA genotype was absent in the four breeds.

Tanmankaurd *et al.* (2008) revealed that three different patterns (AA, AC and CC) were created by two *Msp1* cut sites present in exon 1 and in intron1and investigated that AA genotype birds were higher in body weight than the CC genotype birds at 5 week age. Shahnaz *et al*. (2008) suggested two *Msp1* sites yielded five fragments which is correlated with age at first egg (AFE), body weight and egg production. Strains of White Leghorn chicken revealed that three *Msp1* sites (PM1, PM2, and PM3) were created in exon 1 and suggested that these alleles could be related to body weight and egg production Kuhnlein *et al.* (1997). Ipe *et al.* (2001) reported that *cGH*/*Msp1* polymorphism in populations of Chinese native chickens present in intron1 might be linked to laying performance.

Allele G was abundant than allele A in all four chicken breeds, the highest frequency of GG and lowest frequency of AG genotypes were recorded in Mandarah breed while the highest frequency of AG and lowest frequency of GG genotypes were found in Inshas breed (Table 1).

Khoa *et al*. (2013) recorded highest frequency of AA genotype in the Cobb 500 breed and highest frequency of AG genotype in the CTU-BT01 strain of the Tau breed while Tanmankaurd *et al*. (2008) reported high allele frequency for CC genotype and low allele frequency for AA genotype in different chicken strains. Darabi *et al*. (2010) reported three genotypes in Iranian native chicken with high

Table 1. Genotype and allele frequencies of *GH1* gene in four chicken breeds

Chicken breed	Genotype frequencies			Allele frequencies	
	GG	AG	AA	G	A
Dokki 4	0.64	0.36	0.00	0.82	0.18
Inshas	0.46	0.54	0.00	0.69	0.31
El salam	0.63	0.37	0.00	0.81	0.19
Mandarah	0.71	0.29	0.00	0.85	0.15
Total	2.44	1.56	0.00	3.17	0.83

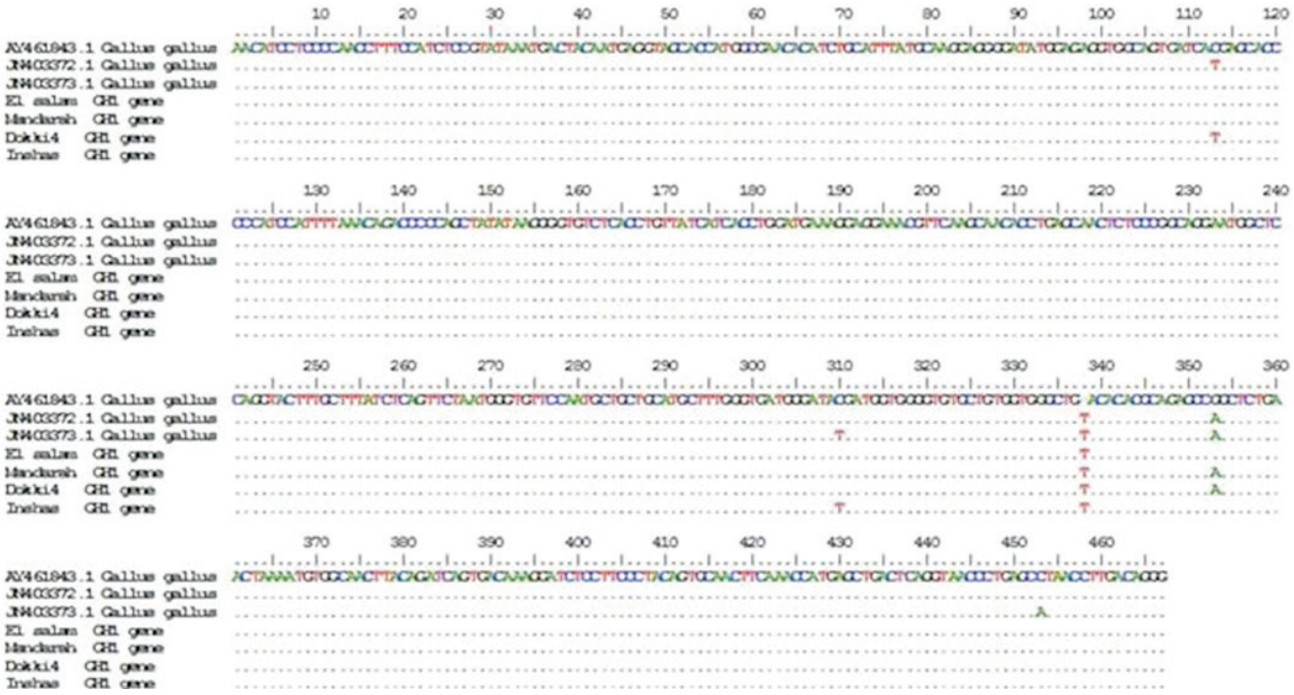

Fig. 1. Blast and alignment of nucleotide sequence of *GH1* gene for four Egyptian chicken breeds with published sequence (accession numbers AY461843.1, JN403372.1 and JN403373.1).

frequency for AB genotype and low frequency for BB genotype. Earlier studies revealed that higher frequencies of certain growth hormone genotypes than others may be indicating that these genotypes are favorable in broiler chicken and there could be election stress against some of the low frequency/ absent genotypes.

*Sequence divergence and PCR-RFLP analysis:*The sequence analysis revealed two substitutions (C/T) at positions 113,310 in Dokki 4 and Inshas breeds, respectively (Supplementary Fig. 4). Also, the alignment revealed insertion of nucleotide T at position 338 in four chicken breeds. Sequence analysis of Mandarah, Dokki4 *GH1* gene showed nucleotide substitution G/A SNP at position 353 (Supplementary Fig. 3) which responsible for generation of two alleles G, A and two genotypes (GG,AG) (Supplementary Fig. 4a, 4b).

The blast and alignment nucleotide sequence of our data with *Gallus gallus* accession no. AY461843, JN403372.1 *Gallus gallus* strain NG growth hormone gene, exon 1, complete sequence and intron 1, partial sequence and JN403373.1 *Gallus gallus* strain IC3 growth hormone gene, exon 1, complete sequence and intron 1, partial sequence were consistent with our results where two substitutions including one C/T at positions 123,320 was found in accession numbers JN403372.1, JN403373.1, respectively in addition to one G/A SNP at position 363 and one insertion of nucleotide T at position 348 (Fig. 1). The sequences of detected SNP were submitted to GenBank database with the accession numbers MG906782–MG906789 in *GH1* gene.

Similarly, Khoa *et al.* (2013) detected (G662A) SNP in chicken *GH1* gene and Lei *et al.* (2007), found that SNP substitution from G to A in the chicken growth hormone (*cGH*) is associated with abdominal fat pad ratio, abdominal fat pad weight and crude fibre content of the breast muscle. Mehdi and Reza (2012), observed a significant correlation between body weight traits and SNP at G662A. Earlier studies reported that SNPs in introns of *GH* gene was associated with growth, egg production and disease resistance (Nie *et al.* 2005, Qui *et al.* 2006 and Lei *et al.* 2007).

Insulin 2 gene

For *insulin* 2 gene, PCR amplification inspects a region of 371 bp (Supplementary Fig. 6a). Only two genotypes were recorded in four chicken breeds namely TT (371 bp), TC (371 bp, 234 bp and 138 bp) while the genotype CC (311 bp and 252 bp) was not detected in breeds studied (Supplementary Fig. 6a,b), the genotype TC was observed only in the Mandarah breed while TT genotype was detected in all chicken breeds. All chicken breeds showed the highest allelic frequency of T Allele and lowest allelic frequency of C Allele (Table 2) which is in concurrence with results recorded by Khoa *et al*. (2013) where low frequency for CC genotype was observed and the Cobb 500 breed recorded no frequency for that genotype, a high frequency for TT was observed in all breeds. In addition, Qui *et al*. (2006) reported that TC and TT genotypes were observed between Chinese native Xinghua chickens and White Recessive Rock chickens that found to be associated with growth traits which in accordance with results reported by Clark (2004) but disagree with results indicated by Morris and Kaplan (2002).

Sequence divergence and PCR-RFLP analysis: The

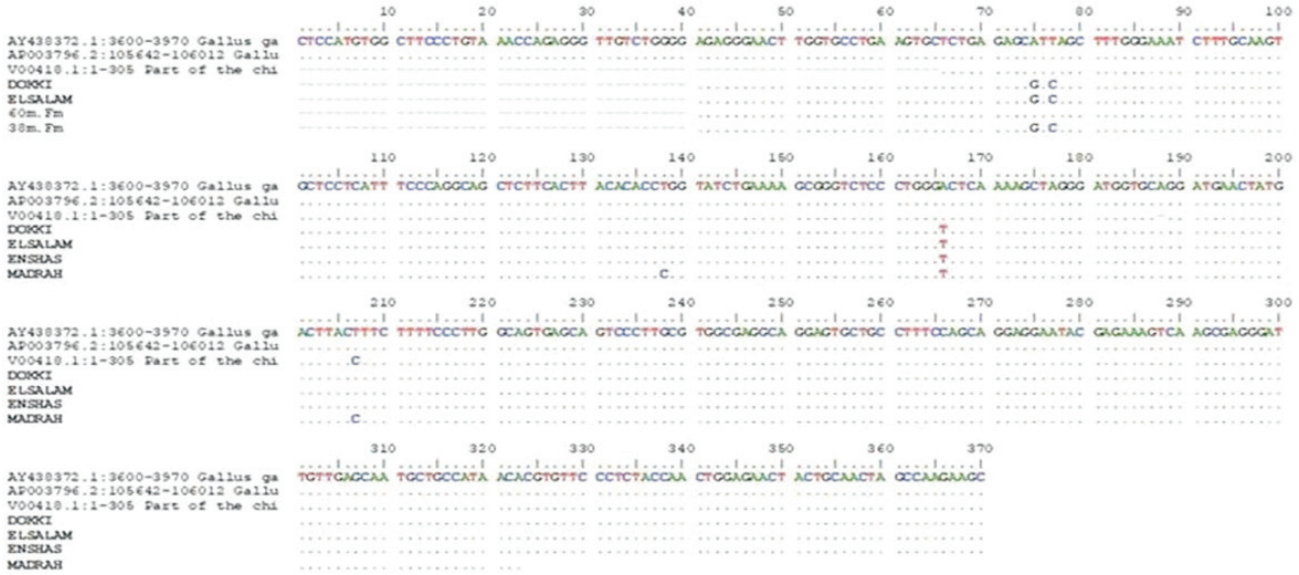

Fig. 2. Sequencing alignment for insulin 2 gene of four Egyptian chicken strains (Dokki4, El-Salam, Inshas and Mandarah) with accession numbers (AY438372.1, AY206845.1 and V00418.1).

Table 2. Genotype and allele frequencies of insulin 2 gene in four chicken breeds

Chicken breed	Genotype frequencies			Allele frequencies	
	TT	TC	CC	T	C
Dokki 4	1.00	0.00	0.00	1.00	0.00
Inshas	1.00	0.00	0.00	1.00	0.00
El salam	1.00	0.00	0.00	1.00	0.00
Mandarah	0.60	0.40	0.00	0.80	0.20
Total	3.60	0.40	0.00	3.80	0.20

sequence analysis revealed nucleotide substitution at nt 207 in a part intron1 (T/C) (SNP position) (Supplementary Fig. 7), two alleles were observed C and T which lead to generation of two genotypes TT and TC in Mandarah breed (Supplementary Fig. 8a,b). In addition, nucleotide substitutions were observed at nt 75 (A/G), nt 77 (T/C) in all Egyptian chicken breeds except Inshas breed and 'transversion' at nt 166 (A/T) in all breeds. While, only in Mandrah breeds nucleotide substitution was detected at nt 207 (T/C).

The blast and alignment nucleotide sequence of our data with *Gallus gallus* accession no. AY438372, AY206845.1 *Gallus gallus* preproinsulin gene, partial cds and V00418.1 part of the chicken *insulin* gene coding for the second exon revealed that SNPs observed in four Egyptian chicken breeds were missed in the published accession numbers AY438372, AY206845.1 and V00418.1 except the SNP at nt 207 (T/C) which also detected in accession number V00418.1 (Fig. 2). The sequences of detected SNP were submitted to GenBank database with the accession numbers MG906790–MG906791 in *insulin* gene.

Qui *et al*. (2006) observed 4 SNPs in insulin gene which were significantly associated with early growth traits. Lei *et al*. (2007) observed a correlation of the insulin gene with muscle fiber density. Wilcox (2005) reported that, insulin plays an important role in lipid and protein metabolism, cellular glucose uptake regulating carbohydrate, as well as promoting cell division and growth.

The result of present investigation revealed that single nucleotide polymorphism (SNP) of *GH1* and *insulin 2* genes along chicken breeds are considered as great genetic powerful resources as the presence of certain polymorphisms could increase the phenotypic variety and will be supportive in selection and breeding programs.

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