

Polymorphism of The Promote Region of Chicken Insulin like Growth Factor-1 and Association With Bone Abnormalities

التغيرات الوراثية في منطقة المشغل لجين *IGF-I* لعينات الدجاج وعلاقتها بتشوه العظام

Farah Thamer Abdullah

Biotechnology Research Center/ Al-Nahrain University

فرح ثامر عبدالله

مركز بحوث التقنيات الأحيائية/ جامعة النهرين

Abstract

The insulin like growth factor-1 (*IGF-I*) is important regulators in stimulating growth, protein synthesis, cell proliferation and differentiation in a variety of cell types. To achieve this goal, blood samples were collected from 50 *Gallus gallus* affected from bone abnormalities (distortion) and 50 samples a control group from the Animal farm/ College of Veterinary medicine/ University of Baghdad. DNA was isolated and the *IGF-I* gene was amplified by using specific primers for promoter region and 5' UTR of this genes, then DNA sequencing was performed by using AB13730XL, (Applied Bio system, Macro Gen company, USA). The DNA sequencing results of flank sense of *IGF-I* gene from a control group was found to be compatible 100% with wild type of *Gallus gallus* from the Gene Bank. On the other hand, sequencing results of the same gene from 35 cases abnormalities (distortion) revealed of 99% compatible and score 1351 and expect 0.0 with the wild type sequences of gene bank. The minor differences could be attributed to two transversion substitutions of (G>T, T>G) and (C>T) on locations 404, 104, and 249 nucleic acid respectability. Fifteen cases of bone abnormalities (Distortion) of *Gallus gallus*, 99% compatibility have one Transversion substitution G>T on position 404 and one transition substitution (C>T) on 249 positions, under number Sequence ID: [gb|JX414253.1](#). In conclusion, our case study suggests that polymorphisms of *IGF-I* gene are strongly associated with vertebrate growth and development of some chicken breeds in Iraq. The aim of this study is to identify the genetic polymorphisms of *IGF-I* gene and its association with vertebrate growth and development of some chicken breeds in Iraq.

Key words: *Gallus gallus*, Expect Value, Score, and *IGF-I*

المخلص

يعتبر عامل نمو الانسولين من المنظمات المهمة في تحفيز النمو، تخليق البروتين، والتكاثر والتميز في مختلف أنواع الخلايا. استخدمت عينات دم من 50 دجاجة جنس *Gallus gallus* من المصابين بتشوهات العظام و 50 عينة من الاصحاء من الحقل الحيواني/ كلية الطب البيطري/ جامعة بغداد. وعزل الحامض النووي الذي اوكسي رايبوزي (DNA) لهذه العينات وتم تضخيم جين عامل نمو الانسولين باستخدام بادانات متخصصه لمنطقه المشغل و 5' UTR لهذا الجين. ثم اجراء تسلسل الاحماض النووية باستخدام جهاز AB13730XL في شركة مايكروجين في الولايات المتحدة الامريكية. أظهرت نتائج تتابع الحامض النووي (الدنا) لجين عامل نمو الانسولين لعينات السيطرة تطابقا بنسبة 100% مع الحمض النووي (الدنا) لجنس *Gallus gallus* عند مقارنتها مع مثيلاتها في البنك الجيني العالمي، وكانت نتائج تسلسل جين عامل نمو الانسولين في 35 عينة من المصابين بتشوه العظام من *Gallus gallus* المعزولة من العراق بنسبة تطابق 99% (وسكور 1351 والمتوقع 0.0 مع بنك الجينات العالمي) ويعزى الاختلاف نتيجة نوعين من الطفرات الانقلابية (G>T and T>G) وطفرة انتقالية (C>T) في المواقع 404, 104 و 249 حامض نووي وعلى التوالي. لوحظ ان 15 عينة من المصابين بتشوهات العظام من جنس *Gallus gallus* ذات تطابق 99% لوجود طفرة انقلابية واحدة G>T ضمن الموقع 404 و طفرة انتقالية (C>T) ضمن الموقع 249. تهدف الدراسة إلى التعرف على التغيرات الوراثية في جين *IGF-I* وعلاقتها بتشوه العظام في بعض السلالات.

الكلمات المفتاحية: دجاج *Gallus gallus*، القيمة المتوقعة، عدد النقاط، *IGF-I* جين

Introduction

The insulin like growth factor gene (*IGF-I*) in chickens is composed of four exons and three introns, spanning more than 50 kb on chromosome 1 [1]. Six exons spanning over a large region of the chromosome from 73-85kb constitute the *IGF-I* gene in humans and rats [2]. The *IGF-I* gene in chickens is composed of four exons and three introns, spanning more than 50 kb on chromosome 1 [1]. Mature *IGF-I* is spanning 210 bp and encodes a single-chain polypeptide of 70 amino acids. The structure of the *IGF-I* gene is variable among chicken breeds, but the association of this variability with

the phenotypic variation is not yet clear [3,4]. One of the major hormones required to support normal growth and muscle development is insulin-like growth factor I (*IGF-I*) [5]. *IGF-I* is a complex system of peptide hormones that bind to the IGF-I receptor (IGFIR) to activate their intrinsic tyrosine kinase domain activities [6]. Biological responses to *IGF-I* have effects on cell growth, proliferation, differentiation, and survival against apoptosis [7,8]. For example, the *IGF-I* gene can influence growth rate, body composition and lipid metabolism in poultry [9]. The objective of the present study was to identify genetic polymorphisms of IGF-I gene and to find a correlation between the genotypic polymorphisms and the phenotype of vertebrate growth and development of some chicken breeds in Iraq.

Material and Method

Approximately, 3ml venous blood was collected from 50 *Gallus gallus* affected from bone abnormalities (distortion) and 50 samples a control group from the Animal farm / College of Veterinary Medicine / University of Baghdad. DNA was extracted from samples by DNA extraction kit (Genomic DNA Mini Kit, USA, Catalog #:GB 100) according to the manufacturer's protocol. Detection of IGF1(chicken insulin like growth factor 1) gene was conducted by using primers for amplification of *IGF1* gene. A fragment 814bp of *IGF1* was amplified using a forward primer (IGF1F:5'-CATTGCGCAGGCTCTATCTG-3') [10] and a reverse primer (IGF1R:5'-TGAAGAGAAGCCCTTCAAGC-3'). These primer sets were supplied by IDT(Integrated DNA Technologies) company, Canada. The PCR amplification was performed in a total volume of 25 μ l containing 1.5 μ l DNA, 5 μ l Taq PCR PreMix (Bioneer, Korea), 1 μ l of each primer (10 pmol) then distilled water was added into tube to the total volume of 25 μ l. PCR amplification was conducted under the following conditions: 5 min at 95°C, followed by 35 cycles of 94 °C for 1min, 56°C for 45s, and 72 °C for 1min and a final extension of 72°C for 10 min using a thermal Cycler made by Labnet (Labnet international, Inc, Multi Gene OptiMax, Catalog #: TC9610-230, USA). The PCR products were separated on a 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after ethidium bromide staining. Sequencing of IGF1 gene was performed by national instrumentation center for environmental management (nicem) online at http://nicem.snu.ac.kr/main/?en_skin=index.html, biotechnology lab, machine is DNA sequence 3730XL, Applied Biosystem. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at ([http:// www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and Bio Edit program.

Results

The results shown in Figure (1) indicated that a yield of single band of the desired product with a molecular weight of 814bp for promoter region and 5' UTR for IGF1 gene was obtained. The sequencing of IGF1 gene amplified product from 50 *Gallus gallus* (control cases) appeared 100% compatibility with standard *IGF1* of Gene Bank results as shown in Figure (2A), (Sequence ID: [gb|JF831880.1|](#)), there was no any polymorphism in promoter region and 5' UTR, however, 35 cases of bone abnormalities (Distortion) of *Gallus gallus*, showed 99% compatibility as shown in Figure (2B), (Sequence ID: [gb|JX414253.1|](#)), and have low number score (1351) bits than control cases (1352) bits. The bit Score is defined as statistical measure of the moral similarity and the higher value indicates that the high degree of similarity, and if dropped from the class of 50 points, the sense that there is no similarity, shown in Table (1). There was polymorphism in the promoter region, where as two transversion (G>T and T>G) and one transition (C>T) on location 404, 104, and 249, respectability Table (2). And 15 cases of bone abnormalities (Distortion) of *Gallus gallus*, 99% compatibility have one Transversion G>T on position 404 and one transition (C>T) on 249 positions, (Sequence ID: [gb|JX414253.1|](#)). (Figure 2C and Table 1 and 2). All 50 samples of control and 50 bone abnormalities (Distortion) of *Gallus gallus* have the same expectation value (0.0). The expectation value is defined to give an estimate of the number of times expected to get the same similarity coincidental and the lower the value of E. This indicates that the degree of similarity was high between a sequence which gives greater confidence. The value of a very close to zero means that these sequences are identical.

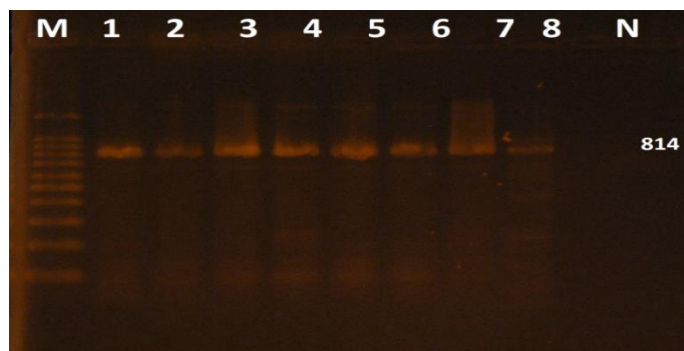


Fig. (1): Amplified *IGF-I* geneproduct run on a 1.5% agarose gel electrophoresis. Bands were fractionated by electrophoresis on a 1.5 % agarose gel (2 h., 5V/cm, 1X Tris-acetic buffer) and visualized under U.V. light after staining with ethidium bromide staining. Amplico sizes are 814 of the specific *IGF-1* gene of the promoter region from chicken .Lanes :positive controls from 1,2,3.Lanes 4-8, samples from bone abnormalities from chicken.

A:Gallus gallus IGF-I gene, promoter region and 5' UTR, Sequence ID: [gb|JF831880.1](#)

Score	Expect	Identities	Gaps	Strand
1352 bits(732)	0.0	732/732(100%)	0/732(0%)	Plus/Minus
Query 1	ATTTAGAGAAAATCCTCACATTTATCTACATTACACAGACACTGTAGACAGGAAACAGCT			60
Sbjct 752	ATTTAGAGAAAATCCTCACATTTATCTACATTACACAGACACTGTAGACAGGAAACAGCT			693
Query 61	GGGGGAGCATTTCCTTctctctctcCCTCTTCTGGCAAAGTTACCGAGTAAGGACTT			120
Sbjct 692	GGGGGAGCATTTCCTTCTCTCTCTCTCCCTCTCTGGCAAAGTTACCGAGTAAGGACTT			633
Query 121	TTTGGGCATGGTGACAAATAACATCATACTTTGCATTTTAAACTAGAGCACAGAAGC			180
Sbjct 632	TTTGGGCATGGTGACAAATAACATCATACTTTGCATTTTAAACTAGAGCACAGAAGC			573
Query 181	ATATTTTTTCCCCTTTAAAAAGAATGTGAATTAGTGACTGAGGGGTTAGCAGGCAAAAAAG			240
Sbjct 572	ATATTTTTTCCCCTTTAAAAAGAATGTGAATTAGTGACTGAGGGGTTAGCAGGCAAAAAAG			513
Query 241	CTTACGCTGCCACGGAAAATAAGGGAATGTATTCTGGTTAACTTTCGGGTGGCTGTGTGT			300
Sbjct 512	CTTACGCTGCCACGGAAAATAAGGGAATGTATTCTGGTTAACTTTCGGGTGGCTGTGTGT			453
Query 301	ATATTTGCATTTTGTGTGTATGTGTCTGCTTTTCAAATAGACAAAAACTTCCATAGGTG			360
Sbjct 452	ATATTTGCATTTTGTGTGTATGTGTCTGCTTTTCAAATAGACAAAAACTTCCATAGGTG			393
Query 361	AAGACATTGTCTGTATACCTTTATATTCTGTGTACATCTGTGCACATTCATTCATGCA			420
Sbjct 392	AAGACATTGTCTGTATACCTTTATATTCTGTGTACATCTGTGCACATTCATTCATGCA			333
Query 421	GAGACACAGGTATTTTATTATTTTCATTTTTTTTTTAACTTAGAGAGACAGGCAGGCAG			480
Sbjct 332	GAGACACAGGTATTTTATTATTTTCATTTTTTTTTTAACTTAGAGAGACAGGCAGGCAG			273
Query 481	TTTACTTTGTTTTAAATGCATCTTACGTTATTAACCTTGGCAGCGCTACATGCTGACTA			540
Sbjct 272	TTTACTTTGTTTTAAATGCATCTTACGTTATTAACCTTGGCAGCGCTACATGCTGACTA			213
Query 541	ACAATTTAAACCTCTGACTCTCTGTGCTATTAACCCTAAAATAGCAGTTTGTAAATTTGCT			600
Sbjct 212	ACAATTTAAACCTCTGACTCTCTGTGCTATTAACCCTAAAATAGCAGTTTGTAAATTTGCT			153
Query 601	AAAAGTAAAAGAGTTGTTGAGCACTGCTTGTAATAGAGCAAAAACAGCGCTGCGATCCTT			660
Sbjct 152	AAAAGTAAAAGAGTTGTTGAGCACTGCTTGTAATAGAGCAAAAACAGCGCTGCGATCCTT			93
Query 661	TAGCAACCACACAGAAGTCATGCAATTTCTAGAATTTACAGTACTATGAATGTAGTAAC			720
Sbjct 92	TAGCAACCACACAGAAGTCATGCAATTTCTAGAATTTACAGTACTATGAATGTAGTAAC			33
Query 721	TCAAGTAGCCCA			732
Sbjct 32	TCAAGTAGCCCA			21

B:Gallus gallus IGF1-2 gene, promoter region and 5' UTR, Sequence ID: [gb|JX414253.1](#)

Score	Expect	Identities	Gaps	Strand
1351 bits(731)	0.0	737/740(99%)	0/740(0%)	Plus/Minus
Query 1	GGGATTTAGAGAAAATCCTCACATTTATCTACATTACACAGACACTGTA			60
Sbjct 753	GGGATTTAGAGAAAATCCTCACATTTATCTACATTACACAGACACTGTA			694

Query 61 GCTGGGGGAGCATTTCCTTctctctctctcCCTCTTCTGGCAAAGTTACCGAGTAAGGA 120
 Sbjct 693 GCTGGGGGAGCATTTCCTTCTCTCTCTCCCTCTTCTGGCAAAGTTACCGAGTAAGGA 634
 Query 121 CTTTTTTGGGCATGGTGACAAATAACATCATAACCTTTGCATTTAAAACTAGAGCACAGA 180
 Sbjct 633 CTTTTTTGGGCATGGTGACAAATAACATCATAACCTTTGCATTTAAAACTAGAGCACAGA 574
 Query 181 ATCATATTTTTTCCCCTTTAAAAGAATGTGAATTAGTGACTGAGGGGTTAGCAGGCAAAA 240
 Sbjct 573 ATCATATTTTTTCCCCTTTAAAAGAATGTGAATTAGTGACTGAGGGGTTAGCAGGCAAAA 514
 Query 241 AAGCTTACGCTGCCACGGAAAATAAGGGAATGTATTCTGGTTAACTTTCGGGTGGCTGTG 300
 Sbjct 513 AAGCTTACGCTGCCACGGAAAATAAGGGAATGTATTCTGGTTAACTTTCGGGTGGCTGTG 454
 Query 301 TGTATATTTGCATTTTTGTGTGTATGTGTCTGCTTTTCAAATAGACAAAACTTCCATAG 360
 Sbjct 453 TGTATATTTGCATTTTTGTGTGTATGTGTCTGCTTTTCAAATAGACAAAACTTCCATAG 394
 Query 361 GTGAAGACATTGTCTGTATACCTTTATATTCTGTGTACATCTGTGCACATTTTCATTCA 420
 Sbjct 393 GTGAAGACATTGTCTGTATACCTTTATATTCTGTGTACATCTGTGCACATTTTCATTCA 334
 Query 421 GCAGAGACACAGGTATTTTATTATTCAttttttAACCTAGAGAGACAGGCAGGCA 480
 Sbjct 333 GCAGAGACACAGGTATTTTATTATTCATTTTTTTTAACCTAGAGAGACAGGCAGGCA 274
 Query 481 GTTACTTTGTTTTAAATGCATCTTACGTTATTAACCTTGGCACGCCTACATGCTGACT 540
 Sbjct 273 GTTACTTTGTTTTAAATGCATCTTACGTTATTAACCTTGGCACGCCTACATGCTGACT 214
 Query 541 AACAAATAAAACCTCTGACTCTCTGTGCTATTAACCCTAAAATAGCAGTTTGTAAATTTGC 600
 Sbjct 213 AACAAATAAAACCTCTGACTCTCTGTGCTATTAACCCTAAAATAGCAGTTTGTAAATTTGC 154
 Query 601 TAAAAGTAAAAGAGTTGTTGAGCACTGCTGTAAATAGAGCAAAACAGCGCTGCGATCCT 660
 Sbjct 153 TAAAAGTAAAAGAGTTGTTGAGCACTGCTGTAAATAGAGCAAAACAGCTCTGCGATCCT 94
 Query 661 TTAGCAACCACACAGAAGTCATGCAATTTCTAGAATTTACAGTACTATGAATGTAGTAA 720
 Sbjct 93 TTAGCAACCACACAGAAGTCATGCAATTTCTAGAATTTACAGTACTATGAATGTAGTAA 34
 Query 721 CTCAAGTAGCCCAGCTTGAA 740
 Sbjct 33 CTCAAGTAGCCCAGCTTGAA 14

C:Gallus gallus IGF1-2 gene, promoter region and 5' UTR, Sequence ID: [gb|JX414253.1](#)

Score	Expect	Identities	Gaps	Strand
1352 bits(732)	0.0	736/738(99%)	0/738(0%)	Plus/Minus
Query 1	AGGGATTTAGAGAAAATCCTCACATTTATCTACATTACACAGACACTGTAGACAGGAAAC			60
Sbjct 754	AGGGATTTAGAGAAAATCCTCACATTTATCTACATTACACAGACACTGTAGACAGGAAAC			695
Query 61	AGCTGGGGGAGCATTTCCTTctctctctctcCCTCTTCTGGCAAAGTTACCGAGTAAGG			120
Sbjct 694	AGCTGGGGGAGCATTTCCTTCTCTCTCTCTCCCTCTTCTGGCAAAGTTACCGAGTAAGG			635
Query 121	ACTTTTTTGGGCATGGTGACAAATAACATCATAACCTTTGCATTTAAAACTAGAGCACAG			180
Sbjct 634	ACTTTTTTGGGCATGGTGACAAATAACATCATAACCTTTGCATTTAAAACTAGAGCACAG			575
Query 181	AATCATATTTTTTCCCCTTTAAAAGAATGTGAATTAGTGACTGAGGGGTTAGCAGGCAAA			240
Sbjct 574	AATCATATTTTTTCCCCTTTAAAAGAATGTGAATTAGTGACTGAGGGGTTAGCAGGCAAA			515
Query 241	AAAGCTTACGCTGCCACGGAAAATAAGGGAATGTATTCTGGTTAACTTTCGGGTGGCTGT			300
Sbjct 514	AAAGCTTACGCTGCCACGGAAAATAAGGGAATGTATTCTGGTTAACTTTCGGGTGGCTGT			455
Query 301	GTGTATATTTGCATTTTTGTGTGTATGTGTCTGCTTTTCAAATAGACAAAACTTCCATA			360
Sbjct 454	GTGTATATTTGCATTTTTGTGTGTATGTGTCTGCTTTTCAAATAGACAAAACTTCCATA			395
Query 361	GGTGAAGACATTGTCTGTATACCTTTATATTCTGTGTACATCTGTGCACATTTTCATTCA			420
Sbjct 394	GGTGAAGACATTGTCTGTATACCTTTATATTCTGTGTACATCTGTGCACATTTTCATTCA			335
Query 421	TGCAGAGACACAGGTATTTTATTATTCAttttttAACCTAGAGAGACAGGCAGGC			480
Sbjct 334	TGCAGAGACACAGGTATTTTATTATTCATTTTTTTTAACCTAGAGAGACAGGCAGGC			275
Query 481	AGTTACTTTGTTTTAAATGCATCTTACGTTATTAACCTTGGCACGCCTACATGCTGAC			540
Sbjct 274	AGTTACTTTGTTTTAAATGCATCTTACGTTATTAACCTTGGCACGCCTACATGCTGAC			215
Query 541	TACAATAAAACCTCTGACTCTCTGTGCTATTAACCCTAAAATAGCAGTTTGTAAATTTG			600
Sbjct 214	TACAATAAAACCTCTGACTCTCTGTGCTATTAACCCTAAAATAGCAGTTTGTAAATTTG			155

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Query 601 CTAAAAGTAAAAGAGTTGTTGAGCACTGCTTGTAATAGAGCAAAACAGCGCTGCGATCC 660
|||||
Sbjct 154 CTAAAAGTAAAAGAGTTGTTGAGCACTGCTTGTAATAGAGCAAAACAGCTCTGCGATCC 95
Query 661 TTTAGCAACCACACAGAAGTCATGCAATTTCTAGAATTTACAGTACTATGAATGTAGTA 720
|||||
Sbjct 94 TTTAGCAACCACACAGAAGTCATGCAATTTCTAGAATTTACAGTACTATGAATGTAGTA 35
Query 721 ACTCAAGTAGCCCAGCTT 738
|||||
Sbjct 34 ACTCAAGTAGCCCAGCTT 17

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Fig. (2): A: Sequencing of sense flanking the partialIGF1-2 gene, promoter region and 5' UTR for 50 cases *Gallus gallus* as compared with standard IGF1-2 gene, promoter region and 5' UTR obtained from Gene Bank. B: Sequencing of sense flanking the partialIGF1-2 gene, promoter region and 5' UTR for 35 cases Bone Abnormalities (Distortion) of *Gallus gallus* as compared with standard IGF1-2 gene, promoter region and 5' UTR obtained from Gene Bank. C: Sequencing of sense flanking the partialIGF1-2 gene, promoter region and 5' UTR. for15 cases Bone Abnormalities (Distortion) of *Gallus gallus* as compared with standard IGF1-2 gene, promoter region and 5' UTRobtained from Gene Bank. Query represents of sample; Subject represent of database of National Center Biotechnology Information (NCBI).

Table (1): Sequencing ID in gene bank, score, expect and compatibility of DNA sequences obtained.

	Organism	Sequence ID	Score	Expect	compatibility	No.Nucleotide
1	<i>Gallus gallus</i> (Control group)	gb JF831880.1	1352	0.0	100	21-752
2	<i>Gallus gallus</i> (Bone Abnormalities)	gb JX414253.1	1351	0.0	99	14-753
3	<i>Gallus gallus</i> (Bone Abnormalities)	gb JX414253.1	1352	0.0	99	14-753

Table (2): Represent type of polymorphism in promoter region, 5UTR of IGF1 gene for Bone Abnormalities (Distortion) of *Gallus gallus*.

	Change	Location of substitution	Type of substitution	No. of sample	Frequency 100%
1	G>T	704	Transversion	15	30
2	C>T	249	Transition	50	100
3	T>G	104	Transversion	50	100

Discussion

The *IGF* are important regulators in stimulating growth, protein synthesis, and cell proliferation and differentiation in a variety of cell types [11,12]. Single nucleotide polymorphisms (SNP), one base change including deletion, insertion, and substitution, play an important role in the transcription and translation of genes and affect function of protein, Laere, *et al.*, [13] reported that a mutation in the *IGF2* gene acts as a major QTL that affects muscle growth in the pigs, Nagaraja, *et al.*, [14] to polymorphism in 5'-untranslated region of the chicken *IGF1* gene is significantly associated with lower egg weight and higher egg shell weight. Amills, *et al.*, [4] reported that polymorphism in the promoter region was associated with growth rate and feed efficiency. Zhou, *et al.*, [15] showed that polymorphism in the promoter and 5'-untranslated region (5'-UTR) of the *IGF-I* gene was associated with growth and carcass traits. Also, Bian, *et al.*, [16] reported that haplotypes based on three *IGF-I* polymorphisms (5'-flanking, exon 3, and 3'-flanking regions) were associated with body weight traits, and Promwatee and Duangjinda [17] and Promwatee *et al.*, [18], found that polymorphism at 5'-UTR of the *IGF-I* gene was associated with body weight and carcass traits of synthetic chickens. Gouda and Essawy, [19], shown evaluate the transcript expression pattern of avian *IGF-I* polymorphism, and their effect on the growth traits of chickens by SSCP analysis of RT-PCR products. Consequently, the purpose of the present study was to investigate the possibility of using the *IGF-I* gene at promoter and the 5'-UTR for 1) studying the variation of the *IGF-I* gene; and 2) studying the associations of *IGF-I* gene polymorphism with vertebrate growth and development.

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