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RESEARCH ARTICLE

The Polymorphism of the Bone Morphogenetic Protein *BMP5* Gene, Intron 1 in Bone Abnormalities (Distortion) of *Gallus gallus* Iraqi

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Abstract

The chicken has been widely used in experimental research given its importance to agriculture and its utility as a model for vertebrate biology and biomedical pursuits for over 100 years. The aim of this work was study the polymorphism in *BMP5* gene in Bone Abnormalities (Distortion) of *Gallus gallus* Iraqi**.** To achieve this goal, blood samples were collected from 50 *Gallus gallus* affected from bone abnormalities (distortion) and 20 samples a control group from the Animal farm / college of veterinary / university of Baghdad, the *BMP5 gene* (A 182 bp fragment) were amplified by using specific primers for intron 1 of this gene, and then found the sequence of this region. The DNA sequencing results of flank sense of *BMP5* gene from control was found to be compatible 98% with wild type of *Gallus gallus* from the Gene Bank, appear frequency 100%, and Our results showed that the polymorphism AA were diagnosis of Gallus gallus genotype of Iraqi was highly significant (X^2=100, P>0.001, while (95% ; 96%; 98%; 96%; 97% and 96%)compatibility were found for that gene from first , second , three, four, five, and six groups respectively) of 50 cases with wild type of gene**.** The difference may be attributed to two deletion (A) at position +142 and +152, two transition mutations, and six transversion mutations. Deletion A at position 152 have high frequency from all groups, however single nucleotide polymorphism (143 A/C, 233 C/G and 234 G/C), have low frequency. Conclusions: variability BMP5 intron 1 polymorphism demonstrated association to Bone Abnormalities (Distortion) of *Gallus gallus* Iraqi**.**

Introduction

Bone morphogenetic protein 5 is a protein that in humans is encoded by the *BMP5* gene [1]. The protein encoded by this gene is member of the TGFβ superfamily. Bone morphogenetic proteins are known for their ability to induce bone and cartilage development. Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-beta (TGF-β) family of secreted signaling molecules [2]. These proteins are synthesized as prepropeptides, cleaved, and then processed into dimeric proteins. This protein may act as an important signaling molecule within the trabecular meshwork and optic nerve head, and may play a potential role in glaucoma pathogenesis. This gene is differentially regulated during the formation of various tumors [3]. When BMP genes were first discovered and assayed for expression in vertebrates, individual members of the family were initially proposed to promote general steps in the differentiation of all skeletal tissue [4]. Although Bmp5 is expressed in a continuous fashion in the perichondrial layer surrounding many developing skeletal structures [5 and 6], our enhancer surveys do not show evidence for general enhancers in the Bmp5 gene that drive expression around the surface of all cartilage or all bones. Instead, distinct Bmp5 enhancers regulate expression in individual skeletal structures. Although previous studies have revealed much about the important role of *BMP*s in skeletal patterning in embryogenesis, many of these studies were limited by two issues. First, since BMPs are required for multiple aspects of organogenesis, loss of function mutations often produce animals with prenatal lethality due to pleiotropy [7 and 8]. Second, multiple coexpressing BMPs can produce functional redundancy and mask the effect of loss of function of a single BMP [9

and 10]. In this study, we aimed to further categorize the association of variants within intron 1 of *BMP5* with bone abnormalities (distortion) of *Gallus gallus* Iraqi through an expanded genetic association study of the intron and subsequent functional analysis of associated polymorphisms.

MATERIALS AND METHODS

Samples and DNA extraction

Approximately, 3ml venous blood was collected from 50 *Gallus gallus* affected from bone abnormalities (distortion) and 20 samples a control group from the Animal farm / College of Veterinary / University of Baghdad (Figure1). Whole blood was collected (3ml) into an EDTA-tube; the samples were stored at -20°C until further processing. DNA was extracted from the samples by DNA extraction kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, WI, USA) according to the manufacturer's protocol.

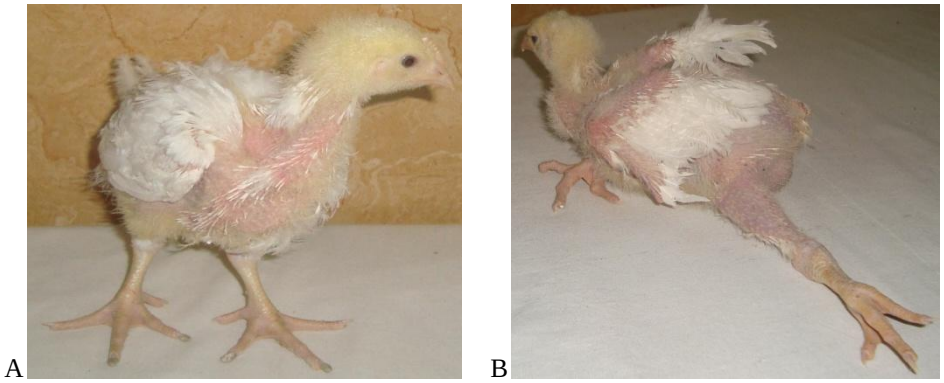

Figure (1): (A): *Gallus gallus* was a control group, (B): *Gallus gallus* was affected from Bone Abnormalities (Distortion).

Detection of Gene *BMP5* by Using PCR

Detection of *BMP5* gene was conducted by using primers for amplification of for *BMP5* gene. A fragment 170 bp of *BMP5* was amplified using a forward primer (BMP5F: 5′-AGCGACCATCATTGTAGCCA-3′) and a reverse primer (BMP5R:5′-GAGAGTGCAGACATGACGCT-3′) (Primers set supplied by alpha DNA Company, Canada). The PCR amplification was performed in a total volume of 25µl containing 1.5µl DNA, 12.5 µl Go Taq green master mix 2X (Promega corporation, USA), 1µl of each primer (10 pmol) then the volume was completed with 25µl of nucleases free water. The thermal cycling conditions were done as follows: Denaturation at 94 °C for 7min, followed by 35 cycles of 94 °C for 30s, 62°C for 30s, and 72 °C for 30s with final incubation at 72°C for 5 min using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after Ethidium bromide staining.

Sequencing and Sequence Alignment

Sequencing of *BMP5* gene were done by Macro gen company, USA for sequencing of products through used individual up and downstream primer were used in each sequencing reactions. Homology searches were conducted between the sequence of standard gene BLAST program which is available at the national center biotechnology information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and using BioEdit program.

Statistical analysis

The statistical analysis is a very important final step in the research to analyse and evaluate the obtained results. Medical statistics of this study was conducted via computer based statistical program which was: $\mathbf{X^2}$ for Windows computer package. The statistical analysis tests which used in this were as follows: P value <0.001 is considered a significant correlation.

Results and discussion

Blood collection and DNA extraction: Collected from 50 *Gallus gallus* affected from bone abnormalities (distortion) and 20 samples a control group from the Animal farm / College of Veterinary / University of Baghdad, the DNA was extracted from 70 blood samples efficiently by using (Wizard Genomic DNA purification kit). Purity and concentration measured by using the standard method [11]. DNA was successfully extracted. The range of DNA concentration extracted from whole blood was (2.7–3.3 µg\µl) and the purity range was (1.2-1.6). *BMP5* gene from genomic DNA were amplified by using specific PCR primers results shown in figure (2) indicated that a yield of single band of the desired product with a molecular weight about 180 bp for BMP5 gene was obtained.

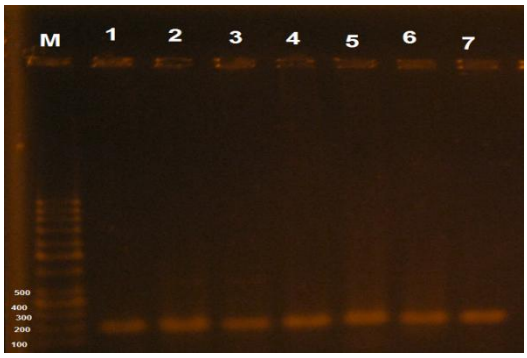

Figure (2) : Agarose gel electrophoresis for amplified *BMP5* gene of *Gallus gallus*. Bands were fractionated by electrophoresis on a 1.5 % agarose gel (2 h., 5V/cm, 1X Tris-acetic buffer) and visualized under U.V. light after staining with Ethidium bromide staining. Lane M: 1000 bp ladder. Lane: 1, 2, 3, 4, 5 (Abnormalities), Lane: 6, 7. (Control).

BMP5 gene was successfully amplified using specific PCR primers for intron 1, Figure (2) showed PCR amplification of intron 1 of the *BMP5* where a specific product at 182 bp was observed. Sequencing of this gene was performed to detect variant and polymorphism which related to development of skeletal muscle. Sequences alignment using BLAST and BioEdit showed that the 98% similarity of 20 healthy sample and 50 defect sample with wild type of the *BMP5* gene of *Gallus gallus* from the Gene Bank may be attributed to (AA) polymorphism in 146 and 147 position of intron 1 in *Gallus gallus* Iraq (Figure 3), appear frequency 100%, and Our results showed that the polymorphism AA were diagnosis of Gallus gallus genotype of Iraqi was highly significant (X^2=100, P>0.001). The *BMP5* gene from 50 *Gallus gallus* affected from bone abnormalities (distortion) divided to six group by using alignments of NCBI, first, second and three group had two deletion A at position (143 and 152) of *BMP5* intron1. However, four group have one deletion A at position 143, but five and six group have deletion A at 152 position. First group (8 sample) shows 95% compatibility with the wild type sequences of *BMP5* gene from Gene Bank as shown in figure (3), one transversion at position +233 C/A single nucleotide polymorphism. Second group (12 sample) shown 96% compatibility with wild type, two transversion at position +231 T/A and +233 C/T. Three group (6 sample), have two transversion at position +231 T/A and +232 A/T and one transition at position + 233 C/T. Five group (9 sample) have one transition at position +143 A/G, however six group (7 sample) have two transversion at position + 143 A/C and +233 C/G, and one transition at position +234 G/A, shown figure 3, Alignment of the partial nucleotide sequences from sex group *Gallus gallus* that carried a polymorphism, and table 1, shown polymorphism and frequency of BMP5 intron 1 in *Gallus gallus* Iraq, appear deletion A at position + 152 were high frequency 60% than other polymorphism.

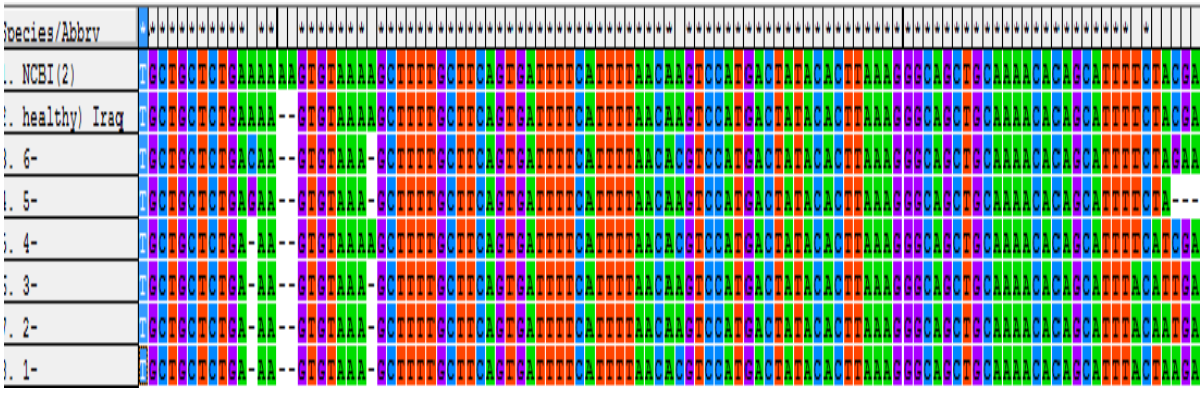

Figure (3): Alignment of the partial nucleotide sequences from sex group *Gallus gallus* that carried a polymorphism, compared with wild type (NCBI) of *BMP5* gene fragment inton 1.

Table (1): Polymorphism detected in partial *BMP5* gene of Bone Abnormalities (Distortion) of *Gallus gallus* Iraqi.

No.	Location of polymorphism	No. of sample	Type of mutation	Name of group	Frequency %
1	143 A	34	Deletion	1,2,3,5,6	48.57
2	152 A	42	Deletion	1,2,3,4	60
3	+233 C/A	8	Transversion	1	11.42
4	+231T/A	26	Transversion	2,3,4	37.14
5	+ 233 C/T	18	Transversion	2,3	25.71
6	+232 A/T	14	Transversion	3,4	20
7	+143 A/G	9	Transition	5	12.85
8	+143 A/C	7	Transversion	6	10
10	+233C/G	7	Transversion	6	10
11	+234G/A	7	Transition	6	10

1: first group ; 2: second group ; 3:three group; 4: four group ; 5: five group; 6: six group

Previous studies demonstrate that secreted signaling molecules in the bone morphogenetic protein (BMP) family play a key role in both formation and repair of skeletal structures [12]. Conversely, mouse mutants missing members of the BMP family show defects in subsets of bone and cartilage elements. A large number of spontaneous and induced short ear mutations suggest that the Bmp5 locus is surrounded by large regulatory regions required for developmental expression patterns in bones and other tissues [13 and 14]. In addition, gain and loss of regulatory elements in *BMP* genes may provide a simple genomic mechanism for evolutionary modification of skeletal structures. While null mutations in *BMP* genes often have pleiotropic defects, adaptive changes in specific regulatory sequences could localize effects to particular skeletal structures, making it possible to alter vertebrate anatomy while preserving viability and fitness [12]. In a previous study the genotyping of 36 microsatellite markers from within a narrow interval of chromosome 6p12.3-q13 and for association to female hip osteoarthritis, with the most compelling association observed for marker D6S1276 located within intron 1 of the bone morphogenetic protein 5 gene (BMP5) [15]. *BMP5* is a member of the TGF-β superfamily of secreted proteins whose family members are involved in synovial joint development and joint tissue homeostasis [16]. Polymorphisms located within the transcribed region of *BMP5* and within its proximal promoter had previously been excluded for

association with osteoarthritis [5], so our association to intron 1 of *BMP5* was unlikely to be explicated by linkage disequilibrium (LD) between D6S1276 and polymorphisms within the coding region or promoter of the gene. Thus, it seems plausible that the osteoarthritis susceptibility mapped to intron 1 of *BMP5* may be due to polymorphisms in *cis*-regulatory elements that act by quantitatively altering gene expression as opposed to amino acid substitutions that qualitatively alter the structure of the encoded protein [6]. Two *BMP5* intron 1 polymorphisms demonstrated association in the combined case control cohort of individuals : microsatellite D6S1276 and SNP rs921126 . Functional analyses in osteoblastic, chondrocytic, and adipocytic cell lines indicated that allelic variants of D6S1276 have significant effects on the transcriptional activity of the BMP5 promoter in vitro [17].

Conclusions: The association of variants within intron 1 of *BMP5* with development of skeletal muscle (bone abnormalities) through an expanded genetic association study of the intron 1, through this analysis, we identified a SNP and associated with bone abnormalities and show that allelic variants of intron are responsible for altered transcriptional activity of the *BMP5* promoter, which implies that polymorphism in cis-regulation of *BMP5* is involved in development of skeletal muscle susceptibility.

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