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Research Article

Diversity of the Chicken Growth Hormone Gene and Effects on Growth Desi and Fayoumi Chicken Traits at District Kashmore, Sindh-Pakistan

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Abstract

Chicken growth hormone (cGH) gene, one of the candidate genes for economic traits that control body weight and fat deposition, is associated with regulating both growth-axon patterning and metabolism. The growth hormone gene located on the chromosome 2 it enhances and encodes growth hormones as well as protein which are responsible for regulating of growth and development of body tissues. Growth hormone gene as economically very important because of growth rate, efficiency of feeds, body weight, and also for egg production. It also used for the Genetic Engineering for production of desirable GH-related genes. Genomic DNA from four different chicken breeds were screened for single nucleotide polymorphisms (SNPs) of cGH gene by denaturing high-performance liquid chromatography, as well as sequencing. SNPs and Nucleotide Diversity of the cGH Gene PCR amplification of 700 bp region from each individual from two chicken breeds was performed, which covered partial exons/introns encoding fragments corresponding to the investigated translated peptide. An average of one SNP was found every 86 bp, with a total of eight SNPs discovered. Nine SNPs were in introns, while 4 and one each was in the 5'UTR and 3'UTR respectively of these, five of them (01 from GWAS linkage signals) had significant associations.

Keywords

Animal Husbandry, Desi Chickens, Fayoumi Chicken, Phenotype, Plumage

1. Introduction

The common chicken also known as *Gallus gallusdomesticus*, is predominantly derived from its predecessor, the red jungle fowl, however, it has also undergone genetic modification from other jungle fowl species [13]. In a flock of chickens, each bird assumes a dominant role, creating a hierarchical structure, and when a chicken faces danger from predators, and experiences anxiety or health issues, it could exhibit fluffing up its feathers. Most developing countries are facing fierce scarcity of animal protein including Pakistan with a gap of approximately (10g) in per capita availability [2]. This scarcity is high in denser rural areas which cover the major part of the population. The rapid source of the protein is only poultry chicken production. The implementation of crossbreeding programs, which involve the enhancement of local breeds with appropriate exotic breeds, has been recognized as a notable approach, and offspring resulting from crossbreeding demonstrated superior characteristics in terms of growth rate, meat quality, and feed conversion [3]. The

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speedy transmission of chicken benefits to the commercial mixed offspring is primarily attributed to genetic choice [4]. The increasing demand for the production of Desi chickens is due to self-caring, least investment, useful for degradation of environmental hazards, and harmful wastes and Desi chicken products are much better than poultry chickens [12]. It is imperative to undertake genetic improvement measures for Desi chicken and an approach to achieve this through cross-breeding of improved Desi chicken and imported exotic breeds, with a focus on the conservation of desirable genes [6].The harsh environmental conditions in their feeding management, overcrowding, dust, litter, and pathogens are relaying the harmful effects on valuable eco-friendly avifauna communities [18]. The breeding Desi and Fyoumi chickens play a significant role in the food industry and valuable economy but climatic changes make it increasingly challenging to survive [8]. The chicken industry has immerged rapidly during the last three decades in Pakistan. The supply of commercial poultry yields in rural areas is much low as compared to urban areas. Their survival ability and adequate environmental adaptation make chickens a successive indicator of poultry production in harsh ecological niches because in different parts of the world including Pakistan [11]. The native chicken breeds give a prior population for better diversification and production so for sufficient meat production and profitability the domestic chicken breeds can be enhanced genetically [9].In many aspects of poultry products, some local traits having much better competence like the weight of eggs, egg laying capacity and rapid rate of expansion can be helpful for the economic effectiveness of rural poultries [15]. Under rural conditions, the chances of survival for chickens become much more difficult due the climatic changes, however, cross-breeds between different types of chickens are carried out under rigorous and improved environmental conditions [1]. Rapid trait production of different breeds may be crossed to increase in their population, efficient sustainability in respect of harsh ecological conditions, and increase in meat production which contributes a great part of income for local farmers [16]. The variation in weight gain, growth rate, and body organ size was analyzed between purebred chicken breeds. The possible outcomes of this research will help in identification of distinguish morphological characteristics of Desi and Fayoumi selected chicken breeds [5].

2. Material and Methods

To establish the resource population, cGH SNPs were used for genetic analysis of chicken growth and phenotype traits by crossing Desi with Fayoumi breeds. The housing and care of the mice were in compliance with National Research Council guidelines for dietary manipulation waves.FloatField(assigns$) At all ages, body weight (BW) and shank length(SL), hatch weight(HW) were recorded; Weight gain was calculated as the average of monthly gain from 90 days up to6 months. EDTA-anticoagulated blood from a total of 24 individuals was used for isolationof genomic DNA. A resource population was established originally through a cross-bred family of Desi and Fayoumi to detect the associations between cGH SNPs with chicken growth and phenotype traits.

2.1. Polymerase Chain Reaction Amplification, SNP Detection

Table 1. *Nucleotide diversity of the chicken growth hormone gene and others reported in chicken.*

No	SNPs	Location	RFLP enzyme
01	G-230A	5'UTR	
02	G+237A	Intron 1	Taq1
03	G+351A	Exon 1	
04	T+419C	Intron 2	
05	G+532A	Exon 2	
06	G+525A	Intron 3	Msp I
07	C+596T	Exon 3	
08	T+682C	3'UTR	

Genes	No. of SNPs	Base pairs	Individuals (n)	Adjusted Ø	References
cGH	08	700	24	1.6	Present study

Genes	No. of SNPs	Base pairs	Individuals (n)	Adjusted Ø	References
GHR	33	4007	40	1.9	Song *et al*,.2018
IGF-I	15	2578	40	1.4	Liu *et al*,. 2012
IGF-II	04	1681	35	0.6	Kazemi *et al*,.2018
LEPR	09	1085	40	2.1	El-Magd et al,.2016

Primers 115-700 were used to amplify full-length cGH gene; Primer PM3 was employed in the identification of polymorphisms within the Gene fragment. Briefly, polymerase chain reaction (PCR) reactions were implemented and assessed as mentioned before; selected PCR products with different mutations particular to each region like COL1A2 ex5 + 151 G->T mutation resulted in a missense amino acid substitution glycine to cysteine that affects the twining repeat domain included for surrerive purifications while others have been sequenced by outsourcing Macrogen Company-Korea. For each PCR product, sequencing was bidirectional. Sequencing output was BLASTed by the ENSEMBL program.

2.2. PCR-RFLP Analysis

G-230A, G+237A, and T+419C were among the eight SNPs identified in the cGH gene that were located at restriction sites for Taq I, Msp I, and Taq 1. Following primer pairs 101 (for G-230A), 112 (for G+237A), and 124 (for T+419C) amplification, the PCR products were digested overnight at 37°C using Taq 1 and Msp I, respectively. The digestion mixture held 20µL of PCR results, of which 7µL were the master, 2µL were the forward and reverse primers, 5µL were the DNA template, and 4µL were PCR grade water.

2.3. Statistical Analysis

To estimate the nucleotide diversity of the cGH gene, the normalized numbers of variant sites (h) was calculated as the number of observed nucleotide changes (K) divided by the total sequence length in base pairs (L), corrected for sample size (n).

3. Results

Nucleotides and SNPs ThecGH gene was amplified using PCR to examine a 700 bp area in two different breeds of chicken, with 36 individuals from each breed. Eight SNPs, or an average of one SNP per 86 bp, were discovered. With four in the 5#UTR, one in the 3#UTR, and five in coding exons, the majority of these SNPs (08) were found in introns. Amino acid alterations were caused by two of the five coding SNPs. Two non synonymous coding SNPs changed an amino acid in the cGH precursor (T+419C) and the mature cGH (R59H), with one (G-230A) changing an amino acid and the other (G+237A) changing an amino acid.Twonon synonymous coding SNPs changed an amino acid in the cGH precursor (T+419C) and the mature cGH (R59H), with one (G-230A) changing an amino acid and the other (G+237A) changing an amino acid.Overall, the adjusted nucleotide (h) diversity of the cGH gene was 2.7 10H3, but within introns, it was 3.1 10H3 [7]. Even within similar base populations, the nucleotide diversity of the cGH gene was somewhat higher than that of some other chicken genes, including GHR, ghrelin, the growth hormone secretagogue receptor (GHSR), IGF1 and IGF2, the insulin-like growth factor binding protein 2 (IGFBP-2), the leptin receptor (LEPR), the pituitary-specific transcription factor-1 (PIT-1), the bone morphogenetic protein receptor type II (BMPR2), and the phosphoenolpyruvatecarboxykinase-C (PEPCK-C) gene [10].

3.1. Associations of Single SNP with Chicken Growth and Carcass Traits

In a F 2 reciprocal cross between the Desi and Fyoumi breeds, an association analysis of the cGH SNP with chicken growth and carcass traits revealed that genotypes at C-121T were substantially (P, 0.05) linked with LA and LFC and with SL at the ages of 70 and 84 days. SNP G-230A showed a strong correlation (P.01) with SIL and a substantial correlation (AFW). There were no discernible relationships between C+596T and any characteristics linked to growth. Growth gene SNPs were highly significant with BW21, 28, 70, 84, and ADG0-4, and strongly linked with BW at the ages of 14, 35, 42, 49, 63, and 77 days, and with SL at the ages of 49, 56, and 84 days. Lastly. SNP showed a strong correlation with SL70 and SL84 and a substantial correlation with HW. Although in certain instances the heterozygous (AG) trait measure did not substantially vary from one or both homozygote's, the influence of the SNP phenotype on the various BW, SL characteristics, and ADG0-4 appeared to be additive. The AA homozygote differed at the substantial or very significant level from the GG homozygote for every attribute given.

3.2. Haplotype Reconstruction and Linkage Analysis

In our F 2 reciprocal cross, we identified 44 diplotypes and 15 haplotypes of H1–H15 when taking these five cGH SNPs collectively. H1 (C/G/T/G/G) and H2 (C/A/T/G/G) were the most prevalent of these 15 haplotypes, with frequencies of 32% and 23%, respectively; minor haplotypes included H8

(C/G/T/G/T), H9 (T/G/T/G/G), H10 (T/G/T/G/T), H11 (C/A/T/A/T), and H12 (C/G/T/A/G); rare haplotypes included H13 (C/G/C/G/T), H14 (T/A/T/G/G), and H15 (C/G/C/A/G). Diplotypes based on all haplotypes were substantially linked with only BW14 (P=.05) according to linkage analysis. When taking into account the major haplotypes with frequencies greater than 5 percent, substantial relationships between diplotypes and SL84 (P>.05) and LA (P<.01) were found.

Table 2. *The probability of associations (P value) of polymorphisms in five single SNPs (single nucleotide polymorphisms) and their haplotypes with growth and carcass traits.*

Single SNPs				**Haplotypes**	
Traits	**G-230A**	**G+237A**	**T+419C**	**≥5%**	**In total**
Body Height (cm)	43.4±3.2	37.8±2.4	38.1±3.4	23.8	14.5
Neck length (cm)	20.4±1.9	15.3±1.4	19.3±1.6	11.0	8.1
Shank Length (cm)	12.5±0.8	10.3±0.6	10.2±0.8	6.60	4.5
Beak Length (cm)	3.6±0.2	3.2±0.17	3.3±0.21	2.02	1.6
Wing Span (cm)	23.7±1.6	20.3±1.4	21.3±1.9	13.1	9.7

Table 3. *Differences in growth and body composition traits between chickens with different genotypes of G1705A.*

Genotypes					
	AA (18)	**AG (85)**	**GG (348)**	**GC (415)**	**AT (652)**
Body Height (cm)	45.6 ± 3.6	42.4 ± 2.1	40.3 ± 2.4	43.6±2.2	44.4±1.8
Neck length (cm)	18.5±1.4	18.2±1.2	19.6±1.3	18.7±1.3	18.8±1.2
Shank Length (cm)	8.6±0.34	8.4±0.32	8.3±0.38	8.2±0.31	8.4±0.34
Beak Length (cm)	3.1±0.3	3.4±0.2	3.2±0.2	3.5±0.3	3.4±0.2
Wing Span (cm)	21.3±1.8	22.4±1.5	21.6±1.3	22.3±1.4	21.8±1.3

4. Discussion

The cGH gene diversity found in this study was quite high. Only a few cGH SNPs (G-230A, C+596T, and T+419C) had previously been identified, and the 5#-regulatory area had not been shown to vary. 46 point mutations across the whole cGH gene were found in this study in four different chicken breeds (Table 1). Even when those other chicken genes were sampled from the same four breeds, the cGH gene's nucleotide diversity was larger than that of numerous other chicken genes (Table 2). Moreover, the cGH gene's h value was larger than those found in hens after adjusting for sample size.A preliminary genome-wide scan for chicken SNPs revealed 2.8 million SNPs throughout the whole chicken genome, including 23 SNPs located in the region of the cGH gene, which seems to confirm the high diversity of the cGH gene.

It was intriguing to note that practically all growth traits had a substantial correlation with G+237A, with the A allele showing a usually beneficial effect on chicken growth. This was in line with the fact that Desi's F 0 chickens had greater A frequencies than X's, despite the fact that G remained the dominant allele in both breeds and that fewer F 2 birds carrying the AA genotype (18) were produced. Prior research on further polymorphisms in the cGH gene's introns revealed correlations with the growth, fat accumulation, and egg output of chickens. According to a recent study, a significant QTL influencing pig muscle growth was encoded by a single mutation in intron 3 of the IGF2 gene. Similar to this instance, the G-230A mutation in cGH's intron 3 may directly impact chicken growth by affecting the expression of the cGH gene. However, it's possible that the G+237A cGH polymorphism and another causal mutation that affects growth features in our resource population are in linkage disequilibrium.

Because of their distinct histories within the two parental breeds used, the other four cGH SNPs that we investigated might not show this degree of disequilibrium with the growth trait QTL. It will take more investigation to distinguish between these alternatives.

5. Conclusion

According to the current study, at 70 and 84 days of age, the genotypes at C-121T were significantly (P>.05)linked with SL and LA and LFC, as well as with characteristics in a F 2 reciprocal cross between the Desi and Fyoumi breeds. Significant (P<.01) associations were found between SNP G-230A and AFW, and SIL. There were no discernible relationships between C+596T and any characteristics linked to growth. Diplotypes based on all haplotypes were substantially related with only BW14 (P >.05), according to linkage analysis.

6. Recommendations

1) The investigation of the relationship between carcass features and the variability of the chicken growth factor gene.

2) Research ought to focus on the genetics and hormonal differences between breeds with the fastest and slowest rates of growth at District Kashmore, Sindh, Pakistan.

3) Research on the relationship between growth performances and polymorphisms in the insulin-like growth factor gene in Desi chickens.

4) Genetic marker regulation and its implications in Fayoumi and Desi chicken.

Abbreviations

cGH	Chicken Growth Hormone
NPs	Single Nucleotide Polymorphisms
PCR	Polymerase Chain Reaction
PEPCK-C	Phosphoenolpyruvatecarboxykinase-C
BW	Body Weight
SL	Shank Length
HW	Hatch Weight
Taq1	Taq Polymerase Enzyme 1
IGF1	Insulin Like Growth Factor 1
GHSR	Growth Hormone Secretagogue Receptor
IGFB	Insulin Like Growth Factor Binding Proteins
LEPR	Leptin Receptors
PIT	Pituitory Specific Transcription Factor
BMPR2	Bone Morphogenetic Genetic Protein Receptor 2

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Conflicts of Interest

The author declares no conflicts of interest.

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