The Pharma Innovation Journal 2022; SP-11(10): 78-81

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(10): 78-81

www.thepharmajournal.com
Received: 08-07-2022
Accepted: 12-08-2022

Prashant Maru
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

FP Savaliya
Poultry Research Station, Anand Agricultural University, Anand, Gujarat, India

SJ Jakhesara
Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

AC Patel
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, India

Corresponding Author:
Prashant Maru
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

SNPs Identification in production related genes in strains of white leghorn chicken

Prashant Maru, FP Savaliya, SJ Jakhesara and AC Patel

DOI: https://doi.org/10.22271/tpi.2022.v11.i10Sb.16009

Abstract
This is the first comprehensive study aimed to determine Single Nucleotide Polymorphisms (SNPs) in candidate genes (GHR, IGF1, OCX-32 and GDF9) reported to associated with egg production at the age of 64 weeks (EP64) in strains of White Leghorn chicken (Anand Synthetic White Leghorn and Anand Bantamised White Leghorn by sequencing the coding region of respective genes. Both the strains of White Leghorn chicken were categorized into four different groups *viz*., Anand Synthetic White Leghorn High Production (ASHP), Anand Synthetic White Leghorn Low Production (ASLP), Anand Bantamised White Leghorn High Production (ABHP) and Anand Bantamised White Leghorn Low Production (ABLP) to find out SNPs in coding region of candidate genes and its association with EP64. The genomic DNA was extracted for 96 chickens and then further sequenced with the designed custom amplicon panel on the Illumina MiSeq platform. Raw data collected after sequencing was used for the bio in formatic analysis to confirm its association with EP64. Consequently, over 99% target regions were amplifed and 87 SNPs were available after quality filtering. Only thirty-three variants were novel while majority of the identified variants were catalogued in dbSNP database as known variants. The T21912084G, C21912423A and rs318030570 of OCX-32 and C13415980T of GHR gene was found to have a significant association (p<0.05) with EP64. The present study revealed that four SNPs, including T21912084G, C21912423A, rs318030570 of OCX-32 and C13415980T of GHR would be useful as a genetic marker for breeding to increase chicken EP64.

Keywords: SNP, GHR, IGF1, OCX-32, GDF9, white leghorn

Introduction
Globally, the poultry industry is gaining significant importance among the agricultural and its allied sectors. Poultry industry is one of the fastest-growing segment of the agricultural sector. Poultry industry play a significant role in boosting the economy of many developing countries, providing nutrition and also con attributing to the livelihood of many, particularly in rural areas (Demeke, 2004) [1].

Egg production is an important economic trait in poultry. Endocrine factor and many other factors such as the length of photoperiod, environmental factors and different feeding allowances could influence egg production. Nevertheless, the genetic factor is the prerequisite. Egg production is a polygenic inheritance trait with low to moderate heritability, which depends on the period involved (Luo *et al*., 2007) [2].

In avian, egg production is a complex process controlled by many hormones. Today, many candidate genes such as Growth Differentiation Factor 9 (GDF9), insulin like growth factor (IGF-I), Growth Hormone Receptor (GHR), Ovocalyxin-32 (OCX-32) were reported to be associated with egg production and its quality (Li *et al*. 2008; Li *et al*. 2009; Uemoto *et al*. 2009) [3, 4, 15]. Insulin like growth factor 1 (IGF-I) as a paracrine regulator of follicular faith initiating the maturation of a follicle as in traovarian regulator. Ovocalyxin-32 (OCX-32), a 32-kDa protein, is present at high levels in the uterine fluid during the terminal phase of eggshell formation and is localized predominantly in the outer eggshell suggesting that it may play a role in the completion of the eggshell (Gautron *et al*., 2001) [7]. Growth Differentiation Factor 9 (GDF9) is a secreted oocyte glycoprotein belonging to the transforming growth factor beta (TGFβ) superfamily, expressed in the oocytes and granulosa cells of ovarian follicles in chickens and is a central regulator of folliculogenesis and ovulation rate (Johnson *et al*., 2005) [6]. Increasing egg yield and quality by applying traditional selection methods is challenging, since they possess low heritability and are controlled by many genes. Today, however, thanks to developing molecular techniques, genes related to egg yield and quality could be detected at molecular level. By supplementing traditional selection methods with Marker Assisted.

Selection (MAS), the frequency of desired genotypes associated with higher egg yield and quality could be increased in chicken populations.

Since the release of the chicken genome sequence in 2004 (Hillier *et al*., 2004) [8], the tool box available for genetic improvement of the multiple traits that define egg quality has improved tremendously. Genome sequence comparisons between mammalian and avian species have allowed for the identification and subsequent annotation of genes. The recent sequencing of multiple breeds of chickens, both research and commercially utilized lines, revealed the presence of millions of genetic variants (Kranis *et al*., 2013) [9]. To aid in the analysis of the large amounts of genetic information that is generated, various bioinformatics tools have been developed that allow for the integration and eventual application of this information into a breeding programme (Wolc *et al*., 2016) [10]. The objective of the present study was to identify Single Nucleotide Polymorphisms (SNPs) of candidate genes *viz*., GHR, OCX-32, IGF^{-1} and GDF9 by sequencing the genomic DNA to detect those DNA polymorphisms in strains of White Leghorn chicken (Anand Synthetic White Leghorn and Anand Bantamised White Leghorn). In particular, we searched for a SNPs present in the four candidate genes, and analysed the effects of SNPs on the relationship between these polymorphisms and egg production at the age of 64 weeks (EP64).

Material and Methods

Sample collection and genomic DNA isolation

The blood samples of two strains of White Leghorn chicken *viz*., Anand Synthetic White Leghorn and Anand Bantamised White Leghorn were collected that were maintained at Poultry Research Station, Anand. Blood samples were collected from wing vein in sterile 10 ml EDTA vacationer and kept at 4 °C until the genomic DNA isolation.

Quality and Quantity analysis of genomic DNA

All the reagents and plastic ware prepared using Milli-Q water and autoclaved at 121 °C for 15 min. at 15 lbs pressure. Genomic DNA isolation was done according to (John *et al*. 1991) [20]. Quality of isolated DNA was evaluate using 0.8% gel electrophoresis. For the quality and quantity of extracted genomic DNA ND-1000 Spectrophotometer (Nano Drop Technologies, Inc. USA) was used. The concentration of genomic DNA for the library preparation was evaluated through Qubit 3.0 Fluor meter as per the protocol.

Custom Amplicon design and ampliseq library preparation

A BED file format containing candidate genes chromosome number and coordinates details specific to *Gallus gallus* 4.0 reference genome assembly was uploaded on Illumina Design Studio (Illumina, San Diego, CA, USA) to generate Ampliseq custom panel. Ampliseq library preparation kit with 96 CD index was utilized for library preparation as given in Illumina Ampliseq library preparation protocol. Sequencing run was kept on Illumina Miseq using v2 reagent kit.

Bioinformatics analysis

Raw sequencing data were collected in Fastq format and visualized using FASTQC v0.11.7 [https://www.bioinformatics.babraham.ac.uk/projects/fastqc/]. After visualization the sequenced reads were mapped to its particular reference genome *Gallus gallus* 4.0 (*Galga*l4) using Burrows Wheeler Alignment (BWA) v0.7.17 [https://bio-bwa.sourceforge.net/bwa.shtml]. Variant calling was performed with the help of free bayes v0.9.9 [https://www.github.com/ekg/freebayes]. Mapping percentage and coverage metrics were derived using Picard tools v2.25.6 [https://www. github.com/broadinstitute/picard]. The annotation part of data analysis was performed using SnpEff v5.0 and Snp Shift v4.3 [https://snpeff.sourceforge.net/SnpEff.html, https://snpeff.source.net/SnpSift.htmal].

Association of variants with the egg production at the age of 64 weeks (EP64) was done using Plink v1.07.

Results and discussion

The study entitled "SNPs identification in production related genes in strains of White Leghorn chicken" was initiated to unleash polymorphism in four genes *viz*., GHR, IGF-1, OCX-32 and GDF9 to assess their association with egg number at 64 weeks (EP64) of age in Anand Synthetic White Leghorn (ASWLH) and Anand Bantamised White Leghorn (ABWLH) chicken. Blood samples were collected from ASWLH and ABWLH strain from low and high egg producing chicken (48 samples from each) maintained at Poultry Research Station, Anand Agricultural University, and Anand. The genomic DNA was isolated from the blood samples using John's method. The quantity and quality of DNA were checked using the NanoDrop-1000 and Qubit 3.0 fluoro meter. The agarose gel electrophoresis image of representative samples of DNA extracted from blood samples is shown in Fig. 1. Illumina Custom Amplicon panel was designed for selected genes, which included 35 Amplicon [375 base pairs (bp)] that covered 99.89 percentage of the exon's regions of the selected genes. Details of custom amplicon design is mentioned in Table 1.

Table 1: Details of custom amplicon design by Illumina Design Studio for target exons

Genome build	*Galgal4*
No. of Amplicon	35
Total target bases	5633
Total target bases covered	5627
Percent target bases covered	99.89
Maximum amplicon length (bp)	375
Minimum amplicon length (bp)	125

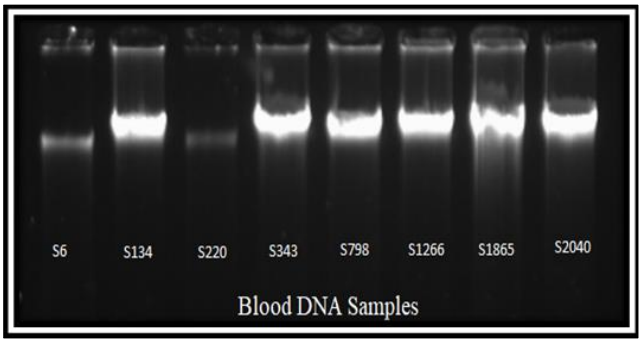

Fig 1: Representative image of qualitative evaluation of genomic DNA using agarose gel electrophoresis under UV transilluminator

Bioinformatics analysis

Sequencing run on Illumina Miseq platform generated 6175.7 Mb of raw data with 97% cluster passing filter and 644 K/mm cluster density. A total of 5.9 GB data was achieved with quality score of 30 (Q-30). Raw sequence data was generated

in FASTQ format which contained both the nucleotide sequence and corresponding quality score.

The quality of each sample was checked by using FastQC v0.11.9. (Fig. 2). The raw data was quality filtered using TRIMMOMATIC v0.20.4. DNA sequence of minimum length of 70 bp and mean quality score of 30 or above were mapped to the reference genome (Galgal4) using Burrows Wheeler Aligner v0.7.17. The SAM files generated after alignment were converted to BAM files using SAM tools v1.12.

The Picard tool v2.25.6 was used to generate alignment metrics of reads *viz*., total number of reads, number of reads aligned to reference genome, number of reads having 2x, 10x and 40x coverage and percentage of target metrics. For variant calling at least 10x coverage is required. The lowest coverage at 10x coverage was observed to be 47.6%, whereas, the highest coverage was observed to be 90.78%. The mean coverage at 10x coverage was 87.33%.

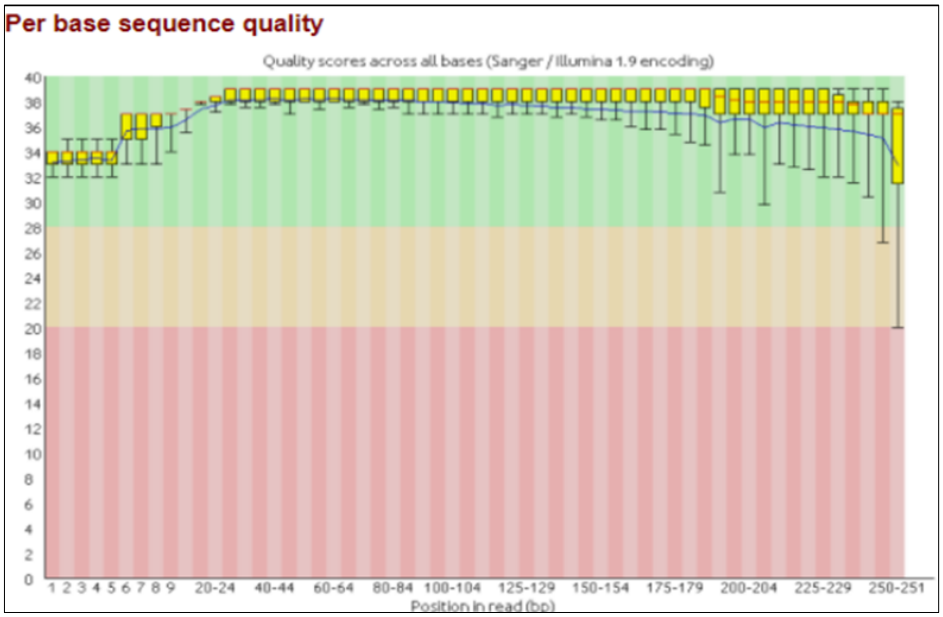

Fig 2: Representative image of per base quality of a random sample generated by Fast QC

Variant calling

The aim of variant calling is to detect how many bases out of the total are different to a reference genome. A total of 87 SNPs were identified from VCF files generated by Free Bayes v1.12. From these 87 SNPs, 33 SNPs were previously reported in dbSNP138 database. Among all identified SNPs, 45 SNPs were identified in OCX-32, 15 SNPs in IGF-1, 24 SNPs in GHR whereas, three SNPs were identified in GDF9 gene. Details of SNPs identified in different region of selected genes generated by SnpEff v5.0 is mentioned in the Table 2.

Table 2: SNPS identified in selected genes of ABWLH and ASWLH chicken by SnpEff

Gene	Region	Number of SNPs
IGF-1	Exon 2	11
	Exon 4	4
OCX-32	5' UTR	3
	Exon 1	9
	Exon 2	6
	Exon 3	2
	Exon 4	4
	Exon 5	4
	Exon 6	17
GDF9	Exon 2	3
GHR	Exon 1	1
	Exon 2	7
	Exon 3	1
	Exon 4	1
	Exon 5	4
	Exon 6	3
	Exon 8	1
	Exon 9	6

Missense variants are of greater concern since these variants change the amino acid sequence in translated protein. Amongst 87 SNPs, 43 SNPs were annotated as missense SNPs. Details of missense SNPs are presented in Table 4.8.

Association of identified SNPs with EP64

In the present study, EP64 was considered as trait to be associated with the SNPs. The association analysis was performed using PLINK v1.07. Total four SNPs were found to be significantly (p<0.05) associated with EP64. Details of SNPs which are significantly associated with EN64 presented in the Table 3.

Table 3: Details of SNPs having significant association with EP64 in ASWLH and ABWLH chicken

Strain	Gene	SNPs	Reference SNP Identifier	P value
ABWLH	GHR	C13415980T	Novel	0.04
	OCX-32	C21912308T	rs318030570	0.02
ASWLH	OCX-32	T21912084G	Novel	0.02
	OCX-32	C21912423A	Novel	0.04

Discussion

The present study was conducted with a total of 96 birds from ASWLH and ABWLH chicken. Out of 87 SNPs, only four were observed to have significant (p<0.05) association with egg production. Only the SNPs present in *OCX-32* and GHR genes were shown to have significant (p<0.05) association with EP64; the rest of the SNPs present in IGF-1 and GDF9 genes had no such significant association. The present findings were in accordance with the results of Uemoto *et al*. (2009) [15], Khan (2011) [1], Kulibaba (2015) [13], Kazemi *et al*. (2018) [11] and Lee *et al*. (2014) [14] who observed significant

(p<0.05) association between OCX-32 and *GHR* gene polymorphism and egg production. However, Kim *et al.* (2004) [18], Li *et al*. (2008) [17], Thaker *et al*. (2012) [16] and Huang *et al*. (2015) [19], on the other hand, revealed a significant association between IGF-1 and GDF9 gene polymorphism and egg production.

References

1. Demeke S. Egg production performance of local and White Leghorn hens under intensive and rural household conditions in Ethiopia. Livest Res Rural Dev. 2004; 16(2):2004.
2. Luo PT, Yang RQ, Yang N. Estimation of genetic parameters for cumulative egg numbers in a broiler dam line by using a random regression model. Poult. Sci. 2007;86:30-36.
3. Li HF, Zhu WQ, Chen KW, Wu X, Tang QP, Gao YS. Associations between GHR and IGF-1 Gene polymorphisms, and reproductive traits in Wenchang Chickens. Turkish Journal of Veterinary and Animal Science. 2008;32:281-285.
4. Li HF, Zhu WQ, Chen KW, Wu X, Tang QP, Gao YS, *et al*. Polymorphism in NPY and IGF-I genes associate with reproductive traits in Wenchang chicken. African Journal of Biotechnology. 2009;8:4744-4748.
5. Uemoto Y, Suzuki C, Sato S, Ohtake T, Sasaki O, Takahashi H, *et al*. Polymorphism of the ovocalyxin-32 gene and its association with egg production traits in the chicken. Poultry Science. 2009;88:2512-2517.
6. Johnson PA, Dickens MJ, Kent TR, Giles JR. Expression and function of growth differentiation factor-9 in an oviparous species, Gallus domesticus. Biol. Reprod. 2005;72:1095-1100.
7. Gautron J, Hincke MT, Mann K, Panhéleux M, Bain M, McKee MD, *et al*. Ovocalyxin^{-32}, a novel chicken eggshell matrix protein: isolation, amino acid sequencing, cloning, and Immunocytochemistry localization. Journal of Biological Chemistry. 2001;276(42):39243-39252.
8. Hillier LW, Miller W, Birney E, Miner TL. 'Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution', Nature. 2004;432:695-716.
9. Kranis A, Gheyas AA, Boschiero C, Turner F, Yu L, Smith S, *et al*. Development of a high density 600K SNP genotyping array for chicken. BMC genomics. 2013; 14(1):1-13.
10. Wolc A, Kranis A, Arango J, Settar P, Fulton JEO, Sullivan NP, *et al*. Implementation of genomic selection in the poultry industry. Animal Frontiers. 2016;6(1):23-31.
11. Kazemi H, Rezaei M, Hafezian H, Mianji GR, Najafi M. Genetic analysis of SNPs in GH, GHR, IGF-I and IGFBPII genes and their association with some productive and reproductive traits in native breeder hens. Gene Technol. 2018;7(1):145.
12. Khan AA. Effect of IGF-I, IGF-II, OCX-32 and PRL gene Polymorphism on Feed Consumption and Egg production in Bantamised White Leghorn Birds (Doctoral dissertation, AAU, Anand); c2011.
13. Kulibaba RA. Polymorphism of growth hormone, growth hormone receptor, prolactin and prolactin receptor genes in connection with egg production in Poltava clay chicken. Сельскохозяйственная биология, (2 (eng); c2015.
14. Lee JY, Choi SY, Kim CD, Hong YH, Jeong DK, Lee SJ. Effects of c. 494A> C and c. 267T> G SNPs in OCX-32 Gene of Korean Native Chicken on Egg Production Traits. Korean Journal of Poultry Science. 2014;41(3): 191-196.
15. Uemoto Y, Suzuki C, Sato S, Ohtake T, Sasaki O, Takahashi H, *et al*. Polymorphism of the ovocalyxin-32 gene and its association with egg production traits in the chicken. Poultry science. 2009;88(12):2512-2517.
16. Thaker MD, Savaliya FP, Khanna K, Joshi CG, Parmar DJ, Ghosh A. Effect of IGF-I gene polymorphism on various economic traits of synthetic White Leghorn. Indian Journal of Poultry Science. 2012;47(1):10-13.
17. Li HF, Zhu WQ, Chen KW, Wu X, Tang QP, Gao YS. Associations between GHR and IGF-1 gene polymorphisms, and reproductive traits in Wenchang chickens. Turkish Journal of Veterinary and Animal Sciences. 2008;32(4):281-285.
18. Kim MH, Seo DS, Ko Y. Relationship between egg productivity and insulin-like growth factor-I genotypes in Korean native Ogol chickens. Poultry Science, 2004;83(7):1203-1208.
19. Huang HY, Liang Z, Li SF, Li CM, Zhao ZH, Wang QB. Polymorphism identification in BMP15 and GDF9 genes and their association with egg production in chickens. British poultry science. 2015;56(3):277-283.
20. John S, Wang J. Quantum optics of localized light in a photonic band gap. Physical Review B. 1991 Jun 1;43(16):12772.