RESEARCH ARTICLE

Screening of SNPs Associated with Egg Production in Two Strains of White Leghorn

Ankit V. Kachchhi[1*], Ashish C. Patel[1], Glory S. Parmar[2], Devanshi V. Patel[2], Prakash G. Koringa[2], F. P. Savaliya[3]

Abstract

The poultry industry in India has become a prominent and rapidly expanding sector of the agro-animal industry, and is recognized as an important source of animal protein in the country. Through genetic analysis, Single Nucleotide Polymorphisms (SNPs) can help identify variations in DNA sequences that are linked to traits such as egg quality, quantity, fertility, hatchability, and other important aspects of egg production in poultry or other egg-laying species. The aim of the present study was to screen the SNPs in the candidate genes associated with egg production, *viz.,* MC4R, MTNR1A, DRD1, PRLR and VLDLR and their association with egg production at the age of 64 weeks in two strains of White Leghorn, *viz.,* Anand Bantamized White Leghorn (ABWLH) and Anand Synthetic White Leghorn (ASWLH). The amplicon-sequenced data of a total of 96 birds for these genes were collected in 'fastq' format and various SNPs were identified. A total of 143 detected SNPs were filtered based on read depth and QUAL score and 60 SNPs were discarded after filtering variants. Out of the remaining 83 SNPs, 69 SNPs were previously reported, while 14 were found as novel. Association analysis showed that one of the SNPs found in the MC4R gene was significantly associated with the egg production in both ABWLH and ASWLH population.

Key words: Egg production, SNPs, White Leghorn.

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Introduction

The poultry industry in India has become a prominent and rapidly expanding sector of the agro-animal industry, and is recognized as an important source of animal protein in the country. The country produced about 45.2 billion eggs in 2004-05, which increased to 138.38 billion in 2022-23. The primary focus of breeding programs for layers is on egg productivity, as it is an important economic trait (Burt, 2005). In recent years, several candidate genes, *viz.,* Very Low-Density Lipoprotein Receptor (VLDLR), Dopamine Receptor D1 (DRD1), Melanocortin Receptor 4 (MC4R), Melatonin Receptor 1A (MTNR1A) and Prolactin Receptor (PRLR) were found to be associated with egg production (Kelly *et al*., 2001; MacNeil *et al*., 2002; Hansen *et al*., 2005; Sundaresan *et al*., 2009; Meng *et al*., 2013).

Any alteration in the expression or function of the VLDLR gene, responsible for the uptake of two essential yolk precursors, vitellogenin (VTG) and VLDL, can significantly impact oocyte development and egg production. In moulted hens, the level of ovarian VLDLR expression is associated with the degree of restoration of reproductive performance (Meng *et al*., 2013). Dopamine is a neurotransmitter exerts its function by selectively binding to dopamine receptors, and at least five different receptor subtypes, DRD1-DRD5, have been identified. In the hypothalamus and pituitary, the DRD1 gene is highly expressed and its expression is linked to the reproductive system (Hansen *et al*., 2005).

Until now, five melanocortin receptor subtypes (MCR1–5) have been identified. The chicken MC4R gene is expressed in various tissues, including the brain, adrenal, gonads, spleen, and adipose tissues (MacNeil *et al*., 2002). Melatonin regulates various biological processes by binding to the MTNR1A, MTNR1B, and MTNR1C receptor subtypes. The expression of MTNR1A, MTNR1B, and MTNR1C transcripts in the chicken ovary was identical to the previously discovered chicken brain receptors, suggesting a direct influence of melatonin on the female reproductive functions of domestic chickens (Sundaresan *et al*., 2009). Prolactin (PRL) is a protein peptide hormone which exerts its functions by selectively binding to the prolactin receptor (PRLR) of cytokine receptor family,

[1]Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat-388001, India

[2]Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat-388001, India

[3]Poultry Research Station, Kamdhenu University, Anand, Gujarat-388001, India

Corresponding Author: Ankit V. Kachchhi, Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat-388001, India. e-mail: ankitkachchhi1998@gmail.com

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in the cell membrane of target cells and their expression patterns are widely distributed. In chickens, PRLR is located on the Z chromosome and is closely linked with reproductive efficiency (Kelly *et al*., 2001).

Next-generation sequencing (NGS) and bioinformatics has revolutionized the field of genomics making the detection of DNA markers easier. Single nucleotide polymorphisms (SNPs) are the most common type of genetic polymorphism, which arise from a single base pair variation in the DNA sequence. SNPs can have functional consequences, such as altering gene expression or protein structure, and are often associated with various traits and diseases (Ko *et al*., 2016). Conventional breeding methods, such as family selection and individual selection, have been used to develop modern chicken breeds that emphasize enhanced production (Qin *et al*., 2015). However, estimating the level of genetic improvement in egg production is challenging due to its polygenic nature along with its low to moderate heritability (Luo *et al*., 2007). So, combining these traditional methods with marker assisted selection can increase the accuracy of selection of birds for meat and egg production. By considering all these facts, the present study was carried out to identify an association of SNPs in the coding region of various genes, *viz.,* VLDLR, DRD1, MC4R, MTNR1A and PRLR in Anand Synthetic White Leghorn and Anand Bantamized White Leghorn chicken.

Materials and Methods

Experimental Birds and Amplicon Sequencing Data

Two strains of poultry, *viz.,* Anand Bantamized White Leghorn (ABWLH) and Anand Synthetic White Leghorn (ASWLH) developed by Poultry Research Station, Veterinary College, Anand Agricultural University, Anand (Gujarat, India) by crossing Bantam chickens with two strains of White Leghorn and by breeding four different commercial strain-crosses: BV-300, Shaver Starcross-280, Hisex white and B.H.78 chickens, to enhance feed efficiency and productivity, respectively, were utilized for this study.

Amplicon sequencing data of Anand Synthetic and Anand Bantamized White Leghorn chicken already generated using Illumina MiSeq sequencer at Department of Animal Biotechnology of the College were utilized for the present study. The details of custom amplicon designed by Illumina Design Studio for target exons are given in Table 1.

Collection of Production Data

The data on egg production of 24 high egg producer and 24 low egg producer ABWLH and ASWLH birds, whose amplicon sequenced data were available, were collected from Poultry Research Station, AAU (now Kamdhenu University), Anand. Noteworthy aspects of the production performance for both ASWLH and ABWLH breeds are presented in Table 2.

Table 1: The details of custom amplicon designed by Illumina Design Studio for target exons

Genome build	*Galgal4*
Number 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

Table 2: Production performance of both ASWLH and ABWLH breeds

Production Traits	ASWLH	ABWLH
Average age at first egg (days)	148.77±1.66	144.25±1.07
Average body weight (g) at 20th weeks	1221.07±58.48	1309.04±26.38
Average no. of eggs produced up to 64th weeks	233.95±1.88	252.34±4.51
Average no. of eggs produced up to 72nd weeks	279.14±4.17	302.38±6.51
Average egg weight (g) at 40th weeks	54.50±0.61	50.42±0.63
Average egg weight (g) at 64th weeks	58.24±0.90	52.63±0.55
Average egg weight (g) at 72nd weeks	59.14±0.76	53.76±0.49

Bioinformatics Analysis

The amplicon sequenced data was obtained in 'fastq' format which was visualized using FASTQC v0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The data with a minimum length of 70 bp and a minimum quality mean of 35 were filtered using PRINSEQ standalone lite v0.20.4 (https://sourceforge.net/projects/prinseq/ files/). Following filtration, the remaining data was mapped against a reference genome *Galgal4* (Gallus_gallus-4.0) using Burrows Wheeler Alignment (BWA v0.7.17) (https://sourceforge.net/ projects/bio-bwa/files/). After mapping, the data was obtained in 'sam' file format which was further converted into 'bam' file format followed by its sorting using SAMTools (v1.12) (https://www.htslib.org/ doc/samtools.html). This sorted bam files were used for variant calling which was also done using SAMTools (v1.12). The Picard tool (v2.25.6) (https://broadinstitute.github.io/picard/) was used to create alignment metrics of the readings recorded in the BAM files. Using Integrative Genomics Viewer (IGV v2.10.0), each sample's variants and mapping properties were examined (https://software.broadinstitute.org/software/igv/igvtools). The variants were then filtered and annotated using SnpSift (v5.0e) (https://snpeff.sourceforge.net/ SnpSift.html) and SnpEff (https://snpeff.sourceforge.net/SnpEff. html). Plink v1.07 (https://zzz.bwh.harvard.edu/plink/) was used to associate variants with egg production at 64 weeks of age.

Results and Discussion

Quality of each and every sample data was visualized using FASTQC v0.11.9. Figure 1 showcases an illustrative representation of the data quality assessment conducted by FastQC. The analysis of bam files using Picard Tool in present investigation revealed all the finding related to reads alignment exhibited a remarkable alignment of at least 99.9% with the reference genome. Notably, 19 out of the 96 samples achieved a perfect alignment of 100% with the reference genome.

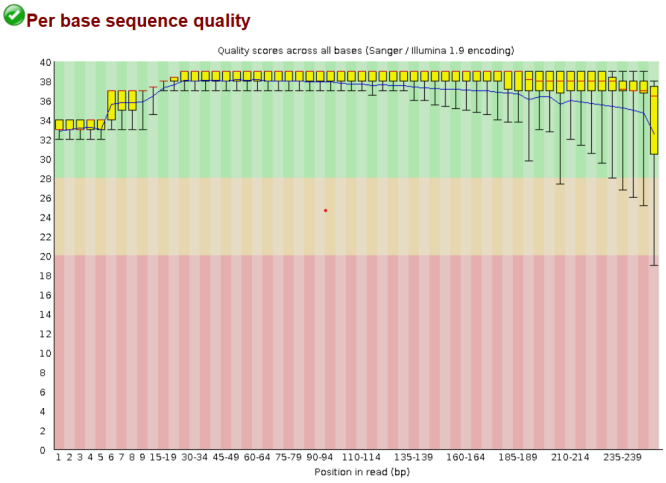

Fig. 1: An illustrative representation of the data quality assessment conducted by FastQC

Variant Calling

A total of 143 SNPs were detected from the VCF files containing data from 96 samples. Based on read depth of 20 and QUAL score of 30, 60 SNPs were discarded. Out of the remaining 83 SNPs, 69 SNPs were previously reported in dbSNPbuild145 database. A total of 14 SNPs were found as novel. Number of variants in different exons of various genes in ASWLH and ABWLH chicken identified by SAMTools is given in Table 3.

Table 3: Number of variants in different exons of various genes in ASWLH and ABWLH chicken identified by SAMTools

Gene	Region	Number of SNPs
MC4R	Exon_1	5
MTNR1A	Exon_1	2
	Exon_2	7
DRD1	Exon_1	3
VLDLR	Exon_3	1
	Exon_6	2
	Exon_9	1
	Exon_10	2
	Exon_13	1
	Exon_15	2
	Exon_17	5
	Exon_18	9
PRLR	Exon_1	1
	Exon_3	1
	Exon_4	1
	Exon_5	1
	Exon_6	3
	Exon_7	3
	Exon_8	2
	Exon_10	11
	Exon_12	9
	Exon_13	1
	Exon_14	10

Transition and transversion both can lead to change in amino acid sequence which ultimately leads to change in protein. However, transversions have a more significant impact on regulatory DNA as emphasized in the study conducted by Guo *et al.* (2017). In the present study, transitions accounted for approximately 67% of all the identified SNPs, with transversions making up the remaining 33%. The most frequent substitutions in the event of transitions were G-to-A (20%), followed by C-to-T (17%), T-to-C (14.50%), and A-to-G (13.25%). The present finding is fully in agreement with the findings reported by Pal *et al.* (2023) and Weng *et al.* (2020), who also noted that transitions were more common than transversions and that G-to-A and C-to-T substitutions were the most common types of transitions.

Association of SNPs with Egg Production

One SNP of MC4R gene was found to have a significant association ($p<0.05$) with high egg number at 64 weeks of age (EN64) in both ABWLH and ASWLH population. On the other hand, SNPs of MTNR1A, DRD1, VLDLR and PRLR genes did not show significant association with egg number at 64 weeks of age. Details of previously reported SNP which have a significant association with EN64, and novel SNPs which have non-significant association with egg production in ABWLH and ASWLH are presented in Table 4.

Among all variants, missense variants can impact the function of genes associated with various aspects of egg production, including reproductive physiology, egg quality, and overall productivity. Annotation revealed that the rs737145363 of MC4R gene leads to glutamic acid to lysine. The present findings agreed with the results reported by Yan *et al.* (2013), who identified a significant association between MC4R polymorphism and egg production, while Karim and Aggag (2018) reported non-significant association of MC4R gene polymorphism with egg production.

In the present study, a total of nine SNPs were identified for MTNR1A gene, but none of the SNP was associated with EN64. These findings agreed with Al-Jaryan *et al.* (2021), who also reported non-significant association between MTNR1A polymorphism and egg production. However, on the contrary, other studies conducted by Li *et al.* (2015), Alsiddig *et al.* (2017) and Feng *et al.* (2018) reported significant associations of MTNR1A gene polymorphism with egg production.

Table 4: Details of previously reported and novel SNPs with their association with egg production at 64 weeks of age of ABWLH and ASWLH chicken

Gene	SNP	Reference SNP Identifier	P Value
MC4R	C69137030T	rs737145363	0.02/0.01
MTNR1A	A61275888G	NOVEL	0.11
	G61275936T	NOVEL	0.56
	C61275965T	NOVEL	0.15
PRLR	G10491831A	NOVEL	0.77
	A10488377T	NOVEL	0.47
	T10482086G	NOVEL	0.42
	T10482090C	NOVEL	0.48
	T10475391C	NOVEL	0.09
	A10472039G	NOVEL	0.36
	T10470347C	NOVEL	0.95
	A10470796T	NOVEL	0.91
VLDLR	G26426138A	NOVEL	0.49
	A26426204T	NOVEL	0.26
	T26427566A	NOVEL	0.35

Three SNPs were identified within the chicken DRD1 gene in present study, however we did not find any significant association between DRD1 gene's polymorphism with EN64, which agreed with findings by Demir *et al*. (2020) and Karsli *et al*. (2020), while Gholami *et al*. (2020) and Guo *et al*. (2021) found a significant association between DRD1 polymorphism and egg production.

A total of forty-three SNPs were identified within the chicken PRLR gene in this study. and none of the SNP was association with egg production, which agreed with findings of Hong *et al*. (2007) and Kulibaba (2015), while Vinh *et al*. (2021) and Liu *et al*. (2021) found significant association between PRLR gene polymorphism and egg production.

In the present study, a total of 23 SNPs were identified for VLDLR gene. However, no significant association was observed between VLDLR gene polymorphisms and EN64, which disagreed with findings of Zhou *et al*. (2020), Liu *et al*. (2021) and Al-Hassani *et al*. (2023), who observed the significant association between VLDLR gene polymorphism and egg production.

Conclusions

The present study is the first of its kind which was conducted to detect SNPs in the candidate genes, *viz.,* MC4R, MTNR1A, DRD1, VLDLR and PRLR in Anand Bantamized White Leghorn (ABWLH) and Anand Synthetic White Leghorn (ASWLH) birds. It was observed that the SNP rs737145363 of MC4R gene was significantly associated with EN64 in high egg producing birds of ABWLH and ASWLH population, while no SNPs were found to be significantly associated with egg production at 64 weeks for MTNR1A, DRD1, PRLR or VLDLR genes. Therefore, MC4R gene can be used as genetic marker for the selection of high egg producing birds at 64th week of age in ABWLH and ASWLH population.

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