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Study of Dopamine Receptor Gene Polymorphisms and Their Association with Growth and Egg Production Traits in West Azerbaijan Native Chicken

Research Article

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ABSTRACT

The objective of this study was to search for single nucleotide polymorphism (SNP)-type polymorphisms in the dopamine D1 receptor in West Azerbaijani native chicken and look for their association with egg production and body weight traits of chickens by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). For this purpose 180 blood samples were taken from native chickens in a poultry breeding station in West Azerbaijan, Iran. DNA was extracted from blood samples using the salting-out procedure. Specific primers were designed for PCR amplification of the 283 base segment of the dopamine D1 receptor gene. In order to demonstration of gene polymorphism, Single-stranded conformation polymorphism followed by sequencing was performed. Four samples from each banding pattern were sent for sequencing. The polymorphism was found in the D1 receptor gene (G123A). We detected three genotypes in *DRD1* gene. The frequencies of three genotypes were 0.42 (AA), 0.49 (AG), and 0.09 (GG), respectively. The analysis of variance was performed using of SAS software. The Result shows that there was a significant relationship between genotypes of dopamine D1 receptor gene with egg production traits. This finding indicates that the dopamine D1 receptor gene was polymorphic and could be a candidate locus or linked to a major gene that influences reproductive traits in chickens.

KEY WORDS body weight, dopamine, native chicken, number of eggs, SNP.

INTRODUCTION

Indigenous chickens are particularly important in livelihoods of rural people (Mahammi *et al*. 2015). On the other hand, due to reduced efficiency in Indigenous chickens (such as late sexual maturity, poor egg production, and slow growth) there are some evidence that show fast decrease in the population of worldwide native breeds of chicken, some of which are in danger of extinction.

In order to increase productivity and improve production efficiency in the native chickens, the breeding stations have been established in six different regions of Iran (West Azarbaijan, Mazandaran, Esfahan, Yazd, Fars, and Khorasan). Native fowls breeding center of West Azerbaijan located in the North West of Iran, which was established in 1984 for the breeding purposes of reproductive and productive traits of the native chicken. The station has focused its phenotype improvement based on genetic programming on egg production and growth traits.

Some breeding programs are underway in West Azerbaijan station flows to improve the economic characteristics of the native chicken. The aims of these programs are improving economic traits through the analysis of recorded data and through the selection of appropriate molecular markers.

Generally, any country's indigenous varieties of animals are considered as their national capital and strategic reserves. Native animal's preservation and reproduction are of paramount importance as they managed to survive after plenty of years of natural and artificial selection, overcoming many adverse environmental conditions. Proliferations for many generations have adopted native chickens to many environmental constraints. Indigenous chickens of Iran are the primary genetic supply for breeding programs in their habitats. Therefore, accurate recognition of these genetic reserves can provide a more precise basis for future breeding programs, and optimize the use of available resources to increase production (Mahammi *et al*. 2015).

Due to the importance of egg productive and growth traits in chickens breeding industry, it is essential to take these traits into consideration as parts of breeding objectives. Egg production and growth traits in chickens are complex quantitative traits involving numerous genes and their interactions (Walling *et al*. 2004; Khani *et al*. 2017).

In the other word these traits are usually controlled by quantitative trait loci (QTLs) and environment. A number of candidate genes have been implicated in egg production and growth traits. Some study suggests that dopamine receptor gene is a candidate gene related to growth and egg production traits in chickens (Xu *et al*. 2010). It is assumed that prolactin play a vital role in the onset and maintenance of incubation behavior in birds. Numbers of factors including dopamine play a pivotal role in prolactin secretion (Xu *et al*. 2010).

Dopamine may be one of the putative neurotransmitters responsible for the activity of GnRH and have both inhibitory and stimulatory influences on gonadotropin release in birds. Dopamine has two exons and one intron and is located in the chromosomal position of 5q35.2 (Xu *et al*. 2011). Jiang *et al*. (2009) investigated the polymorphisms in the 5'-flanking region of the prolactin gene and their effects on reproduction traits in geese.

To date, at least five distinct dopamine receptors subtypes, DRD1-DRD5, are known and classically divided into two categories including D1-like (DRD1 and DRD5) and D2-like (DRD2, DRD3, and DRD4) receptors. These receptors division are based on their pharmacological, biochemical, and physiological characteristics. To our knowledge, there has been no previous study on Dopamine receptor D1 gene polymorphisms and their relation with growth and egg production traits in West Azerbaijan local fowls. Therefore, the objective of this study was an attempt to investigate associations between polymorphisms of Dopamine receptor D1 gene and egg production in West Azerbaijan local fowls by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) technique.

MATERIALS AND METHODS

Sampling and extraction of DNA

In this study, 180 chickens were selected from the native fowls breeding center of the Agricultural and Natural Resources of West Azerbaijan breeding center. Sample collection from studied animals was performed in accordance with animal ethics and approved by the Animal Use Committee in the Department of Animal Science at Urmia University. Blood samples were taken from wing vein and then were stored at -20 ˚C until DNA was extracted. DNA was extracted using a modified salting out procedure (Miller *et al*. 1988) and the purity and concentration of DNA samples were evaluated using UV-visible range spectrophotometer and gel electrophoresis.

PCR amplification, SSCP, and sequencing

The PCR primer pairs were developed according to *DRD1* gene sequence of *Gallus gallus* (GenBank accession no: NM_001144848). Two primer pairs were designed to amplify the two exons fully containing the coding sequence and the genetic variation was investigated using polymerase chain reaction -single-strand conformation. The primer sequences are given in Table 1. The PCR amplification of a 283 bp fragment to the *DRD1* gene were conducted under the procedure: 95 ˚C for 3 min, 35 cycles at 95 ˚C for 40 s, 72 ˚C for 50 s and an extension at 72 ˚C for 5 min. The PCR amplification was performed in the total reaction volume of 25 µL, which contained 2 µL of template DNA (100 ng/µL), 0.51 µL dNTPs at a concentration of 0.2 µm, 15/90 µL H_2O, 0.75 µL $MgCl_2$ at a concentration of 1.5 mM, 0.75 µL $MgCl_2$ at a concentration of 1.5 mM, 2.50 µL PCR buffer at a concentration of 10x, and 1.5 µL of each primer (10 pmol/µL).

To perform the SSCP analysis, the PCR products were finally denatured at 98 ˚C for 10 min and then placed on ice for 10 min. The PCR products were mixed with SSCP buffer (98% deionized formamide, 0.025% bromophenol blue, 00.25% xylene cyanole,10 mmol/L ethylenediaminetetraacetic acid (EDTA), and 10% glycerol). Electrophoresis was run at 300 V/cm on an 8% polyacrylamide gel for 24 h at 8 ˚C. Gels were stained using silver staining. Individual PCR-SSCP banding pattern was determined under visible light. To investigate the single nucleotide polymorphism (SNP) underlying SSCP, four samples of each banding pattern were sent for direct sequencing (Microsynth AG, Switzerland). BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html/) was used to edit the sequence data and MEGA 6.0 software (Tamura *et al*. 2013) was used to find information on the different banding pattern.

Table 1 Primers used for SNP detection of chicken's *BMP15* and *DRD1* genes

Gene	Primer sequences (5'-3')	Annealing temperatures	Fragments
DRD1-F	CACTATGGATGGGGAAGGGTTG	61	283
DRD1-R	GGCCACCCAGATGTTGCAAAATG		

Statistical analysis

PopGene (version 32) software (Yeh *et al.* 1999) was applied to estimate allelic and genotype frequencies and homozygosity and heterozygosity. The Hardy-Weinberg equilibrium (HWE) was tested based on Chi-square test ($\chi 2$). Data were analyzed using the generalized linear model (GLM) of SAS software (SAS Institute Inc., Cary NC). The genetic effects were also analyzed by a general mixed procedure in the SAS package (SAS, 2003). The statistical model for growth trait was as follow:

$$Y_{ijk} = \mu + C_i + HN_j + G_k + e_{ijkl}$$

Where:
Y: phenotypic record (growth trait) in the chickens.
μ: population mean.
C: fixed effect of ecotype.
HN: fixed effect of hatch number.
G: fixed effect of DRD-1 genotype.
e: random error.

The statistical model for egg production traits was as follow:

$$Y_{ijk} = \mu + C_i + HN_j + G_k + AM_l + e_{ijklm}$$

Where:
Y: phenotypic record (egg production traits) in the chickens.
μ: population mean.
C: fixed effect of ecotype.
HN: fixed effect of hatch number.
G: fixed effect of *DRD1* genotype.
AM: fixed effect of puberty month.
E: random error.

RESULTS AND DISCUSSION

PCR amplification

PCR products were detected by running a 1.5% agarose gel electrophoresis (Figure 1). The amplified products a 283 bp fragment to the *DRD1* gene was consistent with the target fragments and had a good specificity, which could be directly analyzed by SSCP.

SSCP analysis

A total of 180 chickens were genotyped for polymorphism in the *DRD1* gene. A PCR-SSCP technique was successfully developed for screening the nucleotide replacements in the piece 283 bp of chicken *DRD1* gene. Based on SSCP analysis of PCR product with polyacrylamide, we detect one SNP at G123A position by directly sequencing the polymorphic fragment based on SSCP banding pattern in *DRD1* gene (Figure 2). The allelic and genotypic frequencies of *DRD1* gene of West Azerbaijan native chicken are shown in Table 2.

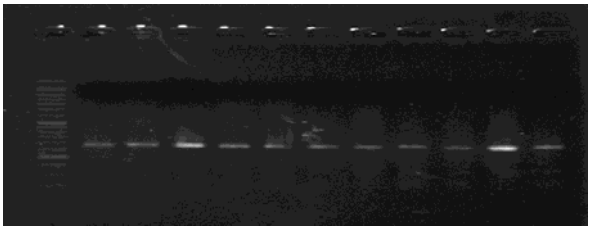

Figure 1 PCR Amplification of chicken *DRD1* gene by running at 1.5% agarose gel electrophoresis

The result of Chi-square test (Table 3) showed that the population of West Azerbaijan native fowls of the *DRD1* gene was in Hardy-Weinberg equilibrium and Shannon's information index was calculated 0.64 which show high genetic variation for *DRD1* gene in this population.

Analysis of egg production traits in different genotypes of *DRD1* gene

The phenotypic record of body weight at 1 week, body weight at 8 weeks, body weight at 12 weeks, average egg weight in 30 weeks of age and total number of eggs laid during first 84 days after flocks maturity in breeder hens of native fowls.
Effects of DRD1 SNP (G123A) on these traits are presented in Tables 4 and 5. There were no significant associations between this SNP and body weight at 1 week, body weight at 8 weeks, body weight at 12 weeks and average egg weight in 30 weeks of age whereas there were significant associations between DRD1 SNP (G123A) on total number of eggs.

Dopamine, a neurotransmitter in the central nervous system, has a high secretion frequency and plays an important role in awareness, emotions, and endocrine in mammals (Xu *et al.* 2011). Dopamine is involved in regulating the reproductive behavior of birds, and it has been shown that in birds, dopamine in the brain reduces prolactin levels.

Figure 2 Detect of SNP at position G123A (GenBank accession no: NM_001144848)

Table 2 Distribution of *DRD1* genotypes in West Azerbaijan native fowls

Genotypic frequency			Allelic frequency	
AA	AG	GG	A	G
0.42	0.49	0.09	0.665	0.335

Table 3 The estimation of population diversity indexes of the *DRD1* gene in West Azerbaijan native fowls[1]

Ob-Hom	Ob-Het	Exp-Hom	Exp-Het	Na	Ne	χ^2	I
0.511	0.488	0.550	0.449	2	1.81	1.40	0.64

Ob-Hom: observed homozygosity; Exp-Hom: expected homozygosity; Ob-Het: observed heterozygosity; Exp-Het: expected heterozygosity; Na: observed number of alleles; Ne: effective number of alleles; χ2: Chi-square and I: Shannon's information index.

Table 4 Least squares means of genotypic patterns on body weight traits

Traits	Genotype			P-value
	AA	AG	GG	
BW1	43.8	43.2	44.3	P > 0.05
BW8	849.3	854.6	811.2	P > 0.05
BW12	1431.1	1421.9	1356.6	P > 0.05

BW1: body weight at first week of age; BW8: body weight at 8 weeks of age and BW12: body weight at 12 weeks of age.

Table 5 Least squares means of genotypic patterns on egg production traits

Traits	Genotype			P-value
	AA	AG	GG	
EW1	48.4	49.3	50.3	P > 0.05
EW30	54.01	54.5	53.6	P > 0.05
EGGNO	54.7[a]	45.3[b]	42.06[b]	P < 0.01

EW1: weight of the first egg; EW30: average egg weight in 30 weeks of age and EGGNO: number of eggs at 84 days of age.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Xu *et al*. (2011) observed that treatment of hens with dopamine receptor antagonist or receptor-blocking agent terminated maintenance of broodiness by inhibiting secretion of prolactin.

Xu *et al*. (2010) observed another domain of dopamine gene in addition to the identification of two alleles G and A, C, T allele and CT, TT and CC genotypes, which is in line with the findings of this study. The relationship between *DRD1* gene polymorphism and growth trait of chicks (Table 4) showed that there was a positive correlation between CC and CT genotypes in eight weeks of age. Wang *et al*. (2012) observed that there were two alleles of A and T on duck.

The researchers reported three genotypes of AA, TT, and AT, indicating that the polymorphism of the gene and the genotypes were significantly related to the body weight and the weight of the first egg production. These findings were similar to the results found in this study as there was a significant correlation between genotypes and weights of the bird in eight weeks and twelve weeks and the weight of the first egg.

Zhaozheng *et al*. (2016) study analyzed polymorphism in the exon 2 of the *DRD2* gene of pigeon and the relationship between polymorphism and reproductive traits. Analyses demonstrated that there were three genotypes (i.e., AA, BB, and AB) in exon 4 and two genotypes (AA and AB) in exon

1, which confirms the results found in the present study. The genotype AA was always dominant.

Hunrahan *et al*. (2004) and Galloway *et al*. (2002) note that various mutations in the *BMP15* and *GDF9* gene, increase the rate of ovulation and infertility in sheep. These mutations increase ovulation, double-breeding rates, and tri-collisions in heterozygotes and cause homozygote defects in complete follicles and infertility in some fertile sheep breeds.

Huang *et al*. (2015) investigated the polymorphisms of GDF9 and BMP15 and their effects on egg production in chicken. The study was aimed at investigating the roles of these genes in the progression of follicle oocyte and chicken egg production rate. The results indicated that these genes had significant effects on the egg production traits.

CONCLUSION

SSCP polymorphisms can be quickly and economically detected single nucleotide polymorphism at genome in a population of farm animal and birds and their relations with quantitative and economic traits studied. We have applied this method to detect of SNPs in *DRD1* gene and then to investigate associations between polymorphisms observed in this gene with growth and egg production traits in breeder hens of West Azerbaijan native fowls breeding station in Iran. In summary, in present study we detected significant association between genotypes of dopamine D1 receptor gene with egg production trait in West Azerbaijan native chicken. It is concluded that the dopamine D1 receptor gene was polymorphic and could be a candidate locus or linked to a major gene that affects egg production traits in chickens and proposes that this SNP could be suitable for selecting birds based on difference at the genome.

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REFERENCES

Galloway S.M., Gregan S.M., Wilson T., McNatty P., Jungle J.L., Ritvos O. and Davis G.H. (2002). BMP15 mutations and ovarian function. *Mol. Cell. Endocrinol*. **191,** 15-18.

Huang H.Y., Liang Z. and Wang Q.B. (2015). Polymorphism identification in *BMP15* and *GDF9* genes and their association with egg production in chickens. *British Poult. Sci*. **56,** 277-283.

Hunrahan J.P., Gregan S.M., Mulsant P., Mullen M., Davis G.H., Powell R. and Gallway S.M. (2004). Mutation in genes for oocyte derived growth factor gene GDF9 increased ovukation and BMP15 are associated with both increased ovulation rate and sterilityin Cambridge and Belclare sheep. *Biol. Reprod*. **70,** 900-909.

Jiang R.S., Zhang L.L., Geng Z.Y., Yang T. and Zhang S.S. (2009). Single nucleotide polymorphisms in the 5'-flanking region of the prolactin gene and the association with reproduction traits in geese. *South African J. Anim. Sci*. **39,** 83-87.

Khani K., Abdolmohammadi A., Foroutanifar S. and Zebarjadi A. (2017). Assessment of polymorphisms in mysostatin gene and their allele substitution effects showed weak association with growth traits in Iranian Markhoz goats. *J. Agric. Sci*. **155(3),** 519-526.

Mahammi F.Z., Gaouar S.B., Laloe D., Faugeras R., Tabet-Aoul N., Rognon X., Tixier-Boichard M. and Saidi-Mehtar N. (2015). A molecular analysis of the patterns of genetic diversity in local chikens from Western Algeria in comparison with commercial lines and wild jungle fowls. *J. Anim. Breed. Genet*. **133(1),** 59-70.

Miller S.A., Dykes D.D. and Pole Sky H.F. (1998). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids. Res*. **16,** 1215-1219.

SAS Institute. (2003). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Tamura K., Stecher G., Peterson D., Filipski A. and Kumar S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*. **30,** 2725-2729.

Walling G.A., Visscher P.M., Wilson A.D., Mcteir B.L., Simm G. and Bishop S.C. (2004). Mapping of quantitative trait loci for growth and carcass traits in commercial sheep populations. *J. Agric. Sci*. **82,** 2234-2245.

Wang C., Li S., Li C., Feng Y., Peng X. and Gong Y. (2012). Molecular cloning, expression profile, polymorphism and the genetic effects of the dopamine D1 receptor gene on duck reproductive traits. *Mol. Biol. Rep*. **39,** 9239-9246.

Xu H.P., Shen X., Zhou M., Luo C.L., Kang L. and Liang Y. (2010). The genetic effects of the dopamine D1 receptor gene on chicken egg production and broodiness traits. *BMC Genet*. **11,** 17-25.

Xu H.P., Zeng H., Luo C.L., Zhang D.X., Wang Q. and Sun L. (2011). Effects of polymorphisms in candidate genes and the QTL region on chicken age at first egg. *BMC Genet*. **12(1),** 27-33.

Yeh F.C., Yang R.C., Boyle T.B.J., Ye Z.H. and Mao J.X. (1999). POPGENE, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada.

Zhaozheng Y., Xinyang D., Youzhi M. and Dingjuan D. (2016). Association of dopamine D2 receptor gene polymorphisms with reproduction traits in domestic pigeons (*Columba livia*). *J. Poult. Sci*. **37,** 106-112.