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Polymorphisms in candidate genes associated with egg yield and quality in brown layer pure lines*

Kahverengi yumurtacı saf tavuk hatlarında yumurta verim ve kalitesi ile ilişkili aday genlerdeki polimorfizmler

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ABSTRACT

The aim of the present study was to detect a total of 11 polymorphisms of 6 genes (*OCX32, GHR, DRD, VIP, VIPR, MR*) related to egg yield and quality in six brown layer lines namely Rhode Island Red-I (RIRI), Rhode Island Red-II (RIRII), Barred Rock-I (BARI), Barred Rock-II (BARII), Colombian Rock (COL) and Line-54 (L5-4) raised in Ankara Poultry Research Institute. A total of 208 samples belonging to RIRI (n= 32), RIRII (n= 32), BARI (n= 40), BARII (n= 32), COL (n= 32) and L-54 (n= 40) were genotyped by PCR-RFLP method. For this reason, a total of 11 polymorphic regions were amplified by PCR and then PCR products were digested with specific restriction endonucleases. Results of the present study showed that polymorphisms and adequate genetic variations were found in *OCX32-ex4/Nco*I, *OCX32-ex2/HpyCH4IV*, *DRD2/BseG*I, *VIPR-1/Hha*I, *VIPR-2/Taq*I and *MR1C/Mbo*I genes which previously reported to associated with egg quality and yield in different chicken lines. However, conducting the association analysis between these genes and egg yield and quality will be useful. On the contrary, it has been revealed that *GHR-intron 2/Hind*III, *GHR-intron 5/Nsp*I, *VIP-501/Vsp*I, *DRD1/BseN*I and *VIP-I2/Hinf*I polymorphisms cannot be used to increase egg yield and quality due to no detection of desired genotypes or homozygous excess.

MAKALE BİLGİSİ

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PCR-RFLP

ÖZ

Bu çalışmanın amacı Ankara Tavukçuluk Araştırma Enstitüsünde yetiştirilen Rhode Island Red-I (RIRI), Rhode Island Red-II (RIRII), Barred Rock-I (BARI), Barred Rock-II (BARII), Colombian Rock (COL) ve Line-54 (L-54) isimli altı kahverengi saf yumurtacı tavuk hattında yumurta verim ve kalitesi ile ilişkili olan 6 gende (*OCX32, GHR, DRD, VIP, VIPR, MR*) toplam 11 polimorfizmin belirlenmesidir. RIRI (n= 32), RIRII (n= 32), BARI (n= 40), BARII (n= 32), COL (n= 32) ve L-54 (n= 40) hatlarına ait toplam 208 örnek PCR-RFLP yöntemiyle genotiplendirilmiştir. Bu amaçla, toplam 11 polimorfik bölge PCR ile çoğaltılmış ve daha sonra PCR ürünleri özgün restriksiyon enzimleriyle kesilmiştir. Mevcut çalışmanın sonuçları daha önce farklı tavuk ırklarında yumurta verimi ve kalitesiyle ilişkili olduğu bildirilen *OCX32-ex4/Nco*I, *OCX32-ex2/HpyCH4IV*, *DRD2/BseG*I, *VIPR-1/Hha*I, *VIPR-2/Taq*I ve *MR1C/Mbo*I genlerinde polimorfizmin ve yeterli genetik varyasyonun bulunduğunu göstermiştir. Bununla birlikte yumurta verim ve kalite özellikleri ile bu genler arasında ilişki analizlerinin yapılması faydalı olacaktır. Aksine, *GHR-intron 2/Hind*III, *GHR-intron 5/Nsp*I, *VIP-501/Vsp*I, *DRD1/BseN*I ve *VIP-I2/Hinf*I gen bölgelerinde istenilen genotiplerin tespit edilememesi ya da homozigot fazlalığından dolayı bu gen bölgelerinin kullanılamayacağı tespit edilmiştir.

*This research was approved by the Ankara Poultry Research Institute Animal Experiments Ethics Committee, Ankara, Turkey (Protocol No: 23.01.2015/03).

1. Introduction

Healthy life of societies depends on consuming animal-derived protein (milk, egg, and meat) at adequate levels (Landi et al. 2017). A large part of protein requirements in Turkey, in which a large part of population lives in centre rather than rural areas, are met by poultry sector due to some advantages of chickens compared to other livestock species. Indeed, chicken meat and egg are not only produced in short period of time but also chickens can be reared with more animals in same unit of area. Chicken egg, containing balanced fatty acids composition, high vitamin, mineral and phospholipids, is one of the main protein resources consumed by children, teenagers, and adults in worldwide (Akbay et al. 2000; Çelebi and Karaca 2006). Studies on breeding material production in poultry sector are performed only by Ankara Poultry Research Institute in Turkey. Six brown layer pure chicken lines (RIRI, RIRII, BARI, BARII, COL and L-54) are raised by Ankara Poultry Research Institute (Göger et al. 2017; Karslı and Fidan 2019).

In avian, egg production is a complex process controlled by many hormones. Decreased level in blood circulation of Prolactin (*PRL*), one of these hormones, increases egg production. Vasoactive intestinal peptide (*VIP*), released by hypothalamus gland, leads to releasing *PRL* hormone. In addition, Dopamine, and its receptors such as Dopamine D1 (*DRD1*) and D2 (*DRD2*) control *PRL* hormone by provoking *VIP* releasing (Xu et al. 2010; Zhou et al. 2010; Xu et al. 2011). On the other hand, eggshell quality is also important for poultry sector, since consumers demand for products without any damages. Eggshell functions as an antimicrobial barrier in order to protect nutrition content of egg. Delivering eggs without any damages to consumers is an important step in poultry sector.

In chickens, many economically important traits including egg yield and quality show quantitative inheritance. These traits are shaped by genetic structure and many environmental factors such as feed intake and photoperiod length (Lewis and Gous 2006). Today, many candidate genes such as *PRL*, *VIP*, dopamine, gonadotropin releasing hormone (*GnRH*), insulin-like growth factor (*IGF-I*), growth hormone (*GH*), neuropeptide Y (*NPY*), Ovocalyxin-32 (*OCX-32*), melatonin and their receptors were reported to be associated with egg yield and quality (Lewis and Gous 2006; Li et al. 2008; Li et al. 2009; Uemoto et al. 2009; Xu et al. 2010; Zhou et al. 2010; Xu et al. 2011; Li et al. 2013; Abdi et al. 2014).

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. Hence, this study aimed to determine a total of 11 polymorphisms in 6 genes and their receptors [*OCX32-ex4*, *OCX32-ex2*, *GHR-intron 2*, *GHR-intron 5*, *DRD1*, *DRD2*, *VIP-501*, *VIP-I2*, *VIPR-1*, *VIPR-2* and *MR1C* (Melatonin Receptor 1C)] related to egg yield and quality in six brown layer lines by using PCR-RFLP (Polymerase Chain Reaction- Restriction Fragment Length Polymorphism).

2. Material and Methods

2.1. Ethic statement

This research was approved by the Ankara Poultry Research Institute Animal Experiments Ethics Committee, Ankara, Turkey (Protocol No: 23.01.2015/03).

2.2. Blood samples and DNA extraction

A total of 208 blood samples were randomly collected from six brown pure layer lines namely RIRI (n= 32), RIRII (n= 32), BARI (n= 40), BARII (n= 32), COL (n= 32) and L-54 (n= 40) raised in Ankara Poultry Research Institute. Approximately 0.5-1 ml blood samples taken from *venous cutenea ulnaris* to vacuum tubes containing EDTA as anticoagulant, were collected and transported to the laboratory and then stored at -20°C until DNA extraction was performed. DNA was isolated from blood samples by using a salting-out method with minor modifications (Miller et al. 1988). DNA quality and quantity were checked using agarose gel (1%) and spectrophotometer (NanoDrop-SD 1000). DNA concentration was adjusted to 50 ng μL^{-1} for PCR-RFLP analysis.

2.3. PCR-RFLP analysis

In this study, a total of 6 different candidate genes together with their receptors were amplified with primer sets reported in previous studies (Table 1). PCR was performed in 20 µL reaction volume containing 1.2 µL HQ buffer (GeneAll), 2.5 mM dNTPs, 10 pM of each primer, 2.5 U *Taq* DNA Polymerase (GeneAll), 50 ng template DNA and 11.4 µL ddH_2O. PCR amplifications were applied in initial denaturation at 94°C for 5 mins, followed by 30 cycles at 94°C for 45 s, at 50-62°C (Table 1) for 45 s and at 72°C for 50 s. The final extension were carried out at 72°C for 5 mins.

In order to genotype the individuals, amplificated PCR products were digested with restriction endonucleases. A total of 16 µL RFLP mixture containing 7 µL of amplified PCR products, 2.5-5 U restriction enzymes (Table 1), 1.4 µL 10X buffer and 7 µL nuclease free water, were incubated at 37-65°C for at least 12 hours according to manufacturer instructions (Thermo Scientific Inc.). PCR products were separated on %1 agarose gel electrophoresis at 80 volts for 45 minutes, while RFLP products were separated on %3 agarose gel electrophoresis at 70 volts for 60 minutes.

2.4. Statistical analysis

In this study, to calculate the genotype and allele frequencies in the studied candidate genes, Popgene V. 1.32. (Yeh et al. 1997) package program was used. This software was also used to test the HW equilibrium.

3. Results and Discussion

It has been reported in previous studies that the polymorphisms in studied candidate genes related to egg yield and quality, were SNPs (Li et al. 2008; 2009; 2010; 2013; Uemoto et al. 2009; Zhou et al. 2010; Xu et al. 2011). Therefore, PCR-RFLP was conducted to detect polymorphisms in candidate genes. For this reason, candidate genes were

Table 1. Some descriptive information of studied candidate genes.

Gene	Chr.	Primers (5'-3')	Ann.Temp. (°C)	PCR Size	Restriction Enzyme	Expected Product Size	References
GHR-intron 2	Z	F:GGCTCTCCATGGGTATTAGGA R: GCTGGTGAACCAATCTCGGTT	59	718	*Hind*III	A_1A_1: 428-290, A_2A_2:170-258-290	Li et al. 2008
GHR-intron 5	Z	F: ACGAAAAGTGTTTCAGTGTTGA R: TTTATCCCGTGTTCTCTTGACA	56	740	*Nsp*I	CC: 550-190, CD:740, 550, 190, DD:740	Li et al. 2008, 2010
DRD1	13	F:CACTATGGATGGGGAAGGGTTG R: GCCACCCAGATGTTGCAAAATG	62	283	BseNI	AA:111-172, AG:111-172-283, GG: 283	Xu et al. 2010
DRD2	24	F:TGCACATAAAAGCCCACTCACTG R:GCCTGAGCTGGTGGGGGG	60	248	*Bse*GI	CC:248, TC: 248-196, TT: 196	Xu et al. 2011
VIP-501	3	F:GAAACCCATCTCAGTCATCCTA R:ACCACCTATTTTTCCTTTTCTACA	55	306	*Vsp*I	II: 306, DI: 306-154-152, DD: 154-152	Zhou et al. 2010
VIP-I2	3	F: GCTTGGACTGATGCGTACTT R: GTATCACTGCAAATGCTCTG	58	520	*Hinf* I	CC: 480, CT:520-480, TT: 520	Zhou et al. 2010
VIPR-1	2	F:CCCCGTTAAACTCAGCAGAC R:CCCAAAGTCCCACAAGGTAA	58	434	*Hha*I	TT:434, TC: 434-253-181, CC 253-181	Xu et al. 2011
VIPR-2	2	F:CTCCTCAGGCAGACCATCATG R:CTTGCACGTATCCTTGGGTAGC	58	486	*Taq*I	TT:486, TC: 486-310-176, CC 310-176	Xu et al. 2011
OCX32-ex4	9	F: TGTTTCTGATGAAGAGCCAGA R: CTTTGCCACTCTGTAGGCTGT	58	250	*Nco*I	AA:250 AC:250-194 CC:194	Uemoto et al. 2009
OCX32-ex2	9	F: GCCCACTGGTCAGAAAAGAA R: CCTGCAGAGGAAAAGAGCTG	58	405	*HpyCH4*IV	TT:237-169, TG: 237-169-151, GG: 237-151	Uemoto et al. 2009
MR1C	4	F: GGTGTATCCGTATCCTCTAA R: GACAGTGGGACAATGAAGT	50	372	*Mbo*I	AA:372, AG:372-333, GG:333	Li et al. 2013

amplified by PCR and then digested with specific restriction endonucleases to detect genotypes. Amplified and digested products for DRD2 gene were given as an example in Figure 1 and Figure 2, respectively.

All chicken lines were monomorphic in *GHR-intron 2/Hind*III (A_1A_1 genotype) and *VIP-501/Vsp*I (II genotype) (data not shown). Similarly, RIRI, RIRII, BARI, BARII and COL populations were monomorphic (CD genotype) in *GHR-intron 5/Nsp*I polymorhism, while CD and DD genotype frequencies were 0.53 and 0.47, respectively in L-54 line. Additionally, all individuals belonging to BARI, BARII, COL and L-54 were monomorphic (CC genotype) in *VIP-I2/Hinf*I polymorphism. T allele frequency ranged from 0.47 (RIRI) to 0.50 (RIRII) in *VIP-I2/Hinf*I polymorphism (Table 2).

In total nine regions, which correspond a large part of studied 11 sites, were found to be polymorphic. In addition, most of studied chicken lines were in Hardy-Weinberg equilibrium for related genes. This situation indicates genetic diversity in chicken lines. Similarly, high genetic diversity was reported in these chicken lines by previous studies based on different molecular marker techniques (Karslı et al. 2017; Karslı and Balcıoğlu 2019). Despite of conducted selection process for a long time in these chicken lines, high genetic diversity was detected. It is thought that adequate population size and controlled mating process may be the main reason for high genetic diversity, which is promising for sustainable use of these chicken lines and possible selection studies in the future.

It has been reported that polymorphisms in *OCX32* gene are related to egg quality including eggshell colour, albumen height and yolk weight (Fulton et al. 2012). In this study, all chicken lines were detected to be polymorphic in *OCX32-ex4/Nco*I and *OCX32-ex2/HpyCH4IV* polymorphisms (Table 2). The highest G and T allele frequencies were detected in RIRI (0.64) and COL (0.74) lines, respectively, while the highest GG, GT and TT genotype frequencies were observed in RIRI (0.45), BARI (0.52) and COL (0.52), respectively in *OCX32-ex2/HpyCH4IV* polymorphism. AA genotype frequency ranged from 0.15 (L-54) to 0.78 (BARI), while the highest AC and CC genotype frequencies were observed in RIRI (0.50) and L-54 (0.47) lines, respectively, in *OCX32-ex4/Nco*I polymorphism. CC, AC and AA genotype frequencies were reported as 0.37, 0.44 and 0.19, respectively in Rhode Island Red breed (Uemoto et al. 2009). Genotype frequencies detected in RIRI and RIRII in *OCX32-ex2/HpyCH4IV* polymorphism were accordant with the results reported by Uemoto et al. (2009). The present study showed that six brown chicken lines conserve genetic diversity in OCX32 gene.

It has been reported that *DRD1* gene is associated with total egg production and broodiness frequency, while *DRD2* gene is related to egg number at 300 days of age in chicken (Xu et al. 2010; 2011). Chickens with AA genotype reported being shown superior number of total egg than chickens with GG and AG genotypes in *DRD1/BseNI* polymorphism (Xu et al. 2010). Additionally, individuals with TT genotype reported being shown superior egg number at 300 days of age than individuals with CC and TC genotypes in *DRD2/ BseG*I polymorphism (Xu et al. 2011). Although PCR products were amplified successfully, no genotype was detected in BARI, BARII and L-54 lines due to unexpected RFLP fragments in *DRD1/BseN*I

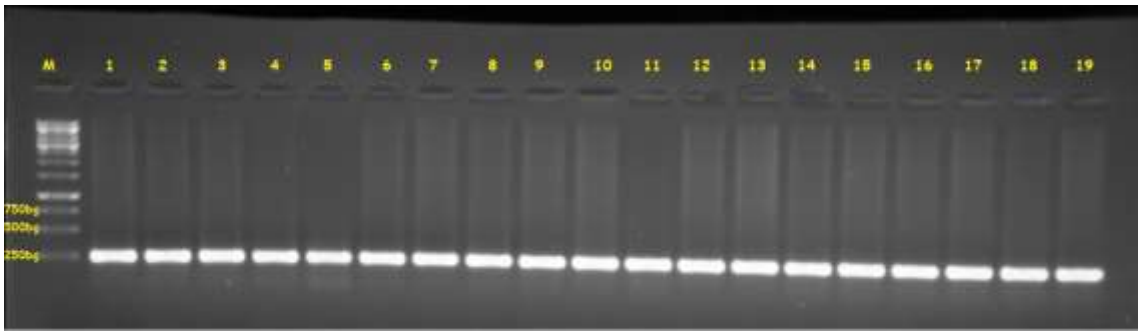

Figure 1. Agarose gel image of PCR products for DRD2 gene (Marker: Thermo, 1 kb, Cat. No: SM0311; 1% agarose gel, PCR band size 248 bp).

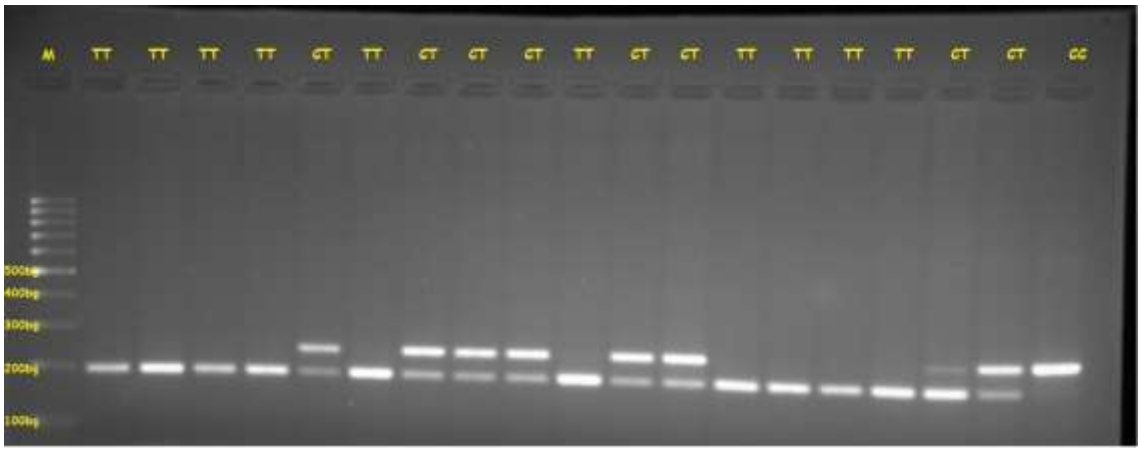

Figure 2. Agarose gel image of digested PCR products of DRD2 gene with *Bse*GI restriction enzyme (Marker: Thermo, 100 bp, Cat. No: SM0241; 3% agarose gel, Digested band size CC: 248 bp, CT: 196-248 bp, TT: 196 bp).

Table 2. Allele and genotype frequencies of candidate genes in six brown pure chicken lines.

RIRI								BARI								COL							
Loci	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2	**Loci**	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2	**Loci**	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2
GHR-intron2	32	A_1/1.00	A_2/0.00	A_1A_1/1.00	A_1A_2/0.00	A_2A_2/0.0	-	**GHR-intron2**	40	A_1/1.00	A_2/0.00	A_1A_1/1.00	A_1A_2/0.00	A_2A_2/0.0	-	**GHR-intron2**	32	A_1/1.00	A_2/0.00	A_1A_1/1.00	A_1A_2/0.00	A_2A_2/0.0	-
GHR-intron5	32	C/0.50	D/0.50	CC/0.00	CD/1.00	DD/0.00	-	**GHR-intron5**	40	C/0.50	D/0.50	CC/0.00	CD/1.00	DD/0.00	-	**GHR-intron5**	32	C/0.50	D/0.50	CC/0.00	CD/1.00	DD/0.00	-
DRD1	32	A/0.00	G/1.00	AA/0.00	AG/0.00	GG/1.00	-	**DRD1**	-	-	-	-	-	-	-	**DRD1**	32	A/0.03	G/0.97	AA/0.03	AG/0.00	GG/0.97	32.00[b]
DRD2	32	C/1.00	T/0.00	CC/1.00	TC/0.00	TT/0.00	-	**DRD2**	40	C/0.69	T/0.31	CC/0.47	TC/0.42	TT/0.10	0.00[a]	**DRD2**	32	C/0.95	T/0.05	CC/0.91	TC/0.09	TT/0.00	0.08[a]
VIP-501	32	I/1.00	D/0.00	II/1.00	DI/0.00	DD/0.00	-	**VIP-501**	40	I/1.00	D/0.00	II/1.00	DI/0.00	DD/0.00	-	**VIP-501**	32	I/1.00	D/0.00	II/1.00	DI/0.00	DD/0.00	-
VIP-I2	32	C/0.53	T/0.47	CC/0.38	CT/0.31	TT/0.31	4.44[b]	**VIP-I2**	40	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-	**VIP-I2**	32	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
VIPR-1	32	C/1.00	T/0.00	CC/1.00	TC/0.00	TT/0.00	-	**VIPR-1**	40	C/0.86	T/0.14	CC/0.73	TC/0.27	TT/0.00	1.02[a]	**VIPR-1**	32	C/1.00	T/0.00	CC/1.00	TC/0.00	TT/0.00	-
VIPR-2	32	C/0.16	T/0.84	CC/0.00	CT/0.31	TT/0.69	1.09[a]	**VIPR-2**	40	C/0.69	T/0.31	CC/0.61	CT/0.17	TT/0.22	14.05[b]	**VIPR-2**	32	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
OCX32-ex4	32	A/0.56	C/0.44	AA/0.31	AC/0.50	CC/0.19	0.01[a]	**OCX32-ex4**	40	A/0.89	C/0.11	AA/0.78	AC/0.22	CC/0.0	0.64[a]	**OCX32-ex4**	32	A/0.64	C/0.36	AA/0.41	AC/0.47	CC/0.12	0.01[a]
OCX32-ex2	29	G/0.64	T/0.36	GG/0.45	GT/0.38	TT0.17	0.93[a]	**OCX32-ex2**	35	G/0.63	T/0.37	GG/0.37	GT/0.52	TT/0.11	0.36[a]	**OCX32-ex2**	25	G/0.26	T/0.74	GG/0.04	GT/0.44	TT/0.52	0.51[a]
MR1C	32	A/0.55	G/0.45	AA/0.31	AG/0.47	GG/0.22	0.09[a]	**MR1C**	40	A/0.64	G/0.36	AA/0.30	AG/0.68	GG/0.02	8.48[b]	**MR1C**	32	A/0.42	G/0.58	AA/0.16	AG/0.53	GG/0.31	0.25[a]
RIRII								**BARII**								**L-54**							
GHR-intron2	32	A_1/1.00	A_2/0.00	A_1A_1/1.00	A_1A_2/0.00	A_2A_2/0.0	-	**GHR-intron2**	32	A_1/1.00	A_2/0.00	A_1A_1/1.00	A_1A_2/0.00	A_2A_2/0.0	-	**GHR-intron2**	40	A_1/1.00	A_2/0.00	A_1A_1/1.00	A_1A_2/0.00	A_2A_2/0.0	-
GHR-intron5	32	C/0.50	D/0.50	CC/0.00	CD/1.00	DD/0.00	-	**GHR-intron5**	32	C/0.50	D/0.50	CC/0.00	CD/1.00	DD/0.00	-	**GHR-intron5**	38	C/0.26	D/0.74	CC/0.00	CD/0.53	DD/0.47	4.85[b]
DRD1	32	A/0.00	G/1.00	AA/0.00	AG/0.00	GG/1.00	-	**DRD1**	-	-	-	-	-	-	-	**DRD1**	-	-	-	-	-	-	-
DRD2	32	C/1.00	T/0.00	CC/1.00	TC/000	TT/0.00	-	**DRD2**	32	C/0.53	T/0.47	CC/0.25	TC/0.56	TT/0.19	0.54[a]	**DRD2**	39	C/0.46	T/0.54	CC/0.26	TC/0.41	TT/0.33	1.19[a]
VIP-501	32	I/1.00	D/0.00	II/1.00	DI/0.00	DD/0.00	-	**VIP-501**	32	I/1.00	D/0.00	II/1.00	DI/0.00	DD/0.00	-	**VIP-501**	40	I/1.00	D/0.00	II/1.00	DI/0.00	DD/0.00	-
VIP-I2	31	C/0.50	T/0.50	CC/0.19	CT/0.62	TT/0.19	1.58[a]	**VIP-I2**	32	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-	**VIP-I2**	38	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
VIPR-1	32	C/1.00	T/0.00	CC/1.00	TC/0.00	TT/0.00	-	**VIPR-1**	32	C/1.00	T/0.00	CC/1.00	TC/0.00	TT/0.00	-	**VIPR-1**	40	C/1.00	T/0.00	CC/1.00	TC/0.00	TT/0.00	-
VIPR-2	32	C/0.41	T/0.59	CC/0.31	CT/0.19	TT/0.50	11.96[b]	**VIPR-2**	32	C/0.59	T/0.40	CC/0.44	CT/0.31	TT/0.25	3.97[b]	**VIPR-2**	40	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
OCX32-ex4	32	A/0.55	C/0.45	AA/0.33	AC/0.42	CC/0.25	1.04[a]	**OCX32-ex4**	32	A/0.69	C/0.31	AA/0.53	AC/0.31	CC/0.16	2.38[a]	**OCX32-ex4**	39	A/0.35	C/0.65	AA/0.15	AC/0.38	CC/0.47	0.88[a]
OCX32-ex2	22	G/0.39	T/0.61	GG/0.14	GT/0.50	TT/0.36	0.06[a]	**OCX32-ex2**	27	G/0.57	T/0.43	GG/0.37	GT/0.41	TT/0.22	0.75[a]	**OCX32-ex2**	33	G/0.41	T/0.59	GG/0.21	GT/0.39	TT/0.40	1.13[a]
MR1C	32	A/0.42	G/0.58	AA/0.19	AG/0.47	GG/0.34	0.05[a]	**MR1C**	32	A/0.91	G/0.09	AA/0.81	AG/0.19	GG/0.00	0.34[a]	**MR1C**	39	A/0.58	G/0.42	AA/0.26	AG/0.64	GG/0.10	3.82[a]

$\chi^2_{0.05;1}$: 3.84; a: Deviation from HWE is non-significant; b: Deviation from HWE is significant.

polymorphism. RIRI and RIRII lines were of GG genotype and desired genotyped (AA) was observed in only COL line at very low frequency (0.03) (Table 2). Except RIRI and RIRII lines, desired genotype (TT) was detected in BARI (0.10) and BARII (0.19) chicken lines at low frequencies in *DRD2/BseG*I polymorphism. However, higher TT genotype frequency (0.33) was observed in L-54 chicken line. These TT genotype frequencies were higher than the value (0.004) reported in Ningdu Sanhuang chicken breed (Xu et al. 2011). It is not surprising, since selection studies have been conducted for studied chicken lines for many years. Additionally, L-54 contains approximately 15% White Leghorn blood, which may cause higher TT genotype frequency. Similarly, Demir et al. (2020) previously reported higher TT frequency (0.00-0.68) in five white pure layer chicken lines obtained from White Leghorn, raising in the same institute.

It is also reported that *VIPR-1* and *VIPR-2* genes are associated with egg number at 300 days in chickens (Xu et al. 2011). Superior values for egg number at 300 days were reported for CC and TC genotypes than TT genotype in *VIPR-1/Hha*I polymorphism and for TT genotype than CC and TC genotypes in *VIPR-2/Taq*I polymorphism (Xu et al. 2011). In the present study, no TT genotype was detected in studied lines, while CC genotype was detected in all chicken lines except for BARI. On the contrary, no desired genotype (TT) was detected in COL and L-54 chicken lines in *VIPR-2/Taq*I polymorphism. However, TT genotype was observed at high frequency in RIRI (0.69) and RIRII (0.50) and at low frequency in BARI (0.22) and BARII (0.25). CC, TC and TT frequencies were reported as 0.935, 0.060 and 0.005, respectively in Ningdu Sanhuang chicken (Xu et al. 2011); 0.64, 0.29 and 0.07, respectively in Vietnam Voi chicken (Ngu et al. 2015) in *VIPR-1/Hha*I polymorphism. CC, TC and TT frequencies were reported as 0.698, 0.209 and 0.093, respectively in Ningdu Sanhuang chicken (Xu et al. 2011); 0.48, 0.33 and 0.19, respectively in Vietnam Voi chicken (Ngu et al. 2015) in *VIPR-2/Taq*I polymorphism. The higher TT frequency was observed RIRI, RIRII, BARI and BARII than Ningdu Sanhuang (Xu et al. 2011) and Vietnam Voi (Ngu et al. 2015) chickens.

Li et al. (2013) reported that *MR1C/Mbo*I polymorphisms are associated with both age at first egg and egg number at 300 days and in chickens in which individuals with AG genotype showed superior egg number at 300 days than individuals with GG and AA genotypes. In the present study, no GG genotype and low AG frequency (0.19) was detected in BARII line, whereas AG frequency were ranged from 0.47 (RIRI and RIRII) to 0.68 (BARI) in other brown chicken lines in *MR1C/Mbo*I polymorphism (Table 2). Also, the highest A and G allele frequencies were 0.91 (BARII) and 0.58 (RIRII and COL), respectively. Similar AG frequency (0.60) was reported in Wenchang chicken (Li et al. 2013).

Today, genes associated with higher egg production and quality traits can be detected at molecular level by using developing molecular techniques. Additionally, the frequency of desired genotypes with higher egg production and quality traits can be increased by supplementing traditional selection methods with MAS. In this context, the present study revealed that *OCX32-ex4/Nco*I and *OCX32-ex2/HpyCH4IV* polymorphisms may be used in order to increase egg quality, while *DRD2/BseG*I, *VIPR-1/Hha*I, *VIPR-2/Taq*I and *MR1C/Mbo*I polymorphisms may be chosen in order to increase egg yield in brown chicken lines reared in Ankara Poultry Research Institute. On the contrary, *GHR-intron 2/Hind*III, *GHR-intron 5/Nsp*I, *VIP-501/Vsp*I, *DRD1/BseN*I and *VIP-I2/Hinf*I cannot be used in MAS due to lack of desired genotypes (or at very low frequencies) in studied chicken lines. Still, *GHR-intron 5/Nsp*I polymorphism may be used for only L-54 line; *VIP-I2/Hinf*I polymorphism for only RIRI and RIRII chicken lines in MAS.

4. Conclusion

This is the first comprehensive study aimed to determine polymorphisms in a total of 11 polymorphisms of 6 genes related to egg yield and quality in six brown layer lines including RIRI, RIRII, BARI, BARII, COL and L-54 reared in Ankara Poultry Research Institute by using PCR-RFLP. The results of this study can be helpful for MAS studies conducted in six brown chicken lines reared in Ankara Poultry Research Institute in future. In the future MAS studies may be carried out based on loci which were found only to be polymorphic in this study. However, we highly recommended that conducting the association analysis between polymorphic loci and egg yield and quality with adequate number of chickens before applying MAS studies.

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References

Abdi M, Seyedabadi H, Gorbani A (2014) Prolactin and NPY gene polymorphism and its associations with production and reproductive traits in west-Azarbaijan native chicken. Bulletin of Environment, Pharmacology and Life Sciences 3: 39-45.

Akbay R, Yalçın S, Ceylan N, Orhan E (2000) Türkiye tavukçuluğunda gelişmeler ve hedefler. Türkiye Ziraat Mühendisliği 5. Teknik Kongresi, Cilt II, Ankara, s. 795-810.

Çelebi Ş, Karaca H (2006) Yumurtanın besin değeri, kolesterol içeriği ve yumurtayı n-3 yağ asitleri bakımından zenginleştirmeye yönelik çalışmalar. Atatürk Üniversitesi Ziraat Fakültesi Dergisi 37: 257-265.

Demir E, Karslı T, Fidan HG, Argun Karslı B, Aslan M, Aktan S, Kamanlı S, Karabağ K, Şahin Semerci, E, Balcıoğlu MS (2020) Polymorphisms in some candidate genes associated with egg yield and quality in five different white layer pure lines. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 26: 735-741.

Fulton JE, Soller M, Lund AR, Arango, Lipkin E (2012) Variation in the ovocalyxin-32 gene in commercial egg-laying chickens and its relationship with egg production and egg quality traits. Animal Genetics 43: 102-113.

Göger H, Demirtaş ŞE, Yurtoğullari Ş, Taşdemir AN, Şenkal UE, Boyali B (2017) Breeding studies on pure lines at Poultry Research Institute. Tavukçuluk Araştırma Dergisi 14: 30-38.

Karslı T, Balcıoğlu MS, Demir E, Fidan HG, Aslan M, Aktan S, Kamanlı S, Karabağ K, Şahin E (2017) Determination of polymorphisms in IGF-I and NPY candidate genes associated with egg yield in pure layers chicken lines reared in the Ankara Poultry Research Institute. Turkish Journal of Agriculture-Food Science and Technology 5: 1051-1056.

Karslı T, Balcıoğlu MS (2019) Genetic characterization and population structure of six brown layer pure lines using microsatellite markers. Asian-Australasian Journal of Animal Sciences 32: 49-57.

Karslı T, Fidan HG (2019) Assesment of genetic diversity and conservation priorities among five White Leghorn Lines based on SSR markers. Animal Science Papers and Reports 37: 311-322.

Landi F, Calvani R, Tosato M, Martone AM, Picca A, Ortolani E, Savera G, Salini S, Ramaschi M, Bernabei R, Marzetti E (2017) Animal-derived protein consumption is associated with muscle mass and strength in community-dwellers: Results from the Milan Expo survey. Journal of Nutrition, Health and Aging 21: 1050-1056.

Lewis PD, Gous RM (2006) Effect of final photoperiod and twenty-week body weight on sexual maturity and early egg production in broiler breeders. Poultry Science 85: 377-383.

Li HF, Zhu WQ, Chen KW, Wu X, Tang QP, Gao YS (2008) Associations between GHR and IGF-1 Gene polymorphisms, and reproductive traits in Wenchang Chickens. Turkish Journal of Veterinary and Animal Science 32: 281-285.

Li HF, Zhu WQ, Chen KW, Wu X, Tang QP, Gao YS, Song WT, Xu HL (2009) Polymorphism in NPY and IGF-I genes associate with reproductive traits in Wenchang chicken. African Journal of Biotechnology 8: 4744-4748.

Li HF, Zhu WQ, Chen KW, Song W, Shu J, Han W (2010) Effects of the polymorphisms of GHR gene and IGF-1 gene on egg quality in Wenchang chicken. Research Journal of Poultry Science 3: 19-22.

Li DY, Zhang L, Smith DG, Xu HL, Liu YP, Zhao XL, Wang Y, Zhu Q (2013) Genetic effects of melatonin receptor genes on chicken reproductive traits. Czech Journal of Animal Science 58: 58-64.

Miller S, Dykes D, Plesky HA (1988) Simple salting out procedure for extracting DNA from human cells. Nucleic Acids Research 16: 1215.

Ngu NT, Xuan NH, Vu CT, An NT, Dung TN, Nhan NTH (2015) Effects of genetic polymorphisms on egg production in indigenous Noi chicken. Journal of Experimental Biology and Agricultural Sciences 3: 487- 493.

Uemoto Y, Suzuki C, Sato S, Ohtake T, Sasaki O, Takahashi H, Kobayashi E (2009) Polymorphism of the ovocalyxin-32 gene and its association with egg production traits in the chicken. Poultry Science 88: 2512-2517.

Xu HP, Shen X, Zhou M, Fang M, Zeng H, Nie Q, Zhang X (2010) The genetic effects of the dopamine D1 receptor gene on chicken egg production and broodiness traits. BMC Genetics 11: 1-10.

Xu HP, Zeng H, Zhang DX, Jia XL, Luo CL, Fang MX, Nie QH, Zhang QX (2011) Polymorphisms associated with egg number at 300 days of age in chickens. Genetics and Molecular Research 10: 2279-2289.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997) "POPGENE, The user-friendly shareware for population genetic analysis". Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Zhou M, Du Y, Nie Q, Liang Y, Luo C, Zeng H, Zhang X (2010) Associations between polymorphisms in the chicken VIP gene, egg production and broody traits. British Poultry Science 51: 195-203.

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