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Polymorphisms in Some Candidate Genes Associated with Egg Yield and Quality in Five Different White Layer Pure Lines [1]

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Abstract

This is the first comprehensive study aimed to determine polymorphisms in candidate genes (OCX32-exon4, OCX32-exon2, GHR-intron 2, GHR-intron 5, DRD1, DRD2, VIP-501, VIP-I2, VIPR-1, VIPR-2 and MR1C) reported to associated with egg yield and quality in white layer pure chicken lines (Black, Brown, Blue, Maroon and D-229) raised by Ankara Poultry Research Institute by using PCR-RFLP technique. A total of 11 gene regions were amplificated for 192 chickens and were then digested with specific restriction endonucleases to determine the genotypes. Although all chicken lines were monomorphic for VIPR-1/*Hha*I and VIP-501/*Vsp*I gene region, polymorphisms were detected in the rest of nine gene regions. The present study revealed that GHR-intron 2/*Hind*III and GHR-intron 5/*Nsp*I polymorphsims can be integrated with MAS studies to increase egg quality in white layer chicken lines (D-229 excluded) raised in Ankara Poultry Research Institute in the future. In addition, in order to increase egg yield DRD2 and MR1C gene may be used in MAS for all studied chicken lines, while VIP-I2/*Hinf*I polymorphism can be used for Maroon line and VIPR-2/*Taq*I polymorphism for Brown and D-229.

Keywords: *Candidate genes, PCR-RFLP, Polymorphism, Pure chicken lines*

Beş Farklı Beyaz Yumurtacı Saf Hatta Yumurta Verimi ve Kalitesi İle İlişkili Bazı Aday Genlerdeki Polimorfizmler

Öz

Mevcut çalışma Ankara Tavukçuluk Araştırma Enstitüsü tarafından yetiştirilen beyaz yumurtacı saf tavuk hatlarında (Black, Brown, Blue, Maroon ve D-229) daha önce yumurta verimi ve kalitesiyle ilişkili olduğu bildirilen aday genlerdeki polimorfizmlerin PCR-RFLP yöntemiyle belirlenmesini amaçlayan ilk kapsamlı çalışmadır. Toplam 11 gen bölgesi 192 tane tavuk için çoğaltılmış ve genotiplerin belirlenmesi için özgün restriksiyon endonükleazlar ile kesilmiştir. Bütün tavuk hatları VIPR-1/*Hha*I and VIP-501/*Vsp*I gen bölgeleri için monorfik olmasına rağmen geriye kalan dokuz gen bölgesinde polimorfizm belirlenmiştir. Bu çalışma Ankara Tavukçuluk Araştırma Enstitüsü'nde yetiştirilen beyaz yumurtacı saf hatlarda (D-229 hariç) yumurta kalitesini iyileştirmek için gelecekte yapılacak MAS çalışmalarında GHR-intron 2/*Hind*III and GHR-intron 5/*Nsp*I polimorfizmlerinin kullanılabileceğini ortaya çıkarmıştır. Ayrıca, yumurta verimini arttırmak için DRD2 ve MR1C genlerinin çalışılan bütün tavuk hatlarında, VIP-I2/*Hinf*I polimorfizminin sadece Maroon hattında, VIPR-2/*Taq*I polimorfizminin ise Brown ve D-229 hatlarında MAS çalışmalarında kullanılabileceği belirlenmiştir.

Anahtar sözcükler: *Aday genler, PCR-RFLP, Polimorfizm, Saf tavuk hatları*

INTRODUCTION

Although, poultry sector is of the highest intensification rate compared to other sectors related to livestock in Turkey, studies on breeding material production have been carried only by Ankara Poultry Research Institute. The institute

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holds a total of 11 pure chicken layer lines composed of 5 white (Black, Brown, Blue, Maroon and D-229) and 6 brown (Rhode Island Red-I, Rhode Island Red-II, Barred Rock-I, Barred Rock-II, Colombian Rock and Line-54) layer lines. Black, Brown, Blue and Maroon lines were imported from Canada in 1995, while D-229 was provided from Czechia in 2010. Since then, selection processes have been carried out to decrease age and body weight at first egg besides feed intake in these chicken lines. Thanks to studies on white chicken layer lines, a hybrid line called ATABEY was obtained [1-3].

In livestock species, traits that are of economic importance show generally quantitative inheritance. Therefore, the expression of these traits in phenotype depends on both numerous loci and environment conditions. In poultry, economically important traits such as egg yield and quality are of low heritability besides showing polygenic inheritance [4,5].

Today, many genes and their receptors such as Gonadotropin Releasing Hormone (*GnRH*) [4], Growth Hormone Receptor (GHR) [6], Prolactin (PRL) [7], Dopamine (DA) [8], Neuropeptide Y (NPY) [9], Vasoactive Intestinal Peptide (VIP) [10], Ovocalyxin-32 (OCX32) [11] and Melatonin [12] were reported to associate with egg yield and quality in chickens. According to previous studies, single nucleotide mutations in these genes are related to egg yield and quality in chickens [6,8].

Today, Restriction Fragment Length Polymorphism (RFLP) based on Polymerase Chain Reaction (PCR) is one of the commonly used molecular techniques to detect known mutations in livestock species [7,13,14]. Using mutations in these candidate genes separately or together in Marker Assisted Selection (MAS) may increase the success in the selection and genetic improvement in chickens [4,9,11].

Hence, this study aimed to determine polymorphisms in a total of 11 candidate genes (OCX32-exon4, OCX32-exon2, GHR-intron 2, GHR-intron 5, DRD1, DRD2, VIP-501, VIP-I2, VIPR-1, VIPR-2 and MR1C) reported to associated with egg yield and quality by using PCR-RFLP technique. The results obtained in the present study may be used by Ankara Poultry Research Institute for traditional selection studies supplemented with MAS in the future.

MATERIAL and METHODS

Ethical Approval

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

Animal Sampling

A total of 192 blood samples were randomly collected from five different white pure layer chicken lines including Black (n=40), Brown (n=40), Blue (n=40), Maroon (n=32) and D-229 (n=40) raised in Ankara Poultry Research Institute. The blood samples were taken from *venous cutenea ulnaris* into vacuum tubes containing EDTA as an anticoagulant. Blood samples were stored at -20°C until the DNA extraction step.

DNA Extraction

A salting-out method described by Miller et al.[15] was used to extract genomic DNA from blood samples. DNA quality and quantity were checked using agarose gel (1%) and spectrophotometer (NanoDrop-SD 1000). DNA concentration was adjusted to 50 ng/µL for PCR-RFLP analysis.

PCR-RFLP Analysis

In total, 11 candidate genes and/or receptors (OCX32-exon4, OCX32-exon2, GHR-intron 2, GHR-intron 5, DRD1, DRD2, VIP-501, VIP-I2, VIPR-1, VIPR-2 and MR1C) reported in previous studies *(Table 1)* were selected. PCR was performed in 20 µL reaction volume containing 1.2 µL HQ buffer (GeneAll), 2 µL 10X 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 H_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 was carried out at 72°C for 5 min.

Amplificated PCR products were digested with restriction endonucleases *(Table 1)* to genotype the animals. Hence, 8 µL of amplified PCR products were mixed with 2.5 U restriction enzymes *(Table 1)* and 8 µL 10X buffer. After incubation, digested RFLP fragments were visualized on agarose gel electrophoresis to detect the genotypes.

Statistical Analysis

Popgene V. 1.32. [17] package program was used to calculate the genotype and allele frequencies in the studied candidate genes. Deviation from Hardy-Weinberg equilibrium was tested by chi-square (χ^2) statistics.

RESULTS

In this study, polymorphisms in a total of 11 candidate genes reported to be associated with egg yield and quality were assessed in five different white layer pure lines by using the PCR-RFLP technique. Primer sets given in *Table 1* were used to amplify studied gene regions. PCR products were digested with specific restriction endonucleases *(Table 1)* after the PCR process in order to detect genotypes. Both amplified and digested products for MR1C gene were given as an example in *Fig. 1*, *Fig. 2*.

All chicken lines were monomorphic for VIPR-1/*Hha*I and VIP-501/*Vsp*I polymorphisms. All individuals were

Table 1. *Some descriptive information about PCR-RFLP process*

Gene	Primers (5'-3'	Chr.	Ann. Temp. (°C)	PCR Size	Restriction Enzyme	Expected Product Size	Ref.
GHR-intron 2	F:GGCTCTCCATGGGTATTAGGA R: GCTGGTGAACCAATCTCGGTT	Z	59	718	*Hind*III	A_1A_1: 428-290 A_2A_2:170-258-290	[6]
GHR-intron 5	F: ACGAAAAGTGTTTCAGTGTTGA R: TTTATCCCGTGTTCTCTTGACA	Z	56	740	*Nsp*I	CC: 550-190, CD:740, 550, 190 DD: 740	[6,16]
DRD1	F:CACTATGGATGGGGAAGGGTTG R: GCCACCCAGATGTTGCAAAATG	13	62	283	BseNI *Cfr*I	AA: 111-172, AG: 111-172-283 GG: 283	[8]
DRD2	F:TGCACATAAAAGCCCACTCACTG R:GCCTGAGCTGGTGGGGGG	24	60	248	*BseG*I	CC: 248, TC: 248-196, TT: 196	[4]
VIP/501	F:GAAACCCATCTCAGTCATCCTA R:ACCACCTATTTTTCCTTTTCTACA	3	55	306	*Vsp*I	II: 306, DI: 306-154 DD: 154	[10]
VIP/I2	F: GCTTGGACTGATGCGTACTT R: GTATCACTGCAAATGCTCTG	3	58	520	*Hinf* I	CC: 480, CT: 520-480 TT: 520	[10]
VIPR-1	F:CCCCGTTAAACTCAGCAGAC R:CCCAAAGTCCCACAAGGTAA	2	58	434	*Hha*I	TT: 434, TC: 434-253-181 CC 253-181	[4]
VIPR-2	F:CTCCTCAGGCAGACCATCATG R:CTTGCACGTATCCTTGGGTAGC	2	58	486	*Taq*I	TT: 486, TC: 486-310-176 CC: 310-176	[4]
OCX32-exon4	F: TGTTTCTGATGAAGAGCCAGA R: CTTTGCCACTCTGTAGGCTGT	9	58	250	*Nco*I	AA: 250 AC: 250-194 CC: 194	[11]
OCX32-exon2	F: GCCCACTGGTCAGAAAAGAA R: CCTGCAGAGGAAAAGAGCTG	9	58	405	*HpyCH4*IV	TT: 237-169, TG: 237-169-151 GG: 237-151	[11]
MR1C	F: GGTGTATCCGTATCCTCTAA R: GACAGTGGGACAATGAAGT	4	50	372	*Mbo*I	AA: 372, AG: 372-333 GG:333	[12]

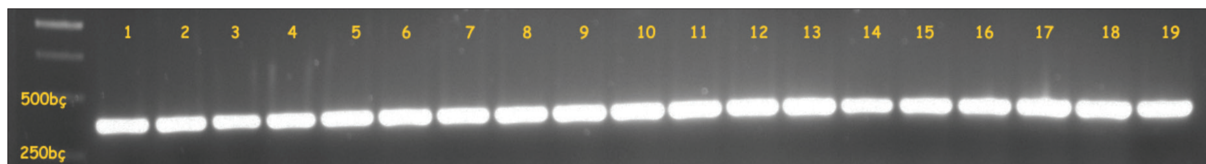

Fig 1. Agarose gel image of PCR products for MR1C gene
(Marker: Thermo, 1kb, Kat. No: SM0311; 1% agarose gel, fragment size 372 bp)

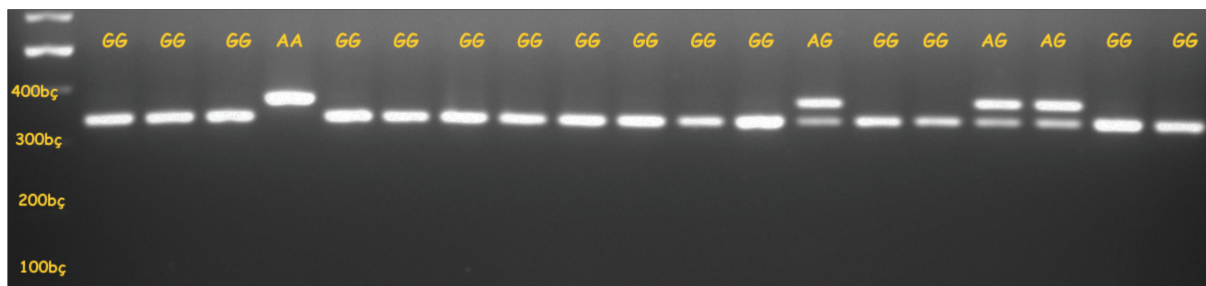

Fig 2. Agarose gel image of digested PCR products of MR1C gene with *Mbo*I restriction enzyme
(Marker: Thermo, 100 bp, Kat. No: SM0241; 2% agarose gel, fragment size GG: 333 bp, AG: 333-372 bp, AA: 372 bp)

with CC and II genotype for VIPR-1/*Hha*I and VIP-501/*Vsp*I polymorphisms, respectively. Except for VIPR-1 and VIP-501, the rest of the nine genes or receptors were found polymorphic. Allele and genotype frequencies for nine polymorphic gene regions are given in *Table 2*.

D-229 line was monomorphic for both GHR-intron 2/*Hind*III and GHR-intron 5/*Nsp*I polymorphisms. A_1A_1 genotype frequency ranged from 0.05 (Blue) to 0.72 (Maroon) in GHR-intron 2/*Hind*III polymorphism, whereas DD genotype frequency varied from 0.43 (Brown) to 0.95 (Blue) in GHR-intron 5/*Nsp*I polymorphism.

Black, Blue, Maroon and D-229 lines were detected to be monomorphic (GG genotype) in DRD1/*BseN*I polymorphism. On the contrary, AA and GG genotype frequencies were 0.10 and 0.90, respectively in Brown lines with significant deviation from HW equilibrium. In DRD2/*BseG*I polymorphism, all chicken lines were polymorphic with significant deviation from HW equilibrium. The lowest

Table 2. *Allele and genotype frequencies of studied genes in five white pure chicken lines*

Chicken Line	Loci	n	Allele Frequency		Genotype Frequency			χ^2
BLACK	GHR-intron2	39	A_1/0.33	A_2/0.67	A_1A_1/0.33	A_1A_2/0.00	A_1A_2/0.67	39.00[b]
	GHR-intron5	38	C/0.30	D/0.70	CC/0.16	CD/0.29	DD/0.55	3.75[a]
	DRD1	40	A/0.00	G/1.00	AA/0.00	AG/0.00	GG/1.00	-
	DRD2	26	C/0.31	T/0.69	CC/0.04	TC/0.54	TT/0.42	1.81[a]
	VIP/I2	40	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
	VIPR-2	39	C/0.96	T/0.04	CC/0.96	CT/0.02	TT/0.02	16.65[b]
	OCX32-exon4	39	A/0.19	C/0.81	AA/0.05	AC/0.28	CC/0.67	0.33[a]
	OCX32-exon2	32	G/0.61	T/0.39	GG/0.56	GT/0.09	TT/0.35	00.64[b]
	MR1C	39	A/0.33	G/0.67	AA/0.05	AG/0.56	GG/0.39	2.83[a]
BLUE	GHR-intron2	40	A_1/0.05	A_2/0.95	A_1A_1/0.05	A_1A_2/0.00	A_2A_2/0.95	40.00[b]
	GHR-intron5	40	C/0.02	D/0.98	CC/0.00	CD/0.05	DD/0.95	0.03[a]
	DRD1	40	A/0.00	G/1.00	AA/0.00	AG/0.00	GG/1.00	-
	DRD2	40	C/0.42	T/0.58	CC/0.15	TC/0.55	TT/0.30	0.63[a]
	VIP/I2	40	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
	VIPR-2	40	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
	OCX32-exon4	40	A/0.40	C/0.60	AA/0.00	AC/0.80	CC/0.20	17.78[b]
	OCX32-exon2	23	G/0.11	T/0.89	GG/0.09	GT/0.04	TT/0.87	13.84[b]
	MR1C	40	A/0.09	G/0.91	AA/0.02	AG/0.12	GG/0.86	1.89[a]
D-229	GHR-intron2	40	A_1/0.00	A_2/1.00	A_1A_1/0.00	A_1A_2/0.00	A_2A_2/1.00	-
	GHR-intron5	40	C/0.51	D/0.49	CC/0.02	CD/0.98	DD/0.00	36.19[b]
	DRD1	40	A/0.00	G/1.00	AA/0.00	AG/0.00	GG/1.00	-
	DRD2	40	C/0.17	T/0.82	CC/0.02	TC/0.30	TT/0.68	0.06[a]
	VIP/I2	32	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
	VIPR-2	40	C/0.75	T/0.25	CC/0.70	CT/0.10	TT/0.20	21.51[b]
	OCX32-exon4	39	A/0.23	C/0.77	AA/0.03	AC/0.41	CC/0.56	0.944[a]
	OCX32-exon2	28	G/0.39	T/0.61	GG/0.29	GT/0.21	TT/0.50	8.49[b]
	MR1C	40	A/0.40	G/0.60	AA/0.17	AG/0.45	GG/0.38	0.16[a]
BROWN	GHR-intron2	40	A_1/0.57	A_2/0.42	A_1A_1/0.57	A_1A_2/0.00	A_2A_2/0.42	40.00[b]
	GHR-intron5	40	C/0.29	D/0.71	CC/0.00	CD/0.57	DD/0.43	6.51[b]
	DRD1	40	A/0.10	G/0.90	AA/0.10	AG/0.00	GG/0.90	40.00[b]
	DRD2	40	C/0.26	T/0.74	CC/0.07	TC/0.37	TT/0.56	0.04[a]
	VIP/I2	40	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
	VIPR-2	40	C/0.99	T/0.01	CC/0.98	CT/0.02	TT/0.50	0.01[a]
	OCX32-exon4	40	A/0.54	C/0.46	AA/0.12	AC/0.82	CC/0.06	17.40[b]
	OCX32-exon2	33	G/0.35	T/0.65	GG/0.21	GT/0.27	TT/0.52	5.26[b]
	MR1C	40	A/0.11	G/0.89	AA/0.02	AG/0.17	GG/0.81	0.61[a]
MAROON	GHR-intron2	32	A_1/0.72	A_2/0.28	A_1A_1/0.72	A_1A_2/0.00	A_2A_2/0.28	32.00[b]
	GHR-intron5	31	C/0.61	D/0.39	CC/0.09	CD/0.34	DD/0.57	0.45[a]
	DRD1	32	A/0.00	G/1.00	AA/0.00	AG/0.00	GG/1.00	-
	DRD2	32	C/0.69	T/0.31	CC/0.37	TC/0.63	TT/0.00	6.61[b]
	VIP/I2	29	C/0.78	T/0.22	CC/0.66	CT/0.24	TT/0.10	2.71[a]
	VIPR-2	40	C/1.00	T/0.00	CC/1.00	CT/0.00	TT/0.00	-
	OCX32-exon4	32	A/0.40	C/0.60	AA/0.12	AC/0.36	CC/0.52	0.89[a]
	OCX32-exon2	24	G/0.60	T/0.40	GG/0.50	GT/0.21	TT/0.29	7.65[b]
	MR1C	32	A/0.40	G/0.60	AA/0.00	AG/0.53	GG/0.47	4.19[b]

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

and the highest C allele frequency were observed in D-229 (0.17) and Maroon (0.69) lines, whereas T allele frequency ranged from 0.31 (Maroon) to 0.82 (D-229). No TT genotype was detected in Maroon line, while it was observed in D-229 line with a high frequency (0.68).

Polymorphism was detected in Black, Brown and D-229 lines, while Blue and Maroon lines were monomorphic (CC genotype) in VIPR-2/*Taq*I polymorphism. The frequency of desired genotype (TT) for the number of egg at 300 days of age were 0.50, 0.20 and 0.02 in Brown, D-229 and Black line, respectively. Only Maroon line showed polymorphism in VIP-I2/*Hinf*I polymorphism. CC, CT and TT genotype frequencies were 0.66, 0.24 and 0.10, respectively in Maroon line. All individuals from other lines were with CC genotype in VIP-I2/*Hinf*I polymorphism.

AA genotype frequency ranged from 0.00 (Maroon) to 0.17 (D-229), whereas GG genotype frequency varied from 0.38 (D-229) to 0.86 (Blue) in MR1C gene. The frequency of desired genotype (AG) for the number of egg at 300 days of age were 0.56, 0.12, 0.45, 0.17 and 0.53 in Black, Blue, D-229, Brown and Maroon line, respectively. In addition, all populations except Maroon line were in HW equilibrium for MR1C gene region.

All studied chicken lines were polymorphic in OCX32-exon2/*Hpy*CH4IV and OCX32-exon4/*Nco*I polymorphisms. G allele frequency ranged from 0.11 (Blue) to 0.61 (Black), while T allele frequency varied from 0.39 (Black) to 0.89 (Blue) in OCX32-exon2/*Hpy*CH4IV polymorphism. The highest GG, GT and TT genotype frequencies were observed in Black (0.56), Brown (0.27) and Blue (0.87) line, respectively. An allele frequency ranged from 0.19 (Black) to 0.54 (Brown), while C allele frequency varied from 0.46 (Brown) to 0.81 (Black) in OCX32-exon4/*Nco*I polymorphism. The lowest and the highest AA genotype frequencies were detected in Blue (0.00) and Brown (0.12) lines, respectively. The highest AC and CC genotype frequencies were detected in Brown (0.82) and Black (0.67) line, respectively. A significant deviation from HW equilibrium was detected in Brown and Blue lines for the OCX32-exon4 gene, while significant deviation from HW equilibrium was observed in all chicken lines for the OCX32-exon2 gene.

DISCUSSION

Li et al.[6] have been reported that GHR-intron 2/*Hind*III polymorphism is related to egg quality in Wenchang chickens, in which individuals with A_1A_1 genotype had higher number of double yolk eggs. Additionally, A_2A_2 genotype frequency was reported as 0.94 in Wenchang chickens by Li et al.[6]. In the present study, no A_1A_1 genotype was detected in D-229 line, whereas its frequency was varied from 0.05 (Blue) to 0.72 (Maroon) in other chicken lines. In addition, Li et al.[16] have been reported that GHR-intron 5/*Nsp*I is associated with eggshell thickness in Wenchang chickens, in which individuals with DD genotype were advantageous in terms of eggshell thickness. In Wenchang chickens, C and D allele frequencies were reported as 0.20 and 0.80 [16], respectively while in Mazandaran native chickens C and D allele frequencies were 0.72 and 0.28 [18], respectively for GHR-intron 5/*Nsp*I polymorphism. In the present study, no DD genotype was detected in D-229 line, while it was observed in other chicken lines with high frequencies (0.43-0.95)

The present study revealed that polymorphisms detected by *Hind*III and *Nsp*I restriction enzymes in intron 2 and intron 5 regions of GHR gene may be used in MAS studies in White chicken lines excluding D-229 raised in Ankara Poultry Research Institute. No desired genotype was detected in D-229 line for both GHR-intron 2/*Hind*III and GHR-intron 5/*NspI* polymorphism. It is thought to be due to breeding history since D-229 line was imported from Czechia in 2010, while the other lines were provided from Canada in 1995. Also, the frequency of desired genotypes was variable among the other chicken lines. Although these chicken lines originated from the same genetic resource (White Leghorn), the underlying reason for this situation is thought to be the long-term selection process applied in these lines.

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 [4,8]. Chickens with AA genotype reported being shown a superior number of total egg than chickens with GG and AG genotypes in DRD1/*BseNI CfrI* polymorphism [8]. 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/*BseGI* polymorphism [4].

In the present study, the desired genotype (AA) was observed in only Brown line with very low frequency (0.10) in DRD1/*BseN*I polymorphism, whereas the desired genotype (TT) was detected in all chicken lines with high frequency except Maroon in DRD2/*Bse*GI polymorphism. It was observed that DRD2 gene polymorphism may be used for MAS studies in the future, while DRD1 gene cannot be used in MAS due to a lack of polymorphism.

It has been reported that VIPR-1 and VIPR-2 genes are associated with egg number at 300 days in chickens [4]. 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 [4]. CC, TC and TT frequencies were reported as 0.935, 0.060 and 0.005, respectively in Ningdu Sanhuang chicken [4]; 0.64, 0.29 and 0.07, respectively in Vietnam Voi chicken [19] 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 [4]; 0.48, 0.33 and 0,19, respectively in Vietnam Voi chicken [19] in VIPR-2/*Taq*I polymorphism.

In the present study, all chicken lines were monomorphic (CC genotype). Although similar CC genotype frequency (0.934) was reported in Ningdu Sanhuang chicken [4], lower CC genotype frequency (0.64) was reported in Wietnam Voi chicken [19]. The main reason of monomorphism could be attributed that these chicken lines were derived from the same genetic origin (White Leghorn). Although all chicken lines were of the desired genotype for egg number at 300 days, VIPR-1/*Hha*I polymorphism cannot be used in MAS studies due to lack of polymorphism.

The desired genotype (TT) frequency for VIPR-2/*Taq*I polymorphism was reported as 0.09 and 0.19 in Ningdu Sanhuang and Vietnam Voi chicken, respectively [4,19]. In the present study, the higher TT genotype frequency (0.50) was detected in the Brown line conserving enough genetic variability. Hence, it is determined that VIPR-2/*Taq*I polymorphism may be used in MAS studies for the Brown line. Although significant deviation from HW equilibrium was observed in D-229 line, TT genotype frequency (0.20) was sufficient. VIPR-2/*Taq*I polymorphism may be used in MAS studies for D-229 line but it is crucial not to decrease genetic variability in this chicken line.

Zhou et al.[10] reported that "AGG" indel was associated with both total and the qualified number of eggs from 90 to 300 days of age in Nindu Sanhuang chickens. In contrast, C+338T was reported to be associated with the duration of broodiness [10]. D allele was reported to be shown superior values for the total number of qualified eggs from 90 to 300 days of age in VIP-501/*Vsp*I polymorphism [10]. CT genotype was reported to be an advantageous genotype for egg production and broodiness traits in VIP-I2/*Hinf*I polymorphism [10]. In the present study, no desired allele (D allele) was detected in five chicken lines of which all individuals were with II genotype in VIP-501/*Vsp*I polymorphism. Similarly, only Maroon line showed polymorphism for VIP-I2/*Hinf*I polymorphism. Hence, it was determined that VIP-501/*Vsp*I cannot be used in MAS studied chicken lines, while *VIP-I2/Hinf*I can be used in MAS for only Maroon line.

Li et al.[12] reported that MR1C/*Mbo*I polymorphism was associated with both age at first egg and egg number at 300 days in chickens, in which individuals with AG genotype showed superior egg number at 300 days than individuals with GG and AA genotypes. A and G allele frequencies were reported as 0.49 and 0.51, respectively, while AA, AG and GG genotype frequencies were 0.17, 0.64 and 0.19, respectively in Wenchang chicken [12]. On the contrary, Padwar and Thakur [20] were reported that Kadaknath and Jabalpur chickens were monomorphich (AA genotype) in terms of MR1C/*Mbo*I polymorphism. In the present study, detected AG genotype frequencies (ranging from 0.12 to 0.56) in five chicken lines were lower than the findings reported by Li et al.[12]. This may be attributed to used different chicken breeds and lines. Significant differences in terms of AG genotype frequency were observed among studied chicken lines, although they were derived from the same breed. This difference is thought to result from the selection process for different purposes.

Uemoto et al.[11] reported that mutations (c.267T>G, c.494A>C and c.381G>C) on OCX32 gene were related to egg production ratio, yellowness and frequencies of meat spot in White Leghorn and Rhode Island Red breeds. G and T allele frequencies were reported as 0.40 and 0.60, respectively in White Leghorn breed for OCX32/*HpyCH4*IV polymorphism [11]. In the present study, similar values were detected in D-229 and Brown lines.

Additionally, C and A allele frequencies were reported as 0.40 and 0.60, respectively in White Leghorn breed for OCX32/*Nco*I polymorphism [11]. In the present study, similar C (0.46) and A (0.54) allele frequencies were detected in the Brown line, while the frequencies were different in other studied chicken lines. This difference could be attributed to many factors such as the selection process and breeding system. This idea was also supported by HW equilibrium values detected for OCX32/*HpyCH4*IV polymorphism. Indeed, a significant deviation from HW equilibrium was detected in only Black lines. Inbreeding and selection processes may cause rapid changes in allele frequencies and deviation from HW equilibrium.

Consequently, this is the first comprehensive study aimed to determine polymorphisms in a total of 11 genes and receptors associated with egg yield and quality in five white layer lines reared by Ankara Poultry Research Institute by using PCR-RFLP. The present study revealed that GHR-intron 2/*Hind*III and GHR-intron 5/*Nsp*I polymorphisms can be integrated with MAS studies to increase egg quality in White chicken lines (D-229 excluded) raised in Ankara Poultry Research Institute in the future. In addition, in order to increase egg yield, DRD2 and MR1C gene may be used in MAS for all studied chicken lines, while VIP-I2/*Hinf*I polymorphism can be used for only Maroon line and VIPR-2/*Taq*I polymorphism for Brown and D-229. It is also important to highlight that association analysis between candidate genes with egg yield and quality should be conducted in five White chicken lines before applying MAS. In addition, the results of the present study showed once again the effects of selection and inbreeding on genetic structure. Despite of deriving from the same genetic origin (White Leghorn), in five chicken lines, allele and genotype frequencies have differentiated due to selection applied for different purposes and inbreeding.

Author Contributions

MSB, TK and SA designed the project. SK provided samples. ED, BAK, MA, HGF and ESS performed DNA extraction and PCR-RFLP stages. ED, TK, MSB and SA performed statistical analysis of data and wrote the article.

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