Associations of *IGF2* and *DRD2* polymorphisms with laying traits in Muscovy duck (#18246)

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Associations of *IGF2* and *DRD2* polymorphisms with laying traits in Muscovy duck

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Insulin-like growth factor 2 (IGF2) and dopamine receptor 2 (DRD2) play important roles in ovarian follicular development. In this study, we analyzed tissue-specific expression of the Muscovy duck *IGF2* and *DRD2* genes and cloned those genes transcripts. Polymorphisms in these genes were tightly linked with egg production traits and both genes were highly expressed in the ovary. Moreover, we identified 5 single nucleotide polymorphisms (SNPs) for *IGF1* and 28 for *DRD2.* Mutations A-1864G and C-1704G of *IGF2* were positively correlated with increased egg laying at 59 weeks (E59W) ($P < 0.05$). The C+7T and C+364G mutations of *DRD2* were highly and significantly associated with first-egg age (FEA) and egg numbers at 300 days (E300D) ($P < 0.01$). Moreover, C+3301G and C+3545G of *DRD2* were highly significantly associated with FEA, E59W and E300D ($P < 0.01$). Other mutations were positively associated with FEA or E300D or E59W ($P < 0.05$). These data suggest specific roles for *IGF1* and *DRD2* polymorphisms in egg production in Muscovy ducks.

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ABSTRACT

Insulin-like growth factor 2 (IGF2) and dopamine receptor 2 (DRD2) play important roles in ovarian follicular development. In this study, we analyzed tissue-specific expression of the Muscovy duck *IGF2* and *DRD2* genes and cloned those genes transcripts. Polymorphisms in these genes were tightly linked with egg production traits and both genes were highly expressed in the ovary. Moreover, we identified 5 single nucleotide polymorphisms (SNPs) for *IGF1* and 28 for *DRD2*. Mutations A-1864G and C-1704G of *IGF2* were positively correlated with increased egg laying at 59 weeks (E59W) ($P < 0.05$). The C+7T and C+364G mutations of *DRD2* were highly and significantly associated with first egg age (FEA) and egg numbers at 300 days (E300D) ($P < 0.01$). Moreover, C+3301G and C+3545G of *DRD2* were highly significantly associated with FEA, E59W and E300D ($P < 0.01$). Other mutations were positively associated with FEA or E300D or E59W ($P < 0.05$). These data suggest specific roles for *IGF1* and *DRD2* polymorphisms in egg production in Muscovy ducks.

INTRODUCTION

Muscovy ducks are an excellent breed species because of their rapid growth, crude feed tolerance and ~~highly priced~~ meat. Although these ducks are raised on a large scale in China, low production performance affects the economic interests of farmers. Breeders have been looking for ways to improve Muscovy ducks egg production. In recent years, with the rapid development

of genome sequencing technologies, molecular marker breeding and transgenic breeding technology have gradually become the mainstream of breeding. Traditional breeding mainly depends on breeding experience, which has a lot of unpredictability. Furthermore, ~~the~~ molecular breeding can significantly improve ~~the~~ breeding efficiency and shorten the breeding period, so using molecular marker breeding has a huge advantage in breeding. Nowadays, molecular markers ~~were~~ widely used in poultry breeding, such as green shell egg related molecular markers (Wang *et al.*, 2013)~~.~~ egg production related molecular markers (Han *et al.*, 2014). Due to the great prospects of molecular markers in breeding, using molecular markers to selecting high laying performance Muscovy ducks is a good decision. Our research focuses on egg production related molecular markers that can be used to improve egg production for the Muscovy duck. Few researchers paid attention to egg production traits in Muscovy ducks ~~that make~~ our research more meaningful. The first egg age (FEA), egg numbers at 300 days (E300D), and egg numbers at 59 weeks (E59W) ~~were~~ ~~very~~ important traits in Muscovy ducks breeding. Muscovy ducks egg peak time is from 35 weeks to 53 weeks, and 59 weeks ~~are~~ the last stage of laying. 300 days ~~are~~ the peak time of laying, and 59 weeks ~~are~~ the end time of laying in Muscovy ducks, ~~W~~which ~~cover~~ most of the egg laying period. Therefore, we ~~can use~~ FEA, E300D and E59W instead of egg production at other time points as important traits.

Insulin-like growth factor 2 (IGF2) plays key roles in animal growth differentiation and proliferation (Kaneda *et al.*, 2007)~~. In addition, these roles also extend to~~ reproduction and the regulation of ovarian follicle development. In mammals, *IGF2* is highly expressed in the

dominant follicle supporting key functions for follicular development (Mao *et al.*, 2004). *IGF2* may ~~effect~~ prolificacy in sows and cattle (Stinckens *et al.*, 2010; Aad *et al.*, 2013), and *IGF2* may regulate ovarian development through follicle-stimulating hormone (FSH) (Baumgarten *et al.*, 2015). But ~~few reports~~ on the regulation of ovarian development by *IGF2* ~~have been found~~ in birds. ~~This is the first time we have reported~~ that IGF2 may be associated with ovarian development. Dopamine (DA) is an essential neurotransmitter and exists in the nerve center and its peripheral tissue. Dopamine receptor 2 (DRD2) may assist with the secretion of reproductive hormones through follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in chicken (Youngren *et al.*, 1996; Youngren *et al.*, 1998). Association studies between single nucleotide polymorphisms (SNPs) of *IGF2* and *DRD2* and reproduction traits have been carried out in poultry (Xu *et al.*, 2011; Wang *et al.*, 2014; Zhang *et al.*, 2015; Zhu *et al.*, 2015). However, until now very few studies have focused on the relevance of these genes to egg production in Muscovy ducks. Therefore, we aim to identify SNPs ~~of~~ these genes, and to reveal their associations with reproduction traits in Muscovy ducks~~, and~~ hope these molecular markers may help to improve the production performance of Muscovy ducks in breeding.

MATERIALS AND METHODS

Ethics Statement

Ethical approval for all animal experiments was granted by the Animal Care Committee of South China Agricultural University (Guangzhou, People's Republic of China) with approval ~~numbe~~

20131019002.

Sample Collection

Eight hundred white Muscovy ducks were offered by Wens Nanfang Poultry Breeding company (Yunfu, Guangdong, China) which were in the same run. All Muscovy ducks were reared under identical conditions of management and feeding. Ducks were maintained outside on the ground from 4 to 12 weeks of age, after which they were transferred to individual cages in a semiconfined house. Feed ~~were~~ provided by Wens company. ~~And the~~ first egg age (FEA), egg numbers at 300 days (E300D), and egg numbers at 59 weeks (E59W) were recorded for each female duck. Genomic DNA from each individual at 59 weeks was isolated from 0.5 ml blood stored with EDTA as an anticoagulant, using E.Z.N.A NRBC Blood DNA Kit (Omega, Norcross, GA, USA) according to the manufacturer's instructions.

RNA Isolation and cDNA Synthesis

Muscovy duck tissues including pituitary, brain, lung, abdominal fat, liver, ovary, subcutaneous fat, spleen, kidney, leg muscle, hypothalamus, cerebellum, heart and breast muscle used for expression pattern analysis of *IGF2* and *DRD2* genes ~~was~~ sampled at first egg age~~, and these~~ ducks were raised under the same conditions, but in different batches from eight hundred Muscovy ducks mentioned above. Total RNA was isolated from tissues using a TRIZOL

Reagent kit (TaKaRa, Dalian, China) according to the manufacturer's protocol. RNA quality was evaluated by 2% agarose gel electrophoresis and then was reverse transcribed using Takara reverse transcription Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. The cDNA was used as template to amplify the coding region of *IGF2* and *DRD2* genes from Muscovy duck.

Cloning of Muscovy duck IGF2 and DRD2 genes

The Muscovy duck IGF2 and DRD2 genes were identified using Mallard duck gene sequences as a reference (Gene Bank accession No. XM_005019778 and XM_013109685). Primers were designed to amplify the coding regions of Muscovy duck *IGF2* and *DRD2* using Primer 5.0 (Primer IGF2-CDS and Primer DRD2-CDS; Table S1). PCR amplifications were conducted in a final volume of 50 µl with 2 µl cDNA, 25 µl 2 × Easy Taq SuperMix (TransGen, Beijing, China), and 0.5 µl each pair of primers, and 22 µl double distilled H_2O. Optimum PCR amplification conditions were programmed as pre-denaturation at 94ºC for 3 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 58ºC for 30 s, and extension at 72ºC for 30 s, and a final extension at 72ºC for 10 min. The PCR products were evaluated by electrophoresis using a 2% agarose gel and then gel purified using a HiPure Gel Pure DNA kit (TransGen, Beijing, China). The amplified fragments were cloned into pMD-18T vector (TaKaRa, Dalian, China), and sequenced by Majorbio, Shanghai, China. Sequence alignment and phylogenetic trees are constructed using MEGA5.

111 **Expression Pattern Analysis of IGF2 and DRD2 mRNA**

112 Total mRNA from 14 different tissues was extracted to investigate the mRNA expression
113 profiles of Muscovy duck *IGF2* and *DRD2* genes using real-time qPCR. Muscovy duck *β-actin*
114 gene was used as the internal reference gene. Primers for *IGF2,* DRD2 and *β-actin* gene were
115 designed using Primer 5.0 (Primer β-actin-duck, IGF2-Q and DRD2-Q; Table S1). The qPCR
116 was performed using a standard SYBR Premix Ex Taq II (TaKaRa, Dalian, China) on a BioRad
117 CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, USA) according to the
118 manufacturer's protocol. The thermal cycling was 95ºC for 2 min, followed by 39 cycles of 95ºC
119 for 15 s, 60ºC for 30 s, 72ºC for 30 s, and final cycle of 72ºC for 7 min. Relative expression of
120 *IGF2* and *DRD2* genes was calculated relative to the expression of *β-actin*. Real-time PCR data
121 were analyzed using the $2^{-\Delta\Delta Ct}$ method.

122

123 **SNPs Detection by Sequencing**

124 We designed 7 primers to identify potential SNPs of *IGF2* and *DRD2* (Primer IGF2-P1, IGF2-P2,
125 DRD2-P1, DRD2-P2, DRD2-P3, DRD2-P4 and DRD2-P5; Table S1). Twenty white Muscovy
126 ducks were sampled and five individuals were selected as a mixed pool. PCR reactions were
127 performed in a 50 μl final volume, containing 2 μl DNA, 25 μl 2 × Easy Taq SuperMix
128 (TransGen Biotech, Beijing, China), 0.5 μl each pair of primers, and 22 μl double distilled H_2O.
129 PCR parameters were 3 min at 94ºC followed by 37 cycles of 94ºC for 30 s, annealing
130 temperature for 60 s, 72ºC for 30 s and a final extension at 72ºC for 10 min. PCR products were
131 evaluated by electrophoresis using 2% agarose gel and sequenced as described above. SNPs were
132 identified by the Seqman program of DNAStar 7.1.0 software.

133

134 **Genotyping and Association Analysis**

135 The SNPs were genotyped in 800 female ducks with egg production records *via* sequencing. We
136 designed 3 primers to ~~Genotyping~~ SNPs of *IGF2* and *DRD2* (Primer IGF2-SNP, DRD2-SNP1
137 and DRD2-SNP2; Table S1)~~, and~~ PCR reactions were identical to those used in SNP detection as
138 described above. Genotypes were tested for Hardy-Weinberg equilibrium with the chi-square test.
139 Linkage analysis was performed using Haploview software. The associations between SNPs and
140 egg production traits were calculated using the general linear model procedure of SAS v. 9.2
141 with the following model:

142 $$Y_{ij} = \mu + G_i + e_{ij}$$

143 ~~Where~~ Y_{ij} is the observed value of different egg production traits, μ is the overall population
144 mean, G_i is the effect of each genotype, and e_{ij} is the random error. For each egg production trait,
145 the least-squares mean was estimated and differences between the genotypes were analyzed
146 using ~~the Bonferroni test. Multiple comparisons were conducted with least square means.~~
147 Difference with P value ≤ 0.05 was considered to be significant in analyses.

148

149 **RESULTS**

150 **Characterization of Muscovy duck *IGF2* and *DRD2* Coding Region**

151 We obtained a 311-bp partial cDNA of the *IGF2* gene that was 98% and 95% identical to *Anas*
152 *platyrhynchos (*XM_013191560.1) and *Anser cygnoides domesticus* (XM_005019778.2),
153 respectively. We obtained the full-length cDNA of *DRD2* including a 52-bp 5'-untranslated

region (UTR), an 1104-bp open reading frame (ORF) containing 368 codons and a 294-bp 3'-UTR. The Muscovy duck *DRD2* cDNA sequence was 98% and 96% to *Anas platyrhynchos* (XM_013109686.1) and *Anser cygnoides domesticus* (XM_013187289.1), respectively. A phylogenetic tree constructed based on the *DRD2* gene also revealed that the Muscovy duck was closely related with both animals above (Figure 1).

Tissue Expression of *IGF2* and *DRD2* Genes

We examined tissue-specific expression of IGF2 and found that it was expressed in most tissues. The highest expression levels were found in the kidney and ovaries (Figure 2). *DRD2* expression was ~~the~~ highest in ovary, but was also expressed in the cerebrum, cerebellum, hypothalamus and pituitary at lower levels. However, other tissues also had expression levels near detection limits including abdominal fat, sebum and breast and leg muscle. Expression in the spleen was negligible (Figure 3**)**.

Polymorphisms of *IGF2* and *DRD2* Genes

We identified 5 SNPs in the 5'flanking region of *IGF2* ~~at~~ a level of one SNP per 449 bp on average. These SNPs were A-1864G, C-1704G, A-584G, A-227G and A-183G (Table 1). We found 28 SNPs in *DRD2* giving rise to one SNP per 317 bp on average. Among them, the C+7T in exon 1 was a missense mutation resulting in P to S amino acid change (Table 1). We selected 2 SNPs of *IGF2* and 11 SNPs of *DRD2* based on mixed pool sequencing results~~,~~ which were more likely to be associated with egg laying traits, for further association analysis.

175

176 **Association of *IGF2* and *DRD2* with egg production traits**

177 Association analysis ~~identified~~ the A-1864G and C-1704G SNPs of *IGF2* gene both significantly
178 associated with E59W ($P < 0.05$) (Table 2), and linkage disequilibrium analysis indicated a high
179 linkage block between A-1864G and C-1704G for *IGF2* (Figure 4). Multiple comparisons of
180 different genotypes showed that the AG genotype individuals of A-1864G had 6-7 eggs more
181 than GG genotype individuals for E59W ($P < 0.01$). The GG genotype individuals of C-1704G
182 had 7-8 eggs more than individuals with the CC genotype for E59W ($P < 0.05$).

183 Association analysis for *DRD2* gene further showed that C+7T and C+364G had highly
184 significant associations with FEA and E300D ($P < 0.01$)~~,~~ and significantly associated with E59W
185 ($P < 0.05$) (Table 3). A+3489G, A+3484T and T+3428C were significantly associated with FEA
186 and E300D ($P < 0.05$)~~,~~ and highly associated with E59W ($P < 0.01$). T+3423C and A+3262G
187 were significantly associated with FEA ($P < 0.05$) and ~~indicate highly significant association~~
188 with E59W ($P < 0.01$). A+3183C was significantly associated with E59W ($P < 0.05$), and
189 T+3024C has no significant association with ~~all~~ three egg production traits. Moreover, it was
190 notable that C+3301G and C+3545G were highly significantly associated with FEA, E59W and
191 E300D ($P < 0.01$). Multiple comparisons among different genotypes showed that the GG
192 genotypes of C+3301G and C+3545G were advantageous for earlier egg laying and egg
193 production. There were two high linkage blocks (C+7T and C+364G, A+3183C and A+3262G)
194 for *DRD2* (Figure 5).

195

DISCUSSION

Muscovy duck is an excellent poultry, but its egg production is low, which has been plaguing farmers and breeders. In recent years, molecular marker breeding ~~have~~ gradually become the mainstream of breeding, and many breeders try to improve egg laying performances through breeding methods of molecular markers in poultry (Wang *et al.*, 2014; Fulton *et al.*, 2012; Uemoto *et al.*, 2009). Using molecular marker to improve Muscovy ducks egg production is an effective method~~,~~ which will greatly improve the economic value of Muscovy ducks. Our study focused on ~~the~~ egg production traits and ~~the~~ related molecular markers, and we tried to find some molecular markers highly related to egg production in Muscovy ducks, ~~and hope~~ they can be used in Muscovy duck breeding. We believe that the relevant personnel of Muscovy ducks industry ~~has~~ a strong interest in ~~the~~ study.

In the present study, we obtained coding ~~region~~ of *IGF2* and *DRD2* in Muscovy duck for the first time, ~~and they will be great help to the further~~ research. *IGF2* and *DRD2* genes in humans, mice and chickens all have transcript variants (Kaalund *et al.*, 2014; Wernersson *et al.*, 2015; Johannessen *et al.*, 2016). However, we only found one transcripts in Muscovy duck. This may be caused by differences between different species.

High expression of *IGF2* in the ovary may be related to follicular development in zebra fish (Irwin *et al.*, 2012). In our study, we found *IGF2* is widely expressed in different tissues with the highest expression in ovary. This suggests that *IGF2* may be associated with ovarian development. The ovarian functions of birds are regulated by luteinizing hormone (LH) and

follicle stimulating hormone (FSH). *IGF2* can stimulate granule cell proliferation and related hormones synthesis and regulate follicle development with FSH in mammals (Lucy, 2011). Previous studies have found that *IGF2* expression in the ovary directly affects the development of dominant follicles in rats (Wang *et al.*, 2002). *IGF1* can inhibit the apoptosis of granulosa cells, while *IGF2* might regulate cell proliferation during follicular development in chicken (Johnson *et al.*, 2009). In addition, *IGF2* expression in the follicles of highly productive chickens are significantly higher than that in lowly productive chickens. Therefore, a relationship exists between the expression of *IGF2* in the ovary and egg production in chickens (Kim *et al.*, 2004). It is also becoming clear from *in vivo* and *in vitro* studies carried out in birds that *IGF2* plays an important role in ovarian follicular development (Wood *et al.*, 2005). All these studies ~~above~~ indicate that *IGF2* is related to the development of ovary. Thus, we deduced that *IGF2* might play a key role in ovarian follicular development of Muscovy ducks and regulate egg production.

In this study, we found that Muscovy *DRD2* also had its highest expression in the ovary. The *DRD2* gene belongs to the catecholamine neurotransmitter receptors that exist widely in central and peripheral nervous tissues. *DRD2* is highly expressed in the ovary and this may be related to follicular and ovarian development in human (Morton *et al.*, 2006). Other studies identified high *DRD2* expression in the regulation of reproductive functions in grey mullet (Nocillado *et al.*, 2007). DRD2 agonist can inhibit the production and secretion of vascular endothelial growth factor protein in human granulosa cells (Ferrero *et al.*, 2014). Together these findings indicate that DRD2 may have a function in follicular and ovarian development. Therefore, we selected *IGF2* and *DRD2* as a candidate gene related to egg laying traits for further study.

IGF2 is important in body growth and development. Most research on *IGF2* has concentrated on growth studies and the association of *IGF2* polymorphisms with growth related traits. Few studies have investigated the association between *IGF2* and egg laying traits. But in the current study, we found the high linkage sites A-1864G and C-1704G of *IGF2* were significantly associated with E59W. This indicated that *IGF2* was positively related to egg laying traits. However, how those two loci of *IGF2* regulate egg laying performance ~~have not been studied~~. ~~Next~~ study should focus on the function of the two loci for egg laying performance. Recently, *DRD2* polymorphisms have been related ~~with~~ poultry egg production. Our previous studies found the chicken *DRD2* gene polymorphisms were correlated with the first egg age and the egg numbers at 300 days in chicken (Xu *et al.*, 2011). SNPs of *DRD2* were significantly associated with egg production at 38 weeks and egg weight at 300 days in chicken (Zhu *et al.*, 2015). These studies suggest that the *DRD2* is indeed associated with the laying performance of birds. In our study, we also found a link between *DRD2* and the laying performance of birds. We found 10 SNPs of *DRD2* gene (C+7T, C+364G, A+3183C, A+3262G, C+3301G, T+3423C, T+3428C, A+3484T, A+3489G and C+3545G) were significantly associated with egg production traits, and two high linkage blocks were found in haplotype analysis. According to our studies, *IGF2* and *DRD2* are indeed related to the laying performance of birds, but the specific functions of these SNPs remain to be studied.

In conclusion, we identified 2 SNPs of *IGF2* and 11 for *DRD2*, which were highly correlated with egg laying performance in Muscovy ducks. These molecular markers highly associated with egg production traits can be used in Muscovy duck breeding. It is conducive to the development of the whole industry of Muscovy ducks. However, the functional mechanisms of these SNPs affecting egg production await further investigation.

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345 **Table 1 SNPs identified in the *IGF2* and *DRD2* genes**

No.	Gene	SNPs [1]	Location [2]	Amino acid change
1	*IGF2*	A-1864G	5' regulatory region	No
2	*IGF2*	C-1704G	5' regulatory region	No
3	*IGF2*	A-584G	5' regulatory region	No
4	*IGF2*	A-227G	5' regulatory region	No
5	*IGF2*	A-183G	5' regulatory region	No
6	*DRD2*	C-300G	5' regulatory region	No
7	*DRD2*	A-251T	5' regulatory region	No
8	*DRD2*	T-237G	5' regulatory region	No
9	*DRD2*	A-194G	5' regulatory region	No
10	*DRD2*	A-84G	5' regulatory region	No
11	*DRD2*	C+7T	Exon 1	Yes (P-S) (ccc-tcc)
12	*DRD2*	C+364G	Intron 1	No
13	*DRD2*	A+476T	Intron 1	No

14	*DRD2*	T+830G	Intron 1	No
15	*DRD2*	T+3024C	Intron 1	No
16	*DRD2*	A+3183C	Intron 2	No
17	*DRD2*	A+3262G	Intron 2	No
18	*DRD2*	C+3301G	Intron 2	No
19	*DRD2*	T+3423C	Intron 2	No
20	*DRD2*	T+3428C	Intron 2	No
21	*DRD2*	A+3484T	Intron 2	No
22	*DRD2*	A+3489G	Intron 2	No
23	*DRD2*	C+3545G	Intron 2	No
24	*DRD2*	T+6859G	Intron 5	No
25	*DRD2*	T+6986C	Intron 5	No
26	*DRD2*	T+7099C	Intron 5	No
27	*DRD2*	T+7295C	Intron 5	No
28	*DRD2*	T+7537C	Exon 6	No

29	*DRD2*	C+7654G	3' regulatory region	No
30	*DRD2*	T+8309G	3' regulatory region	No
31	*DRD2*	A+8442G	3' regulatory region	No
32	*DRD2*	T+8585C	3' regulatory region	No
33	*DRD2*	A+8770G	3' regulatory region	No

Notes.

[1] SNPs means single nucleotide polymorphisms, referred to covered regions, the first nucleotide of the translation start codon was designated +1, with the next upstream nucleotide being -1.

[2] 5’ regulatory region = 5’ flanking and untranslated region; 3’ regulatory region = 3’ flanking and untranslated region.

352 **Table 2 Association of 2 SNPs at *IGF2* gene with egg production traits in Muscovy duck**

SNPs[1]	Traits [2]	Least-squares mean±SEM [3]			*P*-value
		AA(n=204)	AG(n=308)	GG(n=172)	
A-1864G	FEA	276.60±1.41[a]	275.67±1.15[a]	276.77±1.54[a]	0.8087
	E59W	75.35±1.92[a]	76.01±1.57[a]	69.18±2.10[b]	0.0251
	E300D	21.43±1.04[a]	21.91±0.85[a]	20.84±1.14[a]	0.7533
		CC(n=158)	CG(n=310)	GG(n=216)	
C-1704G	FEA	276.50±1.61[a]	276.43±1.15[a]	275.72±1.37[a]	0.9067
	E59W	68.92±2.19[b]	75.33±1.56[a]	76.11±1.87[a]	0.0254
	E300D	20.97±1.19[a]	21.18±0.85[a]	22.33±1.01[a]	0.6050

353 **Notes.**

354 Data are summarized as means ±SEM

355 [1]SNPs means single nucleotide polymorphisms, referred to covered regions, the first nucleotide of the

356 translation start codon was designated +1, with the next upstream nucleotide being -1.

357 [2]FEA = first egg age; E59W = egg number at age 59 weeks; E300D = egg number at age 300 days.

358 [3] Values within a row with no common superscript differ significantly ($P < 0.05$) or are highly significant

359 ($P < 0.01$).

361 **Table 3 Association of 11 SNPs at *DRD2* gene with egg production traits in Muscovy duck**

SNPs[1]	Traits [2]	Least-squares mean±SEM [3]			*P*-value
		CC(n=387)	CT(n=237)	TT(n=31)	
C+7T	FEA	272.95±0.90^{c}	276.30±1.15^{b}	295.94±3.17^{a}	<0.0001
	E59W	74.62±1.35^{a}	75.34±1.73^{a}	61.13±4.78^{b}	0.0187
	E300D	22.90±0.71^{a}	21.81± 0.91^{a}	8.42±2.51^{b}	<0.0001
		CC(n=22)	CG(n=239)	GG(n=394)	
C+364G	FEA	297.00±3.80^{a}	275.64±1.15^{b}	273.79±0.90^{b}	<0.0001
	E59W	62.32±5.67^{b}	77.15±1.72^{a}	73.14±1.34^{ab}	0.0193
	E300D	9.86±3.01^{b}	22.37±0.91^{a}	22.16±0.71^{a}	0.0003
		TT(n=130)	TC(n=160)	CC(n=410)	
T+3024C	FEA	276.27±1.75^{a}	275.21±1.58^{a}	277.19±0.98^{a}	0.5547
	E59W	79.02±2.37^{a}	72.81±2.13^{ab}	72.77±1.33^{b}	0.0594
	E300D	21.94±1.30^{a}	21.37±1.17^{a}	20.80±0.73^{a}	0.7271
		CC(n=143)	AC(n=182)	AA(n=375)	

A+3183C	FEA	276.84±1.67[a]	278.16±1.48[a]	275.67±1.03[a]	0.3816
	E59W	69.38±2.25[b]	72.85±2.00[ab]	76.21±1.39[a]	0.0301
	E300D	21.02±1.24[a]	19.98±1.09[a]	21.75±0.76[a]	0.4124
		GG(n=205)	AG(n=269)	AA(n=226)	
A+3262G	FEA	278.86±1.39[a]	276.85±1.21[ab]	274.15±1.32[b]	0.0466
	E59W	69.75±1.88[b]	73.88±1.64[ab]	77.80±1.79[a]	0.0084
	E300D	19.50±1.03[b]	21.06±0.90[ab]	22.72±0.98[a]	0.0766
		GG(n=132)	CG(n=231)	CC(n=337)	
C+3301G	FEA	272.05±1.72[b]	279.36±1.30[a]	276.42±1.08[a]	0.0033
	E59W	80.70±2.34[b]	73.11±1.77[a]	71.86±1.46[a]	0.0052
	E300D	24.77±1.28[a]	19.26±0.96[b]	21.01±0.80[b]	0.0028
		TT(n=135)	TC(n=245)	CC(n=320)	
T+3423C	FEA	277.77±1.71[a]	278.80±1.27[a]	274.35±1.11[b]	0.0226
	E59W	67.96±2.31[b]	73.13±1.72[ab]	77.08±1.50[a]	0.0038
	E300D	20.13±1.27[ab]	19.89±0.94[b]	22.53±0.82[a]	0.0730

		TT(n=47)	TC(n=154)	CC(n=499)	
T+3428C	FEA	270.64±2.90^{b}	275.08±1.60^{ab}	277.58±0.89^{a}	0.0422
	E59W	85.72±3.92^{a}	76.16±2.16^{b}	72.14±1.20^{b}	0.0022
	E300D	25.87±2.15^{a}	22.16±1.19^{ab}	20.38±0.66^{b}	0.0317
		TT(n=145)	AT(n=141)	AA(n=414)	
A+3484T	FEA	272.65±1.65^{b}	278.96±1.67^{a}	277.13±0.97^{a}	0.0183
	E59W	79.86±2.23^{a}	74.19±2.27^{a}	71.78±1.32^{b}	0.0080
	E300D	24.17±1.22^{a}	19.40±1.24^{b}	20.67±0.72^{b}	0.0141
		GG(n=140)	AG(n=218)	AA(n=342)	
A+3489G	FEA	278.66±1.68^{a}	278.69±1.34^{a}	274.35±1.07^{b}	0.0159
	E59W	67.56±2.27^{b}	72.51±1.82^{b}	77.46±1.45^{a}	0.0008
	E300D	19.99±1.24^{ab}	19.56±1.00^{b}	22.61±0.80^{a}	0.0343
		GG(n=198)	CG(n=180)	CC(n=322)	
C+3545G	FEA	272.70±1.41^{b}	276.78±1.47^{a}	278.81±1.10^{a}	0.0029
	E59W	79.74±1.91^{a}	73.11±2.00^{b}	70.84±1.49^{b}	0.0011

E300D	24.26±1.04[a]	21.02±1.09[b]	19.29±0.82[b]	0.0009

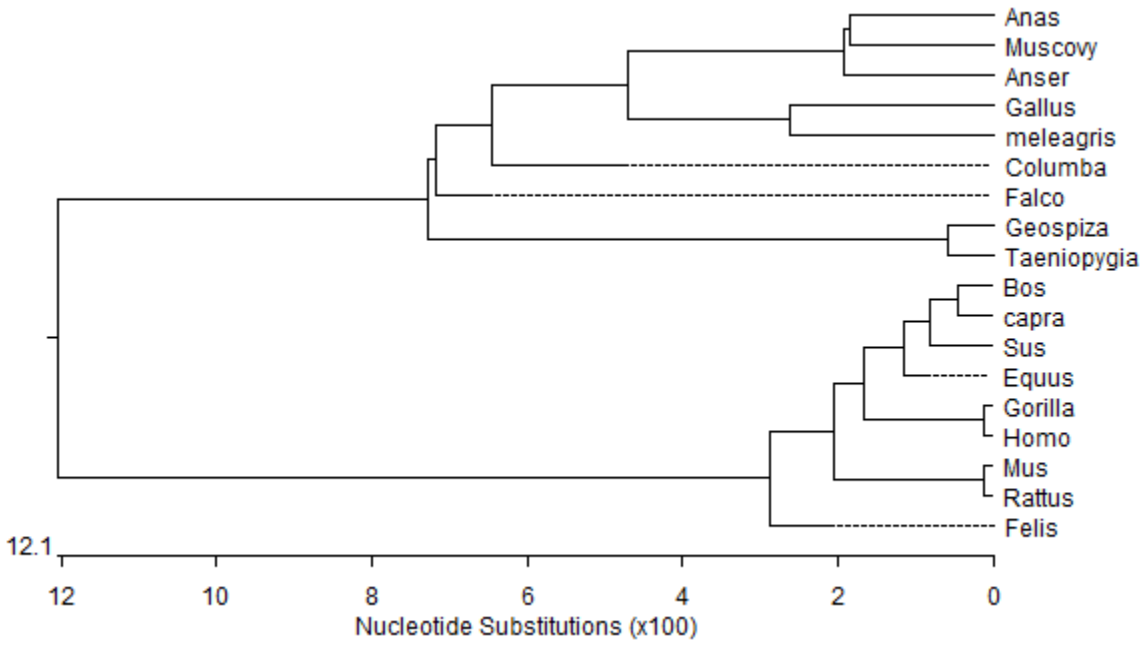
Notes.

Data are summarized as means ±SEM

[1]SNPs means single nucleotide polymorphisms, referred to covered regions, the first nucleotide of the translation start codon was designated +1, with the next upstream nucleotide being -1.

[2]FEA = first egg age; E59W = egg number at age 59 weeks; E300D = egg number at age 300 days.

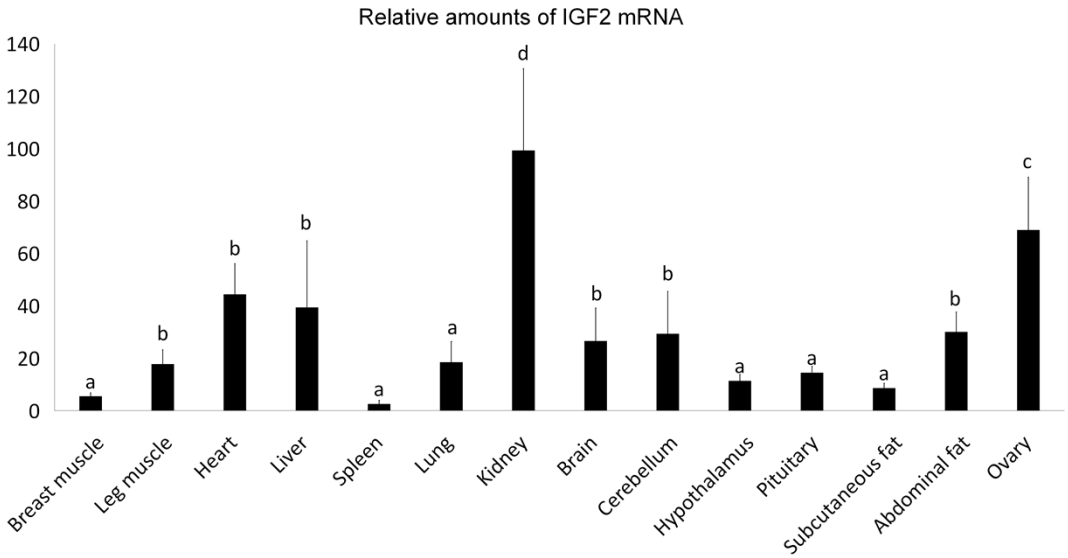
[3] Values within a row with no common superscript differ significantly ($P < 0.05$) or are highly significant ($P < 0.01$).



370

371 **Figure 1 Phylogenetic tree of Muscovy duck *DRD2* aligned amino acid sequences.**

372 **Orthologs were analyzed using Clustal W.**



374

375 **Figure 2 Total mRNA expression of the *IGF2* gene in different tissues of the Muscovy duck.**

376 The value in the Y axis indicated $2^{-\Delta\Delta Ct}$ value.

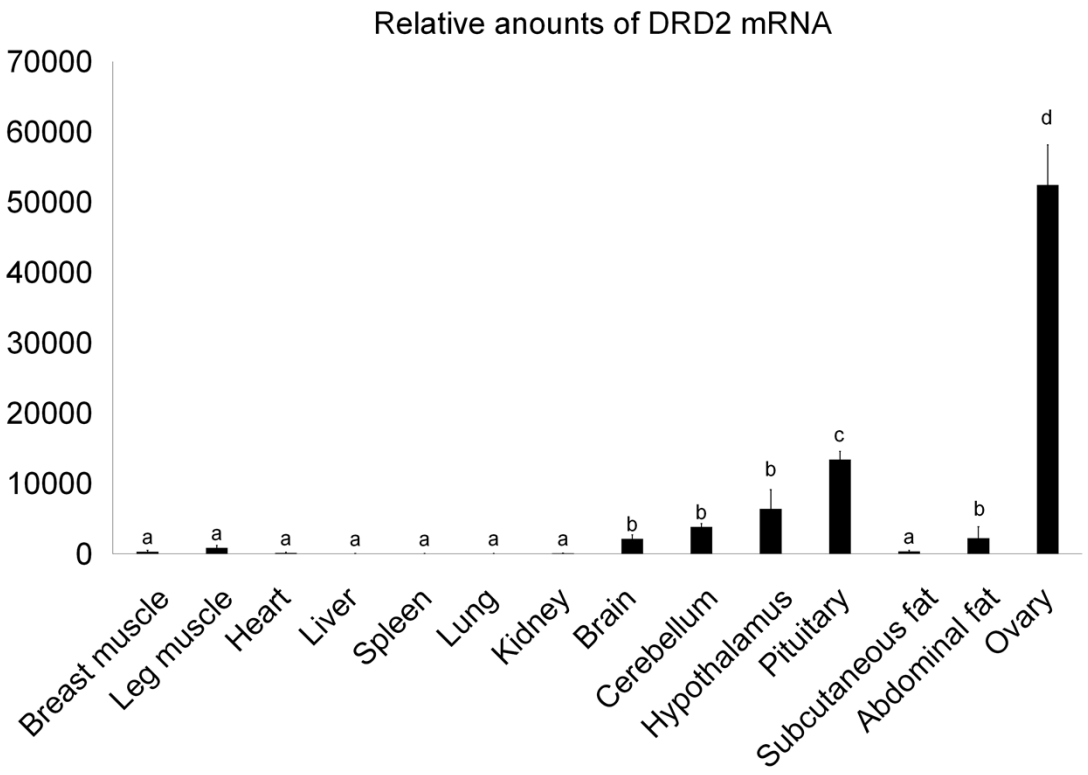
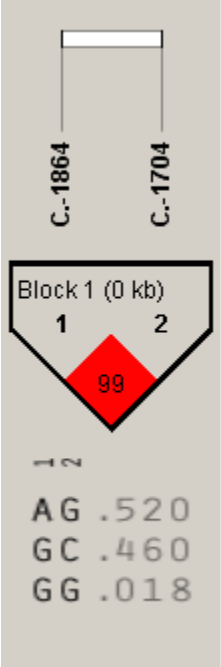


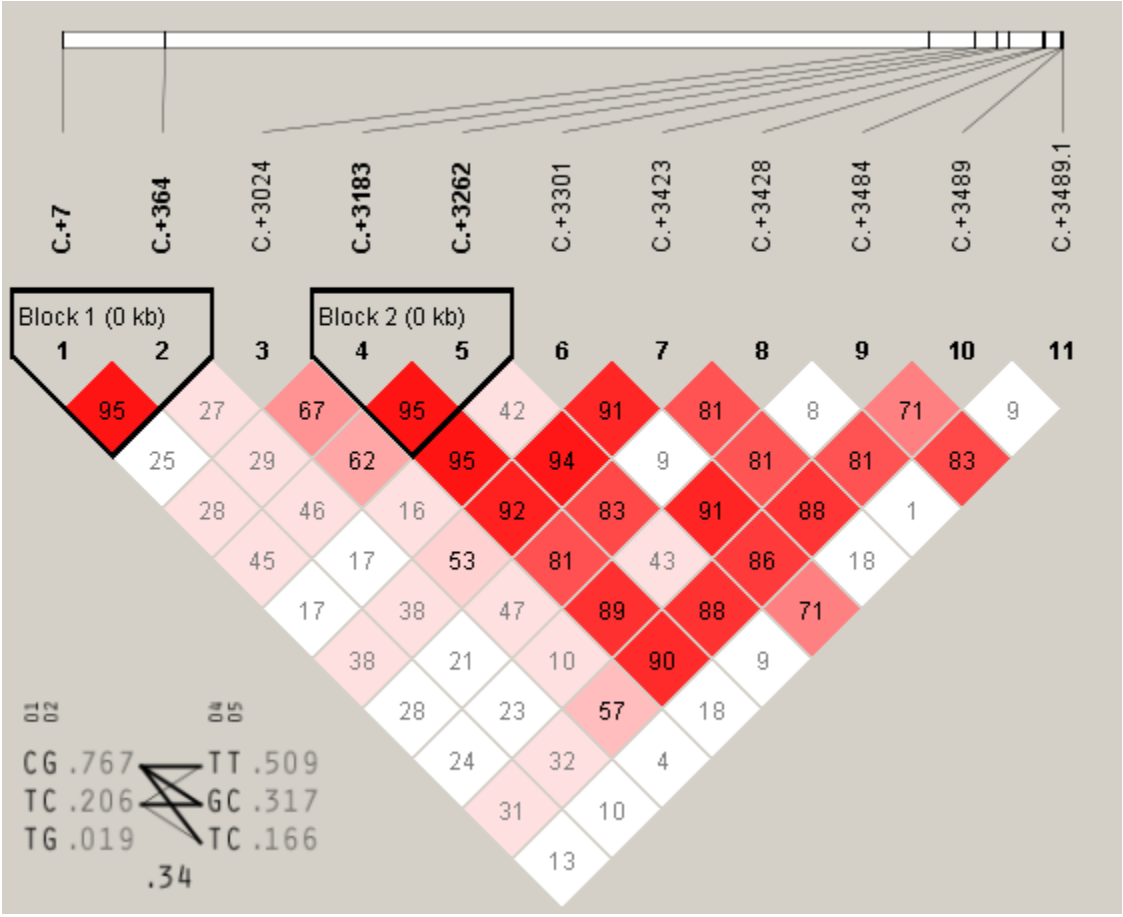
Figure 3 Total mRNA expression of the *DRD2* gene in different tissues of the Muscovy duck. The value in the Y axis indicated $2^{-\Delta\Delta Ct}$ value.



382

383 **Figure 4 The linkage status of 2 identified SNPs in *IGF2* gene.** The color of block indicates

384 the LD status of SNPs; deep red means high linkages between two SNPs.



386

387 **Figure 5 The linkage status of 11 identified SNPs in *DRD2* gene.** The color of block indicates

388 the LD status of SNPs; deep red means high linkages between two SNPs.

389