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.

Keywords Insulin-like growth factor 2, dopamine receptor 2, polymorphisms, association analysis, Muscovy duck, egg production traits

INTRODUCTION

Muscovy ducks are an excellent breed species because of their rapid growth, crude feed tolerance and highly prized meat. Although these ducks are raised on a large scale in China, low production performance affects the economic interests of farmers. Our research focuses on egg

production related molecular markers that can be used to improve egg production for the Muscovy duck. Few researchers pay 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. E300D is the peak time of laying, and E59W is the end time of laying, they cover most of the egg laying period. 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). Dopamine (DA) is an essential neurotransmitter and exists in the nerve center and its peripheral tissue. *DRD2* may assist with the secretion of reproductive hormones (Youngren *et al.*, 1998; Youngren *et al.*, 1996). 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 duck. Therefore, we aim to identify SNPs of these genes, and to reveal their associations with reproduction traits in Muscovy ducks that may help to improve the production performance of Muscovy Duck.

MATERIALS AND METHODS

54 **Ethics Statement**

55 Ethical approval for all animal experiments was granted by the Animal Care Committee of South
56 China Agricultural University (Guangzhou, People's Republic of China) with approval numbe
57 20131019002.

58

59 **Sample Collection**

60 800 white Muscovy ducks were offered by Wens Nanfang Poultry Breeding company (Yunfu,
61 Guangdong, China) which in the same run. All Muscovy ducks were reared under identical
62 conditions of management and feeding. Ducks were maintained outside on the ground from 4 to
63 12 weeks of age, after which they were transferred to individual cages in a semiconfined house.
64 Feed were provided by Wens company. And the first egg age (FEA), egg numbers at 300 days
65 (E300D), and egg numbers at 59 weeks (E59W) were recorded for each female duck. Genomic
66 DNA from each individual at 59 weeks was isolated from 0.5 ml blood stored with EDTA as an
67 anticoagulant, using E.Z.N.A NRBC Blood DNA Kit (Omega, Norcross, GA, USA) according to
68 the manufacturer's instructions.

69

70 **RNA Isolation and cDNA Synthesis**

71 Muscovy duck tissues including pituitary, brain, lung, abdominal fat, liver, ovary, subcutaneous
72 fat, spleen, kidney, leg muscle, hypothalamus, cerebellum, heart and breast muscle used for
73 expression pattern analysis of *IGF2* and *DRD2* genes was sampled at first egg age. Total RNA
74 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 distillation-distillation 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.

Expression Pattern Analysis of IGF2 and DRD2 mRNA

Total mRNA from 14 different tissues was extracted to investigate the mRNA expression profiles of Muscovy duck *IGF2* and *DRD2* genes using real-time qPCR. Muscovy duck *β-actin*

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

SNPs Detection by Sequencing

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

Genotyping and Association Analysis

The SNPs were genotyped in 800 female ducks with egg production records *via* sequencing. We designed 3 primers to Genotyping SNPs of *IGF2* and *DRD2* (Primer IGF2-SNP, DRD2-SNP1

and DRD2-SNP2; Table S1), and PCR reactions were identical to those used in SNP detection as described above. Genotypes were tested for Hardy-Weinberg equilibrium with the chi-square test. Linkage analysis was performed using Haploview software. The associations between SNPs and egg production traits were calculated using the general linear model procedure of SAS v. 9.2 with the following model:

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

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

RESULTS

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

We obtained a 311-bp partial cDNA of the *IGF2* gene that was 98% and 95% identical to *Anas platyrhynchos (*XM_013191560.1) and *Anser cygnoides domesticus* (XM_005019778.2), 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).

144 **Tissue Expression of *IGF2* and *DRD2* Genes**

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

151

152 **Polymorphisms of *IGF2* and *DRD2* Genes**

153 We identified 5 SNPs in the 5'flanking region of *IGF2* at a level of one SNP per 449 bp on
154 average. These SNPs were A-1864G, C-1704G, A-584G, A-227G and A-183G (Table 1). We
155 found 28 SNPs in *DRD2* giving rise to one SNP per 317 bp on average. Among them, the C+7T
156 in exon 1 was a missense mutation resulting in P to S amino acid change (Table 1). We selected
157 2 SNPs of *IGF2* and 11 SNPs of *DRD2* based on Sequencing results for further association
158 analysis.

159

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

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

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

DISCUSSION

Muscovy duck is an excellent poul try, but its egg production is low, it has been plaguing farmers. improving egg production is an effective method for improving the economic benefits of the Muscovy duck. Our study focused on the egg production traits of Muscovy duck, and tried to find the molecular markers related to egg production to provide some theoretical basis for breeding high yield of Muscovy duck. I believe that the relevant personnel of Muscovy duck 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. *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 locate 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 (Irwin *et al.*, 2012). In our study, we found *IGF2* is widely expressed in different tissues with the highest expression found in ovary. This suggest 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 (Lucy, 2011). Previous studies have found that *IGF2* expression in the ovary directly affects the development of dominant follicles (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 in low productivity 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 (Morton *et al.*, 2006). Other studies identified high *DRD2* expression in the regulation of reproductive functions (Nocillado *et al.*, 2007). DRD2 can inhibit the production and secretion of vascular endothelial growth factor protein in 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; 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 cloned the cDNA of *IGF2* and *DRD2* genes and found that they were predominantly expressed in ovary. We identified 5 SNPs of *IGF2* and 28 for *DRD2*. Further analysis showed that SNPs of these two genes were significantly associated with egg production traits in the Muscovy duck. It is conducive to excellent laying performance selection of Muscovy duck in breeding. It is conducive to the development of the whole industry of Muscovy duck. However, the functional mechanisms of these SNPs affecting egg production await further investigation.

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305

307 **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.

314 **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

315 **Notes.**

316 Data are summarized as means ±SEM

317 [1]SNPs means single nucleotide polymorphisms, referred to covered regions, the first nucleotide of the

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

319 [2]FEA = first egg age; E59W = egg number at age 59 weeks; E300D = egg number at age 300 days.

320 [3] Values within a row with no common superscript differ significantly ($P < 0.05$) or are highly significant

321 ($P < 0.01$).

323 **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^{Bb}	276.30±1.15^{Ba}	295.94±3.17^{A}	<0.0001
	E59W	74.62±1.35^{Aa}	75.34±1.73^{Aa}	61.13±4.78^{B}	0.0187
	E300D	22.90±0.71^{Aa}	21.81± 0.91^{Aa}	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^{Bb}	279.36±1.30^{Aa}	276.42±1.08^{a}	0.0033
	E59W	80.70±2.34^{B}	73.11±1.77^{Aa}	71.86±1.46^{Aa}	0.0052
	E300D	24.77±1.28^{Aa}	19.26±0.96^{Bb}	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^{Aa}	274.35±1.11^{Ba}	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^{Aa}	76.16±2.16^{b}	72.14±1.20^{Bb}	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^{Bb}	278.96±1.67^{Aa}	277.13±0.97^{a}	0.0183
	E59W	79.86±2.23^{Aa}	74.19±2.27^{a}	71.78±1.32^{Ba}	0.0080
	E300D	24.17±1.22^{Aa}	19.40±1.24^{Bb}	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^{Bb}	72.51±1.82^{b}	77.46±1.45^{Aa}	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^{Bb}	276.78±1.47^{a}	278.81±1.10^{Aa}	0.0029
	E59W	79.74±1.91^{Aa}	73.11±2.00^{b}	70.84±1.49^{Bb}	0.0011
	E300D	24.26±1.04^{Aa}	21.02±1.09^{b}	19.29±0.82^{Bb}	0.0009

Notes.

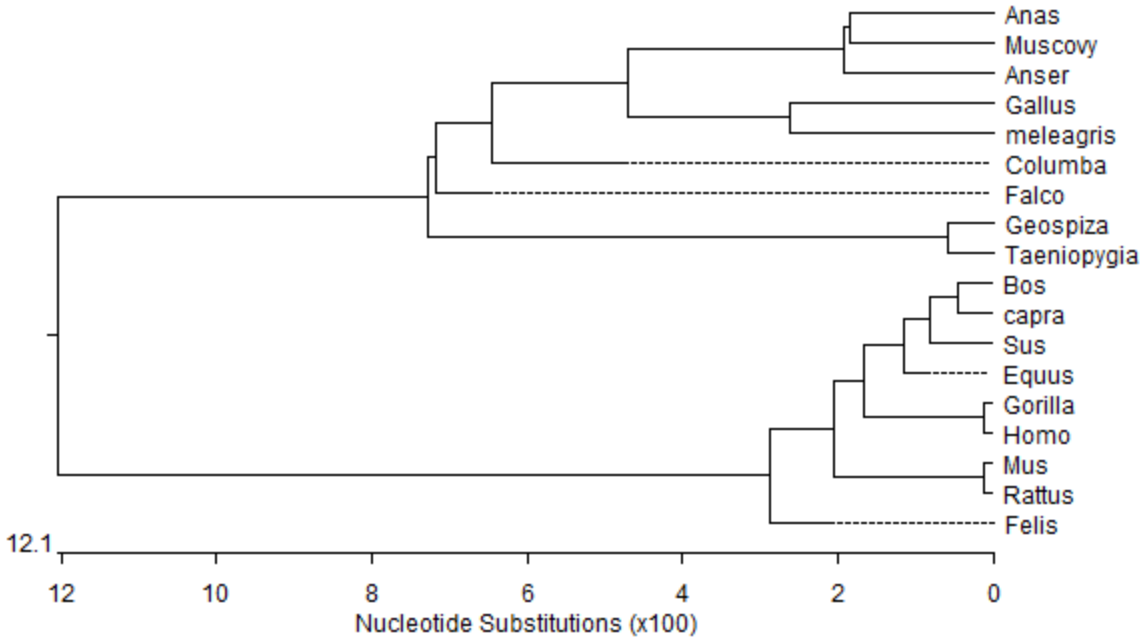
Data are summarized as means ±SEM

[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 ($P < 0.01$).

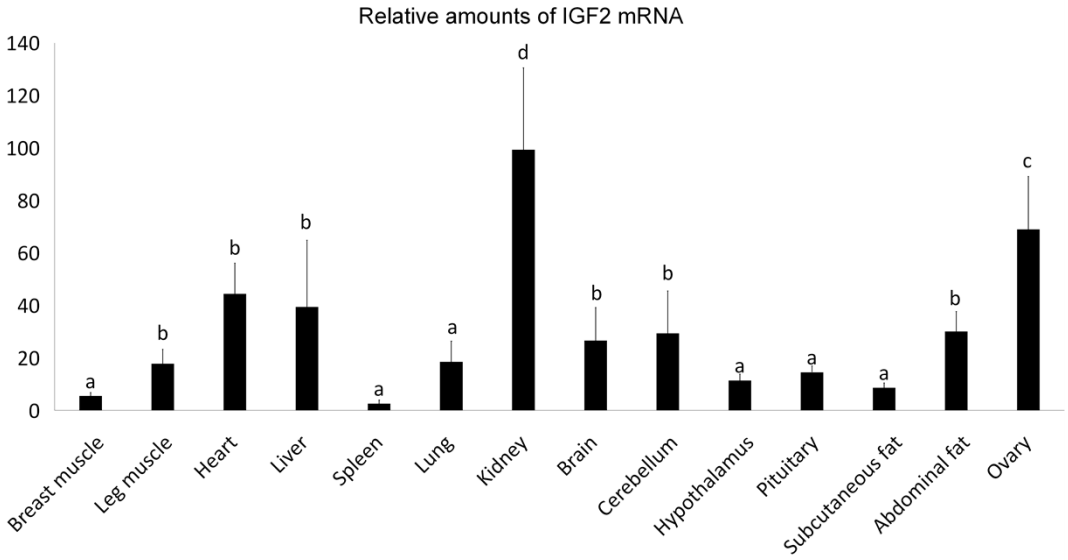
332



333

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

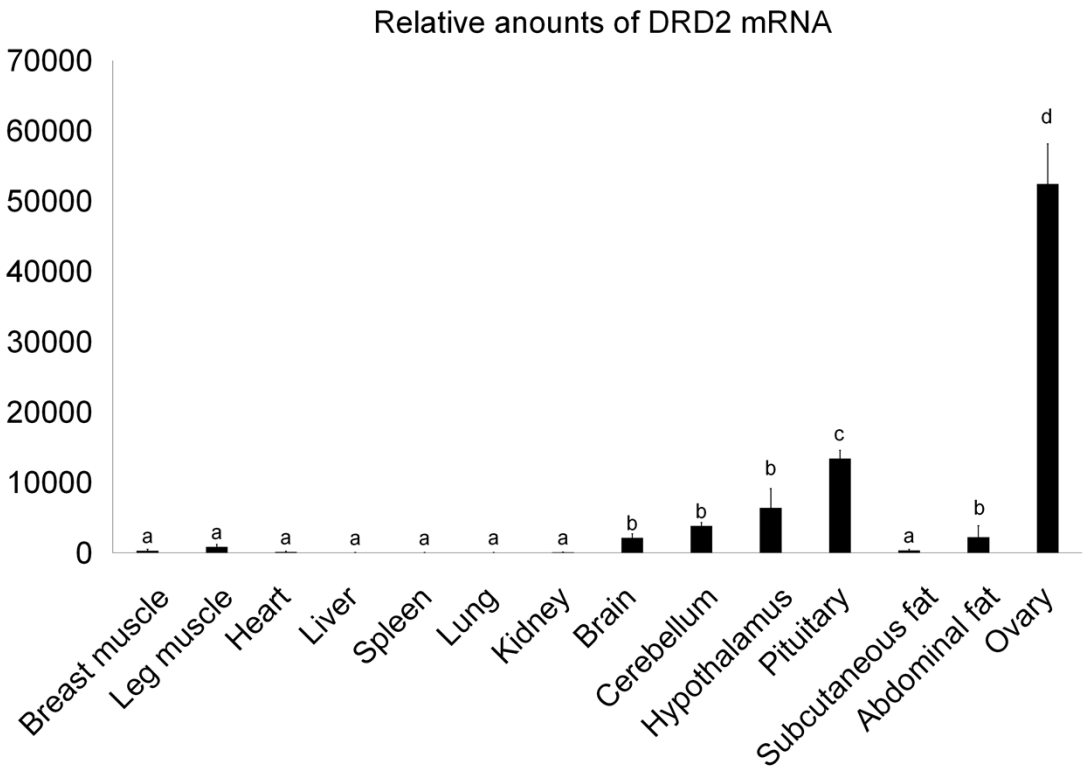
335 **Orthologs were analyzed using Clustal W.**



337

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

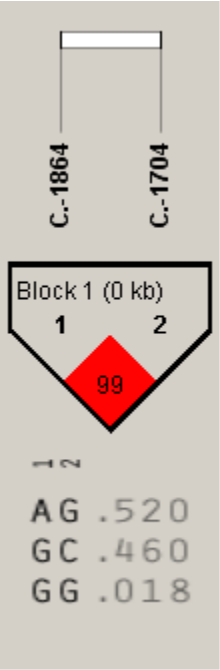
339 The value in the Y axis indicated $2^{-\Delta\Delta Ct}$ value.



341

342 **Figure 3 Total mRNA expression of the *DRD2* gene in different tissues of the Muscovy**

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



345

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

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

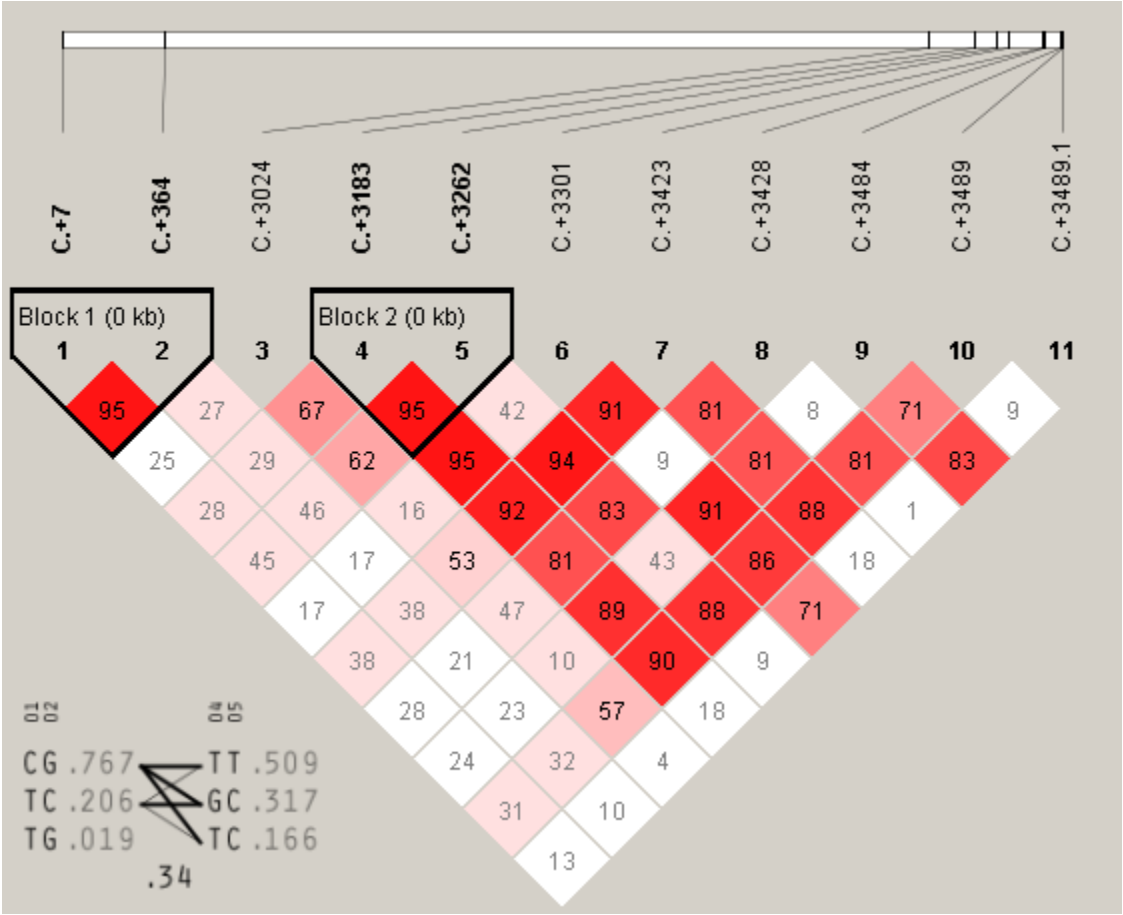


Figure 5 The linkage status of 11 identified SNPs in *DRD2* gene. The color of block indicates the LD status of SNPs; deep red means high linkages between two SNPs.