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Molecular cloning, expression and variation analyses of the dopamine D2 receptor gene in pig breeds in China

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ABSTRACT. The dopamine D2 receptor (DRD2) is a crucial mediator for normal physiological processes. We cloned the pig *DRD2* gene, investigated its distribution in tissues and identified polymorphisms by RT-PCR, quantitative real-time PCR and direct sequencing. Two Yorkshire pigs from Guangdong Academy of Agricultural Sciences (Guangzhou, China) were selected to clone the gene and investigate its expression; 16 individuals from four pig breeds (Yorkshire, Landrace, small-ear spotted, and Xinchang) were used to scan the variations. The two transcripts (*DRD2L* and *DRD2S*), obtained through insertion or deletion of exon 5 and part of 3'UTR, were found to encode 444- and 415-amino acid proteins, respectively. The 574-bp indel in 3'UTR

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comprises five miRNA targeting sites, based on bioinformatics predictions. The pig *DRD2* gene expresses predominantly in the pituitary gland, and then in oviducts and the hypothalamus. Both *DRD2L* and *DRD2S* mRNA were detected in cerebrum, cerebellum, hypothalamus, pituitary gland, back muscle, oviduct, uterus, and testis tissues; *DRD2L* was more abundant than *DRD2S*. The *DRD2* gene is located on chromosome 9 and contains seven exons. Sixty-one different sequences were identified in this gene; among seven in the coding region, only one altered the encoded amino acid. These findings will help us understand the functions of the *DRD2* gene in pigs.

Key words: Pig; *DRD2* gene; Alternative splicing variant; Expression; Polymorphism

INTRODUCTION

As a vital neurotransmitter in the central nervous system and an important hormone in the periphery, dopamine exerts widespread effects on the neuroendocrine secretion, behavioral and physiological functions, such as the control of movement, cognition and emotion (Blasi et al., 2009; Baskerville and Douglas, 2010; Korchounov et al., 2010). Dopamine initiates the biological actions by binding to its receptors on the cell surface. At least 5 dopamine receptor subtypes have been identified so far, including the D1-class (DRD1 and DRD5) and D2-class (DRD2, DRD3 and DRD4) (Missale et al., 1998). All of them are 7-transmembrane domain G protein-coupled receptors (GPCR), but show different ligand-binding characteristics, pharmacological properties and affinities to dopamine. In addition, the second messenger coupling and signal transduction pathways are different between D1- and D2-class receptors (Missale et al., 1998).

DRD2 is a member of Class A GPCR family and possesses a long third intracellular loop with a short C-terminal (Missale et al., 1998; Hearn et al., 2002; Pivonello et al., 2007). It is known that DRD2 can inhibit adenylyl cyclase activity through coupling with Gi protein and subsequently results in a decrease of intracellular cyclic AMP (Obadiah et al., 1999). The *DRD2* gene has been well studied in many species so far, i.e. its cloning and expression in mammals (Bunzow et al., 1988; Grandy et al., 1989; Myeong et al., 2000), turkey (Schnell et al., 1999), bullfrog (Nakano et al., 2010), and fishes (Levavi-Sivan et al., 2005; Pasqualini et al., 2009). Furthermore, different isoforms of *DRD2* have been identified. In humans, alternative splicing generates two isoforms of *DRD2*, D2-short (*DRD2S*) and D2-long (*DRD2L*). They are different with an additional 29 amino acids in the third cytoplasmic loop, a region thought to govern the interaction with different Giα proteins (Dal Toso et al., 1989; Guiramand et al., 1995; Senogles et al., 2004). Moreover, the expression pattern of these two isoforms is different. *DRD2L* is predominately expressed in postsynaptic regions, such as the pituitary gland and the striatum, whereas *DRD2S* is preferentially expressed in presynaptic regions, such as the hypothalamus and the substantia nigra (Sasabe and Ishiura, 2010). It has been well documented that they had distinct functions *in vivo*. DRD2L mainly participates in postsynaptic dopaminergic transmission, whereas DRD2S acts as a presynaptic autoreceptor and inhibits DRD1 receptor-mediated functions (Usiello

et al., 2000; Lindgren et al., 2003).

Ramírez et al. (2009) found that DRD2 might be involved in modulating the boar sperm capacitation and motility. Up to now, there is no report on pig *DRD2* (*pDRD2*) gene. In the present study, RT-PCR was performed to clone *pDRD2* cDNA from pig pituitary and hypothalamus. Then its tissue-specific expression was assessed by quantitative real-time PCR. Finally, polymorphisms were identified in its coding region, 3'UTR and partial introns.

MATERIAL AND METHODS

Animal samples and preparation of cDNA

Two Yorkshire pigs (one male and one female) of 120 d age were obtained from Guangdong Academy of Agricultural Sciences (Guangzhou, China). The tissues of pituitary and hypothalamus were used for cloning *pDRD2* gene. A total of 20 tissues, including cerebrum, cerebellum, pituitary, hypothalamus, liver, spleen, heart, lung, kidney, abdominal fat, foreleg muscle, back leg muscle, back muscle, small intestine, stomach, lymph node, ovary, oviduct, uterus, and testis, were used for quantitative real-time PCR analysis of *pDRD2* gene. All tissue samples were collected immediately after slaughter, frozen in liquid nitrogen, and stored at -80°C until RNA extraction.

Total RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer instructions and then treated with RNAse free-DNAse I (Promega, Madison, WI, USA) to remove any contaminating genomic DNA. First-strand cDNA was synthesized using MMLV SuperScript II reverse transcriptase containing RNase inhibitor (Invitrogen, Carlsbad, CA, USA). The reaction was performed at 42°C for 40 min and then 99°C for 5 min.

A total of 16 individuals from four pig populations (4 from each), Yorkshire pig, Landrace pig, small-ear spotted pig, and Xinchang pig, were selected to scan the variations in the coding region, 3'UTR and partial intron of *pDRD2* gene. Genomic DNA was extracted from ear samples. All animal procedures were handled in compliance with Law of the People's Republic of China on Animal Protection.

Cloning of the pig *DRD2* cDNA

According to the predicted partial cDNA sequences of pig *DRD2* gene (Ensembl ID: ENSSSCT00000016414), three pairs of primers (Table 1) were designed to obtain the full length cDNA. P1 and P2 were designed to amplify partial cDNA containing the start codon and the 3'UTR region containing the stop codon, respectively. P3 was designed to further verify the full-length cDNA sequence. The RT-PCR was performed with an Eppendorf Mastercycler (Eppendorf Limited, Hamburg, Germany). The amplification conditions were as follows: 94°C for 5 min, 30 cycles of 94°C for 30 s, n °C (AT shown in Table 1) for 1 min and 72°C for 1 min, then completed by 72°C for 10 min. The amplified fragment of the predicted size was subcloned into the pMD18-T vector (TaKaRa Biotechnology Co., Ltd., Dalian, China), and then sequenced on automated sequencers ABI 3700 (Invitrogen Biotechnology Co., Ltd., Shanghai, China).

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Table 1. Detail information for primers of the pig *DRD2* gene.

Primer	Primer sequence (5'→3')	Length (bp)	AT (°C)	Application
P1	F: GCCCCGCTGCCCCAATGGATC R: AGGCCTTGCGGAACTCGATGT	1323	68	cDNA amplification
P2	F: CAGCGCCGTGAACCCCATC R: GGGGCTTTGGACGTGACTCTTGT	1173	63	cDNA amplification
P3	F: GCCCCGCTGCCCCAATGGATC R: GGCCACAACCCACGCAGAGGAC	2489	60	cDNA verification
P4	F: TGGCAAAACCCGGACCTCCC R: TGGGGTTCACGGCGCTGTTG	227	61	qPCR
P5	F: CCTGGCTGGCCGGGACCTGA R: GGAGGAGGACGCGGCAGTGG	166	61	qPCR
P6	F: GGCGCAAGCGGGTCAACA R: CTGGGCGGGATGGGGCTGTA	247/160	65	Semiquantitative RT-PCR
E1	F: AGCCCCGGAGCCCTCTGTG R: GCGGGAGGCTGGAGAAGTCAC	672	59	Polymorphism analysis
E2	F: TACCGGGCCCTGACTCTGTGC R:GCGGGGCAGCTTAAGAGGCAAAT	608	59	Polymorphism analysis
E3	F: CCGTTGTGTGCATGTTGTGGAGT R: CACGCCTCACCCATTTCCACTGT	469	59	Polymorphism analysis
E4	F: GGGCTCTCCTCCCCGCAGAC R: GGGCGCCGAGTGCTTTTGTGTTC	461	55	Polymorphism analysis
E5	F: GGCCTCTGTTGTCCTTTGTCCT R: TAATGGGAAGATGGGAGGTGTC	587	59	Polymorphism analysis
E6	F:GGGGGATGGGGAAGAGCTGGGTG R: CCAAACCTCAGTGTCCCCATCT	630	63	Polymorphism analysis
E7	F: GCGGGGCCTGCAGCTGGTGAT R: GGCCACAACCCACGCAGAGGAC	1441	63	Polymorphism analysis

AT = annealing temperature.

Quantitative real-time PCR

The expression patterns of pig *DRD2* gene in each tissue were quantified by quantitative real time PCR using primer P4 (Table 1). Primer P5 was designed to amplify the pig *β-actin* gene (GenBank ID: DQ845171.1), which was used as internal positive control (Table 1). Amplification was run on the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95°C for 3 min; 40 cycles of 95°C for 30 s, 61°C for 30 s and 72°C for 40 s. For each sample, the amplification of *DRD2* gene was run in parallel with the *β-actin* gene. After amplification, dissociation curves were observed to verify the specificity of the assays and one representative product for each gene was sequenced to confirm the correct amplification of fragments. The whole experiment was performed in triplicate. The relative mRNA expression in each tissue was obtained by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). All data were expressed as means ± standard error means.

Semiquantitative RT-PCR analysis of *DRD2L* and *DRD2S* expression

The relative expression of *pDRD2L* and *pDRD2S* was investigated by RT-PCR using P6, which generated a 247- or 160-bp fragment. The PCR reaction program is described above. The amplified fragments were separated by non-denatured polyacrylamide gel (16%, w/v) in 1X Tris-boric acid-EDTA buffer (pH = 8.0). Gels were stained with silver nitrate. Tissues with high or moderate mRNA levels of *DRD2* (*DRD2L* and *DRD2S*), including cere-

brum, cerebellum, hypothalamus, pituitary, back leg muscle, back muscle, oviduct, uterus, and testis, were used to compare *DRD2L* and *DRD2S* expression.

Genomic structure and polymorphism analysis of the pig *DRD2* gene

The obtained cDNA sequences of the *DRD2* gene were used in the draft pig genomic sequence database (http://genome.ucsc.edu/cgi-bin/hgBlat, 2009.9) to reveal its genomic structure. According to genomic sequences, 7 pairs of primers (E1 to E7, shown in Table 1) were synthesized to identify polymorphisms of this gene in the coding region. PCR products amplified from the 16 pigs were sent to sequence directly by the Invitrogen Biotechnology Co. Ltd (Shanghai, China). Forward and reverse reactions were both carried out to avoid false positives. Then all sequences were used to identify the variations of pig *DRD2* gene.

Data analyses

The prediction of miRNA binding sites for the 574-bp deletion in the *pDRD2S* 3'UTR was performed by RNAhybrid2.2 (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) and filtered with RNA22 (http://cbcsrv.watson.ibm.com/rna22_targets.html) (Kruger and Rehmsmeier, 2006). Transmembrane helices prediction was performed with TMHMM 2.0 (http://genome.cbs.dtu.dk/services/TMHMM-2.0). The amino acid sequences of DRD2 in other species used for the homology analysis were retrieved from the GenBank® database. The GenBank® IDs for humans, mouse, cattle, dog, turkey and chicken are NP_000786.1, NP_034207.2, NP_776468.1, NP_001003110.1, AAD03818.1, and ABY28377.1, respectively. Amino acid sequence alignments and homology analysis were accomplished by ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

RESULTS

The full-length cDNA of the pig *DRD2* gene

The cDNA sequence of the pig *DRD2* gene was deduced from two independent clones and the full sequence was further verified by the clone from primer P3. Two different transcripts, *pDRD2L* and *pDRD2S*, were identified and the obtained sequences had been submitted to NCBI database (GenBank® ID: JF412702 and JF412703). The obtained *pDRD2L* and *pDRD2S* are 2489 and 1828 bp long, produced by the presence or absence of an 87-bp fragment in exon 5 and a 574-bp fragment in the 3'UTR. *pDRD2L* contains a 1332-bp open reading frame (ORF) flanked by a 14-bp 5'UTR (incomplete) and a 1,143-bp 3'UTR, whereas *pDRD2S* contains a 14-bp 5'UTR, a 1245-bp ORF, and a 569-bp 3'UTR (Figure 1). *pDRD2L* and *pDRD2S* encode a 444- and 415-amino acid protein with an expected molecular mass of 50.7 and 47.4 kDa, respectively. Protein sequence analysis suggests that pDRD2 contains seven putative transmembrane domains (TMs). The sequences within TM regions are highly conserved among species, whereas the third intracellular loop is the most variable region (Supplementary Figure 1). pDRD2 shows high identity to its counterpart in humans (97.1%), mouse (96.4%), cattle (98.2%), dog (98.0%), turkey (79.1%) and chicken (80.1%).

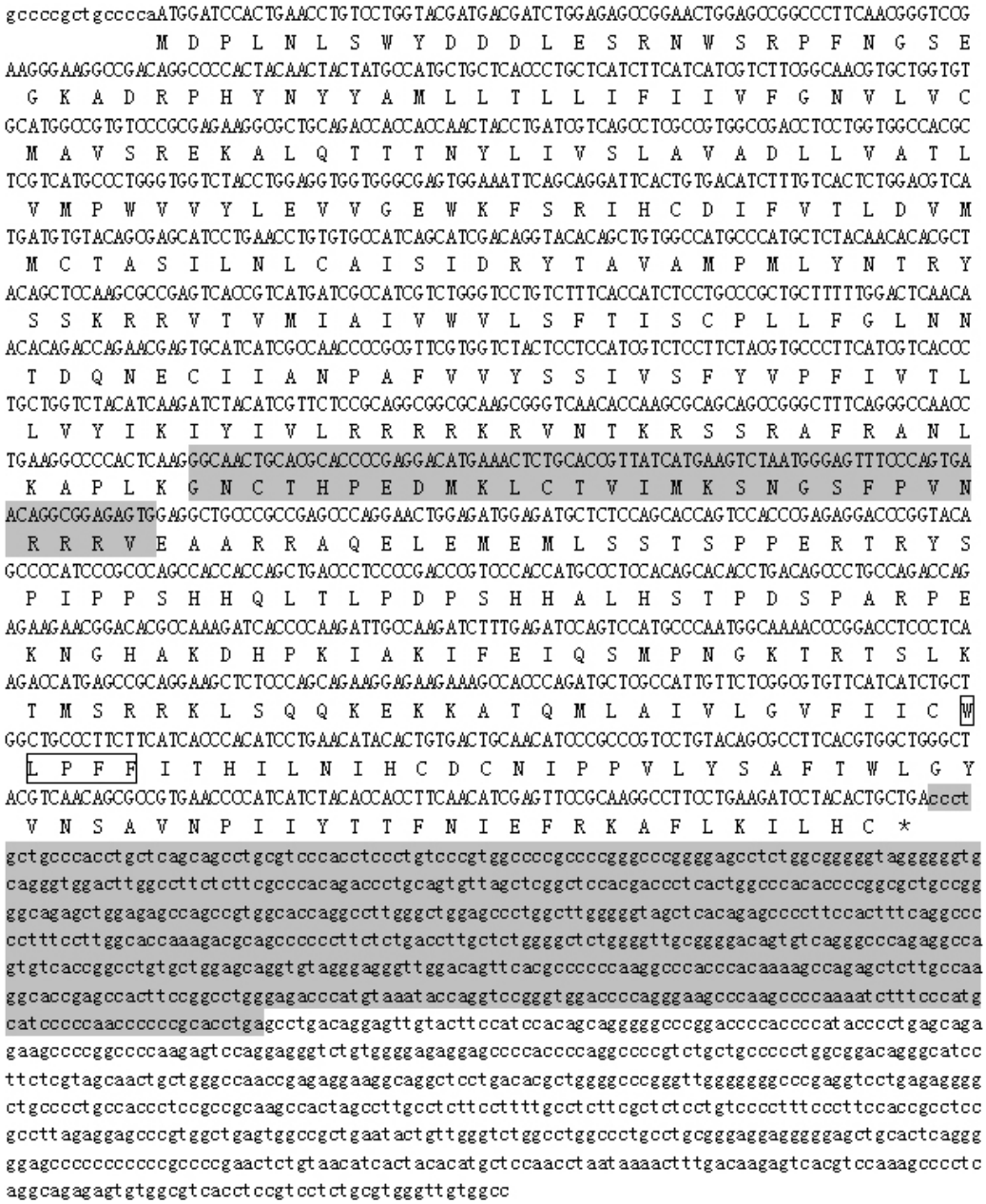


Figure 1. cDNA and its deduced amino acid sequence of pig *DRD2* gene. Nucleotide sequences in capital and lowercase characters refer to open reading frame (ORF) and UTR sequences, respectively. Capital letters below ORF show amino acids for each codon. The stop codon is indicated with an asterisk. Nucleotides in shadow indicate an 87-bp deletion in coding region and a 574-bp deletion in 3'UTR of *pDRD2S* compared to *pDRD2L*. The "WLPFF" in rectangle box indicates the conserved WXXFF motif on TM VI.

Changes of miRNA binding sites by the 574-bp deletion in the 3'UTR

Online prediction showed that five possible binding sites for five miRNAs were observed in the 574-bp segment, which was deleted in the 3'UTR of the pig *DRD2S* gene. These miRNAs are ssc-miR-744, ssc-miR-339-5p, ssc-miR-1307, ssc-miR-1271, and ssc-miR-328.

Tissue-specific expression of the pig *DRD2* gene

The pig *DRD2* gene is expressed at different level in different tissues (Figure 2). The pituitary tissue has the highest mRNA level, which is about 3.8 times higher than oviduct. Moderate expression levels of *DRD2* mRNA were observed in hypothalamus, uterus, back leg muscle, cerebellum, as well as in back muscle. Low or trace quantities were found in other tissues such as cerebrum, heart, liver, spleen, lung, kidney, abdominal fat, foreleg muscle, small intestine, stomach, lymph node, ovary, and testis. In the pituitary gland and foreleg muscle, the mRNA level in male is relatively lower than female (Supplementary Figure 2).

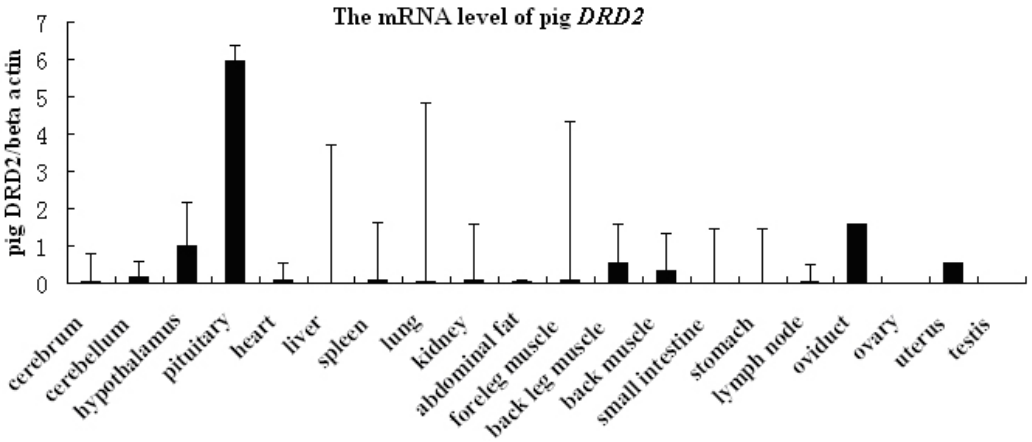


Figure 2. The expression pattern of pig *DRD2* gene in different tissues. The horizontal axis and vertical axis indicate different tissues and $2^{-\Delta\Delta Ct}$ value (mean ± SE), respectively. Each sample was repeated three times.

Differential expression of *DRD2L* and *DRD2S* in pig tissues

The mRNA of both *DRD2L* and *DRD2S* were detected in most of pig tissues that we examined, including cerebrum, cerebellum, hypothalamus, pituitary, back leg muscle, back muscle, oviduct, uterus, and testis. The expression of *DRD2L* is more abundant than that of *DRD2S*, even though the ratio between the long and short isoforms seems to be different among different tissues (Figure 3). *DRD2L* exhibited high or moderate mRNA levels in all tissues examined. *DRD2S* was expressed mainly in cerebrum, cerebellum, hypothalamus, pituitary, and uterus followed by oviduct and testis. In female, *DRD2S* was not detected in back leg muscle (Figure 3).

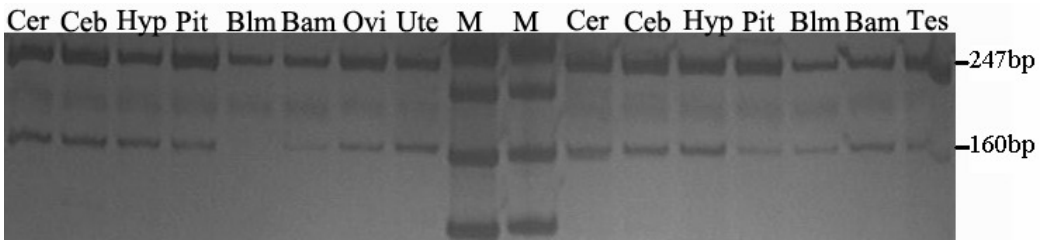
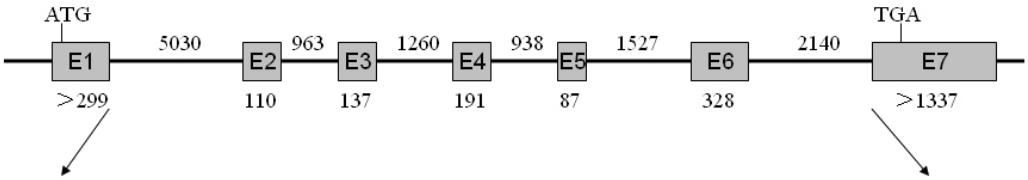


Figure 3. The differential expression of pig *DRD2L* and *DRD2S* in porcine tissues of female (left) and male (right). Cer = cerebrum; Ceb = cerebellum; Hyp = hypothalamus; Pit = pituitary; Blm = back leg muscle; Bam = back muscle; Ovi = oviduct; Ute = uterus; Tes = testis; M = marker (50 bp lander). The eight lanes on the left of markers shows RT-PCR profiles of female, and the seven lanes on the right of markers indicates profiles of male.

Genomic characterization of the pig *DRD2* gene

Alignment showed that pig *DRD2* gene was located at 40,051,792 - 40,066,192 nt of chromosome 9 and spanned approximately 14,400 bp. It contains 7 exons with nucleotide sizes of 318, 110, 137, 191, 87, 328, and 194 bp, respectively. The longest intron is intron 1 (5030 bp) and the shortest one is intron 4 (938 bp). All the consensus sequences of the splice donor and acceptor follow the "GT-AG" rule (Figure 4).



Introns	Span (nt)	Size (bp)	Boundary
1	40052161~40057190	5030	ACCTGGAG**gt**aggtct...gtcccc**ag**GTGGTGGG
2	40057301~40058263	963	ATCGACAG**gt**gagctc...ccttgc**ag**GTACACAG
3	40058401~40059660	1260	CAACACAG**gt**gagtcc...ccccgc**ag**ACCAGAAC
4	40059852~40060789	938	CACTCAAG**gt**cccaag...ctccac**ag**GGCAACTG
5	40060877~40062403	1527	GGAGAGTG**gt**aagtgc...ctttgc**ag**GAGGCTGC
6	40062732~40064871	2140	TGTTCTCG**gt**gagtgg...tcccccc**ag**GCGTGTTC

Figure 4. The genomic organization of pig *DRD2* gene. The gray boxes indicate exons and lines represent introns or flanking regions. Numbers below box show the nucleotide size (bp) for exons, whereas numbers above the line indicate the nucleotide size for introns. The genomic organization is based on the pig genomic sequences released in Nov, 2009 (http://genome.ucsc.edu/). Boundary nucleotides of exons and introns are shown with capital and lowercase characters each. The "gt" and "ag" in bold indicate that the consensus sequences of introns obey the "GT-AG" rule.

Polymorphisms of the pig *DRD2* gene

Fifty-one SNPs, 9 indel variations and a C microsatellite were identified in 4868 bp sequence of the pig *DRD2* gene covering the entire coding region, 3'UTR region, and part of intron region (Table 2). The polymorphism density is one SNP per 80 bp. In these 61 variations, 7 SNPs are located in the coding region and 14 variations in the 3'UTR. The average

density for the coding region and the 3'UTR is one SNP per 190 bp and one per 82 bp, respectively. Among the 7 SNPs in the coding region, only one SNP (T40064902A, Phe390Tyr) is a non-synonymous mutation which is located in transmembrane domain VI (Table 3). Three synonymous polymorphisms, G40052148A, C40057247T, and G40058375A, occur in transmembrane domain II, III, and IV, respectively. The other three variations, C40057199T, G40060801A, and T40062532C, are present in the extracellular domain or the third intracellular loop of *DRD2*.

Table 2. Polymorphisms detected in pig *DRD2* gene.

No.	Variation	Region	No.	Variation	Region	No.	Variation	Region
1	G40052148A	Exon 1	21	T40058497G	Intron3	41	C40061085A	Intron5
2	A40052177G	Intron1	22	T40058519C	Intron3	42	C40061145T	Intron5
3	A40052186G	Intron1	23	G40058595T	Intron3	43	40062398-99TT del	Intron5
4	T40052212A	Intron1	24	C40058596T	Intron3	44	T40062532C	Exon6
5	G40052338T	Intron1	25	T40059861C	Intron4	45	G40062894-	Intron6
6	C40052350-	Intron1	26	T40059905C	Intron4	46	T40064902A	Exon 7
7	40056849C indel	Intron1	27	G40059906A	Intron4	47	C40065074T	3'UTR
8	A40056928G	Intron1	28	C40059915G	Intron4	48	C40065145T	3'UTR
9	A40057006C	Intron1	29	C40059957T	Intron4	49	G40065273A	3'UTR
10	G40057088A	Intron1	30	C40059958G	Intron4	50	A40065426G	3'UTR
11	T40057112C	Intron1	31	C40060017A	Intron4	51	C40065503G	3'UTR
12	40057118G insert	Intron1	32	A40060038G	Intron4	52	A40065504G	3'UTR
13	C40057199T	Exon2	33	G40060055A	Intron4	53	G40065533A	3'UTR
14	C40057247T	Exon2	34	40060067T insert	Intron4	54	C40065685A	3'UTR
15	G40058375A	Exon3	35	G40060659A	Intron4	55	A40065732-	3'UTR
16	C40058415T	Intron3	36	C40060698T	Intron4	56	C40065764T	3'UTR
17	G40058416A	Intron3	37	T40060748C	Intron4	57	G40065768C	3'UTR
18	G 40058441A	Intron3	38	G40060801A	Exon5	58	C40065780-	3'UTR
19	G40058442T	Intron3	39	C40060927T	Intron5	59	C40065857T	3'UTR
20	A40058481T	Intron3	40	C40060985-	Intron5	60	C40066044T	3'UTR

The variations are based on chromosome 9 of the pig genomic sequences released in November 2009 (http://genome.ucsc.edu/).

Table 3. Detail information for SNPs in the coding region of pig *DRD2* gene.

SNP	Region	AA Variation	Location
G40052148A	Exon1	Val91Val	Transmembrane domain II
C40057199T	Exon 2	Gly98Gly	The second extracellular loop
C40057247T	Exon 2	Asp114Asp	Transmembrane domain III
G40058375A	Exon 3	Pro169Pro	Transmembrane domain IV
G40060801A	Exon 5	Thr245Thr	The third intracellular loop
T40062532C	Exon 6	His313His	The third intracellular loop
T40064902A	Exon 7	Phe390Tyr	Transmembrane domain VI

The sites were based on chromosome 9 of the pig genomic sequences released in November 2009 (http://genome.ucsc.edu/).

DISCUSSION

The *DRD2* gene is generally alternative spliced in mammals. Two distinct DRD2-encoding mRNA, *DRD2L* and *DRD2S*, have been characterized in many mammals (Montmayeur et al., 1991; Myeong et al., 2000). Moreover, a novel transcript ($DRD2_{Longer}$) of this gene, with two extra codons in exon 6 than *DRD2L*, was discovered from the human brain (Seeman

et al., 2000). Although $DRD2_{Longer}$ was not found in this study, two other variants, *DRD2L* and *DRD2S*, were characterized in pig. The short isoform (*pDRD2S*) was generated due to an 87-bp deletion in exon 5 and a 574-bp deletion in 3'UTR caused by alternative splicing, which resulted in a 29-amino acid absence in the third intracellular loop. This might lead to DRD2S and DRD2L coupling with distinct subtypes of Giα proteins (Senogles et al., 2004). Moreover, the change of possible miRNA binding sites targeting the deleted 574 bp suggests that this segment might affect gene expression at posttranslational and/or posttranscriptional level, which still needs further investigation. The sequence alignment showed that pDRD2 shared the basic structural features with that of other species, possessing an N-terminal extracellular domain, 7 TMs, a large third intracellular loop and a short C-terminal intracellular domain (Pivonello et al., 2007). In humans, rat and mouse, the *DRD2* gene contains 8 exons, and exon 1 is far away from exon 2 (O'Malley et al., 1990; Mack et al., 1991; Taylor et al., 2006) (Figure 5). However, only 7 exons were found in pig, which was similar to that of cattle and dog (Myeong et al., 2000; Zimin et al., 2009). Whether there is an extra exon in the upstream of pig *DRD2* gene still requires further study.

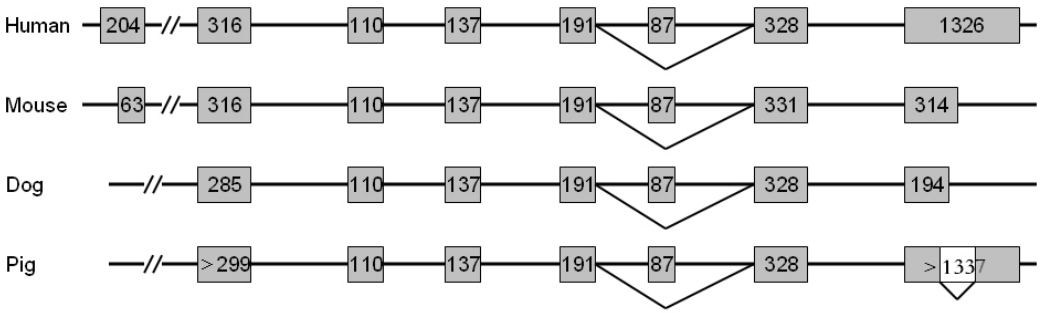


Figure 5. The genomic organization and alternative splicing of *DRD2* gene among humans, mouse, dog and pig. Boxes in gray indicated exons and numbers inside represented the nucleotide sizes (bp) for each exon. "-//-" indicated that there existed partial unknown sequences according to the UCSC BLAT search (http://genome.ucsc.edu/cgi-bin/hgBlat). Alternative splicing, which generated two isoforms with an 87-bp difference, occurred in the same position of *DRD2* gene among humans, mouse and dog. However, in the pig *DRD2* gene, alternative splicing also occurred in the 3'UTR and an additional 574-bp deletion appeared in the short isoform.

In mammals such as humans and mouse, *DRD2* expresses high mRNA level in pituitary and low level in cortex (Montmayeur et al., 1991; Neve et al., 1991). Meanwhile, the canine *DRD2* showed the moderate expression in thalamus and negligible in cerebral cortex (Myeong et al., 2000). In accordance, the pig *DRD2* gene predominantly expresses in pituitary and lowly in the cerebrum. The fact that the *DRD2* gene preferentially expresses in pituitary or hypothalamus tissues in most species is probably related to its important function as a mediator for dopamine. Previous studies in humans have demonstrated that *DRD2L* was preferentially expressed in postsynaptic regions such as pituitary, whereas *DRD2S* preferred to express in presynaptic regions such as hypothalamus (Usiello et al., 2000; Sasabe and Ishiura, 2010). However, in this study, no obvious difference for both *pDRD2L* and *pDRD2S* expression was found between hypothalamus and pituitary tissues. In turkey, even though it is surprising that

the *DRD2S* could not be detected in any of the peripheral tissues, the fact that both *DRD2L* and *DRD2S* are widely distributed in cerebellum, hypothalamus, and pituitary tissues, is consistent with our results (Schnell et al., 1999).

In this study, abundant polymorphisms were observed in pig *DRD2* gene. Wiedmann et al. (2008) reported that the SNP density in pigs was higher than 1:370 bp. Here the SNP frequency of the *pDRD2* gene is one per 80 bp, similar to previous results before correction for sample size (Jungerius et al., 2005). Structure analysis of the human DRD2 showed that, Asp114 in TM III and Phe390 in TM VI were essential for its binding to dopamine (Shi and Javitch, 2002; Kalani et al., 2004). In this study, the mutation Phe390Tyr might have altered the affinity of pig DRD2 to its ligand. Duan et al. (2003) found that synonymous variations in the human *DRD2* can affect mRNA stability and synthesis of the receptor. Although the other 6 mutations in the coding region of *pDRD2* were synonymous SNPs, they might have potential effects on regulating gene expression via the changes of mRNA stability. On the other hand, some intronic SNPs of *DRD2* could modify mRNA expression and splicing and were related with diseases or traits (Zhang et al., 2007; Moyer et al., 2011). In this study, we also observed many mutations in the intron region of the *pDRD2* gene. As a whole, the real effects of all these polymorphisms identified by this study still require further investigation.

In conclusion, the full length cDNA and two variant transcripts of the pig *DRD2* gene were obtained, and it comprised 7 exons and 6 introns. The *pDRD2* gene was predominantly expressed in pituitary tissue and then in oviduct. Both *DRD2L* and *DRD2S* mRNA were detected in cerebrum, cerebellum, hypothalamus, pituitary, back muscle, oviduct, uterus and testis, and among them, *DRD2L* was more abundant than *DRD2S*. In addition, a total of 61 variations were found.

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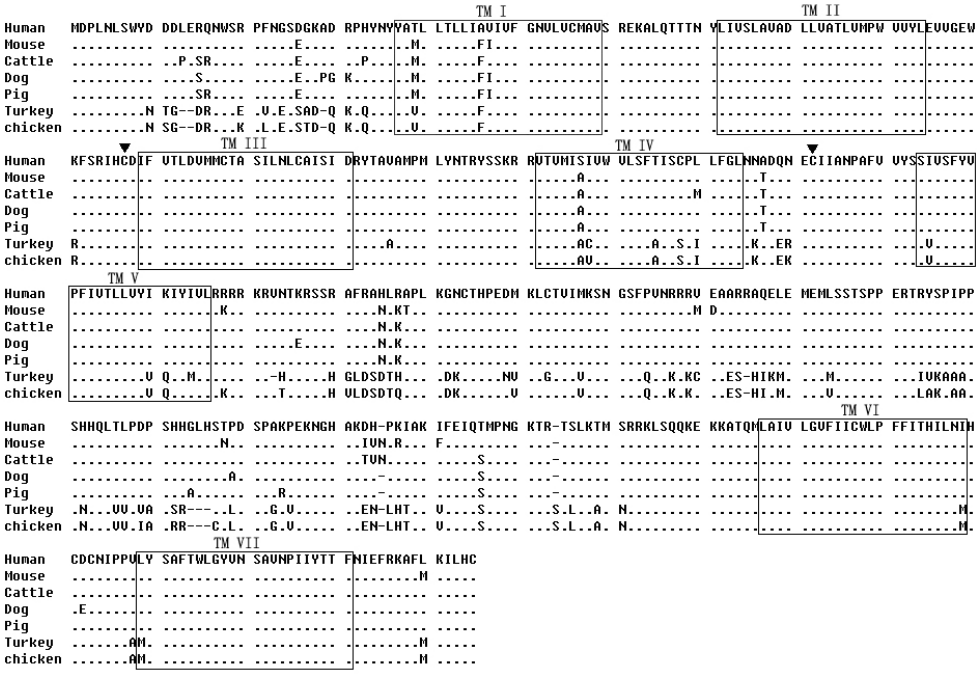
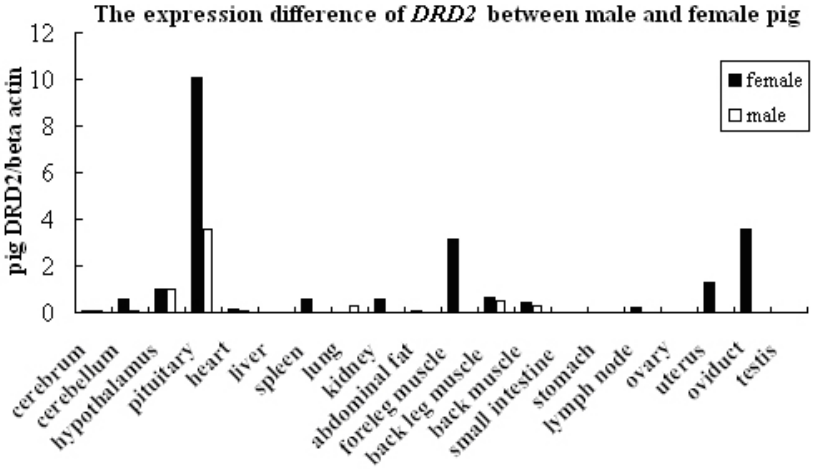
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. The sequence alignment of the DRD2 protein among 7 different species. The alignment was computed by ClustalW. The DRD2 protein sequences of other species were derived from the NCBI GenBank and the accession numbers are as follows: Human (Homo sapiens, NP_000786.1), Mouse (Mus musculus, NP_034207.2), Cattle (Bos taurus, NP_776468.1), Dog (Canis lupus familiaris, NP_001003110.1), Turkey (Meleagris gallopavo, AAD03818.1), chicken (Gallus gallus, ABY28377.1). The seven conserved transmembrane domains (TMs) were numbered and boxed. Sequence between TM V and TM VI is the third intracellular loop. The two cysteine residues for disulphide bond formation were labeled in triangles.



Supplementary Figure 2. The mRNA difference of *DRD2* between male and female pig in each tissue. The horizontal axis and vertical axis indicated different tissues and $2^{-\Delta\Delta Ct}$value, respectively.