ความสัมพันธ์ระหว่าง SNP ของยีน VLDL/VTG receptor กับลักษณะการให้ผลผลิตไข่และการเจริญเติบโตของไก่สี่สายพันธุ์

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISM IN VLDL/VTG RECEPTOR GENE POLYMORPHISM WITH EGG PRODUCTION AND GROWTH PERFORMANCE TRAITS IN FOUR CHICKENS BREEDS

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บทคัดย่อ

ยีนความหนาแน่นต่ำมากไลโพโปรตีนและไวเทลโลจีนินรีเซฟเตอร์ (Very Low Density Lipoprotein/Vitellogenin; VLDL/VTG receptor) เป็นยีนที่มีความสำคัญมากในการให้ผลผลิตไข่ของไก่ งานวิจัยนี้ศึกษาความหลากหลายของยีน VLDL/VTG รีเซฟเตอร์ ในไก่ทั้งหมด 4 สายพันธุ์ ซึ่งประกอบด้วย ไก่พันธุ์โร๊ดไอแลนด์เรด (Rhode Island Red; RR), ไก่พันธุ์เล็กฮอน์ขาว (Single-Comb White Leghorn; WL), ไก่ไข่สายพันธุ์ทางการค้า (HyLine brown; HL) และ ไก่ต่อ โดยใช้เทคนิคพอลิเมอร์เรสเชนรีแอกชั่น (Polymerase Chain Reaction) เพิ่มปริมาณยีน VLDL/VTG receptor และทำการออกแบบไพร์เมอร์ที่มีความจำเพาะต่อยีนดังกล่าว เป็นบริเวณทั้งสิ้น 1 ตำแหน่ง ในส่วนที่เรียกว่า 5´ flanking region ของยีน VLDL/VTG receptor ที่บริเวณตำแหน่ง -1351 ถึง-1599 จากการวิเคราะห์บริเวณ 5´flanking region ของยีน VLDL/VTG receptor พบว่ามี SNPs คือ A-1393G ผลการตรวจสอบความสัมพันธ์ระหว่าง SNPs กับลักษณะการให้ผลผลิตไข่ และการเติบโตของไก่ พบว่า น้ำหนักตัว (BW) น้ำหนักไข่ฟองแรก (EW) ค่าสี b ที่วัดได้จากเปลือกไข่ ใน genotype AAมีค่าสูงกว่า GG อย่างมีนัยสำคัญทางสถิติ (p<0.05) ในขณะที่ ค่าสี (ESC) และค่าสี L ของเปลือกไข่ ใน genotype AA มีค่าน้อยกว่า GG อย่างมีนัยสำคัญทางสถิติ (p<0.05) นอกจากนี้ยังพบความสัมพันธ์เชิงบวกระหว่าง BW และ EW (0.712) BW และ ค่าสี a (0.435) และ EW และ ค่าสี a (0.642) ในขณะที่ ค่าสี a กับ ค่าสี L ที่วัดได้จากเปลือกไข่ ESC และ BW, ESC และ EW มีความสัมพันธ์เชิงลบ โดยมีค่าความสัมพันธ์เป็น -0.833, -0.320 และ -0.545 ตามลำดับ จึงกล่าวได้ว่ายีน VLDL/VTG receptor gene อาจเป็น candidate gene ที่สำคัญที่มีความสัมพันธ์กับลักษณะการให้ผลผลิตไข่และการเติบโตของไก่

คำสำคัญ: ไก่ ยีนความหนาแน่นต่ำมากไลโพโปรตีน/ไวเทลโลจิลิน รีเซฟเตอร์ ซิงเกิลนิวคลีโอไทด์โพลี มอร์ฟิซึม

Abstract

Very Low Density Lipoprotein/Vitellogenin (VLDL/VTG)receptor gene plays crucial roles in laying hen production.In this study, we investigated genetic polymorphism of the VLDL/VTG receptor gene in four chicken breeds:-Rhode Island Red (RR), Single-Comb White Leghorn (WL), Commercial Breeds (HyLine brown chicken, HL) and Hybrid Red Jungle Fowl (Kai-Tor). PCR technique was used to amplify VLDL/VTG receptor gene with three specific primers that were designed to complement the unique 5'flanking region of VLDL/VTG receptor gene at location of -1351to -1599.One SNPs (A-1393G) was identified in the 5′flanking region of VLDL/VTG receptor gene. The association of these SNPs with egg traits and growth performance were examined.The body weight (BW), egg weight at first egg (EW) and b-value of shell color of the AA genotype were significantly higher than those of the GG genotype (p<0.05).In contrast, the total eggshell color (ESC) and L-value of shell color of the AA genotype were significantly lower than those of the GG genotype (p<0.05).Positive phenotypic correlations were found among BW and EW (0.712), BW and a-value (0.435), EW and a-value (0.642).In addition, negative phenotypic correlations were found among a-value and L-value (-0.833), ESC and BW (-0.320), and ESC and EW (-0.545) were negative.This paper confirmed that VLDL/VTG receptor could be a candidate gene related to egg traits and growth performance.

Keywords: chicken, very low density lipoprotein/vitellogenin receptor gene, single nucleotide polymorphism

Introduction

Egg production involves the development of oocytes and ovulation. In this process, the oocytes will take up precursors for yolk formation from the circulation, grow from 6-7 mm to 35 mm and finally undergo ovulation (Bujo et al., 1995). The very low-density lipoprotein receptor (VLDLR), a member of LDL receptor gene superfamily is the key carrier of very low density lipoprotein (VLDL) and vitellogenin (VTG) (Bujo et al., 1994). VLDL is a lipoprotein and VTG is a lipophoshoglycoprotein, secreted by the liver and taken up by the growing oocytes via receptor-mediated endocytosis (Nimpf & Schneider, 1991). It is a 95 kDa plasma membrane protein, the gene of VLDL/VTG receptor which is located on the sex chromosome Z (Barber et al., 1991; Bujo et al., 1995). VLDLR gene encodes a protein with five functional domains: (1) a signal sequence followed by an amino-terminal ligand-binding domain constituted by multiple cysteine-rich repeats (2) an Epidermal Growth Factor (EGF) precursor homologous domain (3) an O-linked sugar domain (4) a transmembrane domain, and (5) a cytoplasmic domain with a FDNPVY sequence (Wyne et al., 1996; Wang et al., 2011). It encodes 863 amino acid residuesand contains 18 exons and 17 introns in its

genomic DNA sequence. VLDLR gene mediates the endocytosis of VTG and VLDL into growing chicken oocytes and plays a major role in controlling the development of oocytes and yolk lipoprotein deposition (Barber et al., 1991; Shen et al., 1993; Takahashi et al., 2003).

The point mutation (G/C) at position 2177 bp of the chicken VLDLR cDNA (mutation named Restricted Ovulation or RO) was reported by Bujo et al. (1994) and the mutation was responsible for reduced egg production. Zhan et al. (2009) found five SNPs in three introns (T3967G in intron2, C809T and A8296G in intron7, A8839T and G9084A in intron 9). In addition, Cao et al. (2012) found one SNP in exon6 (A12321G) and one SNP in intron17 (A13876G). Therefore, the VLDLR gene is a candidate gene for modulating egg production. Specifically, several VLDLR variants show a significant association with egg weight, age at first egg and egg production in chickens, ducks and quails (Wang *et al*., 2011; Cao *et al*., 2012; Wu *et al*., 2015). Hence, the aim of this study was to detect polymorphism of the VLDLR gene promoter in various chicken breeds (Rhode Island Red Breed (RR); Single-Comb White Leghorn (WL); Commercial Breeds (HyLine brown chicken, HL) and Hybrid Red Jungle Fowl (Kai-Tor).

Materials and Methods

Chickens

Blood samples from 66 chickens belonging to four different adult chicken breeds; RR three samples, WL three samples, HL 30 samples and Kai-Tor 30 samples were collected from a commercial and indigenous farm in Phitsalunok Province Thailand. Chicken blood (1 mL) was collected by bleeding the wing vein venipuncture using a sterile syringe and kept in a blood collection tube containing 0.5 mL anticoagulant solution [Ethylene Diamine Tetraacetic Acid; (EDTA)]. All blood collection tubes were kept at 4°C. The genomic DNA from each individual whole blood sample (66 individual tubes) was extracted using QIAamp® DNA Blood Mini Kit (Qiagen, Germantown, MD, US; #51104) according to the manufacturer's instructions.

Phenotypic measurements

The Body weight (BW), egg weight at first egg (EW), and eggshell color (ESC) were measured for each chicken. BW was measured after 12 h with no access to feed. The eggs from each hen were collected during the first egg. EW and ESC were measured within 24 h after laying. ESC was detected by a colorimeter (Konica Minolta Colorimeter CR-300) which established the L-value, a-value, and b-value; representing the brightness, redness and yellowness respectively. Using the L-value, a-value, and b-value taken from the blunt, sharp, and center areas of the egg, ESC was calculated by Equation 1 (Baylan et al., 2017);

$$ESC = (L^2 + a^2 + b^2)^{1/2} \quad \text{.....(1)}$$

PCR amplification

In this study, we used the VLDL/VTG receptor gene in 5′ flanking region for genetic analysis. According to the chicken VLDL/VTG receptor gene sequence (accession no.X80207), a pair of primers (Table 1) was designed to amplify and detect the SNPs for the chicken VLDL/VTG receptor gene in 5′ flanking region. The polymerase chain reaction (PCR) amplification of 5′ flanking region was conducted in a 20 µL final reaction volume. VLDL/VTG gene was amplified using primers VLDL/VTG receptor F1 and R1; VLDL/VTG receptor F2and R2; VLDL/VTG receptor F3 and R3 as shown in Table 1. The 20 µL PCR reaction mix included 13.44 µL of sterile ultrapure water, 2.0 µL of 10X buffer, 1.0 µL of 1.8 mM $MgCl_2$, 0.8 units of Taq polymerase (0.76 µL), 0.4 µL (0.2 mM) of each forward and reverse primer and 2.0 µL of DNA template. The optimized PCR amplification program was composed of 3 min at 94 °C followed by five cycles of 40 s at 94°C, 30 s at 56 °C and 45 s at 72 °C, followed by another 30 cycles of 40 s at 94 °C, 30 s at 58 °C, and 45 s at 72 °C, and finally 7 min at 72 °C. PCR products were visualized under UV light following electrophoresis on an ethidium bromide stained 1% agarose gel.

PCR product analysis

The subsequent electrophoretic analyses of PCR products were identical for all chicken isolates. The amplicons were separated with the QX DNA Screening Kit employing the AM420 method with the following electrophoresis parameters: alignment marker injected at 5kV for 10 s, sample injected at 5 kV for 10s, and separation at 5 kV for 420 s. The alignment markers 15 bp-1kb and the DNA size markers 100 bp-3kb were run simultaneously. The PCR products of VLDL/VTG from each primer (in Table 1) were identified by the QIAxcel Screen Gel Software.

Table 1 Specific primers used in the experiments

Primer names	Sequences 5′ → 3′	nt	Amplicon size	Position
VLDL/VTG receptor F1	TAGCAGCCAATGCCACATCCAAAGC	25	248 bp	-1599
VLDL/VTG receptor R1	ACAAATGATTGTCCAGGCCATCC	23		-1351

SNPs identification

PCR products were purified using QIAquick® Gel Extraction Kit (Qiagen, Germantown, MD, US; catalog # 28740), and directly sent to MacrogenInc., Seoul, Korea for sequencing. The nucleotide sequences of VLDL/VTG in various chickens were retrieved from the NCBI GenBank databases for further analysis using the Genedoc alignment program.

Statistical analysis

The genotypic frequency and *P*-values for Hardy Weinberg equilibrium test were estimated and calculated by the SAS software (SAS Institute, Cary, NC) for λ^2as described earlier (Yao et al., 2010). The following model (Equation 2) was fitted for association of each genotype (SNPs) with egg traits and growth performances.

$$Y_{ij} = \mu + F_i + G_j + e_{ij} \quad \text{.....(2)}$$

where, Y_{ij} represented the dependent variable (Growth performance and egg traits), μ was the population mean,F_i, G_j were the farm and genotype effect of VLDL/VTG receptor gene, e_{ij} was the random error.The correlation procedure was applied to estimate phenotypic correlations among the egg trait and growth performance.

Results and Discussion

Chicken VLDL/VTG receptor 5´flanking region analysis

In order to obtain the partial sequence of chicken VLDL/VTG receptor 5´flanking region, each DNA extract was used as a template for PCR amplification. The primer pairs were designed to complement the unique 5´flanking region of VLDL/VTG receptor gene at location of-1351to -1599. The results showed that the PCR products were found to contain 248 bp (Figure 1), which were the same sizes in all samples.

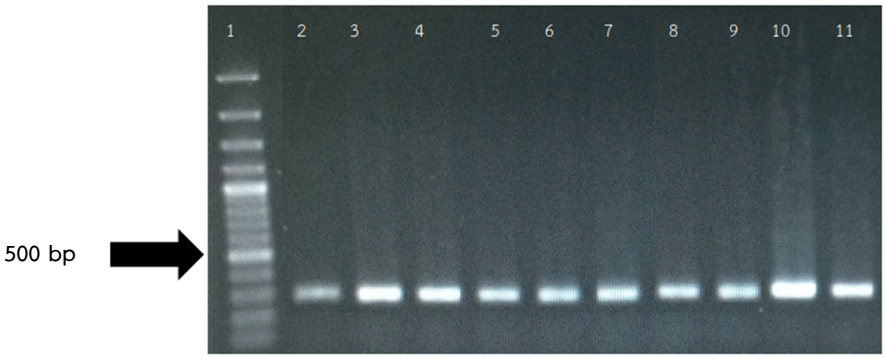

Figure 1 PCR product from all samples; Lane 1 is 100bp plus maker, Lane 2 is Rhode Island Red (RR) chicken, Lane 3 is White Leghorn (WL) chicken, Lane 4-7 are Hybrid Red Jungle Fowl (Kai Tor) number 1-4 and Lane 8-11 are HyLine brown (HL) chickens number 1-4.

The primer pairs showed polymorphism. For primer 1, two alleles (allele A and allele G) and three genotypes (AA, AG and GG) were detected in four different chicken

breeds (Figure 2A). Forward and reverse sequencing identified an A/G single nucleotide change at position 1393 of 5´ flanking region of VLDL/VTG receptor gene (Figure 2B).

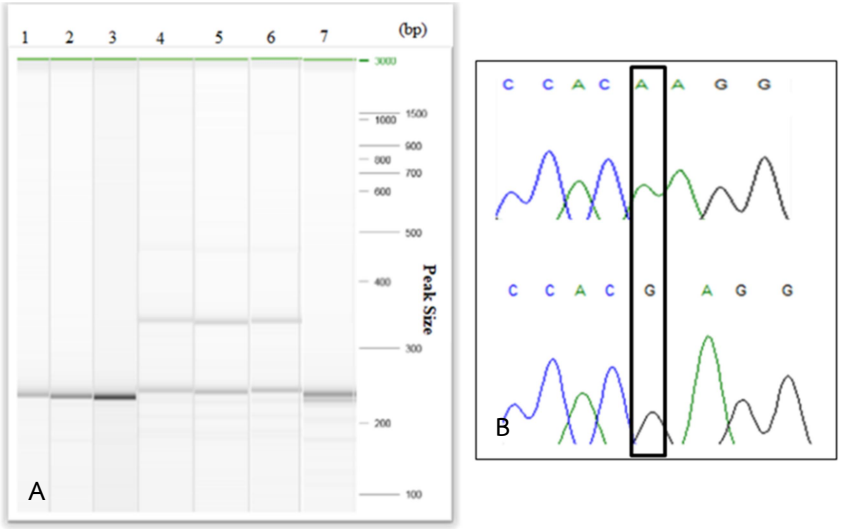

Figure 2 PCR products amplified with primer 1 on QIAxCel A) PCR Screening on QIxcel and Sequencing B) Sequence analysis. (A) Lane 1-3: AA genotype; Lane 4-6: AG genotype; Lane 7: GG genotype; DNA size marker 100-1500 bp. (B) upper, AA; lower GG; Arrow indicates A/G substitution

Genotype and gene frequency of candidate VLDL/VTG receptor were estimated (Table 2). For the SNP A-1351G, allele A is higher than allele G (0.69 and 0.31), respectively.In Chi-square tests, the observed frequencies of the genotypes were not in agreement with Hardy-Weinberg equilibrium (p<0.05), showing that selection might have influenced gene frequencies.

Table 2 Genotypic and allelic frequencies of the SNP (A-1393G) in chicken VLDL/VTG receptor gene

Number of chickens	Genotypic frequency			Allelic frequency			
	AA	AG	GG	A	G	λ^2	*p*-value
N=66	0.4761	0.4278	0.0961	0.69	0.31	11.346	0.023

Association of polymorphisms with egg production traits and growth performances

Associations of the SNPs with egg production traits and growth performances are indicated in Table 3. For SNP A-1393G, least squares means of BW, EW, ESC, L-value, a- and b-value (mean ± SE) of various chickens with genotype AA, AG and GG

differ significantly (p<0.05). In general, AA was the favorable genotype for BW, EW, a- and b-value when compared with the GG genotype in the various chicken populations. However, higher ESC and L-values were found for the GG genotype compared with AA genotype. For the redness of an eggshell color (a-value), genotypes AG and GG had non-significantly different a-values but values were lower than those obtained from AA (p<0.05). There were significant associations between the SNP and BW, EW, L-value, and b-value, except a-value in which AA showed higher values than AG and GG (p<0.05).The phenotype correlations among growth performance and egg traits are summarized in Table 4. The highest positive phenotypic correlation was found between ESC and L-value (0.995), while a-value and L-value (-0.833) was the highest negative correlation. In addition, positive high phenotypic correlations were found among b-value and a-value (0.735), EW and BW (0.712), a-value and EW (0.642). Moreover, b-value and L-value (-0.522), ESC and EW (-0.545), L-value and EW (-0.581), ESC and a-value (-0.777) were negative phenotypic correlations.

Table 3 Least squares analysis between genotype of the SNP (A-1393G) of VLDL/VTG receptor gene and egg traits and body weight in chicken.

Traits	Genotype		
	AA	AG	GG
BW	1,545.78±36.17[a]	1,086.46±45.60[b]	1130.32±67.87[c]
EW	49.59±16.74[a]	29.86±1.02[b]	30.41±1.52[c]
ESC	63.25±1.81[c]	78.34±2.28[b]	82.24±3.39[a]
L-value	59.68±1.94[c]	77.33±2.44[b]	81.45±3.63[a]
a-value	11.76±0.94[a]	1.52±1.18[b]	0.49±1.76[b]
b-value	17.02±1.39[a]	10.46±1.76[b]	9.59±2.62[c]

Remark [a,b] Mean in a row without a common superscript differ significantly (p<0.05).
BW = Body weight (g), EW = Egg weight at first egg (g), ESC = Eggshell color, L-value = brightness, a-value= redness, b-value = yellowness

Generally, AA is the favorable genotype for EW and BW. Selection for the AA genotype will increase EW and BW. Greater BW and EW can increase the economic value. The phenotypic correlations between BW and EW were positive. Moreover, EW and ESC were negative (Table 4).The results showed that the phenotypic correlations of EW with ESC were similar to previous reports by Zhang et al. (2004). The large genetic correlation between BW and EW explained BW was a major factor affecting EW.VLDL/VTG receptor has an important role in the process for VTG and VLDL depositing the yolk membrane which is a 95 KDa plasma membrane protein (Barber et al., 1991; Bujo et al., 1995). Moreover, VLDL/VTG receptor gene plays important roles

in the growth and acts on cardiac fatty acid metabolism, lipoprotein metabolism, and fat deposition (Yagyu et al., 2002). Chicken VLDL/VTG receptor gene was first reported by Novak et al. (1996) and the VLDL/VTG receptor encodes an 863 amino acid protein and contains 18 exons and 17 introns in its genomic DNA. The VLDL/VTG receptor mRNA is highly expressed in the heart, muscle, adipose tissue and barely detectable in the liver (Takahashi et al., 1992).

Table 4 Phenotypic correlations between the egg traits and body weight

Traits	BW	EW	L-value	a-value	b-value	ESC
BW	-	0.712	-0.344	0.435	-0.021	-0.320
EW	0.712	-	-0.581	0.642	0.142	-0.545
L-value	-0.344	-0.581	-	-0.833	-0.522	0.995
a-value	0.435	0.642	-0.833	-	0.735	-0.777
b-value	-0.021	0.141	-0.522	0.735	-	-0.462
ESC	-0.320	-0.545	0.995	-0.777	-0.462	-

Remark BW = Body weight (g), EW = Egg weight at first egg (g), ESC = Eggshell color, L-value = brightness, a-value= redness, b-value = yellowness

Many studies such as Zhan et al. (2009), have reported that VLDL receptor gene had important effects on the age of the first egg, egg production, egg weight and the percentage of yolk. Wang et al. (2011) found SNP sites (T/C) at position 2025 bp of the ORF and G/A in intron 13). They recorded that duck VLDLR gene was significantly associated with egg production age of the first egg and body weight of the first egg. Cao et al. (2012) found the VLDLR SNPs in a Chinese indigenous chicken breed in which A12321G and A13876G were identified. Therefore, they are associated with egg production traits.

Kai-Tor chicken is a hybrid red jungle fowl developed from local chicken, which is characterized by its adaptability to poor quality feed and environment. Yaemkong (2014) reported that many phenotypic characteristics of Thai indigenous chickens were found in Phitsanulok province. In the current study, we screened the VLDL/VTG receptor gene SNPs in various chickens. As a result, A-1393G was identified. The association between the SNP and egg production and growth performance showed the AA genotype was significantly higher than those of the GG genotype ($p<0.05$), whereas ESC and L-value of shell color of the AA genotype were significantly lower than the GG genotype ($p<0.05$). The results of the associated analysis were in agreement with the data reported by other researchers on chickens and were in line with the function of VLDLR gene described in other species (Bujo et al., 1995; Rodriguez et al.,

1996; Cao et al., 2012; Yao et al., 2010). We speculate that VLDL/VTG receptor gene is a potential genetic marker for the studied traits.

Conclusion

In this study, one SNPs in 5′flanking region of VLDL/VTG receptor gene was found in four chicken breeds (RR, WL, HL and Kai-Tor). The identified SNPs have impact on the function of chicken VLDL/VTG receptor gene and the SNPs was also significantly associated with growth performance and egg traits in chickens.The VLDL/VTG receptor gene can be a useful marker for molecular marker-assisted selection of reproductive and growth performance traits in chickens.

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