A Novel Polymorphism of VLDLR Signal Peptide Coding Region and Its Association with Growth and Abdominal Fat Traits of Gaoyou Domestic Ducks

ABSTRACT

The VLDLR gene plays important roles in the growth and adiposity in humans and mice. The purpose of this study was to investigate the relationship between VLDLR gene genetic polymorphisms and growth and abdominal fat traits of the Gaoyou domestic duck. A total of 267 Gaoyou ducks were employed for testing. A 18bp deletion was identified in VLDLR signal peptide coding region. The results of chi-squared test suggested that the genotype frequencies of VLDLR signal peptide coding region were not in Hardy-Weinberg equilibrium. Least squares analysis showed that body weight (BW) of -18bp/-18bp genotype ducks was significantly higher than those of other genotypes from six (BW6) (p<0.05) to ten weeks of age (BW10) (p<0.01). The association analysis was performed taken body weight as covariant for abdominal percentage (AFP). Results showed that there was not interaction between genotype (p>0.05) and body weight for AFP and different genotypes had a significant effect on AFP (p<0.05). The results of Bonferroni t-test revealed that the abdominal fat percentage (AFP) of -18bp/-18bp genotype was significantly lower than those of +18bp/-18bp (p<0.05). Preliminary studies have shown that VLDLR may be a candidate gene for the selection for growth and abdominal fat, and the results of the present study indicate that VLDLR strongly influences carcass abdominal fat content of Gaoyou ducks.

INTRODUCTION

The very-low-density-lipoprotein receptor (VLDLR) is a transmembrane lipoprotein receptor of the low-density-lipoprotein (LDL) receptor family. VLDLR is widely distributed throughout the tissues of the body, including the skeletal muscle, adipose tissue, heart, and the brain, but it is absent from the liver (Nimpf & Schneider, 2000). The VLDLR also exhibits high homology among various species, of up to 95% among rats, mice, rabbits, and humans. There is approximately 84% conservation with the respective protein in chickens (Nimpf & Schneider, 1998; Nimpf & Schneider, 2000).

VLDLR is a peripheral lipoprotein receptor that primarily modulates the extra-hepatic metabolism of triglyceride-rich lipoproteins and acts on cardiac fatty acid metabolism, lipoprotein metabolism, and fat deposition (Yagyu et al., 2002). In addition, VLDLR allows fatty acids to enter the cells, where they are used as an energy source and to regulate the reelin signaling pathway (Takahashi et al., 1995; Tao et al., 2010). The duck VLDLR gene was cloned and characterized by Wang et al. (2011). The deduced amino acid sequence predicts a mature protein of 851 amino acids preceded by a 36-residue signal peptide.

Five structural domains of LDL receptor family are highly conserved: an extracellular N-terminal ligand-binding domain with cysteine-rich
A Novel Polymorphism of VLDLR Signal Peptide Coding Region and Its Association with Growth and Abdominal Fat Traits of Gaoyou Domestic Ducks

Ming-liang C, Cong W, Xuan D, Lu L, Tang-jie Z

A total of 280 hatching eggs were obtained from the elite reservation farm of Jiangsu Gaoyou duck company in Jiangsu province, China. All ducks were raised in floor pens under the same standardized management conditions, fed commercial corn-soybean diets that met the requirements of the NRC (1994).

Blood samples and phenotypic data on growth and carcass traits (hatching weight, weekly body weight (BW) between 3 and 10 weeks of age, whole carcass weight (CW), eviscerated carcass weight, and abdominal fat weight (AFW) of 267 individuals were determined. Birds were slaughtered using appropriate humane methods at 10 weeks of age after 6 h with no access to feed prior to transporting the ducks for slaughter processing.

Eviscerated carcass weight was determined as chilled carcass weight after the removal of feathers, lungs, heart, liver, gastrointestinal tract, kidneys, and abdominal fat. Carcass yield (CY), eviscerated carcass yield (ECY), and abdominal fat percentage (AFP) [(AFP: (AFW / BW) x100] were calculated as the ratio of these traits to body weight at 10 weeks of age (BW10). In this study, intramuscular fat and subcutaneous were not measured owing to experimental limitations.

Genomic DNA was obtained by phenol and chloroform (1:1) extraction, and stored at −80°C.

**Primer design, PCR amplification and identification of gene polymorphism**

The VLDLR genomic sequence (NM_001310401) was obtained from the National Center for Biotechnology Information (NCBI). One pair of primers (5’- ATTACACTGCCAAATGACC -3’ and 5’- CGGGAACTGGGATTCGTT -3’) was designed to amplify the duck VLDLR gene signal peptide region. The product size was 374 bp.

Polymerase chain reaction (PCR) was performed using 50 ng DNA templates, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM MgCl2 and 0.5 U Taq DNA polymerase. Thermal cycling began with an initial denaturation step of 94 °C for 10 min, followed by 35 cycles of 94 °C for 30 s, 53.5°C annealing for 30 s, 72 °C for 30 s, and concluded with a final extension at 72 °C for 10 min. DNA sequencing was performed using an ABI 3130 Genetic Analyzer (Applied Biosystems,
USA). Sequencing variants were detected by visual examination of the sequencing map followed by alignment using DNAMAN.

**Statistical analysis**

Genotype and allelic frequencies, genotypic numbers, effective allele numbers (Ne) and gene heterozygosity (He), polymorphism information content (PIC) were calculated, and their Hardy-Weinberg equilibrium was analyzed using the Chi-square test of the PopGene32 software (version 1.31).

The association of VLDLR gene genotypes with growth and carcass traits, including hatchling weight, BW at weeks 3 to 10, carcass weight, eviscerated carcass weight, abdominal fat weight, carcass yield, and eviscerated carcass yield data were subjected to two-way analysis of variance using the SPSS software (version 16.0), using the following model: Y = μ + G + L + G×L + e, where Y is the dependent variable (analyzed traits), μ is the overall mean, G is the genotype of different variations of the VLDLR gene, L is the duck population, G×L is the interaction between genotype and duck population (fixed effect), and e is the random error. Genotype association analyses were performed using body weight as covariant for AFP. Differences among genotypes were determined by the least square and Bonferroni tests. Significance was assumed at $p<0.05$.

**RESULTS**

**Polymorphism identification and detection**

The sequences amplified with one primer were aligned among Gaoyou ducks. A novel 18bp deletion in VLDLR signal peptide coding region at the nucleotide position 278-295bp was detected, resulting in three genotypes (+18bp/+18bp, +18bp/-18bp and -18bp/-18bp). The sequences presenting variation in signal peptide coding region were submitted to GenBank (accession number: KU317918 for +18bp/+18bp allele and KU317919 for +18bp/-18bp allele).

As shown in Fig. 1 and Fig. 2, a 18bp deletion at locus 278-295 expressed three genotypes: +18bp/+18bp, +18bp/-18bp and -18bp/-18bp. The +18bp/+18bp genotype contains two +18bp single strands (allele +18bp); the -18bp/-18bp genotype lack two -18bp single strands (allele -18bp); the +18bp/-18bp genotype contains one 18bp single strand and one missed 18bp single strand.

**Allele and genotype distribution of the VLDLR gene signal peptide**

Allele and genotype frequencies found at the signal peptide (Table 1) indicated that the frequency of the genotype +18bp/+18bp was higher than those of the +18bp/-18bp and -18bp/-18bp genotypes. The +18bp allele frequency (0.70) was also much higher compared with -18bp which showed a high prevalence in the evaluated Gaoyou duck population. The Chi-square tests showed that the genotypic frequencies were not in Hardy-Weinberg equilibrium ($p<0.05$).

The values of the population genetic indices (He, Ne, and PIC) to evaluate the diversity of the population are also presented in Table 1. The value of He (gene heterozygosity) was above 0.4 and below 0.5, whereas values of Ne (effective allele numbers) approached 2. The PIC values were 0.33.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele</th>
<th>He</th>
<th>Ne</th>
<th>PIC</th>
<th>$\chi^2$</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>+18bp/+18bp</td>
<td>0.65/174</td>
<td>0.11/29</td>
<td>0.24/64</td>
<td>0.70</td>
<td>0.30</td>
<td>0.42</td>
</tr>
</tbody>
</table>

$\chi^2_{0.05}(df=1)=3.841$, $\chi^2_{0.01}(df=1)=6.635$
Association of polymorphism at VLDLR gene signal peptide with growth and carcass traits

The results of association analysis between different genotypes and growth and carcass traits are given in Table 2. The table shows no significant differences in hatchling weight, or in BW at 3, 4, and 5 weeks of age in the three genotypes, as determined by the least squares. However, the genotype -18bp/-18bp presented significantly higher BW at 6 (p<0.05), 7 (p<0.01), 8 (p<0.01), 9 (p<0.01), and 10 (p<0.01) weeks of age compared with the genotypes +18bp/+18bp and +18bp/-18bp.

The covariate association analysis results showed that there was not interaction between genotype (F=0.927, p=0.400) and body weight, and that genotype had a significant effect on AFP (F=6.713, p=0.002). The results of Bonferroni t-test revealed that the ducks of the -18bp/-18bp genotype presented significantly higher BW at 6 (p<0.05), 7 (p<0.01), 8 (p<0.01), 9 (p<0.01), and 10 (p<0.01) weeks of age compared with the genotypes +18bp/+18bp and +18bp/-18bp.

The covariate association analysis results showed that there was not interaction between genotype (F=0.927, p=0.400) and body weight, and that genotype had a significant effect on AFP (F=6.713, p=0.002). The results of Bonferroni t-test revealed that the ducks of the -18bp/-18bp genotype presented significantly lower abdominal fat percentage compared with those of the genotype +18bp/+18bp (p<0.05). Furthermore, no significant association among different genotypes with other traits (carcass yield and eviscerated carcass yield) was detected (p>0.05).

**DISCUSSION**

The allele frequencies showed that the deletion of the 18 bases was polymorphic. The fact that Gaoyou duck population deviated from Hardy-Weinberg equilibrium (p<0.05) may be due to the small sample size and long-term artificial selection for commercial purposes (such as growth, egg production, carcass weight). In selected populations, loci are expected to experience genotypic frequency deviation from the Hardy-Weinberg equilibrium, and that impact traits of the species under selection (Goliasova & Wolf, 2004; Wu et al., 2012; Ismoyowati & Purwantini, 2010).

Abdominal and subcutaneous fat are regarded as the main sources of waste in the processing plant. The results of Table 2 indicate that the VLDLR gene may be a candidate gene for the selection for higher growth rate and lower fat deposition, taking into account the high genetic correlations between abdominal fat weight and subcutaneous fat, while there are almost no genetic correlations between abdominal fat weight and intramuscular fat percentage (Zerehdaran et al., 2004; Lotfi et al., 2011) in poultry. This would be advantageous for human health. The VLDLR gene was associated with fatness traits, like AFP, in ducks, which is consistent with some studies in humans. Many studies have shown associations of VLDLR gene mutations with adiposity and body weight in humans, as previously mentioned in this paper. In a previous study (Zhao et al., 2015), we identified eight SNPs in the homologous domain of VLDLR EGF precursor and showed that there was a significant association between the VLDLR EGF precursor homologous domain and abdominal fat percentage (AFP) in ducks. Similar results were obtained in the present study by analyzing the polymorphism of signal peptide regions. Our findings further support that

**Table 2 – Effects of polymorphism of the VLDLR gene signal peptide on duck growth and carcass traits (n=267)**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+18bp/+18bp(n=174)</td>
</tr>
<tr>
<td>Hatchling weight</td>
<td>47.49±0.50</td>
</tr>
<tr>
<td>BW 3 weeks</td>
<td>610.77±11.00</td>
</tr>
<tr>
<td>BW 4 weeks</td>
<td>855.40±18.67</td>
</tr>
<tr>
<td>BW 5 weeks</td>
<td>1101.42±21.18</td>
</tr>
<tr>
<td>BW 6 weeks</td>
<td>1422.53±24.75</td>
</tr>
<tr>
<td>BW 7 weeks</td>
<td>1763.57±29.00</td>
</tr>
<tr>
<td>BW 8 weeks</td>
<td>1895.26±28.31</td>
</tr>
<tr>
<td>BW 9 weeks</td>
<td>2076.68±34.14</td>
</tr>
<tr>
<td>BW 10 weeks</td>
<td>2247.40±41.56</td>
</tr>
<tr>
<td>CY (%)</td>
<td>90.71±0.43</td>
</tr>
<tr>
<td>ECY (%)</td>
<td>74.56±0.34</td>
</tr>
<tr>
<td>AFP (%)</td>
<td>2.17±0.08</td>
</tr>
</tbody>
</table>

The data are expressed as least square means ± standard errors (mean ± SE)

Only significant associations are shown in this table. Values within a row without a common lowercase and uppercase superscript are significantly different at p<0.05 and p<0.01, respectively. CY = carcass yield, ECY=Eviscerated carcass yield, AFP=abdominal fat percentage
VLDDL may be used as a candidate gene for abdominal fat. We propose that there is a strong linkage existing in the polymorphisms of two regions. Additionally, some polymorphisms in the VLDLR gene were found to be associated with cerebellar ataxia and short stature, disease caused by mutations (Boycott et al., 2009; Schlotawa et al., 2013).

Signal peptides, as short peptides in N-terminus of proteins, direct the proteins from their ribosomal assembly site to extracellular sites or to a particular cellular location. In this study, the amplified fragments was 374bp, containing the 5′-UTR 243bp and 131bp region of the signal peptide, which encoded 37 amino acids of the signal peptide region. The genotype of -18bp/-18bp lacked 6 amino acids (Pro-Arg–Cys-Gly-Ala-Phe) compared with the +18bp/+18bp genotype. It was assumed that this effect may be caused by change of VLDLR synthesis and positioning.

In conclusion, we found one SNP with 2 alleles (+18bp and -18bp) in the signal peptide of the VLDLR gene. The frequency of +18bp allele was higher than that of -18bp allele in the Gaoyou duck breed. The SNP was highly related with growth traits and abdominal fat percentage. Thus, this VLDLR SNP may potentially be used in marker assisted selection for growth rate and fat deposition traits in Gaoyou ducks. This novel SNP was closely related to the QTL affecting body weight and fat deposition traits. However, further investigations with different and larger duck populations are desired to determine how the VLDLR gene affects body weight and fat deposition traits.

ACKNOWLEDGEMENTS

This research was supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions and Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, 225009. Postgraduate Degree innovation projects, Jiangsu Province (SJLX15_0677). Students’ Academic and Scientific Innovation Fund funded project, Yangzhou University (X2015713)

REFERENCES


