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Association of VLDLR haplotypes with abdominal fat trait in ducks

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Abstract. This study aimed to determine the correlation among *VLDLR* (very low-density lipoprotein receptor) gene polymorphisms, body weight and abdominal fat deposition of Gaoyou ducks. A total of 267 Gaoyou ducks from one pure line was employed for testing. The polymorphisms of the *VLDLR* gene were screened by polymerase chain reaction and DNA sequencing. Four novel single nucleotide polymorphisms (SNPs) (g.151G > A, g.170C > T, g.206A > G and g.278–295del) were identified in the 5'-UTR and signal peptide region. Furthermore, eight haplotypes were identified based on the four SNPs. The H8 was the most common haplotype with a frequency of more than 31 %. The four SNPs and their haplotype combinations were shown to be significantly associated with body weight at 6–10 weeks of age ($P < 0.05$ or $P < 0.01$) and abdominal fat percentage (AFP) ($P < 0.05$ or $P < 0.01$). Remarkably, the H1H1 diplotype had an effect on increasing body weight and decreasing AFP from the 6th to the 10th weeks of age. However, increasing positive effects of the H5H8 diplotype were observed for both body weight and AFP. This study suggests that the *VLDLR* gene plays an important role in the regulation of body weight and fat-related traits and may serve as a potential marker for the marker-assisted selection program during duck breeding.

1 Introduction

Genetic markers closely linked to loci for economically important traits can be used to enhance the speed and effectiveness of progress in animal breeding. Once an association between DNA polymorphism and a trait was found, the DNA polymorphism could be considered as a candidate genetic marker for marker-assisted selection (MAS) programs.

Lipoprotein receptor is a member of the low-density lipoprotein receptor (LDLR) family and highly expressed in adipose tissue, heart and skeletal muscles, while it is absent in liver. VLDLR binds apolipoprotein E-triglyceride-rich lipoproteins and plays a critical role in lipid metabolism and the reelin signaling pathway. The *VLDLR* gene contains five functional domains (Willnow, 1998). VLDLR mediates the uptake of very low-density lipoprotein (VLDL) by peripheral tissues through lipoprotein lipase (LPL)-dependent lipolysis and participates in VLDL metabolism (Tacken et al., 2000; Takahashi et al., 1995, 2004; Goudriaan et al., 2004). In addition, VLDLR has protective features against obesity, insulin resistance, premature heart disease, tumour growth, inflammation and angiogenesis (Nguyen et al., 2014; Yuan et al., 2011; Kyosseva et al., 2013). Studies with VLDLR knockout mice have linked VLDLR with obesity and VLDLR mutants exhibit modest reductions in body weight and adiposity (Frykman et al., 1995; Goudriaan et al., 2001; Eppig et al., 2015; Suwa et al., 2010). Patients with VLDLR mutations have abnormally lower body mass index when compared with control subjects (Boycott et al., 2005; Crawford et al., 2008), which is consistent with the results in mice. Furthermore, previous studies showed that VLDLR is related to body weight and adiposity in humans and mice (Brockmann et al., 1998; Kunej et al., 2013; Clemente-Postigo et al., 2011). Thus, VLDLR expression levels are likely associated with the phenotypic biomarkers for obesity (Kim et

al., 2012). To date, most poultry VLDLR studies have focussed on reproduction because VLDLR can develop growing oocytes and deposit yolk lipoprotein (Shen et al., 1993; Wang et al., 2011; Wu et al., 2015), but the relationships between VLDLR polymorphism and fat deposition or body weight have not yet been investigated in poultry. Therefore, based on our previous results that the *VLDLR* gene is probably associated with duck abdominal fat deposition (Zhao et al., 2015), in the present study, we further investigate the association between the *VLDLR* gene and abdominal fat deposition in ducks by screening different polymorphic sites.

Growth rate and carcass lean content are two economically important traits in meat-producing animals. Therefore, a higher growth rate and lower body fat percentage are always to be preferred in breeding programs for commercial breeders. And this is why we screen the polymorphic sites in partial sequences of the *VLDLR* gene and correlate the VLDLR polymorphism with fat deposition and body weight traits. Although association studies cannot determine whether the gene markers are responsible for the variation in a trait or whether the variation is due to a closely linked locus that influences the trait, there is still evidence suggesting that the *VLDLR* gene would affect these traits. This study aimed to identify the polymorphism of the *VLDLR* gene and to analyse the associations among polymorphism, growth and main carcass traits (abdominal fat weight – AFW; abdominal fat percentage – AFP; carcass weight – CW) in Gaoyou ducks.

2 Materials and methods

2.1 Ethics statement

All animal experiments were approved by the Jiangsu Administrative Committee for Laboratory Animals (permission number SYXK-SU-2007-0005) and complied with the guidelines of Jiangsu laboratory animal welfare and the ethics of Jiangsu Administrative Committee of Laboratory Animals.

2.2 Sample collection and preparation

A total of 267 pure-line Gaoyou ducks were obtained from a high-quality Jiangsu Gaoyou duck farm in Jiangsu province that produces pure-bred animals without hybridisation. All ducks were raised in floor pens under the same standardised conditions of management and fed with commercial corn–soybean diets that met NRC nutrient requirements. Blood samples and phenotypic data on growth and carcass traits were collected from 267 individuals. They were euthanised at 10 weeks of age after 6 h with no access to food prior to euthanasia. CW was measured on the chilled carcass after the removal of feathers, heart, lungs, liver, kidneys, the gastrointestinal tract and abdominal fat. The ratio of these traits to BW10 (body weight at 10 weeks of age) was calculated as carcass percentage (CP) and AFP. Genomic DNA was obtained by phenol and chloroform (1 : 1) extraction and stored at −80 °C.

2.3 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′-CGGGAACTGGGATTCTTC-3′) was designed to amplify the signal peptide region of the duck *VLDLR* gene. The size of the product 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 $MgCl_2$ 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 an elongation step at 72 °C for 10 min. DNA sequencing was performed using an ABI 3130 genetic analyser (Applied Biosystems, USA). Sequencing variants were detected by visual examination of the sequencing map followed by alignment using DNAMAN.

2.4 Statistic analysis

The genotype and allelic frequencies, genotypic numbers, effective allele numbers (Ne), gene heterozygosity (He) and polymorphism information content (PIC) were calculated and the Hardy–Weinberg equilibrium was analysed using the χ^2 test of PopGene32 (version 1.31). SHEsis online version (http://analysis2.bio-x.cn/myAnalysis.php) was used to calculate the pairwise linkage disequilibrium. Haplotypes were obtained for each animal using the PHASE computer program, version 2.1. The association between VLDLR genotypes with growth and carcass traits, including the weight of birth, body weight at 2–10 weeks, CW, eviscerated weight, abdominal fat weight, dressing percentage and percentage of eviscerated weight, were evaluated according to the two-way analysis of the software SPSS (version 16.0), using the following model: $Y = \mu + G + L + G \times L + e$, where Y was the dependent variable (analysed traits), μ was the overall mean, G was the genotype of different variation for the *VLDLR* gene, L was the duck population, $G \times L$ was the interaction between genotype and duck population (it is a fixed effect), and e was the random error. The differences between genotypes were determined by least square analysis.

3 Results

3.1 Polymorphisms in the duck *VLDLR* gene

A pair of primers was used to amplify and screen single nucleotide polymorphisms (SNPs) in the entire signal peptide coding region of the duck *VLDLR* gene. PCR amplifi-

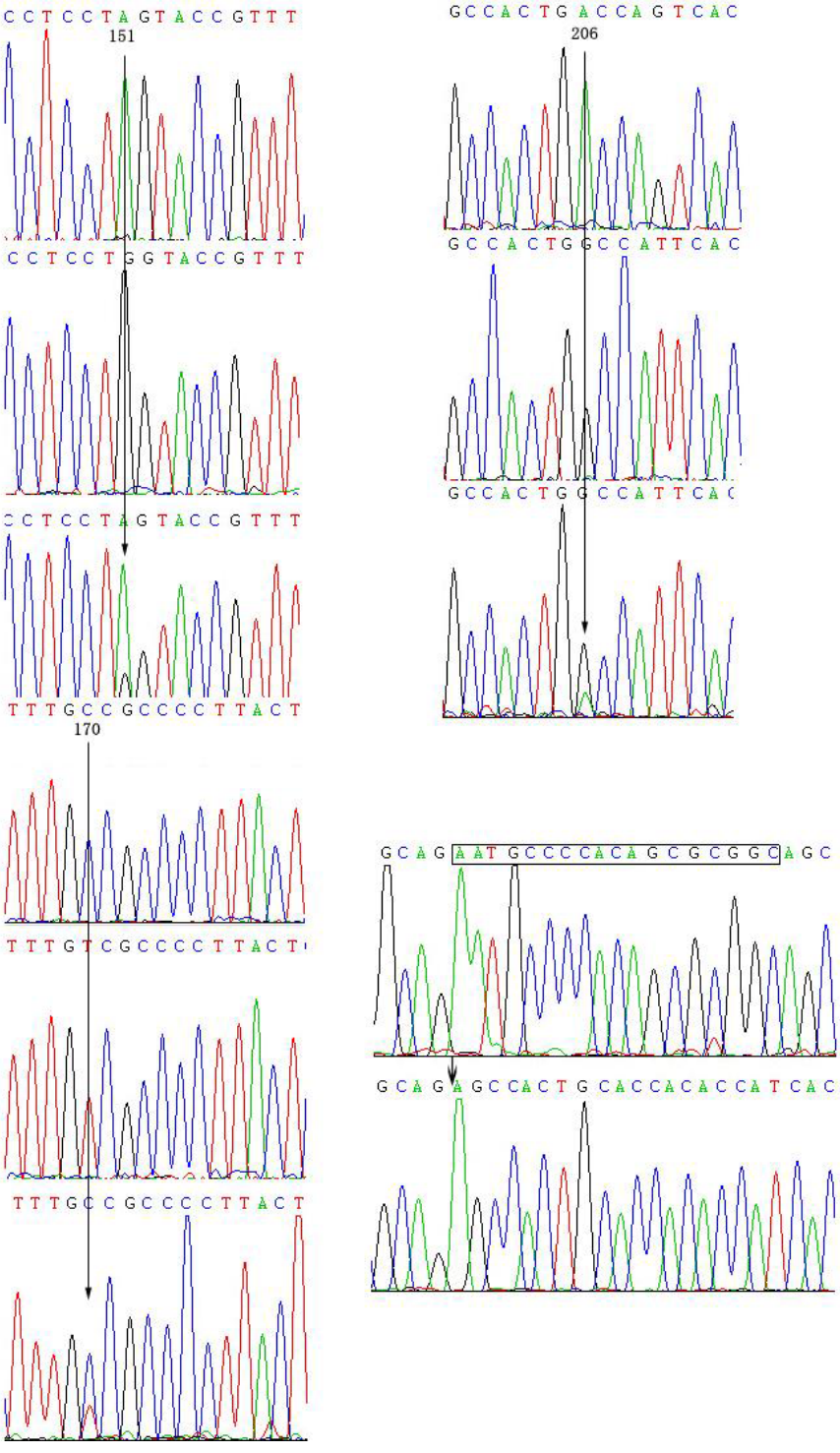

Figure 1. DNA sequencing maps from several DNA templates at four SNPs of duck *VLDLR*.

cation of one *VLDLR* gene fragment yielded a 374-bp fragment including the entire signal peptide coding region and partial exons. The polymorphism type and position were identified by direct DNA sequencing. Multiple sequence alignment showed that four SNPs (g.151G > A, g.170C > T, g.206A > G, g.278–295del) were identified in the duck *VLDLR* gene, all of which were found in the 5'-UTR and the signal peptide coding region. In addition, the four SNPs were all deposited in the GenBank database (KU317918-KU317932). DNA sequencing maps were shown in Fig. 1.

Table 1. Population genetic indexes of four polymorphisms in signal peptide coding of VLDLR.

SNPs	Number of genotypes			Allele		He	Ne	PIC	χ^2	P
g.151G > A	GG (107)	GA (149)	AA (11)	G(0.68)	A(0.32)	0.44	1.77	0.34	21.20	N/A
g.170C > T	CC (133)	CT (59)	TT (75)	C(0.61)	T(0.39)	0.48	1.91	0.36	76.76	N/A
g.206A > G	AA (88)	AG (134)	GG (45)	A(0.58)	G(0.42)	0.49	1.95	0.37	0.25	0.62
g.278–295del	++ (174)	+− (29)	−− (64)	+ (0.70)	− (0.30)	0.42	1.71	0.33	145.56	N/A

Table 2. Haplotype and diplotype frequencies in the duck *VLDLR* gene.

	SNP						
Haplotype	g.151G > A	g.170C > T	g.206A > G	g.278–295del	Frequency (%)	Diplotype	Frequency (%)
H1	G	T	A	−	25.96	H1H1	27.18
H2	A	T	A	+	9.62	H2H8	16.51
H3	G	T	G	+	0.96	H4H8	28.16
H4	G	C	A	+	18.27	H5H8	6.80
H5	G	C	G	+	9.62	H4H4	1.94
H6	A	T	G	+	4.81	H5H5	2.91
H7	A	C	A	+	0.96	H4H5	3.88
H8	A	C	G	+	29.81	H2H5	1.94
						H3H5	0.97
						H2H2	0.97
						H4H7	2.91
						H3H8	0.97
						H6H8	0.97
						H7H8	0.97
						H8H8	2.91

The standardised measure of linkage disequilibrium (LD) denoted as r^2 was calculated for all pairs of the four SNPs (Fig. 1). If $r^2 > 0.33$, the linkage disequilibrium was considered strong (19) (Ardlie et al., 2002). Linkage disequilibrium was often observed between closely positioned loci (20) (Gibbs et al., 2003), as indicated among the four SNPs in the present study. In particular, there was strong linkage disequilibrium between g.206 and g.151, g.170 and g.278–295, and g.170 and g.206.

The g.151G > A, g.170C > T and g.206A > G mutations occurred in the 5'-UTR of the *VLDLR* gene. The g.278–295del lacked 18 bases, which encoding six amino acids of signal peptides.

3.2 Genetic variation in different populations

Minor allelic frequencies, Hardy–Weinberg equilibriums, He, Ne and PIC for each of the four SNPs in the *VLDLR* gene are shown in Table 1. The χ^2 test showed that the genotype distributions at loci g.151, g.17 and g.278–295 displayed deviation from the Hardy–Weinberg equilibrium in the Gaoyou duck population. The genotype distributions at g.206 was consistent with Hardy–Weinberg equilibrium.

3.3 Linkage disequilibrium and haplotype analysis

Haplotypes generally have more information than individual SNPs. Haplotypes were reconstructed with the four SNPs in all 267 ducks by employing the Phase computer program. All eight haplotypes, which accounted for 100 % of all the observations, were listed in Table 2. Among them, five haplotypes, H1(GTA−), H2 (ATA+), H4 (GCA+), H5 (GCG+) and H8(ACG+), were prevalent and counted for 93.28 % of the observations. Fifteen diplotypes were obtained based on these eight haplotypes. Among them, the frequencies of four diplotypes were higher than 5.0 %. Two diplotypes, H1H1 and H4H8, accounted for 55.34 % of them.

The standardised measure of linkage disequilibrium (LD) denoted as r^2 was calculated for all pairs of the four SNPs (Fig. 2). If $r^2 > 0.33$, the linkage disequilibrium was considered strong (Ardlie et al., 2002). Linkage disequilibrium was often observed between closely positioned loci (Gibbs et al., 2003), as indicated among the four SNPs in the present study. Particularly, there was strong linkage disequilibrium between g.206 and g.151, g.170 and g.278–295, and g.170 and g.206.

3.4 Association of diplotypes with duck growth and abdominal fat deposition

Four diplotypes were reconstructed based on haplotypes. The frequencies of all these four diplotypes were higher than 5.0 %. The generalised linear model (GLM) analysis (Table 3) indicated the existence of associations between the

Table 3. Least square means (±SE) of the traits, by diplotype, of the duck *VLDLR* gene.

Traits	H1H1(73)	H5H8(34)	H2H8(52)	H4H8(83)
Weight (g) at birth	48.05 ± 0.79	51.00 ± 1.83	48.70 ± 1.13	48.25 ± 0.59
3 weeks	619.19 ± 17.26	652.00 ± 41.37	576.20 ± 33.42	594.10 ± 16.73
4 weeks	913.00 ± 33.24	948.83 ± 66.39	815.20 ± 44.75	869.95 ± 30.24
5 weeks	1170.71 ± 41.76	1141.17 ± 88.86	1077.10 ± 48.97	1150.95 ± 32.23
6 weeks	1543.14 ± 48.93^{a}	1517.33 ± 96.98^{a}	1365.70 ± 63.53^{b}	1477.30 ± 36.75^{a}
7 weeks	1968.67 ± 59.40^{Aa}	1890.83 ± 108.59^{Aa}	1715.20 ± 67.50^{Bb}	1810.15 ± 43.98^{Ab}
8 weeks	2088.05 ± 53.58^{Aa}	2025.83 ± 126.81^{Aa}	1832.60 ± 65.45^{Bb}	1936.55 ± 42.84^{Ab}
9 weeks	2326.71 ± 55.38^{Aa}	2232.67 ± 173.79^{Aa}	2013.70 ± 59.96^{Bb}	2112.10 ± 50.73^{Ab}
10 weeks	2537.71 ± 51.43^{Aa}	2402.14 ± 215.35^{Aa}	2274.00 ± 77.47^{Bb}	2384.98 ± 32.80^{Ab}
CP (%)	90.05 ± 0.60	90.63 ± 1.14	91.84 ± 0.44	89.74 ± 0.95
EWP (%)	74.36 ± 0.47	74.31 ± 0.63	75.12 ± 0.65	74.38 ± 0.79
AFP (%)	1.86 ± 0.15^{Bb}	2.79 ± 0.24^{Aa}	2.08 ± 0.12^{Bb}	2.22 ± 0.07^{ABb}

The data are expressed as least square means ± standard errors (mean ± SE).
Note that only significant associations are shown in this table. Values within a row without a common superscript letter differ, and values with superscript letters differ significantly ($P < 0.05$ and $P < 0.01$ each). Genotypes with a and b mean they differed significantly ($P < 0.05$), and genotypes with A and B mean they differed very significantly ($P < 0.01$).
EWP: eviscerated weight percentage.

diplotypes and the different traits (body weight (BW) and AFP). The results showed that the weight of ducks with the diplotype H1H1 and H5H8 was significantly higher than that of ducks with the other two diplotypes at 6, 7, 8, 9 and 10 ($P < 0.05$, $P < 0.01$) weeks old, although no significant differences for the weight at birth and 3-, 4- and 5-week weight were found from the least squares means of the four diplotypes. The weight of ducks the diplotype H2H8 was significantly lower than that of ducks with the other three diplotypes from the 6th to the 10th week ($P < 0.05$, $P < 0.01$). The diplotype H1H1 had an effect on increasing body weights, whereas H2H8 had an effect on decreasing body weights ($P < 0.05$).

Ducks with the diplotype H5H8 had a significantly higher abdominal fat percentage than those with the other three diplotypes ($P < 0.01$). Ducks with the diplotype H1H1 had a lower abdominal fat percentage than those with the other three diplotypes ($P < 0.01$). The results showed that the diplotype H5H8 had an increasing positive effect on abdominal fat deposition, and the diplotype H1H1 had an increasing negative effect on abdominal fat deposition. However, there was no significant correlation between the different four diplotypes and other traits

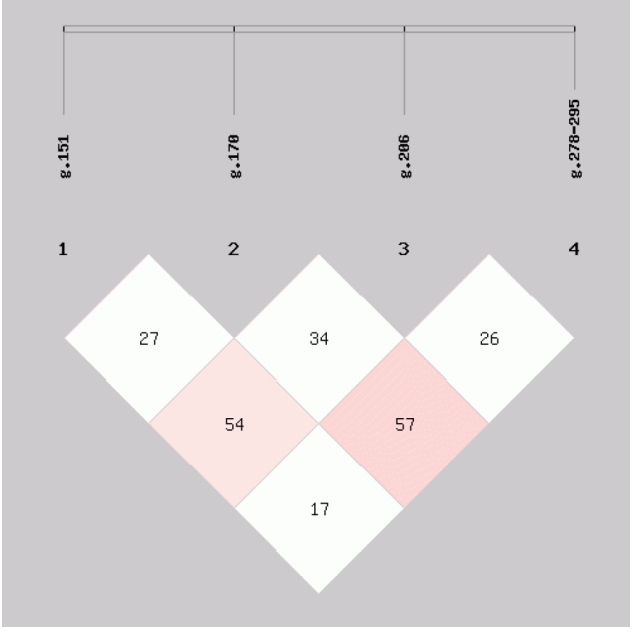

Figure 2. Linkage disequilibrium (LD) plot of the *VLDLR* gene in Gaoyou ducks. The colour scheme is according to SHEsis r^2 scheme. Numbers in each cell stand for the pairwise r^2 value (%) between the corresponding SNPs.

4 Discussion

Three of four SNPs in the Gaoyou VLDLR signal peptide coding region were in Hardy–Weinberg disequilibrium. The Hardy–Weinberg disequilibrium in the Gaoyou duck population may be due to the artificial selection of parents during long-term commercial breeding (e.g., growth, egg appraisal, carcass weight) since genotype frequency deviations from the Hardy-Weinberg equilibrium were expected to happen on loci under selection. The Gaoyou duck population had intermediate levels of genetic diversity ($0.25 <$ PIC $< 0.50 =$ intermediate polymorphism); therefore, there was sufficient genetic diversity for the effective selection on improving growth, egg and other traits during the breeding process.

Through the comparison of the fundamental frequency between different haplotypes, it was demonstrated that mutations are not directional; furthermore, the frequencies of H1 and H5 are not positively related to the complexity of their

respective mutation. In addition, to evolve from haplotype H8, H1 needs a six-step mutation, while the H5 haplotype only needs a one-step mutation. However, the H5 frequency is much lower than that of H1, which reflected that selection also had a role in haplotype frequencies.

In the present study, the duck *VLDLR* gene was investigated as a possible genetic factor in determining the variance of abdominal fat content. Polymorphism-trait association studies cannot determine whether VLDLR is responsible for variation in a trait or whether a closely linked locus influences the trait. Many studies have revealed that obesity candidate genes were associated with genetics, nutritional disease, gastrointestinal and developmental disorders, and cancer (Castro et al., 2017; Ning et al., 2017). Peroxisome proliferator-activated receptor alpha (PPARα) and retinoid X receptor alpha (RXRα) were identified as central nodes, also called hub molecules (Kunej et al., 2013). Several studies have reported that adipose *VLDLR* gene expression can be regulated by PPARγ (Takazawa et al., 2009; Tao et al., 2010; Tao and Hajri, 2011; Gao et al., 2014). All these results suggested that VLDLR is important in regulating fat accumulation (Clemente-Postigo et al., 2011; Go and Mani, 2012; Rankinen et al., 2006).

Most proteins that are completely transported across the cytoplasmic membrane are synthesised with an amino-terminal signal peptide. Signal peptides directly deliver the protein to the proper organelle. In this study, the amplified fragment was 374 bp, containing the 243-bp region of 5′-UTR and the 131-bp region of the signal peptide, which encoded 37 amino acids of the signal peptide region. These mutations in 5′-UTR and signal peptide of the duck *VLDLR* gene implied that this region had effects on duck growth and fat deposition by gene translational efficiency and/or VLDLR synthesis, secretion and position. However, more duck populations and gene expression analysis are required to further confirm our results. Moreover, our results demonstrated that these polymorphisms have a functional effect on fat accumulation. In our previous study, eight SNPs were identified in the VLDLR epidermal growth factor (EGF) precursor homologous domain, and a significant association was revealed between the homologous domain of the VLDLR EGF precursor and the AFP in ducks (Zhao et al., 2015). In the present study, we obtained similar results through the polymorphism of signal peptide regions. Our research further demonstrated that VLDLR can be considered as a candidate gene for abdominal fat deposition. The growth pattern in ducklings shows high and rapid-growth rate during the initial raising period. The growth pattern in ducks approaches the Gompertz curve (Maruyama et al., 2001). The age at the inflection point, at which the curvature changes sign, is approximately 5 weeks for Gaoyou ducks. Ducks grow less rapidly after the inflection point. No significant difference ($P > 0.05$) in body weight was observed across different diplotypes from birth to the age of 5 weeks. A possible reason for this phenomenon was that Gaoyou ducks were in a rapid-growth phase, so body weight differences were not noticeable even though different diplotypes were evaluated.

Clearly, the *VLDLR* gene is worth studying further as a potential candidate gene involved in growth and fat deposition and for the genotyping of these alleles in other resource populations and in elite lines of commercial duck breeders.

In summary, as far as we know, this is the first study to investigate the associations of SNPs in the *VLDLR* gene 5′-UTR and signal peptide region with duck growth and abdominal fat deposition. The results suggest that the *VLDLR* gene plays an important role in the regulation of body weight and abdominal fat deposition and may be used as a potential marker in the molecular MAS program during duck breeding. Further investigations with different duck populations and larger sample sizes are needed to confirm this point.

Data availability. No data sets were used in this article.

Competing interests. The authors declare that they have no conflict of interest.

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References

Ardlie, K. G., Kruglyak, L., and Seielstad, M.: Patterns of linkage disequilibrium in the human genome, Nat. Rev. Genet., 3, 299–309, 2002.

Boycott, K. M., Flavelle, S., Bureau, A., Glass, H. C., Fujiwara, T. M., Wirrell, E., Davey, K., Chudley, A. E., Scott, J. N., McLeod, D. R., and Parboosingh, J. S.: Homozygous deletion of the very low density lipoprotein receptor gene causes autosomal recessive cerebellar hypoplasia with cerebral gyral simplification, Am. J. Hum. Genet., 77, 477–483, 2005.

Brockmann, G. A., Haley, C. S., Renne, U., Knott, S. A., and Schwerin, M.: Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth, Genetics, 150, 369–381, 1998.

Castro, N. P., Euclydes, V. V., Simões, F. A., Vaz-de-Lima, L. R., De Brito, C. A., Luzia, L. A., Devakumar, D., and Rondó,

P. H.: The Relationship between Maternal Plasma Leptin and Adiponectin Concentrations and Newborn Adiposity, Nutrients, 9, E182, https://doi.org/10.3390/nu9030182, 2017.

Clemente-Postigo, M., Queipo-Ortuno, M. I., Fernandez-Garcia, D., Gomez-Huelgas, R., and Tinahones, F. J.: Adipose tissue gene expression of factors related to lipid processing in obesity, PLoS ONE 6, e24783, https://doi.org/10.1371/journal.pone.0024783, 2011.

Crawford, D. C., Nord, A. S., Badzioch, M. D., Ranchalis, J., McKinstry, L. A., Ahearn, M., Bertucci, C., Shephard, C., Wong, M., Rieder, M. J., Schellenberg, G. D., Nickerson, D. A., Heagerty, P. J., Wijsman, E. M., and Jarvik, G. P.: A common VLDLR polymorphism interacts with APOE genotype in the prediction of carotid artery disease risk, J. Lipid Res., 49, 588–596, 2008.

Eppig, J. T., Blake, J. A., Bult, C. J., Kadin, J. A., and Richardson, J. E.: The Mouse Genome Database (MGD): facilitating mouse as a model for human biology and disease, Nucleic Acids Res., 43, 726–736, 2015.

Frykman, P. K., Brown, M. S., Yamamoto, T., Goldstein, J. L., and Herz, J.: Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipo-protein receptor, P. Natl. Acad. Sci. USA, 92, 8453–8457, 1995.

Gao, Y., Shen, W., and Lu, B.: Upregulation of hepatic VLDLR via PPARα is required for the triglyceride-lowering effect of fenofibrate, J. Lipid Re., 55, 1622–1633, 2014.

Gibbs, R., Belmont, J., Hardenbol, P., Willis, T., Yu, F., and Yang, H.: The International HapMap Consortium, The International HapMap Project, Nature, 426, 789–796, 2003.

Go, G. W. and Mani, A. :Low-density lipoprotein receptor (LDLR) family orchestrates cholesterol homeostasis, Yale J. Biol. Med. Mar., 85, 19–28, 2012.

Goudriaan, J. R., Tacken, P. J., Dahlmans, V. E., Gijbels, M. J., van Dijk, K. W., Havekes, L. M., and Jong, M. C.: Protection from obesity in mice lacking the VLDL receptor, Arterioscl. Throm. Vas., 21, 1488–1493, 2001.

Goudriaan, J. R., Espirito Santo, S. M. S., Voshol, P. J., Teusink, B., van Dijk, K. W., and van Vlijmen, B. M. J.: The VLDL receptor plays a major role in chylomicron metabolism by enhancing LPL-mediated triglyceride hydrolysis, J. Lipid Res., 45, 1475–1481, 2004.

Gwang-Woong, G. and Arya, M.: Low-density lipoprotein receptor (LDLR) family orchestrates cholesterol homeostasis, Yale J. Biol. Med., 85, 19–28, 2012.

Kim, O. Y., Lee, S. M., Chung, J. H., Do, H. J., Moon, J., and Shin, M. J.: Arginase I and the very low-density lipoprotein receptor are associated with phenotypic biomarkers for obesity, Nutrition, 28, 635–639, 2012.

Kunej, T., Jevsinek Skok, D., Zorc, M., Ogrinc, A., Michal, J. J., Kovac, M., and Jiang, Z.: Obesity gene atlas in mammals, J. Genomics, 1, 45–55, 2012.

Kyosseva, S. V., Chen, L., Seal, S., and McGinnis, J. F.: Nanoceria inhibit expression of genes associated with inflammation and angiogenesis in the retina of Vldlr null mice, Exp. Eye Res., 116, 63–74, 2013.

Maruyama, K., Vinyard, B., and Akbar, M. K.: Growth curve analyses in selected duck lines, Brit. Poultry Sci., 42, 574–582, 2001.

Nguyen, A., Tao, H., Metrione, M., and Hajri, T.: Very low density lipoprotein receptor (VLDLR) expression is a determinant factor in adipose tissue inflammation and adipocyte-macrophage interaction, J. Biol. Chem., 289, 1688–1703, 2014.

Ning, T., Zou, Y., Yang, M., Lu, Q., Chen, M., Liu, W., Zhao, S., Sun, Y., Shi, J., Ma, Q., Hong, J., Liu, R., Wang, J., and Ning, G.: Genetic interaction of DGAT2 and FAAH in the development of human obesity, Endocrine, https://doi.org/10.1007/s12020-017-1261-1, online first, 2017.

Rankinen, T., Zuberi, A., Chagnon, Y. C., Weisnagel, S. J., Argyropoulos, G., Walts, B., Perusse, L., and Bouchard, C.: The human obesity gene map: the 2005 update, Obes. Res., 14, 529–644, 2006.

Shen, X., Steyrer, E., Retzek, H., Sanders, E. J., and Schneider, W. J.: Chicken oocyte growth: receptor-mediated yolk deposition, Cell Tissue Res., 272, 459–471, 1993.

Suwa, A., Yoshino, M., Yamazaki, C., Naitou, M., Fujikawa, R., and Matsumoto, S. I.: Rmi1 deficiency in mice protects from diet and genetic-induced obesity, Febs Journal, 277, 677–686, 2010.

Tacken, P. J., Beer, F. D., Vark, L. C., Havekes, L. M., Hofker, M. H., and Willems Van Dijk, K.: Very-low-density lipoprotein binding to the apolipoprotein E receptor 2 is enhanced by lipoprotein lipase, and does not require apolipoprotein E, Biochem. J., 347, 357–361, 2000.

Takahashi, S., Suzuki, J., Kohno, M., Oida, K., Tamai, T., Miyabo, S., Yamamoto, T., and Nakai, T.: Enhancement of the binding of triglyceride-rich lipoproteins to the very low density lipoprotein receptor by apolipoprotein E and lipoprotein lipase, J. Biol. Chem., 270, 15747–15754, 1995.

Takahashi, S., Sakai, J., Fujino, T., Hattori, H., Zenimaru, Y., Suzuki, J., Miyamori, I., and Yamamoto T. T.: The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor, J. Atheroscler. Thromb., 11, 200–208, 2004.

Takazawa, T., Yamauchi, T., Tsuchida, A., Takata, M., Hada, Y., Iwabu, M., Okada-Iwabu, M., Ueki, K., and Kadowaki, T.: Peroxisome proliferator-activated receptor gamma agonist rosiglitazone increases expression of very low density lipoprotein receptor gene in adipocytes, J. Biol. Chem., 284, 30049–30057, 2009.

Tao, H. and Hajri, T.: Very low density lipoprotein receptor promotes adipocyte differentiation and mediates the proadipogenic effect of peroxisome proliferator-activated receptor gamma agonists, Biochem. Pharmacol., 82, 1950–1962, 2010.

Tao, H., Aakula, S., Abumrad, N. N., and Hajri, T.: Peroxisome proliferator-activated receptor-gamma regulates the expression and function of very-low-density lipoprotein receptor, Am. J. Physiol. Endocrinol. Metab., 298, E68–E79, 2010.

Willnow, T. E.: The low-density lipoprotein receptor gene family: multiple roles in lipid metabolism, J. Mol. Med., 77, 306–315, 1998.

Wang, C., Li, S. J., Yu, W. H., Xin, Q. W., Li, C., Feng, Y. P., Peng, X. L., and Gong, Y. Z.: Cloning and Expression Profiling of the VLDLR Gene Associated with Egg Performance in Duck (Anas Platyrhynchos), Genet. Sel. Evol., 43, 144–144, 2011.

Wu, Y., Pi, J. S., Pan, A. L., Du, J. P., Shen, J., Pu, Y. J., and Liang, Z. H.: Two novel linkage SNPs of VLDLR gene intron 11 are associated with laying traits in two quail populations, Arch. Anim. Breed., 58, 1–6, https://doi.org/10.5194/aab-58-1-2015, 2015.

Yuan, G., Liu, Y., Sun, T., Xu, Y., Zhang, J., Yang, Y., Zhang, M., Cianflone, K., and Wang, D. W.: The therapeutic role of very

low-density lipoprotein receptor gene in hyperlipidemia in type 2 diabetic rats, Hum. Gene Ther., 22, 302–312, 2011.
Zhao, N., Lin, S., Wang, Z., and Zhang, T.: VLDLR gene polymorphism associated with abdominal fat in Gaoyou domestic duck breed, Czech J. Anim. Sci., 60, 178–184, 2015.