

Full Length Research Paper

Polymorphism in exon 1 of adiponectin gene and its association with water holding capacity, IMF and abdominal fat in duck

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Accepted 29 April, 2010

The objective of the present study was to investigate the effect of polymorphism in adiponectin gene on meat quality traits, serum total cholesterol, serum triglyceride and abdominal fat of 170 individuals from Cherry Valley duck (CV), Jinding duck (JD) and Hybrid duck (CV × JD) (HB) populations. PCR-SSCP technique was developed to analyze a 230 bp region of the adiponectin gene exon 1. Three genotypes (CC, CD and DD), which were the products of two alleles (C and D) were observed. Alignment sequences' results showed that four SNPs (C86T, C104T, C146T and C155T) were found and all of those nucleotide variations were nonsense mutations. Association analysis indicated that all of these traits had significant population effects except meat colour ($P < 0.05$) and then the birds with homozygote (CC) had significant lower than homozygotes (DD) for IMF, water holding capacity and abdominal fat ($P < 0.05$). The research suggested that genotype DD may be an advantage genotype for fat deposition in duck. The adiponectin gene exon 1 polymorphism could be used in marker assistant selection (MAS) as a genetic marker for the birds' fat deposition.

Key words: Duck, adiponectin gene, polymorphism, meat quality, fatness.

INTRODUCTION

Modern strains of ducks exhibit excessive body fat deposition, which is one of the main problems encountered by duck industry today. Excess fat deposition has led commercial breeders to incorporate significant selection for reducing body fatness in breeding programs. Therefore, considerable research effort has been applied to study factors associated with fat deposition and methods to reduce it (Shaping et al., 2000; Wawro et al., 2004). The

use of polymorphic genes as detectable molecular markers is a promising alternative to the current methods of trait selection once these genes are proven to be associated with economic traits in animal. Selection efficiency, however, depends on allelic frequencies in the breeds and the effect of these polymorphisms on selected traits. Understanding the genetic control of meat quality and lipid metabolism in duck will provide an opportunity for genetic enhancement of production performance (Deeb and Lamont, 2002). The combination of traditional genetics and breeding methods and modern molecular biology methods may be preferred for genetic improvement of duck in the future.

Adiponectin is a discovered adipocytokine hormone, which is specifically and highly expressed in mammalian adipose tissue (Maeda et al., 1996). Adiponectin has been found to have a profound effect on glucose utilization, insulin sensitivity, lipid synthesis and energy homeostasis in several mammalian species. Adiponectin,

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Abbreviations: PCR-SSCP, Polymerase chain reaction-single strand conformation polymorphism; **SNPs**, single nucleotide polymorphisms; **PPAR α** , peroxisome proliferator-activated receptor alpha; **IMF**, intramuscular fat; **EDTA**, ethylene diamine tetraacetic acid; **VLDL**, very low density lipoprotein; **QTLs**, quantitative trait loci; **MAS**, molecular assistant selection.

CD DD CD DD CD CD CC CD CC DD CC

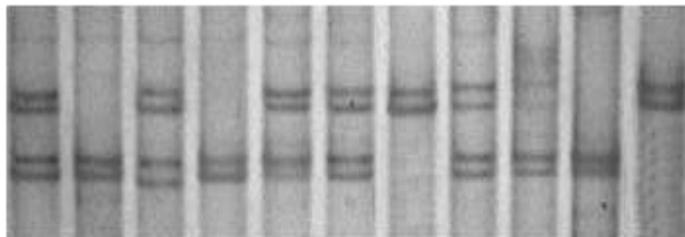


Figure 1. PCR-SSCP band pattern on a 10 % non-denaturing polyacrylamide gel.

also known as apM1, Acrp30, adipoQ and GBP28, which is an approximately 30-kDa polypeptide containing an N-terminal signal sequence, a variable domain, a collagen-like domain and a C-terminal globular domain (Hu et al., 1996; Nakano et al., 1996; Scherer et al., 1995). AdipoR1 and AdipoR2 serve as receptors for globular and full length adiponectin and mediate adiponectin stimulation of AMP kinase, PPAR α ligand activities, fatty acid oxidation and glucose uptake (Yamauchi et al., 2003; Luo et al., 2005; Monika et al., 2006; Chinetti et al., 2004). Adiponectin treatment or overexpression of the adiponectin gene protects overfed rats from gaining weight and from developing cardiovascular diseases by suppressing glucose production and enhancing lipid oxidation (Yamauchi et al., 2002). Overexpression of adiponectin also significantly decreases free fatty acids and triglycerides in genetically obese mice (Zhu et al., 2004). Whereas adiponectin has been well characterized in mammalian species, but non mammalian adiponectin has not been well described in the past. Up to now, the research about association of polymorphisms in duck adiponectin gene with production traits has not been reported. The objectives of the present study were to identify SNPs in the duck adiponectin gene exon 1 and evaluate its association with meat quality, serum total cholesterol, serum triglyceride and abdominal fat in Cherry Valley duck (CV), Jinding duck (JD) and Hybrid duck (CV \times JD) (HB) populations.

MATERIALS AND METHODS

Samples collection and preparation

Blood samples were collected from 170 individuals that belong to three duck populations: Cherry Valley duck (CV) (55), Jinding duck (JD) (55) and Hybrid duck (CV \times JD) (HB) (60). Ducks were reared in the same management system in College of Jiangsu Animal Husbandry and Veterinary located in Taizhou, Jiangsu, P.R. China. The 170 ducks were slaughtered at the end of 70 days. These traits, such as pH, tenderness (shear value)(Kg/cm²), meat colour (OD₅₄₀), water holding capacity (%), intramuscular fat (IMF)(%), serum total cholesterol (TC) (mmol/L), serum triglyceride (TG) (mmol/L) and abdominal fat (%) were collected for statistical analysis (Li et al., 2005; Musa et al., 2007). Genome DNA was obtained by phenol and chloroform (1: 1) extraction and stored at –

20°C.

Primer design and PCR amplification

Based upon duck adiponectin gene sequences (Accession no. DQ452618), the following forward primer: 5'-CACTTCAGGAACGC ACCATG-3' and reverse primer: 5'-ACCTTGCTCTCCTTTCTCTC-3', were designed using the Oligo 6.0 software to amplify a 230 bp fragment of the adiponectin gene exon 1. PCR reactions were carried out in a total volume of 25 μ L with 100 ng of genomic DNA, 5 pmol of each of forward and reverse primer, 2.5 μ L of 10 \times buffers, 1.5 mM of MgCl₂, 0.16 mM of dNTP and 1 U of Taq DNA polymerase (Fermentas). PCR program: initial denaturation for 10 min at 95°C, 30 cycles each 40 s at 94°C, 40s at 58°C and 45 s at 72°C and 10 min final extension at 72°C.

Single stranded conformation polymorphism (SSCP) and sequencing

Aliquots of 5 μ L PCR products were mixed with 10 μ L denaturing solution (98% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and cooled on ice. Denatured PCR products were subjected to 10% acrylamide: bisacrylamide (29: 1) gels in 1 \times TBE buffer and constant voltage (130 V) for 12 - 14 h. The gel was stained with 0.1% silver nitrate. PCR fragments from different SSCP patterns in different populations were sun-cloned to pMD19-T vector (Takara, China). All fragments were sequenced in both directions.

Statistical analysis

χ^2 tests were used to determine whether the individual variant was in accordance with Hardy-Weinberg equilibrium. The genetic parameters of allele gene and genotype frequency, effective number of alleles (N_e), heterozygosity (h) and polymorphism information content (PI_C) were estimated. The effects of the observed adiponectin gene exon 1 genotypes on traits were analyzed using a General Linear Model (GLM). The following statistical model was used:

$$Y = \mu + G + B + G \times B + e$$

where: Y – dependent variable (analyzed traits), μ – overall mean, G – genotype of adiponectin exon-1 (CC, CD and DD), B – duck population, G \times B – interactions between genotype and duck population (all fixed effects) and e – random error. Difference between genotypes was determined by least square analysis.

RESULTS

Single nucleotide polymorphism

A 230 bp region of the adiponectin gene exon 1 was analyzed by PCR-SSCP and its single nucleotide polymorphisms (SNPs) were detected by cloned sequencing. Three genotypes (CC, CD and DD) and two alleles (C and D) were observed in different individuals and populations (Figure 1). Alignment sequences results showed that four SNPs (C86T, C104T, C146T and C155T) were found in exon 1 of adiponectin in 170 duck from three populations (CV, JD and HD) (Figure 2) and had not

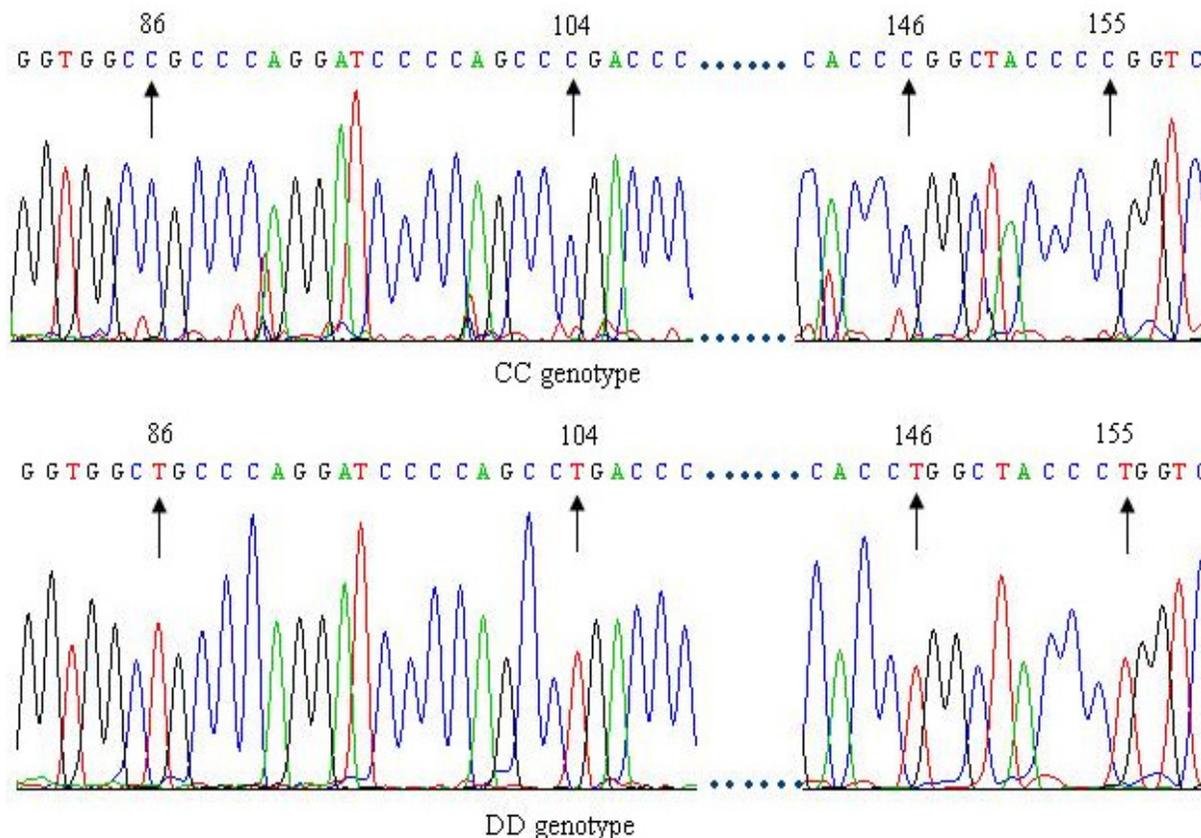


Figure 2. The sequencing result of CC and DD genotypes (the arrows indicate mutation positions).

Table 1. Allele and genotype frequencies of the SNP in exon 1 of adiponectin gene in three duck populations.

Population	Genotype frequencies			Allele frequencies		<i>h</i>	<i>Ne</i>	<i>PIC</i>	χ^2 (HWE)
	CC	CD	DD	C	D				
CV	0.0727 (4)	0.4000 (22)	0.5273 (29)	0.2727	0.7273	0.3966	1.6573	0.3179	0.00
JD	0.4000 (22)	0.3818 (21)	0.2182 (12)	0.5909	0.4091	0.4834	1.9357	0.3665	4.26
HD	0.2000 (12)	0.5667 (34)	0.2333 (14)	0.4833	0.5167	0.4994	1.9976	0.3747	0.54

CV = Cherry valley duck; JD = jinding duck; HB = hybrid duck of CV × JD; *h* = heterozygosity; *Ne* = effective number of alleles; *PIC* = polymorphism information content, and χ^2 (HWE) = Hardy-Weinberg equilibrium χ^2 value.

resulted in any amino acid change.

Allele and genotype distribution

Allele gene and genotype frequency, effective number of alleles (*Ne*), heterozygosity (*h*) and polymorphism information content (*PIC*) for each breed were presented in Table 1. D allele frequencies were higher than C allele in CV and HD populations except JD population. The D allele frequencies for the exon 1 of adiponectin gene were 0.7273, 0.4091 and 0.5167 for CV, JD and HD populations, respectively. In three duck populations, genotypes frequencies were in accordance with Hardy-

Weinberg equilibrium ($P > 0.05$). Simultaneously, the locus belonged to medium polymorphism in three populations ($0.25 < PIC < 0.5$).

Allelic effect of the adiponectin gene exon 1 on meat quality, serum total cholesterol and serum triglyceride

The allelic effects of the adiponectin gene exon 1 on meat quality, serum total cholesterol, serum triglyceride and abdominal fat in 170 samples of three duck populations were showed in Table 2. The birds with homozygote (CC) and heterozygote (CD) had significant lower than homo-

Table 2. Effect of the adiponectin gene exon 1 SNPs genotypes on meat quality, serum total cholesterol and serum triglyceride in duck.

Trait	Genotype CC (Mean \pm S.E) (n = 38)	Genotype CD (Mean \pm S.E) (n = 77)	Genotype DD (Mean \pm S.E) (n = 55)
pH-value	6.05 \pm 0.04	5.99 \pm 0.03	6.06 \pm 0.03
Meat colour (OD ₅₄₀)	1.03 \pm 0.06	1.06 \pm 0.04	1.06 \pm 0.05
Shear value (kg/cm ²)	2.11 \pm 0.10	2.11 \pm 0.08	1.98 \pm 0.08
Water holding capacity (%)	15.69 \pm 1.00 ^a	16.75 \pm 0.75 ^{ab}	18.27 \pm 0.82 ^b
Intramuscular fat (IMF) (%)	7.01 \pm 0.41 ^a	8.12 \pm 0.29 ^b	9.02 \pm 0.34 ^c
Serum total cholesterol (mmol/L)	6.04 \pm 0.21	5.61 \pm 0.15	5.80 \pm 0.17
Serum triglyceride (mmol/L)	1.22 \pm 0.08	1.14 \pm 0.06	1.07 \pm 0.06
Abdominal fat (%)	0.72 \pm 0.14 ^a	1.04 \pm 0.09 ^a	1.34 \pm 0.10 ^b

Parameters with different superscript letters (a, b, c) were significantly different (LSD test, $P < 0.05$) in genotypes CC, CD and DD; n: number of the genotype in the populations.

Table 3. Effects (P-value) of adiponectin gene exon 1 SNPs genotypes on duck meat quality, serum total cholesterol and serum triglyceride.

Trait	Genotype	Population	Genotype \times Population
pH-value	0.328	< 0.001	< 0.001
Meat colour (OD ₅₄₀)	0.911	0.113	0.492
Shear value (kg/cm ²)	0.500	< 0.001	< 0.001
Water holding capacity (%)	0.128	< 0.001	< 0.001
Intramuscular fat (%)	0.001	< 0.001	< 0.001
Serum total cholesterol (mmol/L)	0.277	< 0.001	< 0.001
Serum triglyceride (mmol/L)	0.372	< 0.001	< 0.001
Abdominal fat (%)	0.003	< 0.001	< 0.001

zygotes (DD) for intramuscular fat (IMF) and abdominal fat ($P < 0.05$). However, for water holding capacity, the birds with homozygote (CC) had significant lower than homozygotes (DD) ($P < 0.05$). The birds with heterozygote (CD) alleles had significantly higher intramuscular fat ($P < 0.05$) than homozygotes (DD). Furthermore, no significant association of different genotypes with other traits were detected ($P > 0.05$). The study indicated that the locus linked strongly with the QTL controlling meat quality and lipid metabolism. Therefore, it was presumed that it may have a QTL controlling fatness deposition and meat quality.

Interaction between the adiponectin gene exon 1 and genetic background of three duck populations

The interactions between the genotypes of apoVLDL-II gene exon 1 and genetic background of three duck populations were showed in Table 3. Only the meat colour had no population and genotype \times population effects ($P > 0.05$), while other traits had significant populations and genotype \times population effects ($P < 0.05$). Furthermore, intramuscular fat (%) and abdominal fat (%) had significant genetic effects ($P < 0.05$).

DISCUSSION

Adipose tissue is considered not only to be a tissue passively storing energy under the form of triglycerides, but also to be a hormonally active system participating in the control of whole body metabolism. So far, adiponectin gene has only been found, a negative correlation with the obesity gene. In avian species, a majority of the lipids in the chicken are synthesized in the liver but not in the adipose tissue as in mammals. Furthermore, in poultry, many studies showed that adiponectin gene mRNA was expressed in various tissues but not in the blood cells and its expression was higher in adipose tissue (Sreenivasa et al., 2005; Yuan et al., 2006; Xu et al., 2008; Zhu et al., 2009). So, adiponectin is likely to play a dominant role in carbohydrate and lipid metabolism in avian species because poultry maintain a very high blood glucose concentration such as chicken, duck, goose and so on (Brady et al., 1978). In this study, we firstly found 4 SNPs (C86T, C104T, C146T and C155T) in exon 1 of adiponectin gene and all of those nucleotide variations were nonsense mutations. Allele C was predominant except JD population. In three duck populations, genotypes frequencies were in accordance with Hardy-Weinberg equilibrium ($P > 0.05$) and the locus belonged to medium

polymorphism ($0.25 < PIC < 0.5$). These results have difference with Dong et al. (2007), who found seven nucleotide variations in the whole coding region of adiponectin gene, but all of those mutations, G430A, A457G, C507T, T523C, T540C, C576T and C597T, occurred in exon 2, meanwhile, among those mutations, G430A, A457G and T523C resulted in amino acid changes which were changed into A144T, I153V and Y175H, respectively. Furthermore, the research suggested that in the commercial breeding of ducks, those mutations in exon 1 of adiponectin gene have not been selected directly or indirectly, or it was in accordance with Hardy-Weinberg equilibrium after selecting.

Poultry meat quality is very important to keep up with consumer demand and its final expression is the result of interaction among genetic, nutritional, age and environmental factors. Fatness plays an important role in meat quality. The candidate gene approach is a very powerful method to investigate associations of gene polymorphisms with economically important traits in farm animals (Rothschild and Soller, 1997; Lamont et al., 1996). In this study, the adiponectin gene was selected as a candidate gene to investigate associations of gene polymorphisms with meat quality, serum total cholesterol, serum triglyceride and abdominal fat in three duck populations by using fixed model. The results indicated that that all of these traits had significant population effects except meat colour ($P < 0.05$) and then the birds with homozygote (CC) had significant lower than homozygotes (DD) for IMF, water holding capacity and abdominal fat ($P < 0.05$). Fatness is quite highly heritable in birds. The highest heritability (up to 0.73) was obtained for abdominal fat weight (Zerehdaran et al., 2004). Adipose tissue growth in birds depends mainly on the availability of triglycerides transported by VLDL (Whitehead and Griffin, 1984). In general, body fat accumulation may be considered the net result of the balance among dietary absorbed fat, endogenous fat synthesis (lipogenesis) and fat catabolism via β -oxidation (lipolysis). Carcass fatness had a significant and positive effect on flavor, water holding capacity and juiciness of dark meat. Zhang et al. (2006) reported that IMF content had a greatly negative correlation with water content in chicken. Thus, if the amount of absorbed fat is the same, lower body fat deposition may be attributed to increased fat catabolism of diminished endogenous fatty acid synthesis of both processes (Sanz et al., 2000). Many studies showed that adiponectin had a negative correlation on IMF, TC and TG of serum in the Japanese population (Yamamoto et al., 2002). The polymorphism locus linked strongly with the QTL controlling fatness traits. Therefore, the research conjecture that the adiponectin gene may be a major candidate gene or linked to a major candidate gene that impact duck lipid metabolism and the SNPs could be used in molecular assistant selection (MAS) as a genetic marker for duck fatness traits. However, an explanation for the results was also probably due to the low animal

numbers. We will conduct further tests with higher number of samples in the future.

ACKNOWLEDGEMENTS

This study was supported by Chinese National Key Programs (2006BDA01A09 and 2006BDA14B06) and Modern Agro-industry Technology Research System (nycytx-45-04).

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