Six single nucleotide polymorphisms in adipocyte fatty acid-binding protein (*A-FABP)* gene in Beijing ducks

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ABSTRACT: PCR-SSCP was applied to analyze the polymorphisms of *A-FABP* gene in 4 lines of Beijing ducks (n = 400). The results showed that six SNPs were found in intron 3. There were no polymorphisms found in exon 3 or exon 4. The discovered SNPs were deposited in GenBank (Acc. No.: EU306611 and EU306610). The frequencies of haplotypes A/B in the Z4, Z2, Cherry Valley, Z4 × Z2 populations were 0.745/0.255, 0.764/0.236, 0.552/0.448, 0.672/0.328, respectively. The linkage disequilibrium was stated. The above described SNPs of *A-FABP* gene allow the incoming association analysis.

Keywords: Beijing ducks; *A-FABP* gene; SNPs; PCR-SSCP

Fatty Acid-Binding Protein (*FABP*) is presumed to be an intracellular fatty acid transporter (Gerbens et al., 1998) and includes eight identified members. Fatty acids are bound by FABP at the cell membrane and transported to the sites of fatty acid oxidation, acylglycerol, or phospholipid synthesis (Veerkamp et al., 1993). The adipocyte Fatty Acid-Binding Protein (*A-FABP)* gene which is exclusively expressed in adipocytes can deposit triglyceride (TG) in the cadiocyte and adipocyte, and transfer fatty acids back to the plasma membrane after lipolysis. So the *A-FABP* gene was considered as an important candidate gene affecting intramuscular fat (IMF) content. Microsatellite analysis indicated that *A-FABP* loci were involved in the regulation of intramuscular fat accretion in Duroc pigs, with significant contrast of approximately 1% IMF between certain genotype classes (Gerbens et al., 1998). The study of *Gallus* by SNPs in *A-FABP* gene also proved that meat quality traits were significantly related to different genotypes (Ye et al., 2007). But until now, polymorphisms of the duck *A-FABP* gene were not reported. In this study, the single nucleotide polymorphisms (SNPs) of *A-FABP* gene were screened in four lines of Beijing ducks.

MATERIAL AND METHODS

Primer sequences

Based on *Anas A-FABP* gene (Acc. No. DQ358123), 2 pairs of primers were designed to amplify exon 3, intron 3 and partial exon 4.

The primers were as follows:

Exon 3, Forward: 5'-GTGTGGGATTTGCTACCAGGAA-3', Reverse: 5'-TCTGTCATCTGCTGTGGTCTCAT-3', the primers amplified a product of 166 bp.

Supported by the National 'Eleventh Five Years' Program of China (No. 2006BDA01A09).

Table 1. SNPs and allele frequencies of *A-FABP* gene among four lines of Beijing Ducks (n = 400)[1]

Lines	Genotype frequencies			Allele frequencies	
	AA	*AB*	*BB*	*A*	*B*
Z4	0.564	0.364	0.073	0.745	0.255
Z2	0.611	0.278	0.111	0.764	0.236
Cherry Valley	0.291	0.456	0.253	0.552	0.448
Z4 × Z2	0.413	0.508	0.079	0.672	0.328

[1]in each line 50 males and 50 females

Intron 3, Forward: 5'-GAGACCACAGCAGATGACAGAA-3', Reverse: 5'-CTTTATGATAGTCCCTTTGCCA-3', the primers amplified a product of 319 bp.

PCR conditions

The 20 μl PCR amplification contained 50 ng of genomic DNA, 10 pmol/l of each primer, dNTPs (0.25 mmol/l), $MgCl_2$ (1.5 mmol/l), and 1 U *Taq* DNA polymerase (Tiangen Biotech, Beijing, China). The cycling protocol was 5 min at 95°C, 30 cycles at 94°C for 35 s, annealing at 54°Cor 55°C corresponding to 2 different primer pairs for 30 s, 72°C for 45 s, with final extension at 72°C for 8 min. Polymorphisms of *A-FABP* gene were detected by SSCP after PCR products were denatured 10 min at 98°C. The gel was stained with silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde). The PCR products from individuals which represented different PCR-SSCP patterns were purified and sequenced in both directions.

RESULTS AND DISCUSSION

Complete intron 3 and partial exon 3 (30 bp), exon 4 (72 bp) were cloned and the sequence was deposited in GenBank (Acc. No.: EU306610). In this sequence, six SNPs were found in intron 3 of the *A-FABP* gene among 400 Beijing ducks which belonged to lines Z4, Z2, Cherry Valley, and Z4 × Z2, in all lines 50 males and 50 females. These mutations were located at g.106A>T, g.108T>C, g.123T>C, g.164C>T, g.215C>A and g.231T>C, respectively. Two haplotypes were found, namely haplotype A (AATTCCT), and B (TCCTAC). The frequencies of haplotypes A/B in the Z4, Z2, Cherry Valley, Z4 × Z2 populations are shown in Table 1. The discovered SNPs were deposited in GenBank (Acc. No.: EU306611). The PIC in Z4, Z2, Cherry Valley and Z4 × Z2 showed medial polymorphisms ($0.25 < PIC < 0.5$), which were 0.306, 0.305, 0.375, 0.346, respectively. No mutation was found in exon 3 and exon 4. Previous reports showed that mutations in intron as well as silent mutations in coding regions and other polymorphisms without obvious functional relevance were useful for the evaluation of associations with production traits (Dybus et al., 2006; Jedrzejczak et al., 2006). Hence, the above described SNPs of *A-FABP* gene allow the incoming association analysis.

REFERENCES

Dybus A., Grzesiak W. (2006): *GHRH/Hae* III gene polymorphism and its associations with milk production traits in Polish Black-and-White cattle. Archiv für Tierzucht, 49, 434–438.

Gerbens F., Jansen A., Van Erp A.J.M., Harders F., Meuwissen T.H.E., Rettenberger G., Veerkamp J.H., Te Pas M.F.W. (1998): The adipocyte fatty acid-binding protein locus: characterization and association with intramuscular fat content in pigs. Mammalian Genome, 9, 1022–1026.

Jędrzejczak M., Szatkowska I., Zych S., Grzesiak W., Czerniawska-Piątkowska E., Dybus A. (2006): Evaluation of associations of the polymorphism in the placenta specific promoter 1.1 of the CYP19 gene in Black-and-White and Jersey cattle with milk production traits. Archiv für Tierzucht, 49, 311–314.

Veerkamp J.H., Van Kuppevelt T.H.M.S.M., Maatman R.G.H.J., Prinsen C.F.M. (1993): Structural and func-

tional aspects of cytosolic fatty acid binding proteins. Prostaglandins Leukotrienes and Essential Fatty Acids, 49, 887–906.

Ye M.H., Wen J., Cao H.H., Li H.B., Chen J.L., Zhao G.P., Zheng M.Q. (2007): Study of polymorphisms in adipocyte fatty acid binding protein gene and their relationship with fatness related traits in chick. Chinese Journal of Animal and Veterinary Sciences, 38, 526–532.

Received: 2007–12–13
Accepted after corrections: 2008–11–25

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