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Association Analysis Between Polymorphism of Peroxisome Proliferator-activated Receptor α(*PPARα*) Gene and Slaughter Performance in Goose

Jun He, Xiangyong Qu and Changqing He
College of Animal Science and Technology, Key Laboratory of Animal Genetics and Breeding of Hunan Province, Hunan Agricultural University, Changsha, 410128, China

Corresponding Author: Jun He, College of Animal Science and Technology, Key Laboratory of Animal Genetics and Breeding of Hunan Province, Hunan Agricultural University, Changsha, 410128, China

ABSTRACT

PPAR is one cluster of the most important genes that related with lipid metabolism. And it can used as the index of goose strain selection. In this study, we collected 282 blood samples of 75 days Dongting goose to detect the SNPsof *PPARα* gene by PCR-SSCP. The results showed that in the gene sequence of 215bp had a mutation of C→T and the gene frequency of A,B were 0.393,0.607, respectively, the genotype frequency of AA, AB, BB were 0.126, 0.533, 0.341, respectively. So we can concluded the polymorphism of goose *PPARα* gene and the genotype frequency of heterozygote was higher in the test samples. After genotype detection of all samples, 35 AA and 96 BB genotype obtained. The abdominal fat weight of AA and BB genotype were 23.14±5.08 and 43.69±8.04, the abdominal fat ratio and liver weight ratio of AA and BB genotype were 0.80±0.30, 2.74±0.82 and 1.58±0.55, 2.87±0.69, respectively. By statistics of variance, the *PPARα* genotype was highly significantly associated with abdominal fat weight, abdominal fat ratio and liver weight ratio ($p<0.05$). In general, the abdominal fat weight, abdominal fat ratio and liver weight ratio showed a tendency of BB>AA, indicating that the common allele was the least favorable for abdominal fat deposition. Thus, there is an enormous opportunity to decrease abdominal fat deposition in case this allele is confirmed in other more studies.

Key words: *PPARα*, PCR-SSCP, goose, polymorphism

INTRODUCTION

With the development of living standard, produce and consume low-fat, high quality meat has become mainstream. Lipid metabolism is a very complicated biochemical response process and it cooperated with many regulation factors. *PPAR* gene is known to us that it has important effect to fat deposit and differentiation. Some researches (Kerenzvi *et al*., 1992; Schoonjans *et al*., 1996; Lemberger *et al*., 1996; Xie *et al*., 2005; Ma *et al*., 2011; Meng *et al*., 2002) have showed that activation of *PPAR* gene could induce redistribution of animal external fat and lean tissue, regulate many gene that participated in lipid metabolism. Especially some important enzymes of β-oxidation process, they charge of fat absorption, transportation, formation, decomposition and so on.

Dongting goose is a fine two-line Hybrid Breed. It is breeding and selection by SiChuan white goose and XuPu goose, which are famous goose breeds in China. Dongting goose mainly used its

meat, they have favorable growth rate, however, they also have relative strong ability of deposition external fat. So, if we can select low-fat goose line, it would bring considerable economic and social benefits, fulfill the need of produce and consume.

Now, seldom report about *PPAR* in goose. We screened the SNP of *PPAR* by PCR-SSCP, researched the polymorphism and its relate to production performance. Hope to find the relation of SNP and performance of goose. It will helpful to breeding of low-fat goose line and correlation researches in the future.

MATERIALS AND METHODS

Animals and samples preparation: Selected 282 test samples of 75 days Dongting goose. Each collected 1 mL blood sample from wing vein. Then add into 4% EDTA to prevent the blood from concreting. Adopted saturated phenol-chloroform method extracting genome DNA (Sambrook and Russell, 2001), stored in -20°C.

Primer design: According to the sequence of *PPARα* mRNA in GenBank, designed the prime by Prime Primer 5.0, covered partial coded sequence. Sequence of prime are: F: 5' AATCACCCAGTGGAGCAG 3', R: 5' CAGACCTTGGCATTCGTC 3'.

PCR-SSCP: Used the prime started to PCR and SSCP analysis. The PCR reaction system is: 10×buffer (Mg^{2+}) 1 μL, F, R primer (20 μmol L^{-1}) each 0.15 μL, dNTPs (10 μmol L^{-1}) 0.2 μL, Taq DNA polymerase 1U, DNA (100 ng) 0.7 μL and supplement ddH_2O to 10 μL totally volume.

Take 5 μL PCR production, mix with 10 μL denaturant, denaturalization 10 min at 98°C and put in ice 5 min at once. Then take 10 μL from them, keep voltage at 80~120V to 15% PAGE (He *et al*., 2006). When electrophoresis indicator reach bottom of the gel, terminate it and dyeing by silver staining method, record stripline and statistic every genotype number.

After electrophoresis, we PCR the DNA samples of homozygote and give them sequencing to ShangHai Invitrogen Company.

Statistical analysis: SAS program (8.2 version) was used to statistic and analysis all the test data (Xue *et al*., 2004). Allele and genotypes frequencies were calculated from the genotypes of the 282 goose, respectively. Hardy-Weinberg equilibrium in the studied population was tested by comparing expected and observed genotype frequencies using chi-square test. A linear model was established to analyze the genotypic effects of the locus:

$$y_{ijl} = \mu + s_i + g_j + e_{ijl}$$

where, y_{ijl} is an observation on the slaughter traits, μ is the overall mean, s_i is the effect of sex, g_j is the effect of genotype and e_{ijl} is the random residual. The data were analyzed by GLM procedure.

RESULTS

PCR-SSCP

Result of agarose gel electrophoresis of PCR: We amplified partial sequence of goose *PPAR*. From the result of agarose gel electrophoresis Fig. 1, the band of PCR ranged of 242~331 bp. The result showed that the PCR condition was suitable. The size of PCR fragment and aimed gene fragment is consistent and has nice specificity.

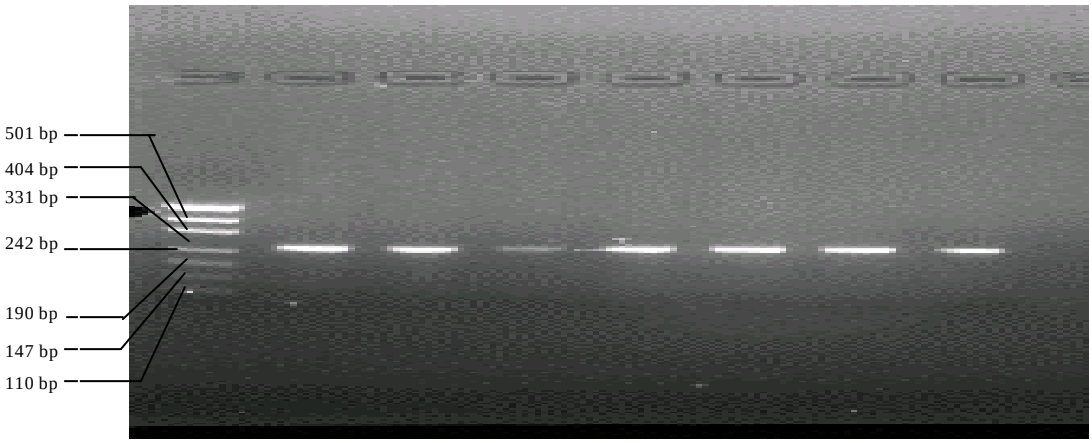

Fig. 1: Agarose gel electrophoresis of PCR product of goose *PPAR* gene

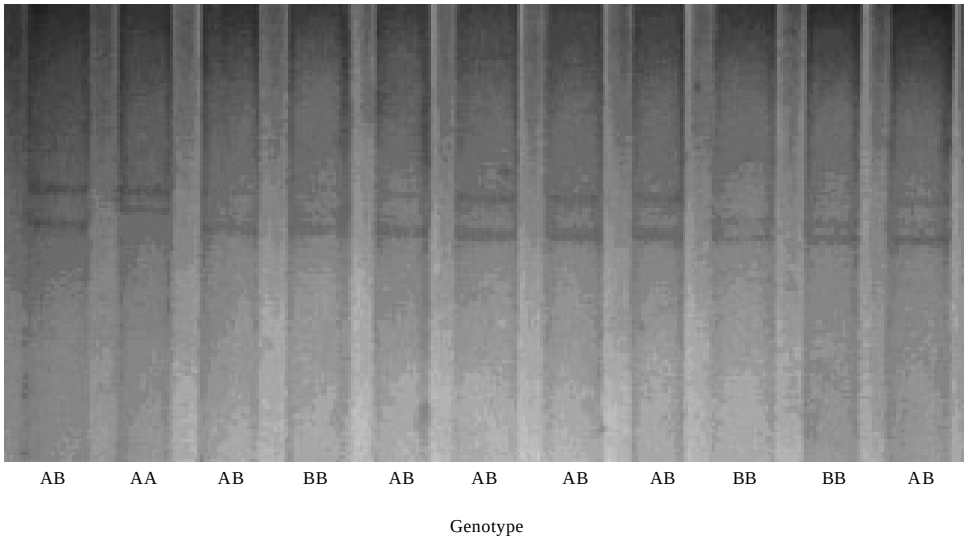

Fig. 2: PCR-SSCP electrophoresis of *PPAR* gene partial sequence

Genotypes 3 had come forth via SSCP: 2 homozygotic type and 1 heterozygotic type. According to the band location of electrophoresis, 2 homozygotic type were defined AA and BB genotype and 1 heterozygotic type was defined AB genotype (Fig. 2). Because the existed of SNP between AA and BB, space conformation of the two homozygotic type were different. This led the different result of electrophoresis. It showed that mutation would change its space conformation and electrophoresis could reflect the change.

Sequence analysis: After sequencing, the sequence of AA and BB gene are as follows:

- AA:ttgtggggataaagcctcaggctaccattacggagtacatgcttgtgaaggttgtaagggtttttttaggagaacaatccgatt gaaactcatctatgataaatgcgatcgcaattgcaaaattcagaaaaaaaaatcgtaataagtgccaatactgtcgttttcagaagt gcctttcagttggaatgtcacataatgcaatacgttttaaaaacaaaaacaggacgaatgccaaggtctg

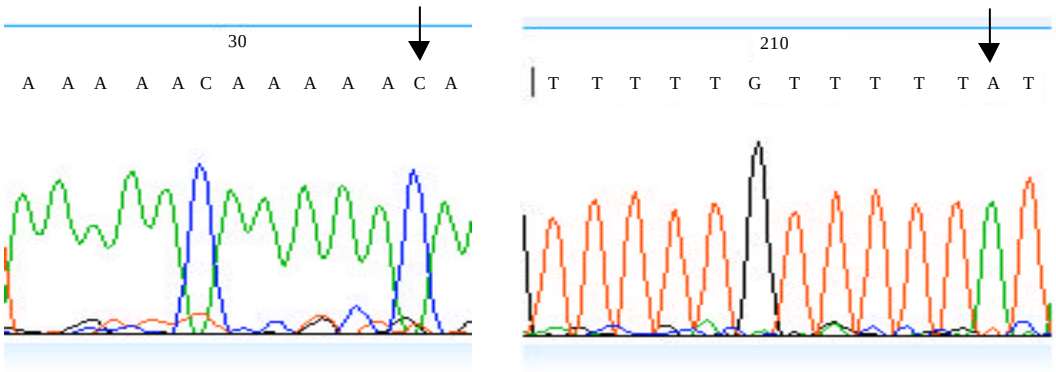

Fig. 3: Mutation site of *PPAR* gene partial sequence

Table 1: Table of gene frequency and genotype frequency

Genotype	Samples	Genotype frequency	Expectation frequency	Gene	Gene frequency	χ^2
AA	35	0.124	0.153	A	0.392	2.527
AB	151	0.536	0.477			
BB	96	0.340	0.370	B	0.608	

$\chi^2_{0.05(2)} = 5.99$

Table 2: Relation of genotype and performance in goose of different gender

Genotype	Gender	No.	Body weight (kg)	Liver weight (g)	Abdominal fat weight (g)	Semi-eviscerated (%)	Eviscerated (%)	Abdominal fat ratio (%)	Liver weight ratio (%)
AA	♂	15	3.82±0.43	82.42±6.23	20.32±4.21^b	88.65±2.67	77.44±3.67	0.69±0.26^B	2.79±0.95^B
	♀	20	3.68±0.35	76.66±4.25	25.26±5.73^a	86.51±3.26	76.96±3.52^a	0.89±0.33^a	2.71±0.73^b
	$\bar{x}$	35	3.74±0.38	79.13±5.10	23.14±5.08*	87.43±3.00*	77.17±3.58	0.80±0.30	2.74±0.82
BB	♂	31	3.79±0.51	84.61±7.64	38.59±7.65^a	85.81±3.92	76.55±4.15	1.33±0.52^A	2.91±0.86^A
	♀	65	3.63±0.46	77.32±6.12	46.12±8.22^b	84.38±4.71	74.68±5.37^b	1.70±0.57^b	2.85±0.61^a
	$\bar{x}$	96	3.68±0.48	79.67±6.61	43.69±8.04	84.84±4.45	75.28±4.98	1.58±0.55*	2.87±0.69*

In the same column, the different capital letters mean significant difference of ♂ in different genotype ($p<0.05$), the different small letters mean significant difference of ♀ in different genotype ($p<0.05$) and *mean significant difference of means in different genotype

- BB:attgtggggataaagcctcaggctaccattacggagtacatgcttgtgaaggttgtaagggttttttaggagaacaatccgat tgaaactcatctatgataaatgcgatcgcaattgcaaaattcagaaaaaaaatcgtaataagtgccaatactgtcgttttcagaagt gcctttcagttggaatgtcacataatgcaatacgttttaaaaacaaaaataggacgaatgccaaggtctg

Selected PCR product of AA and BB, send them to sequencing. From the figure of above, we can deduce that the mutation of C→T in two gene sequence at 215 bp Fig. 3.

Allele frequency and genotype frequency: Upon chi-square test, the difference of our research group was not significant ($p>0.05$). It showed that the population was fitting hardy-weinberg law, belonged to balance population. Gene frequency of A was 0.392 and B was 0.608. the specific results were listed in Table 1.

By way of ANOVA and multiple comparisons, the results were shown in Table 2, the difference of abdominal fat weight, abdominal fat ratio and liver weight ratio of gander, goose and their means in different genotype were significant ($p<0.05$). It indicated that the Dongting goose has different fat deposit performance in different genotype. From this point, we could deduce that SNP

may interrelate with the performance in a certain extent. It can affect the lipid metabolism. Furthermore, the semi-eviscerated percentage of means and eviscerated percentage of goose had significant difference in genotype of AA and BB ($p<0.05$).

DISCUSSION

SSCP is a rapid, simple and sensitive mutation detection method. In order to achieve the best results of SSCP, we should pay attention to electrophoresis voltage and temperature. For maintain a stable conformation of single-strand DNA, SSCP should be carried out under low temperature (4~15°C). In addition electrophoresis process temperature, high voltage caused a rise in temperature is the main reason. With about 100 V voltage electrophoresis, which was mainly due to the beginning of high voltage can separate the different conformation of DNA single strand and the gel will not increase the temperature and then the low-voltage electrophoresis can make it further separation (He *et al*., 2006). How many voltage electrophoresis we can used? It should be based on specific test conditions to determine.

It found in the test that short-chain DNA mutation detection rate higher than the long-chain of SSCP. This may be due to molecules of long-chain DNA, changed single nucleotide plays a small role in maintenance of the conformation. Generally, the midpoint mutation of 300 bp DNA detection rate is over 90%.

Screen SNP of *PPAR* gene in Dongting goose by PCR-SSCP. It found that there is a G→A mutation in the two allele gene sequence of 215 bp. That showed goose *PPAR* gene fragments of DNA has single-nucleotide polymorphism and the heterozygous genotype frequencies of test samples were higher, which could related on the breeding of Hybrid Breed System of Dongting goose.

Research has shown that *PPAR* gene polymorphism related with abdominal fat weight and abdominal fat ratio. The results of this study was similar (Luo *et al*., 2010). We can see the *PPAR* gene mutation consisting of different genotypes of goose have some regulation with fat metabolism and deposition. Whether it could be use as a candidate gene remains to be further studied. If there is obviously related, *PPAR* gene will improve as the candidate genes to goose meat quality trait and apply for marker-assisted selection, provide new ways and means for the scientific breeding of goose.

CONCLUSION

In our detection, the *PPARα* gene frequency of A, B were 0.393, 0.607, respectively and the genotype frequency of AA, AB, BB were 0.126, 0.533, 0.341, respectively the abdominal fat weight, abdominal fat ratio and liver weight ratio showed a tendency of BB>AA. So the genetic marker can be used as one of the indexes of high, low fat goose strain selection.

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