Single Nucleotide Polymorphisms on Peroxisome Proliferator-activated Receptor Genes Associated with Fatness Traits in Chicken

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ABSTRACT : The peroxisome proliferator-activated receptors (PPARs) are members of a superfamily of nuclear hormone receptors. Lots of studies in rodents and humans have shown that PPARs were involved in lipid metabolism and adipocyte differentiation. The main objective of this work was to detect the single nucleotide polymorphisms (SNPs) in whole coding regions of peroxisome proliferator-activated receptor alpha (PPAR-α) and gamma (PPAR-γ) genes with approach of single strand conformation polymorphism (SSCP) in the chicken population of Arber Acres broiler, Hyline layer and three Chinese native breeds (Shiqiza, Beijing You, Bai'er). Two SNPs of C1029T and C297T were found in chicken PPAR-α and PPAR-γ genes respectively and each SNP found three genotypes in the experimental populations. The results showed that the distribution frequency of 3 genotypes in Arber Acres broiler, Hyline layer and Chinese native breeds had significant differences on the PPAR-α and PPAR-γ gene respectively ($p<0.01$). Furthermore, in the PPAR-α gene, the results of least square estimation for genotypes and body composition traits showed the BB genotype birds had higher abdominal fat weight (AFW) and percentage of abdominal fat (AFP) than AA genotype birds ($p<0.05$). From these we conjecture the PPAR-α and PPAR-γ genes were suffered intensive selection during the long term commercial breeding and the PPAR-α gene may be a major gene or linked to the major genes that impact chicken fat metabolism and the SNPs could be used in molecular assistant selection (MAS) as a genetic marker for the chicken fatness traits. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1221-1225)***

Key Words : PPARs, SSCP, SNPs, Fatness traits, MAS, Chicken

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of superfamily of nuclear hormone receptors (Schoonjans et al., 1996) and can be activated by peroxisome proliferators, such as hypolipdemic drugs of fibrates class and some fatty acids. These receptors could increase the size and number of peroxisomes in rodents (Issemann et al., 1990; Dreyer et al., 1992). The PPARs regulate a variety of target genes which involved in intra- and extracellular lipid metabolism such as fat acid absorbed by membrane, fat acid binding to cell, the transportation and combination of lipid protein, particularly those involved in peroxisome β-oxidation (Dreyer et al., 1993; Wahli et al., 1995). These receptors are critical determinants of adipocyte differentiation (Tontonoz et al., 1994a, b) and are also direct targets of antidiabetic drugs of the thiazolidinedione class (Lehmann et al., 1995).

Lots of studies for obesity, diabetes, atherosclerosis and hypertension showed that the variants of PPARs genes influence the metabolism of glucide and lipid in human. The Pro12Ala polymorphism of PPAR-γ gene was found to increase the body mass index (Beamer et al., 1998; Lei et al., 2000) and directly related with the type 2 diabetes (Koch et al., 1999). The patients who had the Pro115Gln polymorphism of PPAR-γ gene were distinctly obesity (Ristow et al., 1998). The same mutation of PPAR-γ gene could induce the coronary heart disease, severe insulin resistance and hypertention (Barroso et al., 1999). In PPAR-α gene, Pro162Val polymorphism associated with plasma lipid levels (Tai et al., 2002) and lower body mass index in patients (Evans et al., 2001). Some researchers consider the variants of PPAR-α gene associated with atherosclerosis and ischemic heart disease (David et al., 2002). A lot of data have shown that there are associations between gene polymorphisms and traits in diverse domestic animals (Jiang et al., 2002a, b).

The metabolism of animal lipid is very complex, and there are many differences in lipid metabolism between poultry and mammals. Yet, the regulation of lipid metabolism in poultry is not completely understood. PPARs have been shown as a potential key regulator of the lipid metabolism and adipocyte differentiation. The main objective of this study was to scan the coding regions of PPAR-α and PPAR-γ gene by single strand conformation polymorphism (SSCP), and to analyze the relationship between the single nucleotide polymorphisms (SNPs) and the fatness trait. Results of this study can be the basis of lipid metabolism studies in poultry and the polymorphisms of PPARs genes may be used as a genetic molecular marker in the breeding of low abdominal fat chicken.

MATERIALS AND METHODS

Animals, genomic DNA and traits

The experimental population were composed of Arber

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Acres (AA) broilers, Hyline layer and three Chinese native chicken breeds (Shiqiza, Beijing You, Bai'er), the Shiqiza is Guangdong province by origin, the Beijing You is Beijing by origin and the Bai'er is Jiangxi province by origin. Blood samples were collected from wing vein with 4% EDTA treated injectors at 7 wk of age. The genomic DNA was extracted from blood by conventional phenol-chloroform methods. Body composition traits of AA male broilers were recorded at 7 wk of age and these measurements included live body weight (BW), carcass weight (CW), semi-carcass weight (SCW), liver weight (LW), abdominal fat weight (AFW) and percentage of abdominal fat (AFP). The carcass weight is a body weight without blood and feather, the semi-carcass weight is a carcass weight without trachea, esophagus, craw, gut, spleen, pancreas and generative organs.

PCR primers

In order to scan the full coding region of PPAR-α and PPAR-γ genes by PCR-SSCP, we had to known the division of exons within region coding of PPAR-α and PPAR-γ genes through comparing the sequences of chicken cDNA with sequences of human genomic DNA. Subsequently, the PCR primers were designed on the diverse exons base on sequences of chicken cDNA by primer premier 5.0 software (Premier, Canada) and the positions base on sequences of chicken PPAR-α (GenBank Accession No: AF470455) and PPAR-γ (GenBank Accession No: AB045597).

PPAR-α:

F 5`-ACCAGCTCTATCCACTTACTCC-3`
R 5`-AAATGGTCCAGGATCTGATG-3`
F 5`-CACCCTTTCACCAGCATCC-3`
R 5`-CCTTCACAAGCATGTACTCCG-3`
F 5`-CCGATTGAAACTCATCTATG-3`
R 5`-ACATTCCAACTGAAAGGC-3`
F 5`-TGGACGAATGCCAAGGTC-3`
R 5`-TTTCCCTGCAAGGATGACTC-3`
F 5`- CCCTGGCTTCTCCAATCTTG-3`
R 5`-TCATCCAGTTCCAGTGCATTG-3`
F 5`-CATGATATGGATACCTTGTGC-3`
R 5`-AGCCAGGGATAGATTTGG-3`
F 5`-TGCAGGAGAGCATTGTGC-3`
R 5`-GATTTCCTGCAGTAAAGGGTG-3`

PPAR-γ:

F 5`-ATGGTTGACACAGAAATG CC-3`
R 5`-TTTGGCAATCCTGGAGCTTG-3`
F 5`-GTGCAATCAAAATGGAGCC-3`
R 5`-CTTACAACCTTCACATGCAT-3`
F 5`-GCTTTTTTCGAAGAACAATC-3`
R 5`-TTATGTGACATTCCAACTGC-3`
F 5`-CCATCAGGTTTGGGCGAATG-3`
R 5`-TGATTTGTCTGTCGTCTTTC-3`
F 5`-CCATTTGTTATTTATGACAT-3`
R 5`-TCATTCAGGTCAAGATTCAC-3`
F 5`-GTTTTGTGAATCTTGACCTG-3`
R 5`-CTCCACTTAGTATAATGACA-3`
F 5`-CCAGGTTTGTTAAATGTG-3`
R 5`-CTTTATAGATTTCTTGTAGG-3`

PCR-SSCP

Polymerase chain reaction (PCR) amplifications were performed using genomic DNA as templets with gene specific primers. Then the SNPs were determined by SSCP in these populations of AA broilers, Hyline layer, shiqiza, Beijing You, Bai'er. The process of PCR-SSCP was as follows: 1 μl PCR products were denaturalized at 95°C for 10 min with the 5 μl buffer (98% formamide, 10 mM EDTA, 0.05% bromophonel blue, 0.05% xylene cyanol blue) and then kept into ice for 5 min, the products of PCR were loaded in to the holes of 14% PAGE gel and performed the electrophoresis under 5V/cm at 16°C for 10-15 h. Subsequently, the gel body was taken out and dipped in to 70% ethanol for 15 min, washed twice at a time for 2 min by distilled water, then dyed 30 min by the 100 ml solution (1 ml NH_3H_2O, 2.1 ml 3.6% NaOH, 1.8 ml 20% $AgNO_3$, 95.1 ml distilled water), washed twice at a time for 2 min, colored 10-15 min by the 200 ml solution (1 ml 1% citrate sodium, 100 μl formaldehyde, 198 ml distilled water), washed by abundant water and then typed as AA, AB, BB based on the silver stain and recorded it.

Statistical analysis

Genotype frequencies in five populations, Hyline layer, shiqiza, Beijing You, Bai'er were calculated and the independent test (X^2 test) was used to test the independence between the genotypes and breeds in the whole breeds. The association between the genotypes of the PPARs polymorphism patterns and body composition traits in the AA broiler at 49 days age was analyzed by the SAS 6.12 software (SAS, USA) with least square estimation. According to the character of the experimental population, the analyses model was described to be as follows:

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

Where Y is the value measured of the body composition trait on each of animal; μ is mean value of the body compound trait of population; G is fixed effect of PPAR genotype, e is random error effect. Considering the all experimental birds were male from the same line and farm as well as slaughtered at the same age, so other effects were not taken in this model as sex, generation and farms.

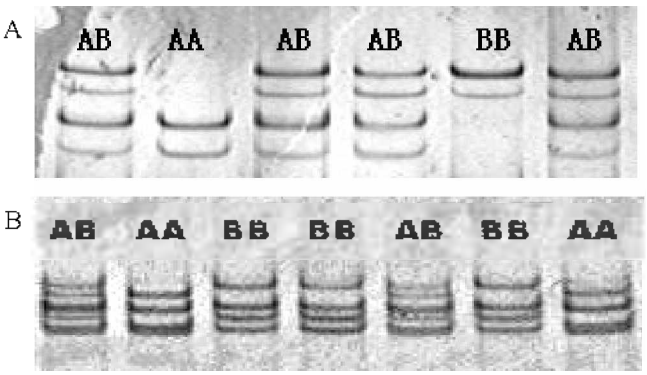

Figure 1. Identification of genotypes on the PPARs gene by PCR-SSCP. A. Genotypes of product with primer 4 amplified on the PPAR-α gene. B. Genotypes of product using primer 2 amplified on the PPAR-γ gene.

RESULTS

Identification of SNPs

The SNPs of the PPAR-α and PPAR-γ genes were determined by the methods of PCR-SSCP. Two polymorphisms were found from the products amplified with primer 4 on the PPAR-α gene and with primer 2 on the PPAR-γ gene, and three genotypes, named as AA, AB and BB respectively, were found in the experimental populations (Figure 1).

The PCR products of the individuals with the genotype AA and BB were cloned and sequenced. A C1029T mutation was found on PPAR-α gene and the sequence of the BB genotype were consistent with the reported sequence at GenBank. A C297T mutation was found on PPAR-γ gene and the sequence of the BB genotype were consistent with the sequence of reported at GenBank. However these mutations don't change the amino acid sequence. We have submitted the two sequences, including the mutation sites found in PPAR-α and PPAR-γ gene, to GenBank, and the GenBank Accession numbers were AF470455 and AB045597 respectively.

Distribution of the genotypes on PPAR genes in five chicken populations

In order to understand the distribution of these SNPs in various populations and to deduce the association between genes and traits, we selected the three populations- AA broiler, Hyline layer and Chinese native breeds, which have great difference in genetic background as experimental population to determine the distribution of the three genotypes. The X^2 test results were showed at Table 1 and 2 on PPAR-α gene and PPAR-γ in three populations. From results of X^2 test for PPAR-α gene we found significant difference at the frequency of genotypes in three populations (AA broilers, Hyline layer and Chinese native breeds) ($X^2 = 113.12 > X^2_{0.01(4)}$, $p<0.01$). Further analysis showed that there were significant differences on genotype distribution between any two populations ($p<0.01$). Chinese native breeds were consisted of Shiqiza, Beijing You, Bai'er and the result X^2 test showed that there was no significant difference among the three breeds on genotype frequency (data not shown). In addition, with regard to the percentage of three genotypes in three breeds, the BB genotype was higher in AA broiler (55.6%) and Hyline layer (87.6%) than in Chinese native breeds (26.9%), the AA genotype was very lower in AA broiler (9.0%) and Hyline layer (0.7%).

In PPAR-γ gene, the X^2 test results were similar to PPAR-α gene that significant difference were existed at the distribution of three genotypes in three populations (AA broiler, layer and Chinese native breeds) ($X^2 = 269.11 > X^2_{0.01(4)}$, $p<0.01$), and between any two populations ($p<0.01$), but distribution of three genotypes have no significant difference among the three breeds of Chinese native breeds (data not shown). Specially, the percentage of BB genotype was higher in Chinese native breeds (62.6%) than in AA broiler (21.1%) and Hyline layer (8.6%).

Analysis of the SNPs associated with body composition traits

The association analysis of the polymorphisms on PPARs genes with the body composition traits measured at 7 wk of age in AA broilers were carried out with least square estimation. The PPAR-α gene had great effect on AFW ($p<0.05$) and AFP ($p<0.05$) (Table 3), and the result of multiple comparisons analysis showed that AA genotype birds had significantly lower AFW and AFP than BB and AB genotype birds ($p<0.05$), but no significant difference

Table 1. X^2 test of the distribution of three genotypes on PPAR-α gene in three populations

Breeds	Numbers	AA genotype	AB genotype	BB genotype	X^2	X^2 0.01(4)
AA broiler	333	30	118	185		
Hyline layer	145	1	17	127	113.12**	13.28
Native breeds	145	39	61	45		

Table 2. X^2 test of the distribution of three genotypes on PPAR-γ gene in three populations

Breeds	Numbers	AA genotype	AB genotype	BB genotype	X^2	X^2 0.01(4)
AA broiler	188	47	90	51		
Hyline layer	105	39	57	9	269.11**	13.28
Native breeds	115	5	38	72		

Table 3. The least square analysis of the polymorphism on PPAR-α gene with growth and body composition traits

Genotype	Stat. parameter	BW	CW	LW	AFW	AFP
AA (12)	Average	2,242.50	2,050.00	55.91	33.38	1.51E-02
	Standard error	190.71	165.68	3.23	4.17	1.81E-03
AB (56)	Average	2,459.80	2,231.71	45.91	49.16	1.99E-02
	Standard error	31.80	29.27	1.08	2.00	7.87E-04
BB (53)	Average	2,416.83	2,189.81	46.93	53.46	2.22E-02
	Standard error	43.55	39.39	1.42	1.83	6.89E-04
	F	1.248	1.125	2.235	4.380	4.527
	p value	0.291	0.328	0.112	0.015*	0.013*

Table 4. Multiple comparisons of fatness traits among three PPAR-α genotypes birds

Genotype	Numbers	AFW	AFP
AA	12	33.38^{a}	0.01508^{a}
AB	56	49.16^{b}	0.01998^{a}
BB	53	53.46^{b}	0.02217^{b}
Total	121	50.62	0.02083

[a, b] Means in the same column with different superscripts significantly differ at $p<0.05$.

between BB and AB genotype birds on the AFW and AA and AB genotype birds on the AFP (Table 4).

On the PPAR-γ gene, the association analysis of polymorphisms with traits was also carried out with least square estimation. Results showed that the PPAR-γ gene polymorphism was not related with growth and body composition traits (data not shown).

DISCUSSION

From the results of independent analysis (X^2 test) we found that the genotypes of the PPAR-α and PPAR-γ gene were associated with various breeds chicken. On the PPAR-α gene, the distribution difference of three genotypes in various breeds (AA broiler, layer and Chinese native breeds) and between any two breeds was significant ($p<0.01$). In addition, the X^2 test analyses showed that there was no significant difference among Chinese native breeds (Shiqiza, Beijing you, Bai'er) on genotype frequency ($p>0.05$). Talk about the genotype's distribution frequency, the Hyline layer and AA broiler had more BB type individuals than Chinese native breeds. Similar to PPAR-α gene, the distribution difference of three genotypes of PPAR-γ gene was also significant among various breeds and between any two breeds ($p<0.01$). The frequency of BB genotype birds was higher in Chinese native breeds than in AA broiler and Hyline layer. From the current study we speculated that PPARs genes were suffered direct or indirect selection with the long commercial breeding in broiler and layer. Specific genotype of PPARs genes such as BB genotype of PPAR-α gene maybe linked to some quantitative trait loci (QTL) that affect economic traits (growth, deposition of abdominal fat and layer performance), and increasing the percentage of beneficial genotypes may bring us selection progress in the production performance of layer and broiler. The native breeds were whole random mated without any commercial breeding; maybe this was the reason why the genotype frequency of PPAR-α and PPAR-γ gene has no significant difference among native breeds.

The results showed that the PPAR-α gene polymorphism was associated with AFW ($p<0.05$) and AFP ($p<0.05$) and there were significant higher AFW and AFP in the BB genotype birds than the AA genotype birds ($p<0.05$). Combined the result with independent analysis (X^2 test) between distribution of three genotypes and breeds, we concluded that PPAR-α gene have great effect on chicken lipid metabolism, and it may be a major gene or linked to a QTL to affect important economic traits, such as growth, fatness and layer performance. The PPAR-α-AA genotype birds had lower abdominal fat, and the polymorphism discovered in the present study could be used as a genetic marker in marker assistant selection (MAS) for the chicken fatness traits.

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