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Correlation Analysis Between Single Nucleotide Polymorphisms in Exon 2 of ADSL Gene and Inosine Monophosphate Acid Content in Chicken

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Abstract: This study was designed to investigate the effect of Adenylosuccinate Lyase (ADSL) gene on Inosine Monophosphate content (IMP) in chicken. The chickens used for the study included exogenous Recessive White chicken and Chinese indigenous chicken breeds such as Silkies, Baier, Tibetan and Xiaoshan chickens. A pair of primer was designed to detect exon 2 of ADSL gene by PCR-SSCP and mutation detected was directly sequenced. A synonymous mutation at 3484 bp in exon 2 was substituted C/T SNP. In five chicken breeds, three genotypes TT, CT and CC were observed in exon 2. Muscle IMP content of individuals with TT genotype in exon 2 had significantly higher than those with CT and CC genotypes ($p<0.05$). In exon 2, Recessive White, Xiaoshan and Baier chickens were in Hardy-Weinberg equilibrium ($p>0.05$) and Silkies and Tibetan chickens were significant departure from Hardy-Weinberg equilibrium ($p<0.01$).

Key words: ADSL gene, SNP, inosine monophosphate content, chicken, genotype, Hardy-Weinberg equilibrium

INTRODUCTION

Meat flavor is one of the most important aspects of the meat quality in chicken. Herein Inosine Monophosphate acid (IMP) is one of the key components for meat flavor, which plays a major role in umami taste as an umami substance (Fuijmura, 1998; Wu and Qi, 2001; Kawai *et al*., 2002). Different IMP contents result in different umami test because of their interaction with Glutamine Sodium (MSG) (Yamaguchi, 1967; Maehashi *et al*., 1999; Kawai *et al*., 2002). Many experiments indicated that breed, age, sex and different parts of muscle may have effects on muscle IMP content in chickens (Davide and Khan, 1967; Khan *et al*., 1968; Chow and Jacobson, 1968; Song *et al*., 2002).

Because it's difficult to improve meat quality of chicken by traditional breeding methods, candidate gene method may be an alternative approach to study these traits presently. However, few researches were reported on polymorphisms of candidate genes and its relation with muscle IMP content in poultry. The de novo synthesis of the nucleotide IMP is 10 steps, which serves as a branch point in the pathway to AMP and GMP. ADSL is an essential enzyme involved in this de novo purine biosynthesis, which catalyzes two steps in the synthesis: the conversion of Succinyl-Amino-Imidazole-Carboxamide Ribotide (SAICAR) into Amino-Imidazole-Carboxamide Ribotide (AICAR) and the conversion of adenylosuccinate into adenosine monophosphate (Aimi *et al*., 1990b; Julie and Riichard, 1995). Chicken ADSL gene was located on chromosome No.1, the full length of its cDNA is 15928 bp and has 13 exons (Aimi *et al*., 1990a).

The objective of this study aimed to identify the single nucleotide polymorphism in 13 exons of ADSL gene in one commercial chicken breed and four important Chinese indigenous chicken breeds using the polymerase chain Reaction-Single Strand Conformation Polymorphism analysis (PCR-SSCP) and direct DNA sequencing, which would pave the foundation of studying its relationship with IMP content of muscle and further improving meat quality of chicken breeds.

MATERIALS AND METHODS

Experimental materials and procedures: Five chicken breeds, including Recessive White chicken, Silkies chicken, Baier chicken, Tibetan chicken and Xiaoshan chicken were used in this study. All breeds were reared under the same management system in Poultry Institute, Academy of Chinese Agricultural Science, Yangzhou, P. R. China. Sixty 12-weeks-old chickens (30 per sex) were randomly selected from each breed. About 2 g of breast muscles was collected from each individual and then muscle IMP content was measured using the method described by Chen *et al*. (2000).

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Genomic DNA was isolated from the whole blood using phenol/chloroform method described by Sambrook and Russell (2001). A pair of primers of Exon 2 of ADSL gene were designed by Oligo 6.0 software, according to chicken genomic sequence in GenBank database (accession number AY665559) as follows: forward primer was 5'-CTT TCT CCT CCG CAG TCA C-3' and reverse was 5'-AGC ACC TTC GTC TTC GTT TT-3'.

PCR was carried out in 25 μL volume containing 50 ng of template DNA, 1.0 μM of each primer, 200 μM of each dNTP, 1.5 mM $MgCl_2$ and 1 U Taq DNA polymerase (TakaRa Biotechnology Dalian Co., Ltd.). Thermal cycling was performed with 5 min denaturation at 95°C, followed by 30 cycles of 30 sec at 95°C, 30 sec at an annealing 56°C and 30 sec at 72°C and a final extension step of 7 min at 72°C. PCR products were checked in 1% agarose gels stained with ethidium bromide. Then for SSCP, 1.5 μL of the PCR product of each individual was mixed with 6 μL formamide loading buffer (95% formamide, 20 mmol L^{-1} EDTA, 0.05% bromophenol blue and 0.05% xylenecyanol FF), denatured at 98°C for 10 min and then chilled on ice for 10 min. The denatured PCR products were subjected to 10% polyacrylmide gel in 1×TBE buffer and electrophoresed at a constant voltage (8 V cm^{-1}) for 12-14 h. The DNA bands on the gel were viewed by silver staining.

Different homozygotes were selected for direct sequencing, according to the PCR-SSCP results. The PCR products of the two different homozygous individuals were purified from excess reaction components with Microcon-PCR device (Millipore Corp., Bedford, MA). Sequencing was performed on an ABI Prism 377 sequencer (Applied Biosystems) using ABI PRISM Big DyeTM Terminator Cycle sequencing Ready Reaction Kit (Perkin Elmer, Foster City, CA).

Statistical analysis: DNASTAR package was used to assemble the sequences and to identify polymorphisms. All values are presented as means±standard error of mean. The chi-square (χ^2) test was used to test if a gene frequency was in Hardy-Weinberg equilibrium. The following model was fitted for association of ADSL genotypes and muscle IMP. Linear model:

$$Y_{ij} = \mu + M_j + e_{ij}$$

Where,

Y_{ij} = Phenotypic value of muscle IMP content
μ = Population mean
M_j = The fixed effect of the ith genotype
e_{ij} = Random error effect of each observation

The model was determined by ANOVA using General Linear Model (GLM), all statistical analysis were performed by SAS 9.0 software.

RESULTS

Single-strand conformational polymorphism analysis: PCR-SSCP analysis was carried out in exon 2 of ADSL gene. Three genotypes TT, CT and CC were observed in exon 2 within five chicken breeds (Fig. 1a). Sequence analysis revealed a C/T substitution at position 3484 bp in exon 2 (Fig. 1b), but this mutation was silent. Allele frequencies and genotype frequencies of ADSL gene exon 2 in chicken breeds and the chi-square (χ^2) test are presented in Table 1. The distributions of allele and genotype frequencies of ADSL exon 2 were differed among populations. In all populations, the C allele was more frequent than the T allele. The results of the chi-square (χ^2) test indicated that allele frequencies in Recessive White, Xiaoshan and Baier chickens were in Hardy-Weinberg equilibrium ($p>0.05$), while Silkies and Tibetan chickens were significant departure from Hardy-Weinberg equilibrium ($p<0.01$).

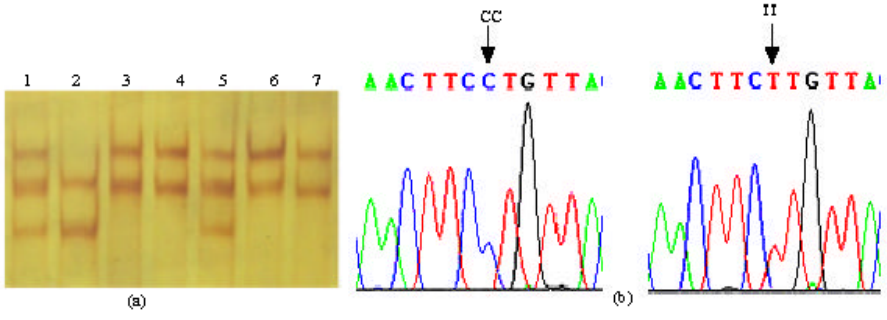

Fig. 1: C3484T polymorphism in exon 2 of ADSL gene. a) Electrophoresis image of PCR-SSCP, CT: 1, 5; TT: 2; CC: 3, 4, 6, 7, b) Sequence analysis of PCR products of CC and TT genotypes

Table 1: Allele frequencies and genotype frequencies of ADSL exon 2 in five chicken breeds

		Genotype frequencies			Allele frequencies		
Breeds	Numbers	TT	CT	CC	T	C	χ^2
Recessive white	60	0.067 (4)	0.067 (4)	0.866 (52)	0.100	0.900	5.23
Silkies	60	0.267 (16)	0.167 (12)	0.567 (32)	0.367	0.632	10.24**
Xiaoshan	60	0.167 (10)	0.566 (34)	0.267 (16)	0.450	0.550	0.55
Baier	60	0.167 (10)	0.500 (30)	0.333 (20)	0.417	0.583	0.04
Tibetan	60	0.083 (5)	0.750 (45)	0.167 (10)	0.458	0.542	9.30**

Values with *differ significantly at $p<0.05$ ($\chi^2_{0.05\,(2)} = 5.99$); Values with **differ significantly at $p<0.01$ ($\chi^2_{0.01\,(2)} = 9.21$)

Table 2: Effect of mutation in exon 2 of ADSL gene on IMP content in breast muscle

Locus	Genotype	Sample size	IMP content (mg g^{-1})
Exon 2	CC	130	3.395±0.112[a]
	CT	125	3.517±0.127[a]
	TT	45	4.275±0.231[b]

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

Effect of mutation in exon 2 of ADSL gene on muscle IMP content: By statistical analysis, breeds did not effect muscle IMP content and the mutation detected in exon 2 of ADSL gene significantly affected muscle IMP content ($p<0.05$).

Muscle IMP content of individuals with TT genotype in exon 2 was significantly higher that of CT (0.758 mg g^{-1}) and CC (0.88 mg g^{-1}) genotype ($p<0.05$). Muscle IMP content of individuals with TC genotype also was high than that of CC genotype, but the difference was not significant ($p>0.05$) (Table 2).

DISCUSSION

To date, researches about the candidate genes or genetic markers related to muscle IMP content are few. Gavalas and Zalkin (1995) noted that GPAT and ARIC genes, which catalyze the first and sixth steps in de novo purine synthesis respectively, were closely linked and divergently transcribed from intergenic regions of approximately, 230 bp in chickens. Marie *et al.* (2002) reported that a -49 T→C mutation occurred in the 5'untranslated region of ADSL gene in human, which might be an unusually frequent cause of ADSL deficiency. Chen *et al.* (2002) found that in same chicken breed, muscle IMP content of muscles of individual was different.

In the present study, a Single Nucleotide Polymorphism (SNP) was observed, which was a C/T at 3484 bp in exon 2. In exon 2, Recessive White, Xiaoshan and Baier chickens were in Hardy-Weinberg equilibrium ($p>0.05$) and Silkies and Tibetan chickens were significant departure from Hardy-Weinberg equilibrium ($p<0.01$). These results may reveal that artificial selection, such as selecting meat traits (Ji *et al.*, 2003; Liu *et al.*, 2005), had a profound effect on the shaping of genetic diversities and their genetic differentiation in chicken breeds. Muscle IMP content of individuals with TT genotype in exon 2 had significant higher than that of CT and CC genotypes ($p<0.05$). Although, the mutation in exon 2 locus was synonymous and did not produce altered coding sequences, but this synonymous mutation might affect exon skipping, alter the predicted mRNA folding, decrease in mRNA stability and translation (Chava *et al.*, 2007; Jubao *et al.*, 2003; Chamary *et al.*, 2006). Ji *et al.* (2005) studied GARS-AIRS-GART gene as candidate gene for muscle IMP content and detected Single Nucleotide Polymorphism (SNP) of C→A at 6545 bp in exon 4 of the gene and muscle IMP content of individuals with AA genotype had significantly higher than that of individuals with CC genotype in Silkies chicken breed ($p<0.01$), but the IMP content was not significantly different between these two genotypes in Recessive White chicken breed. These results reflected that one nucleotide in ADSL gene altered may change manifestation of corresponding character.

CONCLUSION

PCR-SSCP analysis was carried out in exon 2 of ADSL gene in Recessive White chicken, Silkies chicken, Baier chicken, Tibetan chicken and Xiaoshan chicken. A Single Nucleotide Polymorphism (SNP) were screened, which was a C/T at 3484 bp in exon 2. The values of allele frequency varied among five chicken populations in exon 2. Muscle IMP content of individuals with TT genotype in exon 2 of ADSL gene was significantly higher that of CT and CC genotypes ($p<0.05$). This was useful for marker assisted selection of meat quality traits in chicken.

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