RESEARCH NOTES

Sequences and polymorphisms of exons 3 and 4 in porcine UCP_2 gene*

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Abstract Uncoupling proteins are mitochondrial membrane transporters, which regulate metabolic pathways of energy balance, and are associated with biological traits of animal body weight, resting metabolic rates and energy conversion. In this study, a region of the exons 3 and 4 of pig UCP_2 gene was cloned and analyzed, and a new single nucleotide polymorphic site was detected by PCR-SSCP in five pig breeds. This newfound polymorphism results from a T to G substitution at the position of nucleotide 272, which is located in intron3.

Keywords: pig, uncoupling proteins, PCR-SSCP.

Uncoupling proteins (UCPs) have been proved to play a role in dissipating the coupling pathway of oxidation and phosophorylation by knockout mice[1]. They can regulate cellular reaction of energy metabolism and thermogenesis. Four members in UCP gene family have been found, namely, UCP_1, UCP_2, UCP_3 and UCP_4[2~4]. UCP_2 gene is widely expressed in many tissues, including brain, muscle and white adipose. The UCP_2 and UCP_3 genes are tightly linked in the form of a gene cluster[5].

The porcine UCP_2 gene was mapped to 9p21-p24[6]. In the research of the function of UCP_2 gene, we cloned and sequenced a region of UCP_2 gene, and a polymorphic site in the region was identified by PCR-SSCP. Here is the report.

1 Materials and methods

The DNA samples for experiment were from the breeds of Landrace, Yorkshire, Erhualian, Neijiang, and Wuzhishan. For each breed 20 individuals were selected.

According to the human UCP_2 gene sequence (GenBank accession number: AF096289), the primers were designed as UCP_2-34F (5'-AGATGCCAGCATTGGGAGCCGC-3') and UCP_2-34R (5'-ACCTGTCATGAGGTTGGCTTTC-3'). The PCR reaction was performed at 94 ℃ for 5 min, followed by 30 cycles of denaturation at 95 ℃ for 30 s, annealing at 60 ℃ for 30 s and extension at 72 ℃ for 30s, and then the reaction was extended at 72 ℃ for 7 min.

PCR products were ligased to the plasmid vector of PGEM-T (Promega). The recombinant was transformed to the competent cells of *E. coli* DH5α. The plasmid DNA was isolated and sequenced by an ABI PRISM377 DNA sequencer.

PCR-SSCP was carried out as described previously[7].

2 Results

The amplified fragment covers the region of the exon3, exon4 and intron3 of UCP_2 gene. The length of the PCR product is 379 bp (Fig. 1), and its sequence is shown in Fig. 2.

Using PCR-SSCP technique, a new polymorphic

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site was detected within the amplified fragment (Fig. 3 (a)). By sequencing analysis of the two homozygotes (dominant and recessive), the mutant homozygous genotype BB had a T to G substitution at nt272 position in intron 3, which resulted in a single strand conformation polymorphism (Fig. 3(b)).

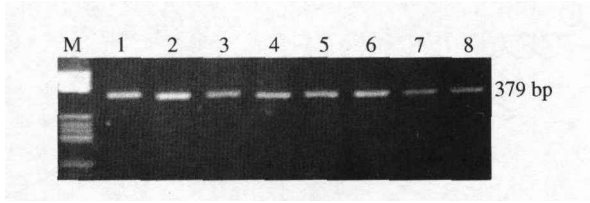

Fig.1. PCR products of pig UCP_2 gene. 1~8, PCR products of pigs from five different breeds; M, molecular size markers.

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1    AGATGCCAGC ATTGGGAGCC GCCTCCTGGC AGGCAGCACC ACGGGGGCCT TGGCTGTGGC
61   CGTGGCCCAG CCAACAGACG TGGTAAAGGT CCGGTTCCAA GCGCAGGCCC GGGCCGGCGG
121  AGGCCGGCGG TACCGGAGCA CTGTCGACGC CTACAAGACC ATCGCCCGAG AGGAGGGGCT
181  GCGGGGCCTC TGGAAAGGTG TGTGCGCAGC TCTCTCCCCT CCTCCTCCTC CCCTACTCCC
241  TGGCCTCACC CAGGCACCCC CCCTTGCTCC CTTAGGGACC TCACCCAATG TCGCTCGTAA
301  TGCCATTGTC AACTGTGCTG AGCTGGTGAC CTATGACCTC ATCAAGGACA CGCTCCTGAA
361  AGCCAACCTC ATGACAGGT
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Fig.2. The sequence of the PCR-amplified fragment. Exon 3, nt3~197; intron 3, nt198~275; exon 4, nt276~377.

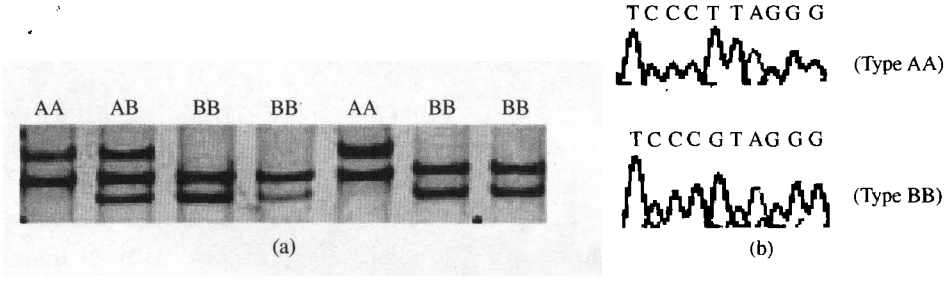

Fig.3. A new SNP in intron3 of pig UCP_2 gene. (a) A genetic polymorphic locus detected by PCR-SSCP in intron 3 of pig UCP_2 (including AA, BB and AB genotypes); (b) the G to T base substitution found in two homozygous genotypes at nt272.

Allele frequencies of UCP_2 gene in five pig breeds are shown in Table 1.

Table 1. Allele frequencies of UCP_2 gene in five pig breeds

Pig breed	Number of animals	Allele frequency	
		A	B
Landrace	20	0.250	0.750
Yorkshire	20	0.275	0.725
Erhualian	20	0.200	0.800
Neijiang	20	0.175	0.825
Wuzhishan	20	0.225	0.775

References

1 Enerback, S. et al. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature, 1997, 387: 90.
2 Fleury, C. et al. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat. Genet., 1997, 15(3): 269.
3 Boss, O. et al. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett., 1997, 408(1): 39.
4 Mao, W. et al. UCP4, a novel brain-specific mitochondrial protein that reduces membrane potential in mammalian cells. FEBS Lett., 1999, 443(3): 326.
5 Solanes, G. et al. The human uncoupling protein-3 gene, genomic structure, chromosomal localization, and genetic basis for short and long form transcripts. J. Biol. Chem., 1997, 272(41): 25433.
6 Werner, P. et al. Characterization of the porcine uncoupling proteins 2 and 3 (UCP2 & UCP3) and their localization to chromosome 9 p by somatic cell hybrids. Anim. Genet., 1999, 30(3): 221.
7 Wang, Q. G. et al. Single nucleotide polymorphism analysis on chicken extracellular fatty acid binding protein gene and its associations with fattiness trait. Progress in Natural Science, 2002, (3): 236.