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GENETIC VARIANTS OF PPAR γ 2 SPECIFIC REGION IN JAPANESE BLACK CATTLE

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Keywords: Japanese Black cattle, genetic variants of PPAR γ 2, PCR-RFLP with a mismatch primer

Background:

In Japan, marbling score is one of the important factors to determine beef carcass grade. Japanese Black cattle, native cattle in Japan, are selected mainly by their marbling property. Therefore, many approaches have been carried out to increase the marbling. The marbling is adipose tissue in muscle, and differentiation and growth of adipocytes seem to be closely related to the formation of the marbling. With the progress of molecular genetics, a participation of many genes has become clear in differentiation of adipocytes . Among these, peroxisome proliferator-activated receptor γ (PPAR γ) is expressed early during the course of adipocyte differentiation and thought to be a key regulator of the adipogenesis(Schoonjans K et al., 1996). The PPAR γ gene contains 9 exons and two isoforms of PPAR γ 1 and PPAR γ 2 are generated by alternative splicing (Zhu Y et al., 1995). In mouse, PPAR γ 1 is expressed in various tissues, whereas PPAR γ 2 is expressed predominantly in adipose tissue(Tontonoz P et al., 1994). Recently, in Norwegian cattle, nucleotide sequence of PPAR γ and expression of their transcripts (PPAR γ 1 and PPAR γ 2) in various tissues have been reported (Sundvold H et al.,1997). In Japanese Black cattle , however, little is known about the nature of PPAR γ .

Objectives:

The objective of this study was to investigate the sequence of PPAR γ 2 in Japanese Black cattle and to detect genetic variants of PPAR γ 2.

Methods:

Partial sequence of PPAR γ 2

Twenty eight month old Japanese Black cattle were used. The cattle were killed by bleeding, and longissimus dorsi muscle was removed and frozen in liquid nitrogen. Total RNAs were extracted from the frozen muscle using Trizol reagents. Synthesis of cDNA was performed with 3' primer (TAGTGCGGAGTGGAAATGCTGGAGAA) , approximately 1 μ g total RNAs and 5 U of reverse transcriptase. After synthesis of cDNA, 5' primer (GCTGTGATGGGTGAAACTCTGGGAGA) and Tag-polymerase (2.5 U) were added and polymerase chain reaction (PCR) was carried out. The template was denatured for 2 min at 94℃, followed by 30 cycles of amplification at 94℃ for 1 min, 55℃ for 1 min and 72℃ for 1 min. Amplified DNA was subcloned into pT7 blue-T and sequenced using ABI 377XL DNA sequencer. The obtained sequence was compared with the reported sequence of Norwegian cattle (Sundvold H et al., 1997) and mutation was detected.

Restriction fragment length polymorphism (RFLP) analysis with a mismatch primer

To detect the mutation easily, PCR-RFLP with a mismatch primer was carried out (Haliasson A et al., 1989). PCR conditions were same as described above except that mismatch 3' primer (GTGAGGTCCTTGCAGACACTGTGTCA) and 5' primer (GCTGTGATGGGTGAAACTCTGGGAGA) were used. The amplified products were digested with *Hin*c II .

Results and discussions:

The partial amino acid sequence of Japanese Black cattle PPAR γ 2 is presented in Fig.1. Compared with the reported sequence (Sundvold H et al., 1997) , mutation was located in the 2nd codon of 18th amino acid, and act to change amino acid from alanine (GCT) to valine (GTT). By PCR-RFLP with a mismatch primer (Fig.2) , this mutation was detected easily by the appearance of mutation specific bands (60bp) . Using this method, we could detect 3 hetro mutants from 47 Japanese Black cattle.

It is important that the mutation (2nd codon of 18th amino acid) was located within the first 30 N-terminal amino acids. This mutation is likely to change the function of PPAR γ 2, as the first 30 N-terminal amino acids are unique to PPAR γ 2 (Zhu Y et al., 1995). Taking into account these findings and PPAR γ is key regulators of lipid metabolisms (Schoonjans K et al., 1996), marbling seems to be affected by this mutation. We are now studying the relationship between this mutation and marbling in Japanese Black cattle.

Conclusions:

Genetic variants of PPAR γ2 specific region in Japanese Black cattle were detected. The mutation was located in the second codon of the 18th amino acid, and act to change amino acid from alanine to valine. Three hetro mutants were detected from 47 Japanese Black cattle.

Pertinent literature:

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M	G	E	T	L	G	D	A	L	I
1							▼		10
D	P	E	S	E	P	F	V	V	T
11									20
V	S	A	R	T	S	Q	E	I	T
21									30
M	V	D	T	E	M	P	F	W	P
31									40
T	N	F	G	I	S	S	V	D	L
41									50
S	M	M	D	D	H	S	H	A	F
51									60
D	I	K	P	F	T	T	V	D	F
61									70
S	S	I	S	T	P	H			
71									

Fig. 1 Partial amino acid sequence of PPAR γ2 mutant. The arrowhead indicates the location of mutation. The mutation changed amino acid from alanine (GCT) to valine(GTT).

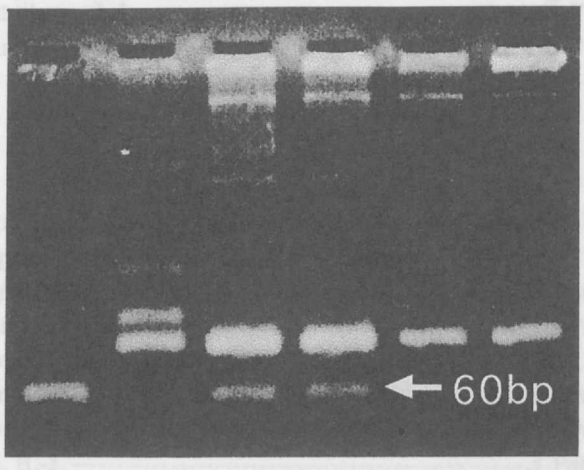

Fig. 2 Acrylamide gel electrophoresis of the PCR products digested with Hinc II .
1, cloning plasmid (mutant)
2, cloning plasmid (wild)
3, Japanese Black cattle(hetro)
4, Japanese Black cattle(hetro)
5, Japanese Black cattle(wild)
6, Japanese Black cattle(wild)
Mutants were detected by the specific band (60bp)