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Genotyping of the polymorphism within exon 1 of *Hormone Sensitive Lipase* (*LIPE*) Gene in three Chinese Yak (*Bos grunniens*) breeds by PCR-RFLP
(Brief Report)

Genotypisierung eines Polymorphismus im Exon 1 des Genes für die hormonsensitive Lipase bei drei chinesischen Yak (*Bos grunniens*) Rassen mittels PCR-RFLP (Brief Report)

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Background

The yak (*Bos grunniens*), a herbivore living on the Qinghai-Tibetan Plateau and its adjacent territories, is one of world's most remarkable domestic animals. Over the past decades, research has been done on properties of yak meat at physiological and biochemical levels. In recent years, some candidate genes associating with meat quality and lipid metabolism in yak have been studied (MA *et al.* 2007, ZHONG *et al.* 2007). The *Hormone Sensitive Lipase (LIPE)* gene has been regarded as a candidate gene associating with lipid metabolism and meat quality. Several studies have reported the genetic variations in the *LIPE* genes of human and pig (TALMUD *et al.* 1998, KNOLL *et al.* 1998, WU *et al.* 1998, HARBITZ *et al.* 1999), whereas the yak *LIPE* gene polymorphism has not been investigated and no information is available. The aim of this study was to identify and characterize the genetic variation in Chinese yak *LIPE* at the DNA sequence level and genotyping of the polymorphism within *LIPE* in different yak breeds.

Procedures

Primer sequences

The primer sequences were adapted from those used by KNOLL *et al.* (1998) to match porcine *LIPE* gene sequence (acc. no. AJ000482 and AJ224692).
Forward primer: 5′-CGC ACA ATG ACA CAG TCG GT-3′;
Reverse primer: 5′-CAG GCA GCG GCC GTA GAA GCA-3′.

PCR condition, PCR-RFLP and sequencing

DNA was isolated from blood samples of 92 domestic yaks (31 Jiulong yaks, 31 Bazhou yaks and 30 Maiwa yaks) using a phenol-chloroform extraction protocol followed by an ethanol precipitation step. The PCR reaction mixture contained 50-100 ng yak genomic DNA, 10 pM of each primer, 0.50 U *ExTaq* DNA polymerase (TakaRa, Dalian, China), 10 ×*ExTaq* Buffer (Mg^{2+} Free), 0.25 mM dNTP, 2.5 mM $MgCl_2$ and ddH_2O in a final volume of

25 µL. The following cycles were applied: 95°C/4 min, followed by 35 cycles at 95°C/45 sec, 60.5°C/1 min, 72°C/1 min, and final synthesis at 72°C/5 min. The 13 µL PCR amplified product was incubated at 30°C for 5-8 h with 1µL of *SmaI* (12U/µL) (TakaRa, Dalian, China), 2µL of 10×T Buffer (330 mM Tris-Ac, PH7.9; 100 mM Mg-Ac; 5 mM Dithiothreitol; 660 mM K-Ac), 0.1% BSA and ddH_2O, respectively. The fragments were separated and visualized by electrophoresis in 2% agarose gels. The different genotypes were scored manually by comparison with a 150 bp DNA ladder (TakaRa, Dalian, China). Chi-square tests were conducted to test the population for Hardy-Weinberg equilibrium. After PCR-*SmaI* analysis purified PCR products from homozygous individuals were sequenced directly with the ABI-3730 automatic DNA sequencer (Applied Biosystems). The sequences alignments, translations, and comparisons were carried out with Bioedit 4.8.10 soft.

Results

The restriction digestion of 498 bp PCR products with *SmaI* enzyme revealed three genotypes AA, AB, and BB (Table 1). Comparing the sequences from different homozygous individuals showed a G>A substitution at position nt70 (acc. no. AY871311 and AY898615). The single nucleotide polymorphisms (SNP) is in exon 1 of yak *LIPE* and results in an amino acid exchange (G→R) (aa27 of the bovine sequence, acc. no. NP_001073689) (Figure 1).

Allele A was most frequent in all three breeds. Animals homozygote for the allele B were not obtained in Jiulong and in Maiwa, but only in Bazhou yaks. The Chi-square test results (1 degree of freedom, $P \leq 0.01$) revealed genetic equilibrium in three yak breeds.

Table1
Different genotypes and allele frequencies and corresponding fragment sizes after digestion of a 498 bp PCR product with *SmaI* restriction enzyme

Genotypen, relative Häufigkeiten der Allele und Fragmentgrößen nach Restriktionsverdau des 498 bp PCR Produkts mit SmaI

Breeds	Animals	Number (frequencies) of genotypes*			Allele frequencies	
		AA	AB	BB	A	B
Jiulong	31	26 (0.839)	5 (0.161)	0	0.92	0.08
Maiwa	30	17 (0.567)	13 (0.433)	0	0.78	0.22
Bazhou	31	14 (0.452)	16 (0.516)	1 (0.032)	0.71	0.29

*corresponding fragments obtained in PCR-RFLP, AA 333 bp, 96 bp, 69 bp, AB 402 bp, 333 bp, 96 bp, 69 bp, BB 402 bp, 96 bp

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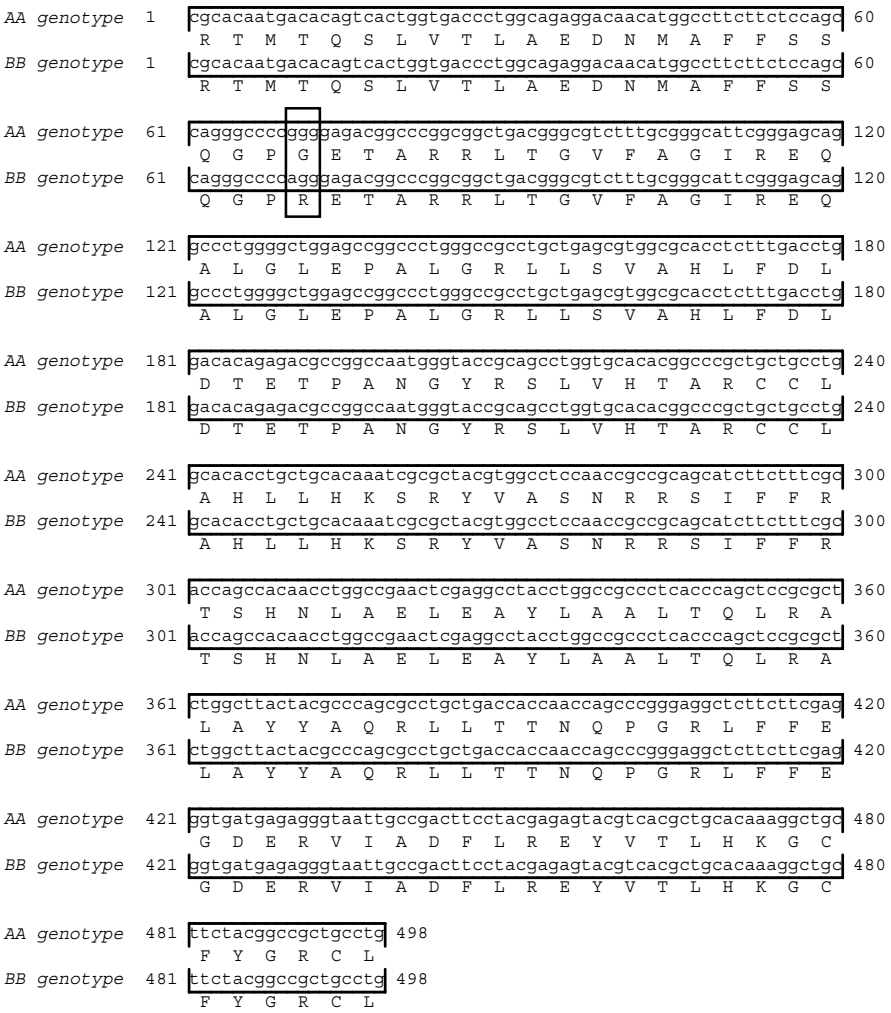

Figure 1

Sequences alignment of the yak *LIPE* gene variants A and B with the GenBank sequence AY871311 and AY898615

Sequenzalignment der LIPE Genvarianten A und B des Yaks mit den Genbanksequenzen AY871311 und AY898615

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