Allele frequencies of Stearoyl CoA desaturase genetic variants in various cattle breeds

Moioli B., Fr. Napolitano, G. B. Congiu, L. Orrù and G. Catillo, Istituto Sperimentale per la Zootecnia, via Salaria 31, 00016 Monterotondo, Italy

Introduction

Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (Ntambi et al.,2002). The cattle genomic sequence of the bovine stearoyl-CoA desaturase gene (accession AY241932, 17088 nucleotides) encodes 6 exons (Medrano J.F. et al., 2003). In this sequence, the authors identified 18 SNPs, all of which are found in the non-coding regions, except three SNPs in exon 5, (10153 bp; 10213 bp; 10329 bp). SNP at position 10329 (C/T) gives rise to a different amino-acid codon: the Alanine residue (allele C) becomes a Valine residue (allele T). Taniguchi et al. (2004) refer that the three SNPs of exon 5 are in linkage disequilibrium, and that the Valine residue may change the enzyme catalytic activity compared to the Alanine; they demonstrate, in fact, that, in Japanese Black cattle, allele C is associated to higher content of monounsaturated fatty acid in carcasses, and suggested that the genotyping for this region is a useful tool for selection of favourable flavored beef carcasses. Purpose of our work was to genotype individuals of various Italian cattle breeds and verify if there are significant differences between breeds in the Alanine/Valine genotypes.

Materials and Methods

DNA of 187 animals was amplified with primers designed on the cattle sequence (GenBank: accession AY241932) in order to produce an amplicon of 212 bp, spanning from 10232 to 10443 bp, encoding the portion of exon 5 containing the targeted C/T SNP, as well as part of the 3' region; the primers used were as follows: Forward: ACCTGGCTGGTGAATAGTGC; Reverse: TGACATATGGAGAGGGGTCA. The Polymerase Chain Reaction (PCR) amplification was performed in a final volume of 50ul, containing 250 ng genomic DNA, 0.2 mM of each dNTP, 40 pmol of each primer, 2 mM $MgCl_2$ and 2,5 U AmpliTaq Gold (Applied Biosystems Foster City U.S.A.). Thirty-five PCR cycles at $T_{annealing}$ of 58 °C were performed. The analysed animals included 22 Chianina, 48 Friesian, 26 Maremmana, 23 Piedmontese, 26 Podolica, 42 Simmental. The detection of sequence variation was performed on the DHPLC Transgenomic WAVE® system, a versatile and powerful tool allowing the resolution of DNA fragments on the basis of differential retention of double-stranded *vs*. single-stranded DNA (Hecker, 2001). At a given temperature, the difference in the melting between homo- and heteroduplex is revealed by differences in retention times. DNA profiles of all animals were compared at the partial denaturation temperature of 60.6 °C. Three amplicons showing the heteroduplex profile, as well as three more samples that showed

a homoduplex profile, were direct sequenced (Perkin Elmer ABI Prism 310 DNA sequencer). The PCR for sequencing was obtained by using ABI Prism BigDye Terminator Cycle Sequencing, Ready Reaction Kits (version 1.1 - Applied Biosystems). The protocol for Single and Double Stranded DNA was optimized in 20 ul of final volume, containing: 4 ul of Terminator Ready Reaction mix, 10-15 ng PCR product and 5 pmol of single primer. The product of sequencing reaction was purified with ethanol/EDTA precipitation method.
Each of the amplicons that had shown the homozygous profile was mixed with one already sequenced homozygous amplicon, and re-detected on the DHPLC Transgenomic WAVE® system: in case the mixture showed a heteroduplex profile, the un-known amplicon was considered of opposite homozygous genotype than the other one used in the mixture. Three of those samples, the genotype of which was inferred by the heteroduplex profile obtained after the mixing, were also direct sequenced to validate this genotyping method.
Allele and genotype frequencies in each breed were calculated by direct counting, and Chi-square analysis of the differences between breed pairs was performed.

Results

Percentage of heterozygous animals was 52.41 %, with a maximum of 65.22 % in the Piedmontese and a minimum of 36.36% in the Chianina (table 1). Alanine homozygous were 38.50 % with a maximum of 59.09 % in the Chianina and a minimum of 21.43 in the Simmental. Valine homozygous were only 9.09 %, with a maximum in the Simmental (16.67 %) while in the Friesian no Valine homozygous was detected. Allele frequency reflects genotype frequency; in fact, frequency of the Valine allele was 35.39 %, with a maximum in the Simmental (47.62%) and a minimum in the Chianina (22.73). Significant differences, in both genotype and allele frequencies, were found between Friesian breed *vs.* Podolica and Simmental, and between Chianina and Simmental (table 1). Between Friesian and Maremmana, only genotype differences were significant.
Genotyping through the DHPLC proved to be efficient and faster compared to direct sequencing. Heterozygous samples (figure 1a) are clearly distinguishable from the homoduplexes (figure 1b) at a partial denaturation temperature of 60.6 °C. Direct sequencing of three samples, the genotype of which was inferred after the heteroduplex profile of the mixture, confirmed that they actually had the opposite genotype of the amplicon they were mixed with.

Conclusions

Because the Valine encoded by allele T at position 10329 is referred to be the ancestral amino-acid of SCD, our results confirm that frequency of this

variant is higher in the ancestral breeds (Maremmana, Podolica, Simmental). Moreover, because it has been referred that the Alanine variant is associated to higher content of monounsaturated fatty acid in carcasses, the low frequency of Valine homozygous in the Chianina and Piedmontese makes to suppose that selection for better flavour of meat, in agreement with Taniguchi et al. (2004), has been performed.

Table 1. Allele frequencies of the Alanine/Valine in the 6 cattle breeds. Significant differences in allele frequencies (P<0.01) between breed pairs are indicated by different letters.

breed	No.	genotype frequency %				allele frequency %		
		Alanine homoz.	Valine homoz.	Heterozygous	P<0.01	Alanine	Valine	P<0.01
Chianina	22	59.09	4.55	36.36	ab	77.27	22.73	ab
Friesian	48	47.92	0	52.08	a	73.96	26.04	a
Maremmana	26	42.31	15.38	42.31	b	63.46	36.54	ab
Piedmontese	23	30.43	4.35	65.22	ab	63.04	39.96	ab
Podolica	26	34.62	15.38	50.00	b	59.62	40.38	b
Simmental	42	21.43	16.67	61.90	bc	52.33	47.62	bc
Total	187	38.50	9.09	52.41		64.71	35.29	

References

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Figure 1-a. DHPLC chromatogram profile of the heteroduplex

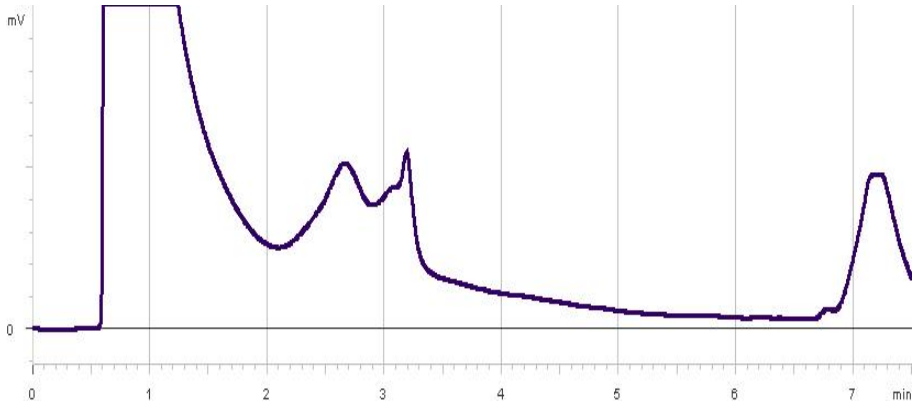

Figure 1-b. DHPLC chromatogram profile of the homoduplex