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Variability of $\mu$-Calpain and Calpastatin genes in cattle

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ABSTRACT - A preliminary analysis on the variability of $\mu$-Calpain (CAPN1) and Calpastatin (CAST) genes in si cattle breeds was carried out, focusing the attention on CAPN1 g.5709C>G, CAPN1 g.6545C>T, and CAST g.282C>G SNPs, which have been suggested to affect beef tenderness in cattle. The results indicate that the two genes are polymorphic in all the analysed breeds, with significant between-breed differences. On the basis of their variability, only CAPN1 g.6545C>T and CAST g.282C>G SNPs seem appropriate to be considered as potential markers for beef tenderness.

Key words: Cattle, Calpain, Calpastatin, SNP.

Introduction – Three mutations, two at the $\mu$-Calpain (CAPN1) and one at the Calpastatin (CAST) gene, have been shown to affect beef tenderness, with the C allele at each SNP being the most favourable (Page et al., 2004; White et al., 2005; Schenkel et al., 2006). Therefore, these markers have been included in commercial tests to classify the animals for tenderness according to their genotype (Van Eenennaam et al., 2007). However, before including a marker into selection programmes, it is important to know the allele frequencies for the specific breed which the markers could be used in. This study is aimed at investigating the variability at the two loci in six cattle breeds for which no data are available, in order to verify their variability and the diffusion of the favourable alleles.

Material and methods – The polymorphism at the CAPN1 and CAST genes was analysed in beef and dairy breeds (Aosta Black Pied, ABP: 20; Aosta Red Pied, ARP: 42; Blonde d’Aquitaine, BA: 48; Belgian Blue, BB: 17; Italian Friesian, IF: 42; Piemontese, PI: 72), collecting samples from individuals as unrelated as possible. DNA was extracted from blood or muscle using NucleoSpin® Blood or NucleoSpin® Tissue kits (Macherey-Nagel). For the SNP genotyping, PCR-RFLP methods were set up, using primers and restriction enzymes shown in Table 1. The PCR mix (20 µl) contained 1.5 mM MgCl_2, and the amplification was carried out at an annealing T of 57°C, 67°C, and 60°C for CAPN1-5709, CAPN1-6545, and CAST, respectively. The digested fragments were electrophoresed on 2.5% agarose gel, stained with ethidium bromide and visualised under UV light.

The allele frequencies, the deviations from Hardy-Weinberg equilibrium, the linkage disequilibrium between the SNPs at CAPN1 gene, as well as the genotypic differentiation for each population

| Table 1. The considered SNPs, with primer sequences and restriction enzymes used. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Gene | Acc. no. | SNP | Primer forward | Primer reverse | Enzyme |
| CAPN1 AF252504 5709C>G 5’gactggggtctctggactt3’ | 5’ggaacctctggctcttgaga3’ | BtgI |
| CAPN1 AF248054 6545C>T 5’catgtcaacctcgtgat3’ | 5’ttaggtcactcgtagacgag3’ | DdeI |
| CAST AY008267 282C>G 5’aagtaaagcacaaggaacac3’ | 5’aggcttttgctgaaaaca3’ | RsaI |

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pair were calculated with the Genepop software (Raymond and Rousset, 1995). The dendrogram was designed on the basis of the Nei’s genetic distances using the Phylip software (Felsenstein, 1995).

**Results and conclusions** – The PCR-RFLP methods set up to analyse the three SNPs gave good results: **amplicons of the expected size and clearly recognizable digested fragments were obtained.** The analysis of **CAPN1-5709** showed the presence of two alleles: C gave two fragments of 272 and 143 bp, while G remained undigested. Two alleles were also observed for the **CAPN1-6545**: C originated two fragments of 216 and 28 bp, while T, characterized by a further restriction site, yielded three fragments of 159, 57, and 28 bp. Similarly, the analysis of the **CAST-282** allowed identifying the G allele, with two fragments of 265 and 168 bp, and the C allele undigested.

The three markers were polymorphic in **all the six breeds, except for Aosta Black Pied** where the **CAPN1-5709 C** allele was not observed (Table 2). In the other breeds, this allele was rather rare, confirming the low variability of the marker already found in all the populations studied so far (Van Eenennaam et al., 2007). As for **CAPN1-6545**, the favourable C allele was prevalent in Belgian Blue, Piemontese and, unexpectedly, in Italian Friesian breed, where it showed the highest frequency. At **CAST** locus, the C allele was the most frequent in Friesian as well as in beef breeds; the highest frequency was found in the Piemontese breed, where a previous investigation had confirmed the favourable association of the C allele with meat tenderness (Di Stasio et al., 2008); the frequencies of this allele in the examined breeds were **slightly lower than those reported for most of the other studied breeds** (Van Eenennaam et al., 2007).

The genotypic distributions were in agreement with the Hardy-Weinberg equilibrium in all the populations. No linkage disequilibrium was observed between the two SNPs at **CAPN1** locus (P=0.08), which indicates the possibility of an independent selection.

**Table 2.** Allele frequencies at three SNPs in the examined breeds.

| Locus | SNP | Allele | ABP | ARP | BA | BB | FI | PI |
|-------|-----|--------|-----|-----|----|----|----|----|
| CAPN1 | g.5709C>G | C | 1.00 | 0.94 | 0.93 | 0.82 | 0.81 | 0.85 |
|       |     | G | 0.06 | 0.07 | 0.18 | 0.19 | 0.15 |    |
| CAPN1 | g.6545C>T | C | 0.38 | 0.39 | 0.32 | 0.59 | 0.81 | 0.53 |
|       |     | T | 0.62 | 0.61 | 0.68 | 0.41 | 0.19 | 0.47 |
| CAST  | g.282C>G | C | 0.48 | 0.50 | 0.57 | 0.59 | 0.59 | 0.64 |
|       |     | G | 0.52 | 0.50 | 0.43 | 0.41 | 0.41 | 0.36 |

The analysis of the genotypic differentiation across loci for each population pair showed significant differences in 7 cases out of 15, after Bonferroni correction (Table 3).

**Table 3.** Genetic differences between breeds: genetic distances (above the diagonal) and P values for genotypic differentiation (under the diagonal).

|     | ABP | ARP | BA | BB | IF | PI |
|-----|-----|-----|----|----|----|----|
| ABP | -   | 0.002 | 0.008 | 0.045 | 0.126 | 0.036 |
| ARP | ns  | -   | 0.005 | 0.034 | 0.112 | 0.025 |
| BA  | ns  | ns  | -   | 0.046 | 0.142 | 0.026 |
| BB  | ns  | ns  | ns  | -   | 0.029 | 0.004 |
| IF  | 0.000 | 0.000 | 0.000 | ns | - | 0.045 |
| PI  | 0.001 | 0.003 | 0.002 | ns | 0.000 | - |
The Italian Friesian and Piemontese breeds significantly differed from all the others, except for Belgian Blue. The same analysis considering the single SNPs (data not reported) indicated that CAPN1-6545 was the main responsible for this differentiation.

The lowest genetic distances (Table 3) were observed between the two Aosta breeds and between Piemontese and Belgian Blue, consistently with previous results obtained with other genes on the same breeds (Di Stasio et al., 2004). The breed clustering seems to be independent from the breed purpose (Figure 1).

The results on the variability of CAPN1 and CAST loci in different cattle breeds indicate that the integration of the CAPN1-5709 marker into selection programmes should be evaluated with caution, because the selection could be too restrictive for other traits, due to the low frequency of the favourable allele. The CAPN1-6545 and CAST-282 mutations seem more appropriate to be used as markers, if associations with tenderness were confirmed in each breed.

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