Investigation on variability of candidate genes for meat quality traits in Piemontese cattle

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ABSTRACT - This study aimed to investigate the variability of 15 genes chosen according to their function as markers of meat quality traits in Piemontese cattle. Meat samples of Longissimus thoracis muscle and data on carcass weight (CW), shear force (SF), cooking loss (CL), and pH collected on 1,208 Piemontese young bulls progeny of 10 AI sires were available for this investigation. For each trait considered (CW, SF, CL, pH), 48 samples were chosen from each tail of normal distribution, and one or more single nucleotide polymorphisms (SNPs) were determined for the following loci: growth hormone (GH), growth hormone receptor (GHR), pro-opiomelanocortin (POMC), pituitary-specific positive transcription factor 1 (POU1F1), melanocortin-4 receptor (MC4R), corticotrophin-realising hormone (CRH), insulin-like growth factor binding protein-3 (IGFBP3), diacylglycerol acyltransferase 1 (DGAT1), thyroglobulin (TG), carboxypeptidase E (CPE), calpain 1 (CAPN1), calpastatin (CAST), cathepsin B and D (CATB, CATD), and protein kinase adenosine monophosphate-activated γ-subunit (PRKAG3). Eight SNPs were characterized by a high, 4 by an intermediate and 2 by a low variability; 6 may be almost fixed. Based on these results, variable loci will be investigated on the entire available data set in order to study their effects on meat quality traits.

Key words: Meat quality traits, Candidate gene, SNP, Piemontese breed.

Introduction - Consumers demand for high quality products has increased over the last decades, thus emphasizing the role of selection in sustaining and improving the quality of beef. Recently, Boukha et al. (2007) estimated genetic parameters for several beef quality traits of Piemontese young bulls. Results from this study indicated that heritability of investigated traits was moderate. Moreover, lab procedures for the assessment of meat quality traits are expensive and burdensome, and therefore not suitable for an implementation into current breeding schemes. It is well-known that genes regulating metabolism and their polymorphisms can be useful to improve meat quality traits through gene-assisted selection (Dekkers, 2004). This study investigated 15 genes with the aim of assessing their variability as a preliminary study focusing on meat quality traits of Piemontese cattle.

Material and methods – In this investigation meat samples of Longissimus thoracis muscle and data on carcass weight (CW), shear force (SF), cooking loss (CL), and pH collected on 1,208 Piemontese young bulls progeny of 10 AI sires were used; samples and data were available from a previous study where details on animals and procedures for meat quality analysis can be found (Boukha et al., 2007). Genomic DNA was extracted from each sample following salting out method.
For each trait considered (CW, SF, CL, and pH), 48 samples were chosen from each tail of the trait distribution. On the basis of literature indications, the following loci were chosen: growth hormone (GH), growth hormone receptor (GHR), pro-opiomelanocortin (POMC), pituitary-specific positive transcription factor 1 (POU1F1), melanocortin-4 receptor (MC4R), corticotrophin-realising hormone (CRH), insulin-like growth factor binding protein-3 (IGFBP3), diacylglycerol acyltransferase 1 (DGAT1), thyroglobulin (TG), carboxypeptidase E (CPE), calpain 1 (CAPN1), calpastatin (CAST), cathepsin B and D (CATB, CATD), protein kinase adenosine monophosphate-activated γ3-subunit (PRKAG3), and one or more SNPs were determined in above loci. Traits, SNPs' name, GenBank Acc. no. and SNPs' position are listed in Table 1. All SNPs were compiled and chosen from sequences of the candidate genes as published in GenBank. Subsequently, primers were constructed using Vector NTI Advance 10 (Invitrogen).

SNPs genotyping was carried out using a PCR-RFLP technique with the only exception of CAPN1:g.6545C>T SNP, where a PCR-ARMS technique was used (Rincón and Medrano, 2006). Allele frequencies, Hardy-Weinberg (HW) equilibrium as well as genic differentiation for sampled populations (i.e., 2 sub-population per trait based on the tails of the corresponding phenotypic distribution) were carried out using Genepop software version 4.0 (Rousset and Raymond, 2007). In light of the fact that number of individuals for each sample was less than 1,000, deviation from HW equilibrium (P-value) was calculated through the complete enumeration method (Louis and Dempster, 1987). In the genic differentiation for population test an unbiased estimate of the P-value of a log-likelihood ratio (G) based exact test was performed.

**Results and conclusions** – In the present study, several genes were chosen based on their biological function to be evaluated for their variability in Piemontese cattle. The SNPs within these genes were examined and analyzed with Genepop, and minor allele frequencies, HW equilibrium and genic differentiation between populations are reported in Table 2. Gh: g.200G>A SNP, CRH:g.145G>A SNP, POMC: g.254C>T SNP, IGFBP3:g.299C>A SNP, TG:g.1696C>T SNP, and CATB: g.540T>C SNP were characterized by a very low variability, and this suggests that the major allele could be almost fixed in Piemontese breed. The test of HW equilibrium revealed a significant deviation (P<0.05) only for CPE and CATD loci (Table 2). Samples from the

### Table 1. Summary of SNPs information and location.  

| Trait | Gene | BTA | SNP name | Location | Acc. no. | SNP |
|-------|------|-----|----------|----------|----------|-----|
| CW    | GH   | 19  | 1547 intron 3 | M57764    | C/T      |
|       | GHR  | 20  | 200 exon 10 | AF140284  | G/A      |
|       |      |     | 257        |           | G/A      |
|       | POMC | 11  | 254 exon 3 | J00021    | C/T      |
|       | POU1F1 | 1  | 208 exon 6 | EF090615  | A/G      |
|       | MC4R | 24  | 1069 exon 2 | AF265221  | C/G      |
|       | CRH  | 14  | 145 exon 2 | AF340152  | G/A      |
|       |      |     | 240        |           | G/C      |
|       | IGFBP3 | 4  | 299 intron 2 | AY306011  | C/A      |
|       | DGAT1 | 14  | 10434 exon 8 | AJ318490  | C/A      |
|       | TG    | 14  | 1696 5'-UTR | M35823    | C/T      |
|       | CPE   | 17  | 601 exon 5 | AY970663  | T/C      |
|       | CAPN1 | 29  | 4558 exon 14 | AF248054  | G/A      |
|       |      |     | 6545 intron 17 | C/T      |
|       | CAST  | 7   | 2870 3'-UTR | AF159246  | A/G      |
|       | SF    | 7   | 2959      |           | A/G      |
|       |      |     | 282 intron 5 | AY008267  | C/G      |
|       | CATB  | 8   | 540 exon 7 | DQ978217  | T/C      |
|       | CATD  | 29  | 77 exon 7 | AB055312  | G/A      |
| pH    | PRKAG3 | 2  | 1609 exon 4 | AY692035  | G/A      |

1Position number.
tails of traits distributions were treated as different populations to investigate any possible difference in allele frequencies. Only $MC4R$ locus showed a significant population difference ($P=0.01$) for CW trait, with high frequency of $C$ allele in the low and high frequency of $G$ allele in the high carcass weight tail, respectively.

In conclusion, several analyzed loci were variable, and they can be considered in further investigations aimed to explore associations with meat quality traits in Piemontese cattle. Based on the results of this prescreening, variable loci will be investigated on the entire available data set in order to study their effects on meat quality traits.

Authors acknowledge financial support from ANABoRaPi.

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