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SHORT COMMUNICATION

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Association of polymorphisms in *calpain 1*, (*mu/I*) *large subunit*, *calpastatin*, and *cathepsin D* genes with meat quality traits in double-muscled Piemontese cattle

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Summary

Five single-nucleotide polymorphisms (SNPs) located in the *calpain 1, (mu/I) large subunit* (*CAPN1*), *calpastatin* (*CAST*), and *cathepsin D* (*CTSD*) genes were analyzed in a large sample of Piemontese cattle. The aim of this study was to evaluate allele and genotype frequencies of these SNPs and to investigate associations of *CAPN1*, *CAST*, and *CTSD* gene variants with meat quality traits. Minor allele frequencies ranged from 30 to 48%. The presence of the A allele at *CAPN530* increased yellowness and drip loss. The *CAST282* G allele was associated with an increased drip loss compared to the C allele, and the *CAST*2959 A allele decreased redness compared to the G allele.

Keywords *calpain 1* (*mu/I*) *large subunit*, *calpastatin*, *cathepsin D*, meat quality, Piedmontese, single-nucleotide polymorphism

The most important postmortem changes for muscle protein degradation are the release of cathepsins and the increased activity of calpains. Their activity influences meat tenderness, and genes coding for these proteins are considered functional candidates for meat quality (MQ) traits (Cafè *et al.* 2010). Some SNPs associated with meat traits are the following: *CAPN530* (AF248054.2:g.4558G>A), located in exon 14; *CAPN4751* (AF245054.2:g.6545C>T), located in intron 17; *CAST2959* (AF159246.1:g.2959A>G), located in the 3′UTR of the gene; *CAST2870* (AF159246.1: g.2870A>G), located in the 3′UTR of the gene; *CAST282* (AY008267:g.282G>C), located in intron 5; and *CTSD* (AB055312:g.77G>A), located in the coding region of *cathepsin D*. The investigated SNPs have been reported to be involved in beef or pork quality traits, and some of them have been incorporated into commercial tests. However, these tests have not been validated for double-muscled beef breeds. The Piemontese is the most important Italian beef cattle breed (Albera *et al.* 2004); it is highly specialized for beef production and exhibits double muscling. This study aimed to estimate allele and genotype frequencies for 5 SNPs located in three different genes, *CAPN1*, *CAST*, and *CTSD,* and to investigate associations between these gene variants and MQ traits using data from 990 young Piemontese bulls.

Details on sample collection and procedures for measurement of MQ can be found in Boukha *et al.* (2011). Traits of interest for this study were muscle pH (pH24h), reflectance coordinates (L*, lightness; a*, redness; b*, yellowness), shear force (SF, kg), drip loss (DL,%), and cooking loss (CL,%) (Table S1).

The investigated genes were genotyped using the ARMS-PCR and RFLP-PCR techniques. Primer sequences, annealing temperatures, enzymes, and fragment sizes are presented in Table S2.

Estimation of allele and genotype frequencies was performed using GENEPOP ver. 4.0 (Rousset & Raymond 2007). An association study for *CAPN1*, *CAST*, and *CTSD* was carried out using Bayesian procedures and by performing numerical integration through the Gibbs sampler on the basis of the following mixed-inheritance linear animal model:

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$$y_{ijk} = \mu + \mathrm{HERD}_i + \mathrm{WLA}_j + \alpha_k + \sum_{i=1}^{n} x_{ijkl}\beta_l + \sum_{l=1}^{n} z_{ijkl}\gamma_l + \varepsilon_{ijk}$$

Where y_{ijk} was a phenotypic measure for a trait, HERD_i was the effect of the fattening herd (124 levels), WLA_j was the effect of week of MQ analysis (92 levels), α_k was the infinitesimal genetic effect of individual k, x_{ijkl} (0,1,2) counts the number of copies of the minor frequency allele at the lth SNP of subject ijk, β_l is the additive effect of the lth SNP, (0,1) equals 1 if subject ijk was heterozygous at the lth SNP or zero otherwise, γ_l is a deviation owing to dominance for the lth locus, and ε_{ijk} was a random residual term. The model was fitted to estimate, for all traits, the contribution of each SNP separately, the aggregated effects of the SNPs within a gene, or the aggregated contribution of all SNPs. Parameters of concern were dispersion parameters, and additive and dominance effects of SNPs as defined by Falconer & Mackay (1996). The proportion of the additive genetic variance explained by all SNPs, single SNP, *CAPN1*, and *CAST* was computed as the percentage reduction of the polygenic variance, estimated with appropriate models (i.e., models with single SNP or pool of SNPs), relative to the estimate provided by a model including polygenic effects only. The posterior median was used as a point estimate of parameters of concern. We computed P1, which was the posterior probability for the estimated SNP effect being >0.1 (for positive effects) or lower than −0.1 (for negative effects) polygenic standard deviation, and P2, which was the posterior probability for the proportion of the additive genetic variance explained by all SNPs, single SNP, *CAPN1*, and *CAST* of being >0. An association and the proportion of variance were considered to be relevant when either P1 or P2 was at least 80%.

Allele and genotype frequencies are listed in Table S3. Minor allele frequency (MAF) at *CAST282* was in agreement with findings of a previous study on Piemontese cattle (Lisa & Di Stasio 2009). The predominance of the A allele at *CAST2959* was also reported by Curi *et al.* (2009). The frequency of the G allele at *CAST2870* was similar to that observed by Corva *et al.* (2007) in an Angus × Hereford crossbred population.

Consistent with Lisa & Di Stasio (2009), MAF at *CAPN4751* was close to 0.5. Minor allele frequency at *CAPN*530 was comparable to the frequency estimated by Allais *et al.* (2011) for Limousin and Blond d'Aquitaine.

The *Cathepsin D* SNP showed a low frequency of the A allele, and it seems to be monomorphic in Piemontese cattle. Across breed, variation in allele frequencies may be related to variation in cathepsin D content and activity (Rosochacki *et al.* 2005).

Estimates of the proportion (%) of the additive genetic variance explained by all SNPs, single SNP, *CAPN1*, and *CAST* are presented in Table 1. The proportion of additive genetic variance explained was smaller than 25% for all traits, with the exception of DL. The probability of the proportion of the additive genetic variance explained being different from 0 was lower than 80%. This means that the investigated SNPs explain a limited amount of the additive genetic variance of MQ traits.

The estimated additive effect for *CAST282* indicated that the G allele was associated with a 0.14% increase in DL (Table 2). *CAST282* is in the intronic region, and its association with DL may be due to linkage with other mutations (possibly the causal mutation) that lie in the coding

Table 1 Estimates of the proportion (%) of additive genetic variance explained by all SNPs, single SNP, *CAPN1*, and *CAST* genes[1,2].

Model (genetic effects other than the polygenic effect)	Trait[3]						
	pH24h	L*	a*	b*	DL	CL	SF
All SNPs in *CAPN1*, *CAST*, and *CTSD* genes	9.8 (55)	−15.0 (40)	19.8 (64)	27.4 (65)	31.7 (75)	−12.7 (43)	1.3 (51)
Single-gene models							
All SNPs in *CTSD*	17.0 (57)	−6.3 (45)	6.7 (55)	18.8 (60)	27.2 (73)	18.1 (60)	8.6 (54)
All SNPs in *CAPN1*	4.8 (52)	−4.4 (47)	2.0 (51)	24.2 (64)	27.8 (73)	17.5 (61)	2.6 (51)
All SNPs in *CAST*	3.4 (51)	−12.3 (42)	13.1 (59)	16.2 (60)	26.3 (72)	17.3 (60)	1.0 (51)
Single-SNP models							
CAST282	15.0 (56)	−7.3 (45)	9.5 (57)	20.0 (61)	28.0 (74)	18.4 (60)	−3.2 (49)
CAST2959	15.4 (57)	−12.7 (40)	7.4 (55)	14.0 (58)	27.0 (73)	14 (58)	10.4 (56)
CAST2870	13.2 (55)	−6.8 (45)	8.7 (56)	21.8 (62)	27.2 (73)	19.3 (61)	0.5 (50)
CAPN530	11.8 (55)	−3.3 (48)	6.9 (55)	25.6 (64)	30.7 (74)	18.6 (60)	5.3 (53)
CAPN4751	14.0 (56)	−7.7 (44)	7.0 (55)	20.4 (61)	29.5 (75)	20.2 (62)	7.8 (54)

[1]The proportion of the additive genetic variance explained by all SNPs, single SNP, *CAPN1*, and *CAST* was computed as the percentage reduction of the polygenic variance, estimated with appropriate models (i.e., models with single SNP or pool of SNPs), relative to the estimate provided by a model including polygenic effects only.

[2]The posterior probability (%) for the proportion of the additive genetic variance explained by all SNPs, a single SNP, *CAPN1*, and *CAST* genes of being >0 is reported within parentheses.

[3]pH24h, pH at 24 h after slaughter; L*, lightness; a*, redness; b*, yellowness; DL, drip loss; CL, cooking loss; SF, shear force.

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Table 2 Estimates of additive and dominance deviations (estimated probability of a deviation being >0.1, if positive, or lower than −0.1, if negative, polygenic standard deviation) by SNP[1].

	Trait[2]						
SNP	pH24h	L*	a*	b*	DL, %	CL, %	SF, kg
CAST282							
Additive deviation *G* vs. *C*	−0.001 (53)	−0.238 (62)	−0.077 (39)	−0.083 (54)	0.139 (81)	0.009 (33)	0.009 (39)
Dominance deviation	0.002 (57)	0.216 (57)	−0.278 (79)	−0.076 (51)	0.058 (49)	−0.149 (56)	−0.080 (92)
CAST2959							
Additive deviation *A* vs. *G*	−0.002 (75)	−0.002 (20)	−0.003 (88)	−0.127 (68)	0.086 (62)	−0.043 (36)	−0.036 (69)
Dominance deviation	−0.004 (81)	0.569 (93)	−0.052 (36)	0.211 (80)	0.182 (86)	−0.316 (78)	−0.007 (40)
CAST2870							
Additive deviation *A* vs. *G*	0.000 (40)	0.096 (37)	−0.175 (63)	0.031 (38)	−0.003 (23)	−0.066 (42)	−0.019 (50)
Dominance deviation	−0.002 (59)	−0.141 (46)	0.141 (54)	0.131 (64)	−0.042 (43)	0.178 (61)	0.009 (26)
CAPN530							
Additive deviation *A* vs. *G*	0.003 (77)	0.190 (53)	0.052 (33)	0.213 (85)	0.143 (82)	−0.154 (59)	−0.009 (40)
Dominance deviation	−0.003 (75)	−0.004 (25)	0.146 (55)	0.115 (61)	−0.094 (63)	−0.148 (57)	−0.010 (42)
CAPN4751							
Additive deviation *C* vs. *T*	0.002 (62)	0.145 (23)	0.157 (59)	0.117 (64)	−0.041 (41)	0.037 (36)	0.025 (57)
Dominance deviation	−0.003 (75)	−0.004 (25)	0.146 (55)	0.115 (61)	−0.094 (63)	−0.148 (57)	−0.010 (42)
CTSD							
Additive deviation *A* vs. *G*	−0.001 (51)	−0.155 (48)	−0.023 (36)	0.057 (48)	0.035 (43)	0.137 (53)	−0.037 (42)
Dominance deviation	0.004 (68)	0.152 (48)	−0.020 (40)	−0.026 (45)	−0.227 (77)	−0.140 (52)	0.118 (90)

[1]Additive deviations were computed as half of the difference between the effects of homozygous genotypes, and dominance deviations as the deviation of the heterozygous genotype from the average of the two homozygous genotypes.
[2]pH24h, pH at 24 h after slaughter; L*, lightness; a*, redness; b*, yellowness; DL, drip loss; CL, cooking loss; SF, shear force.

or regulatory region of *CAST*. Likewise, Krzęcio *et al.* (2005) detected a significant association between a mutation in the intronic region of *CAST* and DL in pork. *CAST2959* exhibited an additive effect on a* and dominance effects on pH24h, L*, b*, and DL. Indirect effects of this gene might be hypothesized, as a consequence of known influences on regulation of glycolysis.

The A allele of *CAPN530* was associated with increased b* and DL (Table 2). As for *CAST*, few studies have detected associations of the *CAPN1* gene with DL, a*, or b*. Conversely, in pork, there is much evidence that *CAPN1* and *CAST* SNPs are related to water-holding capacity traits. Because desmin is a known calpain 1, (mu/I) large subunit substrate, it might be argued that *calpain 1, (mu/I) large subunit* autolysis and activation may explain a portion of the variation of desmin degradation and activation/deactivation of drip channels, thus affecting the extent of DL.

There was no additive effect of *CAPN4751* on the investigated traits, but we observed a dominance deviation for b* and CL. This result might be related to the high myofibril volume in double-muscled cattle, which increases the interstitial liquid and CL.

The results obtained from the association analysis showed an additive effect of three SNPs on important quality traits such as DL and color, but some associations of these SNPs for other cattle populations that were previously published could not be confirmed in our work (e.g., SF). The lack of association with SF might be due to the double-muscle mutation, because it is associated with decreased SF affecting collagen content, proportion of stable non-reducible cross-links, and type of muscle fiber. Further, as demonstrated by King *et al.* (2003), the effects of SNPs on SF were breed specific. In Piemontese cattle, effects of the investigated SNPs on MQ were small, so their impact in a breeding program might be trivial, demonstrating the fundamental role of SNP validation before the usage of polymorphism information in selection procedures.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Descriptive statistics for meat quality traits.

Table S2 Single nucleotide polymorphisms, amplified size, primer sequences, annealing temperature, enzyme and digest product size.

Table S3 Allelic and genotype frequencies for *CAST*, *CAPN1*, and *CTSD* polymorphisms.

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