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Effect of polymorphisms in candidate genes on carcass and meat quality traits in double muscled Piemontese cattle

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ABSTRACT

The aim of this study was to investigate the association between 10 candidate genes and carcass weight and conformation, carcass daily gain, and meat quality (pH, color, cooking loss, drip loss and shear force) in 990 double-muscled Piemontese young bulls. Animals were genotyped at each of the following genes: growth hormone, growth hormone receptor, pro-opiomelanocortin, pro-opiomelanocortin class 1 homeobox 1, melanocortin-4 receptor, corticotrophin-releasing hormone, diacylglycerol O-acyltransferase-1, thyroglobulin, carboxypeptidase E and gamma-3 regulatory subunit of the AMP-activated protein kinase. All the investigated SNPs had additive effects which were relevant for at least one of the traits. Relevant associations between the investigated SNPs and carcass weight, carcass daily gain and carcass conformation were detected, whereas associations of SNPs with meat quality were moderate. Results confirmed some of previously reported associations, but diverged for others. Validation in other cattle breeds is required to use these SNPs in gene-assisted selection programs for enhancement of carcass traits and meat quality.

1. Introduction

Meat quality (MQ) is a big challenge for the beef industry because the main traits that affects MQ variation can be measured only after slaughter, require expensive and time-consuming techniques and are moderately to low heritable (Boukha et al., 2011). Thus, the enhancement of MQ is difficult. A number of DNA markers, found in various candidate genes regions, were incorporated in commercial genetic tests to improve MQ through selective breeding. Validation of these markers across breeds is crucial to establish whether the observed effects are detected in the breed of interest (Van Eenennaam et al., 2007). These tests have never been validated in the Piemontese breed, which is numerically the most important Italian beef cattle population (Albera, Carnier, & Groen, 2004) exhibiting double-muscling.

A total of 20 polymorphisms located in 15 genes, identified on the basis of their biological role, were included in a preliminary study with the aim of assessing their variability (Ribeca et al., 2009). The associations between some of those polymorphisms, located in calpain, calpastatin and cathepsin genes, and MQ in Piemontese cattle were described by Ribeca et al. (2013). In this study, we consider 10 additional candidate gene polymorphisms. The investigated SNPs were located in the following gene regions: growth hormone (*GH*), growth hormone receptor (*GHR*), pro-opiomelanocortin (*POMC*), POU class 1 homeobox 1 (*POU1F1*), melanocortin-4 receptor (*MC4R*), corticotrophin-releasing hormone (*CRH*), diacylglycerol O-acyltransferase (*DGAT*), thyroglobulin (*TG*), carboxypeptidase E (*CPE*) and gamma-3 regulatory subunit of the AMP-activated protein kinase (*PRKAG3*). The *GH*, as well as the *GHR*, is involved in the control of several physiological processes and it has been previously associated with marbling (Barandese, Bunch, Harrison, & Thomas, 2006). The *POMC* gene acts as endogenous ligand for the MC4R, a key molecule underlying energy homeostasis, and is also a positional candidate gene for shipping weight and hot CW (Buchanan, Thue, Yu, & Winklman-Sim, 2005). The *POU1F1* gene is pituitary-specific transcription factor that regulates *GH*, prolactin and thyroid-stimulating hormone β subunit genes. The *CRH* plays an important role in many biological and physiological functions regulating adrenocorticotropin (ACTH) secretion. The *DGAT* gene encodes to a microsomal enzyme that catalyzes the final step of the triglyceride synthesis pathway (Cases et al., 1998). The *TG* gene encodes thyroglobulin, precursor of triiodothyronine (T3) and tetraiodothyronine (T4), signal for fat cells development (Fortes et al., 2009). The *CPE* gene is a peripheral membrane protein that specifically bonds regulated secretory pathway proteins and has been linked to obesity in mice and type 2 diabetes mellitus in Human (Chen et al., 2001). The *PRKAG3* gene, after activation, increases fatty acid oxidation and glucose uptake to satisfy muscle energy demands (Winder, 2001). The aims of the present study were to estimate allele and genotype frequencies at the 10 investigated candidate gene polymorphisms and to investigate the associations between these polymorphisms and carcass quality and MQ in double-muscled Piemontese cattle.

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2. Material and methods

2.1. Animals

The study was carried out on 990 purebred Piemontese young bulls that were progeny of 109 AI sires and of 1170 dams. Animals were fattened in 124 farms and slaughtered at the same commercial slaughterhouse from March 2005 to February 2007 (average age at slaughter: 523 ± 73 days). Pedigree information (10050 animals) was supplied by the Association of Piemontese Cattle Breeders (ANABoRaPi, Carrù, Italy) and included animals with phenotypic records for carcass quality and MQ and all their known ancestors. Each animal with a phenotypic record had at least 6 known ancestors in the pedigree data. Sire-offspring relationships in the pedigree were confirmed through DNA testing based on 19 microsatellites (Budowle et al., 2005) and not confirmed animals were discarded.

2.2. Samples collection and phenotyping

Details on collection of samples and measurement of carcass traits and MQ can be found in Boukha et al. (2011) and Bonfatti, Albera, and Carnier (2013). Briefly, carcass traits of interest were: carcass weight (CW), carcass conformation category, based on the EU linear grading system (Commission of the European Communities, 1982) and rearranged into numerical score (EUS: S+ = 6.33, S = 6, S− = 5.66, E+ = 5.33, E = 5, E− = 4.66, U+ = 4.33, U = 4, U− = 3.66), and gross carcass daily gain (GCDG) computed by relating CW to age at slaughter. Assessment of MQ was performed using individual samples of *Longissimus thoracis* muscle which were collected between the 5th and 6th thoracic vertebra 24 h after slaughter. MQ traits included muscle pH measured at 24 h (pH24h) and 8 d after slaughter (pH8d) as the average of three measures taken at random positions in the sample, drip loss (DL), recorded as the difference between the weight of the packaged sample and the weight of the sample dried by blotting-paper plus the weight of the heat-dried bag, color reflectance coordinates (L*, a*, b*) (Commission International de l'Éclairage, 1976), cooking loss (CL) computed as the weight loss of the steak after cooking expressed as a percentage of the uncooked steak weight, shear force (SF) measured on 5 cross-sectional round cores in each cooked sample steak. With the exception of ph24h, all MQ measurements were performed 8 d after slaughter.

2.3. DNA extraction and genotyping

Individual meat samples obtained from the 990 young bulls were used for total genomic DNA isolation. DNA was extracted from each sample (100 mg), previously stored at −20 °C, using a non-phenolic method: cells were lysed in a cell lysis solution (50 mM Tris, HCl pH 8, 20 mM EDTA and 2% SDS) and proteinase K was added. After a protein precipitation by ammonium acetate (10 M), DNA was isolated by isopropanol and washed in ethanol. The concentration of genomic DNA samples was determined by electrophoresis on 1% agarose gel, stained with SYBR® safe (Invitrogen).

A total of 10 SNPs were genotyped using RFLP-PCR techniques. SNPs, amplicon dimension, primers sequences, annealing temperature, enzyme and digestion product size are presented in Table 1.

Genotyping procedures for SNPs located on *GH* (Zhang, Brown, De Nise, & Ax, 1993), *GHR* (Di Stasio, Destefanis, Brugiapaglia, Albera, & Rolando, 2005), *POMC* (Thue & Buchanan, 2003), *POU1F1* (Woollard, Schmitz, Freeman, & Tuggle, 1994), *MC4R* (Thue, Schmutz, & Buchanan, 2001), and *TG* (Thaller et al., 2003) have been previously described.

Although the primers used to amplify *CRH* gene were designed using Vector NTI® software (Invitrogen) to include 3 SNPs in exon 2 (AF340152:g. 22C > G, AF340152:g.145G > A and AF340152: g.240G > A), in this study samples were genotyped only for *CRH 240* because in a prescreening (Ribeca et al., 2009) *CRH22* and *CRH145* SNPs resulted to be monomorphic in the Piemontese breed. The *DGAT10434* was amplified using forward primer described by Winter et al. (2002) and reverse primer was designed using Vector NTI® software. The *CPE* and *PRKAG3* genes were analyzed using primers designed by Vector NTI® software.

The PCR reactions were optimized as follows: 30 ng of genomic DNA, 0.20 μM of each primer, for *CRH*, *CPE* and *PRKAG3* and 0.40 μM for *DGAT*, 200 μM deoxynucleotide triphosphates (dNTPs), 2 mM $MgCl_2$, 1X Taq reaction buffer and 1 unit Taq Pol (Jena Bioscience), in a final volume of 15 μl. Genotypes at each SNP were validated by direct sequencing of 8 samples using the CEQ8000 sequencer (BeckmanCoulter, Fullerton, CA).

Table 1
Single nucleotide polymorphisms, amplified size, primer sequences, annealing temperature, enzyme and digest product size.

Genetic Polymorphism	Amplified size (bp)	Primer sequence (5′-3′)	Annealing temperature (C°)	Enzyme[a]	Product size (bp)
GH(M57764:g.1547TC > G)	891	Forward primer: ATCCACACCCCCTCCACACAGT Reverse primer: CATTTTCCACCCTCCCCTACAG	67 × 35	MspI	525, 194, 109 and 63 (C allele) 634,194 and 63 (D allele)
GHR(AF140284:g.257A > G)	342	Forward primer: GCTAACTTCATCGTGGACAAC Reverse primer: CTATGGCATGATTTTGTTCAG	53 × 40	AluI	191 and 151 (G allele) 191, 101 and 50 (A allele)
POMC(J00021:g.254C > T)	390	Forward primer: CGTGCATCCGGGCCTGCAAGC Reverse primer: CAG CTC CCT CTT GAA TTC GAG	TD: 68(−1 °C) × 5 63 × 30	BtsI	390 (C allele) 233 and 157 (T allele)
POU1F1(EF090615:g.208A > G)	451	Forward primer: AAACCATCATCTCCCTTCTT Reverse primer: AATGTACAATGTGCCTTCTGAG	56 × 35	HinfI	451 (A allele) 244 and 207 (G allele)
MC4R(AF265221:g.1069C > G)	226	Forward primer: TACCCTGACCATACTGATCG Reverse primer: AGAGCAACAAATGATCTCTTTG	56 × 35	TaiI	226 (C allele) 123 and 103 (G allele)
CRH(AF340152:g.240G > C)	353	Forward primer: CGCCCGCTAAAATGCGACTG Reverse primer: CTGCAGCAGCGCTCGGAA a	65 × 35	TaqI	353 (C allele) 238 and 115 (G allele)
DGAT(AJ318490: g.10434GC > AA)	250	Forward primer: GCACCATCCTCTTCCTCAAG Reverse primer: AGGTTGTCGGGGTAGCTCAC	TD: 63(−0.5 °C) × 10 58 × 25	CfrI	250 (AA allele) 202 and 42 (GC allele)
TG(M35823:g.1696C > T)	545	Forward primer: GGGGATGACTACGAGTATGA CTG Reverse primer: GTGAAAATCTTGTGGAGG CTGTA	60 × 35	PsuI	474 and 71 (T allele) 296, 178 and 71 (C allele)
CPE(AY970663:g. 601C > T)	336	Forward primer: TCTACTTTGCAGTATCAGCAGT GG Reverse primer: CTGTCATCATCATTCTTGCGAC	60 × 35	Hin1II	162, 149 and 25 (C allele) 149, 99 and 25 (T allele)
PRKAG3(AY692035:g. 1609G > A)	339	Forward primer: AAGGGAGACAACAGATGGGA Reverse primer: CATGAAGTGCATGTAGACCTGAG	60 × 35	FokI	339 (A allele) 282 and 117 (G allele)

[a] All enzymes are provided by Fermentas and BioLabs.