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To cite this article: Sebastiana Failla, Antonella Cuteri, Cinzia Marchitelli, Alessandra Crisà, Michela Contò, Chiara Berti, Francesco Filippini, Sergio Gigli & Alessio Valentini (2009) Effect of some candidate genes on meat characteristics of three cattle breeds, Italian Journal of Animal Science, 8:sup2, 81-83, DOI: [10.4081/ijas.2009.s2.81](https://doi.org/10.4081/ijas.2009.s2.81)

To link to this article: <https://doi.org/10.4081/ijas.2009.s2.81>



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Published online: 07 Mar 2016.



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# Effect of some candidate genes on meat characteristics of three cattle breeds

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**ABSTRACT** - With the aim to assess if some molecular markers can help to select animals for meat characteristics, we studied 84 individuals equally representing the Marchigiana, Maremmana, and Holstein Friesian cattle breeds genotyped at 288 SNPs located within candidate genes. Several SNPs were found associated with meat quality parameters but with P which was higher than the Bonferroni threshold. However, several SNPs had a low P at different times during meat maturation, suggesting their involvement in the meat quality variation. Of particular interest for the biological role and potential for selection were: cathepsin G affecting MFI, IGF1R affecting pH and collagen XVIII affecting colour.

*Key words:* Meat quality, SNP, Beef.

**Introduction** - The selection in beef breeds is carried out mostly on growth and morphological traits. However, these traits are quite far away from the marketed products, i.e. meat with characteristics suitable to processing industry and consumers. On the other hand, meat traits imply taking measures post-mortem and killing potential breeding animals. Molecular markers can help overcome these limits since if a marker is found associated with a particular desired characteristic using a set of "training" samples, it can be used in living population to select animals bearing that characteristic. In this paper, we tried to find associations of 288 SNPs located within genes candidate for meat traits.

**Material and methods** - *Animals* - The work was carried out on 28 male individuals each for Marchigiana (MR), Maremmana (MM), and Holstein Friesian (HF) breeds. These were obtained at weaning and reared up to an average of 459 days (SD=26) on a standard diet. *Meat measurements* - At slaughter, 5 steaks were taken from the right side from the 6<sup>th</sup> to 10<sup>th</sup> rib. On these, several measures were taken at different maturation times and here we will report results concerning pH, myofibril fragmentation index (MFI), colour (L\* a\* b\*) and dripping, cooking and thawing losses. *SNPs genotyping* - Two hundred and eighty eight single nucleotide polymorphisms (SNPs) previously characterized in genes candidate for meat traits under the EU Gemqual project (Negrini *et al.*, 2008) were genotyped on individuals belonging to the 3 breeds. SNPs characterization has been described elsewhere (Williams *et al.*, 2009). Genotyping was performed by K Biosciences (www.Kbioscience.com), using Amplifluor<sup>TM</sup> (Serologicals<sup>TM</sup>) and Taqman<sup>TM</sup> (Applied Biosystems<sup>TM</sup>) chemistries. Generally, accuracy greater than 99% was achieved. Quality control criteria were performed (water as negative control, inter plate duplicate testing of a known DNA, intra plate testing of a known DNA). *Statistical analysis* - SNPs with minor allele frequency less than 10% were excluded from the analysis. The remaining SNPs (always more than 200) were used as fixed effects in the model:  $y = \mu + bx + cNF + e$ , where  $y$ =meat characteristic,  $\mu$  =overall mean,  $x$ =SNP genotype,  $NF$ =noise factors (breed, age at slaughter, stress status at slaughter visually assessed as yes/no),  $e$ =residuals.

**Results and conclusions** - None of the SNPs considered was found associated with the meat characteristics at a Bonferroni threshold of  $5.57 \times 10^{-6}$  which is relative to all the multiple comparisons possible. We report in the following tables the SNPs found associated at  $P < 0.02$ , with the caution that further datasets are necessary to confirm the association. However, some genes resulted associated with a quite low P for several samplings carried out at different times during meat maturation and this may suggest a real involvement in the trait variability. Due to space limits, we report full tables for only two traits, and summarise the remaining traits.

In colour measurements, COL18A1\_314GA, CPT1A\_363AG, GJB1\_258CT, IGF2R\_183GA, RYR1\_568TG, and VIM\_728GA resulted to be associated with  $P < 0.02$  with more samplings along meat maturation. In particular, COL18A1\_314GA resulted associated with all three colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ). Considering drip, cooking and thawing losses, no genes were found with  $P < 0.02$  in more samplings. Only FKHR\_149AC was involved in both cooking and thawing losses at 10 days.

Table 1. Probabilities associated to fixed effects on MFI. In bold genes that are present in more sampling times.

	SNP	Age	Stress	HF	MG	MM	Genotype
MFI 0h	SDC4_187GT	0.29	0.16	0.27	0.01	0.28	0.002
	SDC4_230CT	0.26	0.07	0.25	0.02	0.50	0.009
	MMP1_208TC	0.24	0.02	0.09	0.00	0.50	0.011
MFI 24h	<b>PCK2_489GA</b>	0.70	0.31	0.16	0.44	0.63	0.004
	CHRNE_462CA	0.39	0.14	0.05	0.47	0.59	0.013
	IGF1_348CT	0.56	0.13	0.13	0.39	0.68	0.016
MFI 48h	<b>PCK2_489GA</b>	0.75	0.57	0.01	0.20	0.42	0.004
	<b>ACVR2B_115CT</b>	0.94	0.72	0.00	0.12	0.36	0.018
	VTN_81GA	0.73	0.98	0.00	0.15	0.17	0.018
	ATP1B2_322AG	0.48	0.65	0.00	0.19	0.07	0.020
MFI 120h	<b>ACVR2B_115CT</b>	0.99	0.37	0.17	0.00	0.69	0.004
	<b>CTSG_410AG</b>	0.54	0.40	0.34	0.01	0.84	0.014
MFI 10d	FABP4_84CT	0.82	0.66	0.20	0.01	0.48	0.002
	FABP4_372CT	0.82	0.66	0.20	0.01	0.48	0.002
	FABP4_149AG	0.85	0.60	0.23	0.01	0.52	0.003
	GHR_185CT	0.75	0.67	0.00	0.06	0.83	0.004
	FABP4_28GA	0.81	0.58	0.13	0.01	0.52	0.004
	<b>ACVR2B_115CT</b>	0.66	0.73	0.03	0.03	0.93	0.013
	<b>CTSG_410AG</b>	0.71	0.76	0.16	0.02	0.73	0.018

With the caution above stated, several genes were found in association with meat quality traits, often in more samplings along the maturation time. Some gene effects are still present at 10 days after slaughtering, in particular, cathepsin G (CTSG) that plays a major role in late maturation of meat (Caballero *et al.*, 2007). It is quite interesting that polymorphisms in this gene affect MFI since there is a direct correlation between this parameter and meat tenderness (Veiseth *et al.*, 2001).

Sillence (2004) reports that IGFs are positively associated with growth rate, feed intake, and feed:gain ratio; here we find that insulin-like growth factor I receptor also extends its effects post-mortem by affecting pH all along the maturation time. The effect of collagen XVIII on colour confirms what has been found by Okeudo and Mossb (2005) by biochemical analysis. In conclusion, in this experiment we had more factors than measurements. It

was therefore expected that we had a Bonferroni threshold so low that no SNPs were found associated with a lower P. However, several SNPs had a low P at different times during meat maturation, suggesting their involvement in the meat quality variation. Moreover, since several studies are currently conducted with the same SNPs or with other SNPs that may be in close linkage disequilibrium with them, a meta-analysis of different experiments may reveal in the future causative genes that remain hidden if considering only the single experiment.

Table 2. Probabilities associated to fixed effects on pH. In bold genes that are present in more sampling times.

	SNP	Age	Stress	HF	MG	MM	genotype
pH 3h	<b>VIM_728GA</b>	0.00	0.45	0.00	0.03	0.00	0.010
	MYH7_129CT	0.01	0.85	0.00	0.01	0.00	0.011
	IGFBP2_476CT	0.01	0.90	0.00	0.02	0.00	0.013
	<b>IGF1R_540GA</b>	0.01	0.97	0.00	0.01	0.00	0.013
	<b>SDC4_150CT</b>	0.00	0.30	0.00	0.01	0.00	0.019
pH 24h	VIM_312TA	0.04	0.67	0.01	0.96	0.37	0.001
	<b>IGF1R_540GA</b>	0.04	0.41	0.24	0.88	0.74	0.004
	PPARA_232GC	0.13	0.57	0.31	0.78	0.94	0.011
	<b>SDC4_150CT</b>	0.09	0.09	0.62	0.52	0.33	0.011
	PCSK1_33GA	0.05	0.23	0.05	0.79	0.49	0.012
	<b>VIM_728GA</b>	0.07	0.27	0.16	0.97	0.76	0.015
pH 48h	CPT2_181GT	0.04	0.31	0.15	0.53	0.88	0.016
	ACBP_227AT	0.48	0.52	0.26	0.38	0.35	0.005
	PLOD2_640GC	0.25	0.99	0.13	0.38	0.26	0.015
pH 120	MYF6_181CG	0.40	0.99	0.04	0.55	0.85	0.020
	<b>VIM_312TA</b>	0.01	0.81	0.13	0.56	0.53	0.005
	<b>IGF1R_540GA</b>	0.01	0.83	0.41	0.59	0.15	0.007
	PRKAG2_216TC	0.01	0.75	0.45	0.88	0.15	0.007
pH 10d	IGF2R_491TC	0.01	0.55	0.90	0.42	0.02	0.011
	<b>VIM_312TA</b>	0.00	0.65	0.00	0.64	0.85	0.008
	MYL2_581GA	0.02	0.75	0.15	0.27	0.31	0.012
	<b>IGF1R_540GA</b>	0.02	0.94	0.02	0.51	0.57	0.012
	AANAT_302TC	0.00	0.80	0.01	0.23	0.29	0.014

The research was supported by Regione Lazio - Progetto PRAL.

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